THE ASSOCIATION OF VITAMIN D STATUS WITH DISEASE ACTIVITY IN CANADIAN CHILDREN NEWLY DIAGNOSED WITH JUVENILE IDIOPATHIC ARTHRITIS

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ABSTRACT

Introduction: Juvenile Idiopathic Arthritis (JIA) is among the most common chronic diseases of childhood. The cause of JIA is unknown but is suspected to occur in genetically susceptible children with some unidentified environmental exposure. Vitamin D, through its genetic or environmental influences, may regulate inflammation and immune responses in JIA. To date, no 25 hydroxyvitamin D (25(OH)D) concentrations specific to children with JIA have been suggested.

Objective. The overarching goal of this research was to understand how vitamin D affects disease activity in children who are suffering from JIA. The specific objectives were to: (1) Compare vitamin D status between healthy children and patients with JIA. (2) Determine vitamin D status and its association with disease activity and outcomes in children with JIA. (3) Identify potential associations of vitamin D pathway gene polymorphisms and JIA.

Methods. Data from the Biologically-Based Outcome Predictors (BBOP) Study, a prospective multi-center study of newly diagnosed Canadian children with JIA (n=186, 2007-2012) was analyzed. Blood samples were obtained at baseline and 6 months later to measure 25(OH)D)and plasma inflammatory cytokine concentrations. Saliva was collected for genetic analysis. Vitamin D-related factors (milk intake, season of measurement, supplementation and steroid use) and clinical data to define remission were recorded every 6 months for 2 years.

First, BBOP children were compared to healthy children from the Canadian Health Measures Survey (CHMS). 25(OH)D concentrations, vitamin D related factors, measures of inflammation, and anthropometric measurements were evaluated. Longitudinal analysis then explored whether 25(OH)D and related factors could predict disease activity in BBOP children. Genome-Wide Association Studies (GWAS) techniques were then applied to identify frequent gene polymorphisms of potential relevance to the vitamin D pathway in JIA. Significant variables from linear regressions, genes identified through GWAS, vitamin D pathway genes and interactions were selected for further analysis.

Results. Mean 25(OH)D concentration was significantly higher in JIA patients (79 ± 3.1 nmol/L vs. 68 ± 1.8 nmol/L $p < 0.05$) and JIA patients used vitamin D supplements more often (50% vs. 7% $p < 0.05$). Children with JIA were more likely to be born in the fall and winter compared to healthy children. C-reactive protein concentration (CRP) and erythrocyte sedimentation rate

(ESR) decreased significantly over the 2 years ($p<0.05$). Increased 25(OH)D or its associated factors predicted lower ESR, CRP and pro-inflammatory cytokine concentrations. Overall, 36% of children achieved remission on continuing medications; 25% had sustained remission after discontinuing medication. GWAS identified the following genetic components: NOTCH4, C6orf10, HLA-DQA1, LEP, IGFBP4, and GPS1. Interactions between frequent gene polymorphisms and those in the vitamin D pathway (VDR, GC, CYP24A1, and CYP1R1) significantly predicted disease activity-related outcomes. Genes, when included, modified the association between 25(OH)D and indicators of disease activity. With and without genes in the model, drinking milk every day predicted a reduction in indicators of disease activity as measured by CRP, ESR, and interleukin-6.

Conclusion. Using 25(OH)D recommendations suggested for healthy children, 25(OH)D was adequate in the JIA population. A preponderance of JIA patients born in seasons associated with reduced endogenous vitamin D could implicate low vitamin D during gestation and early life as a factor influencing JIA pathogenesis. Milk intake, as a source of dietary vitamin D, is associated with suppression of inflammation in children with JIA. This is the first time gene and environment influences in relation to vitamin D were analyzed together in association with JIA disease activity. Environmental, biochemical, and genetic factors, including their interactions, predict disease activity in children with JIA.

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LIST OF ABBREVIATIONS

Abbreviation	Description
$1,25(OH)_2D$	1,25-dihydroxyvitamin D
25(OH)D	25-hydroxyvitamin D
AI	Adequate intake
ACR	American College of Rheumatology
ANA	Antinuclear antibody
BBOP	Biologically Based Outcome Predictors
BMI	Body mass index
CHAQ	Child Health Assessment Questionnaire
CHMS	Canadian Health Measures Survey
CLARITY	ChiLdhood Arthritis Risk factor Identification sTudY
CRP	C-reactive protein
DNA	Deoxyribonucleic acid
DMARDs	Disease-modifying antirheumatic drugs
DRI	Dietary reference intake
ESR	Erythrocyte sedimentation rate
EULAR	European League Against Rheumatism
F	Female
HLA	Human leukocyte antigen
IL	Interleukin
ILAR	International League of Associations for Rheumatology
IOM	Institute of Medicine
JCA	Juvenile chronic arthritis
JIA	Juvenile idiopathic arthritis
JRA	Juvenile rheumatoid arthritis
M	Male
MAS	Macrophage activation syndrome
NSAIDs	Non-steroidal anti-inflammatory drugs
PGA	Physician Global Assessment

PTP2	protein tyrosine phosphatase
PTPN22	lymphoid tyrosine phosphatase
RDA	Recommended dietary allowance
RF	Rheumatoid factor
RXR	Retinoid X receptor
SNP	Single nucleotide polymorphism
TNF	Tumour necrosis factor
VDR	Vitamin D receptor
VDRE	Vitamin D response element
UVB	Ultraviolet Beta

CHAPTER 1

INTRODUCTION

1.1. Rationale

Juvenile Idiopathic Arthritis (JIA) is one of the most common chronic diseases in children. The prevalence of JIA is one in 1000 in Canada, with over 7000 children and adolescents living with this disease (Bernatsky et al., 2011). Symptoms of JIA, including joint pain, morning stiffness, joint swelling, and problems with mobility, it can interfere with daily activities and overall quality of life. JIA is a cause of short and long-term disability. It has substantial economic impact on patients, their families and the health care system (Bernatsky et al., 2007; Rasu et al., 2014; Ravelli & Martini, 2007; Toupin , Cavallo, Feldman, & Ni, 2012). Recent studies suggest that vitamin D plays a role in the prevention and pathogenesis of JIA by suppressing pro-inflammatory mediators, and immune responses (Von Scheven & Burnham, 2011). Certain deoxyribonucleic acid (DNA) polymorphisms (genetic variants) are associated with susceptibility to some diseases. By determining if a polymorphism occurs more frequently in individuals genetically at risk for a disease, there is the potential for preventative care to be instituted through the development of pharmacological treatments (Israeli, 2013). Specific polymorphisms of VDR genes may be associated with different biologic responses to vitamin D. For example, the Cdx2 polymorphism of the VDR gene, specifically the GG genotype and G allele, have been suggested to be more represented in patients with JIA (Falcini et al., 2013). The environmental role of vitamin D in JIA is impacted by our intake of vitamin D through food and supplements as well as our cutaneous synthesis. These factors combined make up our circulating vitamin D concentrations in the blood. Investigating the genetic and environmental role that vitamin D plays in the prevention and control of JIA over time will help to understand the multifaceted role played by vitamin D in this disease. Understanding how genetic variants increase the risk of disease development will help guide vitamin D management in individual patients and contribute to improving control of disease activity and improve outcomes.

To date and to our knowledge, no study has investigated the relationship between vitamin D status and JIA serially from the time of the first presentation. The ultimate goal of this research was to document the vitamin D status in a JIA population and to gain insight into the association

of vitamin D status with disease activity. I compared the vitamin D status and growth outcomes for children and adolescents with JIA to a healthy age-matched population of children and adolescents. I explored the relationship of vitamin D status on markers of inflammation and measures of disease activity in children and adolescents with JIA and explored a possible genetic association between vitamin D levels and VDR gene polymorphisms. Findings from this research provide valuable information on the association of vitamin D status with disease activity and disease outcomes.

1.2. Study Objectives

1.2.1. Study 1: Vitamin D Intake and Growth Patterns of Children with Juvenile Idiopathic Arthritis

Objective: Evaluate vitamin D status and growth parameters in children and adolescents with JIA and compare with those of healthy children and adolescents.

Hypothesis: Children and adolescents with JIA have lower vitamin D status and growth parameters (height, weight, waist circumference, body mass index [BMI]) than healthy children and adolescents.

1.2.2 Study 2: Vitamin D and Biochemical Markers of Inflammation in Juvenile Idiopathic Arthritis

Objective: Examine the association between plasma levels of 25(OH)D and disease activity and outcomes as determined by clinical and biomarker profiles in JIA.

Hypothesis: Vitamin D status is negatively associated with markers indicative of inflammation and disease activity and outcomes among JIA patients.

1.2.3 Study 3: Vitamin D Gene Polymorphism in Juvenile Idiopathic Arthritis

Objective: Evaluate association between vitamin D pathway gene polymorphisms, vitamin D levels, and JIA disease activity.

Hypothesis: There are specific polymorphisms of vitamin D pathway genes associated with JIA. The rs11568820 polymorphism of the VDR gene, specifically the GG genotype and G allele, are more represented in patients with JIA.

1.3. Significance

The relationship between vitamin D status and JIA over time in newly diagnosed individuals in Canada or abroad has yet to be investigated. Investigating the potential association of vitamin D, from both the genetic and environmental (as a nutrient) perspectives, in the same children with JIA will help to tease out the multifaceted role played by vitamin D in this disease.

CHAPTER 2

LITERATURE REVIEW

2.1. Introduction

Juvenile Idiopathic Arthritis is one of the most common chronic childhood diseases. The prevalence of JIA is one in 1000 in Canada, with over 7000 children and adolescents living with this disease (Bernatsky et al., 2011). Recent studies suggest that vitamin D plays a role in the pathogenesis of JIA by suppressing pro-inflammatory mediators and immune responses (Von Scheven & Burnham, 2011). Certain DNA polymorphisms (genetic variants) are associated with susceptibility to some diseases. Specific polymorphisms of VDR genes may be associated with different biological responses to vitamin D. For example, the rs11568820 polymorphism of the VDR gene, specifically the GG genotype, has been suggested to be more represented in patients with JIA compared to healthy controls who had the GA genotype more often (Falcini et al., 2013). The role of vitamin D in JIA is influenced by vitamin D intake through food and supplements as well as cutaneous synthesis. Together these factors determine our circulating vitamin D concentrations in blood. Investigating genetic, nutritional and environmental factors that influence vitamin D in JIA could help inform ways in which vitamin D status influences the occurrence and activity of JIA. Understanding if genetic variants increase the risk of disease development will help guide vitamin D management in individual patients, contribute to improving control of disease activity, and improve outcomes.

To date and to our knowledge, no study has investigated the relationship between vitamin D status and JIA serially from the time of the first presentation. The purpose of this review is to describe the current knowledge about vitamin D in JIA, then to summarize the evidence of the potential association of vitamin D with the development and disease activity of JIA

2.2. Juvenile Idiopathic Arthritis

JIA is a term that encompasses all forms of continuous inflammation from unknown causes of one or more joints, beginning in children younger than age 16 years that lasts for at least six weeks (Petty, Southwood, & Manners, 2004). JIA is characterized by persistent joint swelling that is due to an accumulation of synovial fluid and thickening of the synovial lining (Prahalad & Glass, 2008). Symptoms of JIA include joint pain, morning stiffness, joint swelling

and decreased joint mobility; these symptoms interfere with daily activities and overall quality of life (Ravelli & Martini, 2007). The pathogenesis and influences on outcomes of JIA are still poorly understood and may include both genetic and environmental components (Ravelli & Martini, 2007).

2.2.1. Classification Systems and Clinical Characteristics

Two previous classification systems to describe chronic childhood arthritis were proposed in the 1970's. The American College of Rheumatology (ACR) (Brewer et al., 1977) classification criteria referred to chronic childhood arthritis as Juvenile Rheumatoid Arthritis (JRA). The second classification system adhered to by the European League Against Rheumatism (EULAR) applied the term Juvenile Chronic Arthritis (JCA) (Woods, 1978). Key differences between the two classifications systems prevented interchangeable use and hindered comparison of research among users of the two systems (Borchers et al., 2006). To reconcile differences between ACR and EULAR criteria, the International League Against Rheumatism (ILAR) criteria were created and the term JIA was adopted. The criteria were further revised in 2001 (Petty et al., 2004). The three classification systems are summarized in Table 2.1. When quoting the literature, the terminology for chronic childhood arthritis that was used at the time of publication of the respective referenced citations will be used in this review.

Juvenile idiopathic Arthritis can be further classified into seven categories based on inclusion and exclusion criteria according to features present within the first six months of disease (Table 2.2). These categories are systemic, oligoarticular, polyarticular with positive rheumatoid factor (RF positive), polyarticular without rheumatoid factor (RF negative), psoriatic arthritis, and enthesitis-related arthritis. A seventh category of patients who do not fit into one category, meet criteria for more than one category, or have exclusion criteria that preclude assigning a category are classified as undifferentiated. Most subtypes of JIA occur more frequently in females; oligoarticular JIA, which constitutes 27-56% of all JIA, is approximately three times more common in females than males (Ravelli & Martini, 2007).

Table 2.1. Comparison of classification systems of chronic childhood arthritis

	American College of Rheumatology 1977	European League Against Rheumatism 1978	International League Against Rheumatism 1994 and 2001
Symptom duration	Minimum 6 week	Minimum 3 month	Minimum 6 week
Classification Title	Juvenile Rheumatoid Arthritis	Juvenile Chronic Arthritis	Juvenile Idiopathic Arthritis
Subtypes	Systemic	Systemic	Systemic
	Polyarticular	Polyarticular	Polyarticular RF negative
		JRA (RF positive Polyarticular)	Polyarticular RF positive
	Pauciarticular	Pauciarticular	Oligoarthritis
			Persistent
			Extended
		Juvenile psoriatic	Psoriatic arthritis
		Juvenile ankylosing spondylitis	Enthesitis-related arthritis
		Arthritis associated with inflammatory bowel disease	
			Undifferentiated arthritis

Adapted from (Borchers et al., 2006)

Table 2.2. Summary of JIA types

Type	Sex Ratio M:F	% of Total JIA cases	Age of Onset	Diagnosis (ILAR)
Oligoarthritis Persistent Extended	1:4	27-56%	Early childhood; peak at 2-4 years	*Definition:* Arthritis is affecting one to 4 joints during the first six months of disease. Two subcategories are recognized: 1. Persistent oligoarthritis: Affecting not more than 4 joints throughout the disease course 2. Extended oligoarthritis: Affecting a total of more than four joints after the first six months of disease *Exclusions:* a, b, c, d, e.
Rheumatoid-factor-positive polyarthritis	1:4	2-7%	Late childhood or adolescents	*Definition:* Arthritis affecting 5 or more joints during the first six months of disease; 2 or more tests for RF at least three months apart during the first six months of disease are positive. *Exclusions:* a, b, c, e.
Rheumatoid-factor-negative polyarthritis	1:3	11-28%	Biphasic distribution; early peak at 2-4 years and later at 6-12 years	*Definition:* Arthritis affecting 5 or more joints during the first six months of disease; a test for RF is negative. *Exclusions:* a, b, c, d, e.
Enthesitis-related arthritis	M>F	3-11%	Late childhood and adolescents	*Definition:* Arthritis and enthesitis, or arthritis or enthesitis with at least 2 of the following:

				1. The presence of or a history of sacroiliac joint tenderness and/or inflammatory lumbosacral pain 2. The presence of HLA-B27 antigen 3. Onset of arthritis in a male over six years of age 4. Acute (symptomatic) anterior uveitis 5. History of ankylosing spondylitis, enthesitis-related arthritis, sacroiliitis with inflammatory bowel disease, Reiter's syndrome, or acute anterior uveitis in a first-degree relative *Exclusions:* a, d, e.
Psoriatic arthritis	M<F	2-11%	Biphasic distribution; early peak at 2-4 years and later peak at 9-11 years	*Definition:* Arthritis and psoriasis, or arthritis and at least 2 of the following: 1. Dactylitis 2. Nail pitting or onycholysis 3. Psoriasis in a first-degree relative *Exclusions:* b, c, d, e.
Undifferentiated arthritis	…	11-21%	….	*Definition:* Arthritis that fulfills criteria in no category, or in 2 or more of the above categories.

Exclusions:

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

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

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

d. The presence of IgM rheumatoid factor on at least two occasions at least three months apart.

e. The presence of systemic JIA in the patient.

Adapted from (Borchers et al., 2006; Petty et al., 2004; Ravelli & Martini, 2007; Rigante, Bosco, & Esposito, 2014)

2.2.2. Epidemiology

2.2.2.1. Global Perspective

Estimates of the incidence and prevalence of JIA have varied. A review of worldwide chronic childhood arthritis (JRA, JCA and JIA) prevalence found that prevalence ranged from 0.07 to 4.01/1000 children, and annual incidence ranged from 0.008 to 0.226/1000 children (Manners & Bower, 2002). This review presented results from 34 studies conducted since 1966 and found a higher prevalence of JIA in studies using a community assessment approach (Manners & Bower, 2002). Ellis et al. (2010) hypothesized that the wide variability in incidence and prevalence rates is due to the presence of undiagnosed children in the community with mild illnesses, who have never presented to clinics. Manners and Bower noted that the studies they summarized used a variety of diagnostic criteria, sampling methods (clinic versus community-based), clinician experience in disease diagnoses, sample sizes and ethnic groupings to estimate the incidence and prevalence of chronic childhood arthritis, this contributes to the variability in their results (Manners & Bower, 2002).

2.2.2.1.1. Geographic Location

Differences have been found in the incidence of various JIA subtypes based on geographic location or ethnicity. There is evidence that latitude as a marker of vitamin D synthesis may potentially influence JIA incidence and sub-type. Latitude and season of measurement are important considerations for vitamin D status and have been explored in relation to other autoimmune diseases (Ellis, Munro, & Ponsonby, 2010). For Western countries, the most common JIA subtype is oligoarthritis; this type is rare in countries such as Costa Rica, India, New Zealand and South Africa where the most commonly diagnosed subtype of JIA is polyarthritis (Ravelli & Martini, 2007).

In Europe, JIA incidence follows a north-south gradient, with a lower annual incidence of JIA in more southern countries (Gäre, 1999). It was also noted by Gäre (1999) that the incidence of JIA in Costa Rica was outside the confidence interval for that of Nordic countries, suggesting an environmental and/or genetic influence of disease occurrence and course. The difference in JIA incidence between Germany (35/100000 children) and the United States (11.7/100000 children) was discussed by Berkun et al. (Berkun & Padeh, 2010). It was hypothesized that the

differences in incidences could be due to environmental, genetic or study methodological differences. A temporal variation in JIA incidence was observed in Minnesota. The incidence of JRA decreased in Minnesota over a 33 year period with the most noticeable reductions in the pauciarticular and systemic subtypes (Peterson et al., 1996). The authors suggested that environmental factors such as infectious agents may be contributing to this pattern (Peterson et al., 1996).

2.2.2.1.2. Prevalence and Incidence in Canada

The majority of studies reviewed in the systematic review performed by Manners and Bower (Manners & Bower, 2002) were conducted in Caucasian populations; however the populations studied that were not Caucasian had a lower prevalence of disease. Considering ethnicity when comparing incidence and prevalence amongst populations would be useful in understanding if the regional differences observed were due to environmental or genetic factors or a combination of the two. A multiethnic cohort study of 1082 children at The Hospital for Sick Children, Toronto, Ontario was conducted to investigate the influence of ethnicity on the risk of developing JIA (Saurenmann et al., 2007). When the diversity of the study population was compared to that of the general Toronto region population, there was an overrepresentation of patients of European and Indigenous descent and an underrepresentation of patients of Black, Asian, or Indian subcontinent origin in their cohort. European descent was significantly associated with an increased risk of developing JIA, including all subtypes except RF positive polyarticular JIA.

A higher prevalence of rheumatic diseases is reported in Indigenous children (Jarvis & Cleland, 2003). Hence, large Indigenous populations in Saskatchewan may explain why Saskatchewan and Manitoba have a higher prevalence of childhood chronic arthritis than other provinces where the prevalence of JIA has been reported (Shiff et al., 2014). Children of Indigenous ancestry were more likely to develop polyarticular RF-positive JIA (Saurenmann et al., 2007).

In 1982 the prevalence of JRA among Western Canadian Indigenous children was reported by Rosenberg et al (Rosenberg et al, 1982). Using data from two pediatric rheumatic disease clinics, one in Winnipeg, Manitoba and one in Vancouver, British Columbia the prevalence of JRA for Indigenous children was 36/100,000 children compared to 20/100,000 for

Caucasian children (Rosenberg et al, 1982). Like Saurenmann et al., (Saurenmann et al., 2007) JRA, polyarticular RF positive subtype was significantly more common in the Indigenous population. A 3-5 year cyclical pattern was also found in Manitoba by Oen et al. (Oen, Fast & Postl, 1995). Peaks in JRA in 1979, 1982, 1986, and 1990-91 corresponded with increases in confirmed Mycoplasma pneumonia infection at those time points (Oen, Fast & Postl, 1995). Using a prospective disease registry, data on 3269 consecutive cases of JRA were collected from the Pediatric Rheumatology Clinic at the University of Saskatchewan in Saskatoon, Saskatchewan. The clinical point prevalence and clinical referral incidence were reported as 35.0/100,000 children and 4.7/100,000 children respectively (Rosenberg, 2005).

Both vitamin D status and JIA have genetic and environmental components, including the impact of latitude. Determining if the differences in JIA prevalence in varying ethnicities is due to genetic or environmental differences in vitamin D status and metabolism will help to focus research on the appropriate risk factors.

2.2.3. Extra-articular manifestations of JIA

Certain subtypes of JIA are more likely to present with specific extra-articular manifestations and some extra-articular manifestations (such as fever with systemic JIA) are included as criteria for JIA category assignment.. Extra-articular manifestations of systemic JIA include a high spiking fever, rash, serositis, lymphadenopathy, hepatosplenomegaly, pericarditis, and growth disterbances (both whole body or localized) (Boros, 2010; Hahn & Kim, 2010). Like systemic JIA, an extra-articular manifestation of psoratic JIA is included in the subtype diagnosis of the disease. The definition of psoratic arthritis includes the extra-articular manifestation of either psoriasis or two of the following: dactylitis, nail pitting, onycholysis, or psoriasis in a first degree relative (Petty et al., 2004). In patients with RF positive polyarthritis, the most common extra-articular manifestation is the development of subcutanious nodules with approximatly 30% of RF positive patients developing nodules within the first year (Cassidy, Petty, Laxer & Lindsley, 2011) .

Three examples of serious extra-articular manifestations that can be associated with certain JIA subtypes are chronic asymptomatic uveitis, macrophage activation syndrome (MAS) and secondary amyloidosis. Chronic asymptomatic uveitis can be present in up to 30% of oligoarticuar JIA patients and can be present before other JIA symptoms present (Borchers et al.,

2006). Uveitis associated with JIA is a potential cause of childhood blindness (Bou et al., 2015). Chronic, asymptomatic anterior uveitis can also occur in children with RF- polyarthritis and psoriatic JIA. Acute, symptomatic uveitis can occur in enthesis-related arthritis (Angeles-Han & Prahalad, 2010; Borchers et al., 2006). Risk factors for chronic, asymptomatic uveitis include young onset age, female sex, oligoarthritis, and a positive antinuclear antibody (ANA) test (Angeles-Han & Prahalad, 2010; A Consolaro et al., 2014). As uveitis associated with JIA is usually asymptomatic, regular screening and monitoring by an ophthalmologist are recommended. Guideliness for follow-up frequency based on the risk of uveitis have been suggested by an interdisciplinary panel of experts with a special interest in uveitis associated with JIA (Bou et al., 2015).

MAS is a potentially fatal complication of systemic JIA (Borchers et al., 2006). MAS is associated with excessive activation and proliferation of T cells and macrophages which lead to a massive systemic inflammatory reaction (Grom, 2004; Yokota et al., 2015). The clinical manifestation of MAS include persistent fever, mental status changes, lymphadenopathy, hepatosplenomegaly, liver dysfunction, and thrombocytopenia (Grom, 2004).

The third condition is secondary amyloidosis (Woo, 2006). Amyloidosis is characterized as abnormal accumulation of amyloid protein deposits in tissues and organs. Secondary amyloidosis frequently affects the kidneys and the gastrointestinal tract (Okuda & Takasugi, 2006). There has been a decline in the incidence of amyloidosis within the past two decades (Immonen et al., 2011). The prevalence of amyloidosis secondary to JIA is reported to be 1.8% in the United States of America (Immonen, Savolainen, & Hakala, 2016). The surveillance of, prevention and management of these complications are crucial for the long-term quality of life of the child (Gowdie & Tse, 2012). To our knowledge, no study has linked these complications to vitamin D status. They are, however, important in describing the severity of the disease and may be important covariates in describing inflammatory status.

2.2.4. Etiology and Pathogenesis

The etiology of JIA is unknown and the pathogenesis of JIA not well understood. Given the clinical heterogeneity of the disease there are most likely different etiologies and pathogenic mechanisms for the various JIA categories. Multiple genetic and environmental interactions are

believed to be involved resulting in the dysregulation of the immune system (Moncrieffe, Prahalad, & Thompson, 2014; Ravelli & Martini, 2007).

Genetic

Ethnic differences in epidemiologic studies and an increased risk of JIA in patients' relatives form evidence for the contribution of genetic factors influencing the risk of developing JIA (Manners & Bower, 2002; Saurenmann et al., 2007). The prevalence of JIA in twins and siblings of JIA patients is 15-30 times higher than that of the general population. There is also concordance in the age of onset, and disease course for diagnosed pairs (Ellis, Munro, & Ponsonby, 2010; Prahalad & Glass, 2008). While a heritable risk component is thought to be present, JIA is believed to be determined by both genetic and environmental factors. JIA does not exhibit classic Mendelian inheritance patterns meaning the cause of JIA cannot be attributed only to variations in a single gene locus (Ellis et al., 2010; Oliver & Silman, 2009; Prahalad & Glass, 2008).

Variants at the human leukocyte antigen (HLA), protein tyrosine phosphatase (PTP2) and lymphoid tyrosine phosphatase (PTPN22) loci are associated with JIA (Angeles-Han & Yeh, 2012; Ellis et al., 2015). Both HLA and PTPN22 play a role in the development of other autoimmune diseases (Ellis et al., 2010). HLA is estimated to account for 17% of the concordance of JIA occurrence in siblings (Prahalad & Glass, 2008). Other genes currently being explored include VTCN1, IL-2RA/ CD25 (encoding IL-2 receptor a), TRAF1-C5 locus (encoding TNF receptor-associated factor 1 and complement component 5), TNFA *MIF* (polymorphisms in the migration inhibitory factor). The *NRAMP1* gene may also play a role in a predisposition to JIA (Ellis et al., 2010; Oliver & Silman, 2009; Prahalad & Glass, 2008). These loci, however, still need to be confirmed. It is important to note that the concurrence rate for JIA development in twins is only 25% (Prahalad, 2006). Therefore, while genetics is likely to have some influence on the occurrence of JIA, additional factors are involved in the pathogenesis of JIA. Exploring genetic interactions between multiple genes involved in the inflammatory pathway in conjunction with the study of potential environmental influences will be required to fully explore the pathogenesis of the disease.

Environmental Factors

Multiple environmental factors have been suggested to impact the development and progression of JIA in children with a susceptible genetic predisposition (Table 2.3). The environmental exposure (or exposures) have yet to be fully explored with very few studies conducted in this area. Therefore, these factors have yet to be proven as causal events in the disease pathway (Burke et al., 2005; Ellis et al., 2010).

Table 2.3. Environmental factors explored for association in the development of JIA

Short duration of breastfeeding
Stress and psychological factors
Infectious agents and microbe exposure
Maternal smoking
Season of birth
Vitamin D status

Adapted from (Berkun et al., 2015; Berkun & Padeh, 2010; Ellis et al., 2010; Neufeld, Karunanayake, Maenz & Rosenberg 2013; Rigante et al., 2014)

Duration of breastfeeding

A JIA biobank study from Melbourne, Australia called CLARITY - ChiLdhood Arthritis Risk factor Identification sTudY, has the broad aim of identifying genomic and environmental disease risk factors associated with JIA (Ellis et al., 2012). This study follows children ≤18 years of age with a diagnosis of JIA by 16 years of age (cases) and healthy children ≤18 years of age, born in the state of Victoria, undergoing a minor elective surgical procedure (controls) (Ellis et al., 2012). In the most recent publication, the CLARITY study reports no significant difference in the commencement of breastfeeding among cases or controls. Additionally, for those who were breastfed no significant difference in duration of breastfeeding has been found. A significant difference in the introduction of cow's milk has been found amongst cases and controls with cases starting cow's milk at a younger age. However, the association was not present after adjustment for covariates (Ellis et al., 2012). Mason et al. (1995) reported a case-control study of 54 mothers of children with JRA and 79 playmates from North Carolina, that children who developed JRA, especially pauciarticular JRA, were less likely to have been

breastfed (Mason et al. 1995). They also noted a lower odds ratio for increased duration of breastfeeding in children with JRA and concluded that breastfeeding might have a protective effect on the development of JRA. A larger longitudinal case-controlled population survey of children in Saskatchewan, Canada found no statistically significant difference in breastfeeding rates amongst 137 JRA cases and 331 controls (Rosenberg, 1996). Additionally, no difference in breastfeeding history and duration was found between the cases and controls.

Stress and psychological factors

Neufeld et al. (2013) investigated patients presenting for their first visit to a pediatric rheumatology clinic in Saskatoon, Canada between 1981 and 2010 for JRA symptoms. Patients were more likely than controls (unrelated child of the same age and sex, living in the same geograhical region at approximately the same time) to have experienced a stressful life event such as a serious upset, a currently ill family member, separated parents, or difficulties with interpersonal relationships (Neufeld et al., 2013). The suggested mechanism for the relationship between experiencing a stressful life event and the expression of juvenile arthritis relates to how certain pro-inflammatory cytokine single nucleotide polymorphisms (SNPs) are associated with heightened cytokine production in response to stress and that some individuals through genetic predisposition may experience an exaggerated stress-induced inflammatory response (Neufeld et al., 2013).

Infectious agents and microbe exposure

It has been suggested that exposure to infectious agents or exposure to certain microbial agents may lead to the development of JIA in genetically susceptible individuals (Carlens et al., 2009; Ellis et al., 2010; Ellis et al., 2012; Rigante et al., 2014). Also, a lack of exposure to microbial agents has been suggested to be associated with immune disorders (hygiene hypothesis). Caesarean delivery has been associated with an increased risk of childhood immune disorders; this may be related to a lack of exposure to vaginal and intestinal flora during birth (Ellis et al., 2010; Neu & Rushing, 2011). A case-control study conducted in Sweden by Carlens et al. (2009) investigating perinatal characteristics, early life infections, and later risk of rheumatoid arthritis and JIA, discovered that being hospitalised for any infection during the first year of life was associated with the risk of later JIA (Carlens et al., 2009). A borderline

statistically significant increase in the risk of JIA development was also observed for individuals born after more than 42 gestational weeks and for those delivered by Caesarean section (Odds Ratio 1.1:1, 95% confidence interval 1.0 to 1.3) (Carlens et al., 2009). The CLARITY study, however, found no risk associated with caesarean delivery and JIA development (Ellis et al., 2012).

A review by Rigante et al. (2014) investigated how different external antigens could stimulate multiple antigen-specific pathways such as cytotoxic T cell responses, to produce proinflammatory cytokines (Rigante et al., 2014). They reviewed the possible role of parvovirus B19, Epstein-Barr virus, Salmonella, Shigella, Campylobacter, Mycoplasma pneumoniae, Chlamydophila pneumonia, Bartonella henselae, and Streptococcus pyogenes for the development of immune-mediated JIA. The review found no clear evidence for any of the viral or bacterial agents investigated (Rigante et al., 2014).

Maternal smoking

Symmons et al. (2005) surmised that smoking might play a role in the development of JIA through a number of mechanisms (Symmons, 2005). Smoking may directly cause immunological abnormality in children. This is hypothesized to increase susceptibility to childhood infections and might trigger arthritis. Maternal smoking, while the child is *in utero*, may be a marker for exposure to second-hand smoke during the early years of life, (the mother may continue to smoke after pregnancy) and that may trigger the autoimmune response.

Smokers have been reported to have abnormalities in T-lymphocyte function, a reduction in the number of natural killer cells, and abnormalities in humoral and cellular immunity. This phenomenon may occur in children exposed to second-hand smoke (Symmons, 2005). Symons also discusses the correlation between maternal smoking and reduced likelihood of breastfeeding which could potentially be the link between maternal smoking and JIA. This controversy will require further investigation. A case-control study of 1196 JIA patients and randomly selected healthy controls from Washington State conducted from 1997-2010 did not observe an increased risk of JIA, overall or in relation to JIA subtypes, with maternal prenatal smoking. They found smoking to occur less often in mothers of JIA cases (Shenoi S, Bell S, & Wallace CA, 2015). The study by Carlens et al. (2009) was conducted using a nationwide registry-based case-control of Swedish inpatients, the early arthritis registry found that maternal smoking did not

significantly alter the risk of developing JIA (Carlens et al., 2009). In the CLARITY study, fewer mothers and fathers of cases reported any smoking at the time of the interview (Ellis et al., 2012). There is still uncertainty as to what role if any maternal smoking plays in the pathogenesis of JIA.

As a link between the above environmental factors is currently being investigated for their role in the development of JIA, they are important covariates to include in the analysis of the impact of other environmental measurements on JIA.

Season of birth

Season of birth has been suggested to have an impact on the risk of developing a number of autoimmune diseases such as multiple sclerosis, type 1 diabetes and celiac disease (Berkun et al., 2015). A recent study investigating month of birth and risk of JIA found a difference in the pattern of the birth month for children with JIA compared to that of the general population (Berkun et al., 2015). Children with JIA were more likely to be born between November to March with the birth month for the general population peaking in the summer months. The study by Carlens et al. (2009) also investigated the relationship between season of birth and the risk of developing JIA and found no increased risk (Carlens et al., 2009). Season of birth may be a marker for vitamin D status *in utero* with children born in the non-vitamin D synthesizing periods being exposed to less vitamin D during the third trimester than those who are born during the vitamin D synthesizing seasons.

Vitamin D status

Recent studies suggest that vitamin D plays a role in the pathogenesis of JIA because vitamin D might be immunosuppressive (Cutolo, Pizzorni, & Sulli, 2011). Therefore, low levels would be associated with inflammation (Cutolo et al., 2011). The role of vitamin D in other autoimmune disorders has been established but has not been fully explored in relation to JIA (Holick, 2005). The CLARITY study explored the use of nutritional supplements during pregnancy and the risk of developing JIA. The use of vitamin D and fish oil during pregnancy was lower in case mothers than controls but the association was not statistically significant following covariate adjustments (Ellis et al., 2012). A case-cohort investigation from Denmark, comparing the 25(OH)D status in children diagnosed with either oligoarthritis or polyathritis

JIA using dried blood spot samples that were collected at birth, did not find any association between 25(OH)D status at birth and JIA risk development risk (Thorsen et al., 2016). Concentrations of 25(OH)D fluctuated statistically significantly by season of birth and year. There was no follow up as to if there was an impact of 25(OH)D status or season during the first year of life impacted risk of JIA or whether other subtypes of JIA were impacted by season of birth of 25(OH)D status at birth

Pathogenesis of JIA

The pathogenesis of JIA is thought to involve both the innate and adaptive immune system (Prahalad & Glass, 2008). In genetically susceptible individuals exposed to an environmental trigger, the synovial tissue comprises various auto-antigens and is infiltrated by inflammatory cells including neutrophils, plasma cells, dendritic cells and activated T-cells. The trigger activates both the innate and adaptive immune system to up-regulate local inflammation (Borchers et al., 2006; Prahalad & Glass, 2008; Prakken, Albani, & Martini, 2011; Reinards et al., 2014).

Activated T-cells have been found in high levels in the synovium of patients with JIA (Borchers et al., 2006). It is believed that autoreactive T-cells (mainly Th1 cells are the initiator of insult that lead to the cascade of inflammation (Prakken & Albani, 2009). Monocytes, macrophages, fibroblasts and T cells within the inflamed synovial fluid in response then secrete mediators that interact directly with the surrounding tissue and have a pro-inflammatory effect (de Jager et al., 2008). T-cells produce pro-inflammatory cytokines including interleukin (IL)-2, IL-6, interferon-γ, and tumour necrosis factor (TNF)-β and-α (Prahalad & Glass, 2008). IL-1 and TNF-α stimulate the release of tissue-destroying matrix metalloproteinases and inhibit the production of the enzymes that inhibit metalloproteinases (Hahn & Kim, 2010). This is what causes joint damage (Hahn & Kim, 2010). Interleukin-1 also stimulates the production of IL-6 which is associated with clinical and laboratory measures of disease activity in children with JIA (Hahn & Kim, 2010; Świdrowska-jaros, Orczyk, & Smolewska, 2016). IL-12 p40 levels also showed a significant positive correlation with CRP and ESR and a negative correlation with disease duration (Borchers et al., 2006).

2.2.5. Diagnosis and Assessment

There are no definitive diagnostic tests for JIA; a diagnosis is made clinically and requires excluding other diagnostic possibilities. The ILAR criteria are then applied to categorize the JIA subtype. (Petty et al., 2004).

2.2.5.1. Biochemical Tests for Diagnosis and Follow-up

There are no specific diagnostic laboratory tests for JIA. Laboratory tests are performed to monitor inflammation status or secondary complications of the disease. Table 2.4 presents the commonly performed laboratory tests. The erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) are non-specific markers of inflammation (Tugal-Tutkun & Quartier, 2014). The Antinuclear Antibody (ANA) test is performed in children with JIA as a positive test indicates a higher risk of developing iridocyclitis (Breda et al., 2012). ANA positive children tend to be female, have asymmetric arthritis, and an early disease onset (Breda et al., 2012). ANA's are detected in approximately 30-50% of JIA patients (Borchers et al., 2006). Testing positive for Rheumatoid Factor is associated with more aggressive joint disease in children and adults (Breda et al., 2012). In children, it can also impact the growing skeleton either by impeding overall growth or by accelerating growth in the affected joint (Prakken et al., 2011). RF positive polyarthritis impacts approximately 5% of JIA patients (Prakken et al., 2011).

Table 2.4. Commonly performed biochemical measurements

Erythrocyte sedimentation rate	Antinuclear Antibodies
C-reactive protein	Rheumatoid Factor

(Borchers et al., 2006; Breda et al., 2012; Ravelli & Martini, 2007; Tugal-Tutkun I, Quartier P, 2014)

2.2.6. Treatment

While there is no cure for the disease, treatments and therapies are used to manage the disease and improve the quality of life. Treatment of JIA is based on pharmacotherapy, physiotherapy, occupational therapy, and psychosocial support (Prakken et al., 2011). In 2011

the ACR published recommendations for the treatment of JIA which provided guidance for the use of therapeutic agents (medications) (Beukelman et al., 2011). The ACR did not use the ILAR sub-types in providing their recommendations. Instead, they grouped children into five distinct "treatment groups". These groups are 1) History of arthritis of 4 or fewer joints, 2) History of arthritis of 5 or more joints, 3) Active sacroiliac arthritis, 4) Systemic arthritis with active systemic features (and without active arthritis) and 5) Systemic arthritis with active arthritis (and without active systemic features). For each treatment group there is a therapeutic treatment pathway recommended, a description of the features of poor prognosis and methods to classify disease activity level (Beukelman et al., 2011). In 2013 updated recommendations were published by the ACR for the treatment of systemic JIA and for screening children receiving biologics for tuberculosis (Ringold et al., 2013).

Most patients are first treated with non-steroidal anti-Inflammatory drugs (NSAIDs). However, early treatment with disease-modifying anti-rheumatic drugs (DMARDS) for certain JIA subsets is required to improve long-term outcomes. (Borchers et al., 2006). Another treatment option occasionally used is corticosteroids, either oral or provided by intra-articular injection (Gowdie & Tse, 2012). DMARDs help prevent irreversible damage and reduce long-term exposure to medications with harmful side effects such as corticosteroids. (Gowdie & Tse, 2012). Biologic agents are a group of medications that blocks the body's inflammation response. They are used mostly in treating systemic and polyarticular JIA. They are occasionally used in the treatment of enthesitis-related arthritis and chronic uveitis. While biologic therapies are effective in treating JIA in patients who do not respond to standard therapies use can be limited in some jurisdictions because of their high costs (Gowdie & Tse, 2012). Each medication has its own side effect profile that can impact quality of life and nutritional status such as gastro-esophageal reflux, nausea, stomach ulcers, elevated blood glucose, increased appetite and risk of infection as shown in Table 2.5. These factors can lead to an imbalance of energy and nutrients and subsequently affect growth and development in children with JIA.

Table 2.5. Summary of side effects of JIA medication classes

Medication	Side effects
NSAIDs	Edema (feet), gastro-esophageal reflux, nausea, stomach ulcers and possibly increased the risk of blood clots, heart attack, and stroke.
Corticosteroids	Cataracts, elevated lipids and blood glucose, increased appetite, weight gain and bone loss.
DMARDs	Nausea and increased susceptibility to infection. (Other side effects vary by drug)
Biologic agents	Injection or infusion site reactions, including redness, swelling and increased risk of serious infections. Other side effects vary by drug.

Adapted from (Borchers et al., 2006; Gowdie & Tse, 2012; Ringold & Wallace, 2007)

2.2.7. Outcome Measures of Disease Activity

Various survey tools have been developed to measure the success of a treatment or define improvement of a patient with JIA. The three terms that are most often used to describe changes in disease activity are disease improvement, disease flare and disease remission (Ringold et al., 2013). As no individual measure is an accurate indicator of disease activity, composite scores that combine a number of indices, have been developed to reflect the multifaceted nature of the disease (Duffy, 2007). These indices can be used to follow changes in response to treatment of individuals or differences in treatment response between groups in clinical trials (Ringold & Wallace, 2007).

The ACR has outlined a core set of outcomes for use when defining clinically important patient improvement in pediatric patients. This is called the ACR Pediatric 30 (Giannini et al., 1997). This set of criteria was initially for use in clinical trials as opposed to monitoring improvement in individuals in a clinic setting The ACR30 outlines six components 1) Physician global assessment (PGA) of overall disease activity (measured on a 10 cm visual analog scale), 2) parent or patient global assessment of overall well-being (measured on a 10 cm visual analog scale), 3) functional ability (Childhood Health Assessment Questionnaire (CHAQ)), 4) number of joints with active arthritis, 5) Number of joints with limited range of motion, 6) ESR as shown

(Table 2.6). Disease improvement is defined as a minimum improvement of 30% from the baseline measure in any three of the six components, with a worsening of no more than one of the components (Giannini et al., 1997). A disease flare was defined by Brunner et al. for patients with polyarticular JRA as a less than 40% worsening of a minimum of two of the six ACR Pediatric 30 components, with no more than one component improving by more than 30% (Brunner, Lovell, Finck, & Giannini, 2002). Inactive disease is defined by Wallace et al. as when all six of the criteria in Table 2.7 are met (Wallace, Ruperto, Giannini, & Arthritis, 2004). There are two types of clinical remission, clinical remission on medication and clinical remission off medication, which are also described in Table 2.7.

A limitation of the ACR Pediatric 30 Criteria is that it does not quantify disease activity of an individual in a way that allows for comparison between individuals. The Juvenile Arthritis Disease Activity Score (JADAS) is a composite score that combines four components and was developed for this purpose. It combines four indices, 1) physician global assessment of disease activity which is measured on a 10 cm visual analog scale, 2) parent or patient global assessment of well-being which is also measured using a 10 cm visual analog scale, 3) active joint count in 10, 27 or 71 specified joints, 4) ESR, which has been normalized to a scale of 0-10 (Consolaro et al., 2012). The JADAS score has not been established as a measure for clinical practice by the ACR; there are however suggested numerical cut-off values for inactive, mild, moderate and highly active disease. The values available are for all types of JIA and specific cut-offs for oligoarthritis and polyarthritis are not defined. Given that the number of joints with active arthritis is a part of the definition of the subtypes as well as a measurement of disease activity, the cut-offs are not appropriate for comparison between subtypes.

Table 2.6. Core set of outcome variables for the ACR 30

1. Physician global assessment of overall disease activity
2. Parent or patient global assessment of overall well-being
3. Functional ability
4. Number of joints with active arthritis
5. Number of joints with limited range of motion
6. ESR

Adapted from (Giannini et al., 1997)

Table 2.7. Criteria for the clinical remission of JIA

1. No joints with active arthritis
2. No fever, rash, serositis, splenomegaly, or generalized lymphadenopathy attributable to JIA
3. No active uveitis
4. Normal ESR and/or CRP
5. Physician's global assessment of disease activity indicates no disease activity
Key Definitions
Inactive disease: all five criteria must be met
Clinical remission on medication: The criteria for inactive disease must be met for a minimum of 6 continuous months while the patient is on medication in order for the patient to be considered to be in a state of clinical remission on medication.
Clinical remission off medication: The criteria for inactive disease must be met for a minimum of 12 continuous months while off all anti-arthritis and anti-uveitis medications in order for the patient to be considered to be in a state of clinical remission off medication.

Adapted from (Wallace et al., 2004)

2.3. Nutrition and Juvenile Idiopathic Arthritis

Nutritional impairment in JIA has been assessed using a number of anthropometric and biochemical methods. However, there is no consensus on the optimal nutritional assessment method for this population (Cleary, Lancaster, Annan, Sills, & Davidson, 2004). Children with JIA often experience nutrition-related concerns such as protein energy malnutrition, micronutrient deficiencies and growth abnormalities (Dinardo et al., 1991). Inadequate intake due to mechanical feeding problems, reduced physical activity, joint pain and inflammation, drug side effects, fatigue, and disease activity status may influence nutrient requirements (Dinardo et al., 1991). Growth can be impacted by a number of interrelated facets including medication side effects, inflammation, poor nutrition, lack of exercise, genetic background, disease severity and duration (see Figure 2.1.) (Marcovecchio, Mohn, & Chiarelli, 2012).

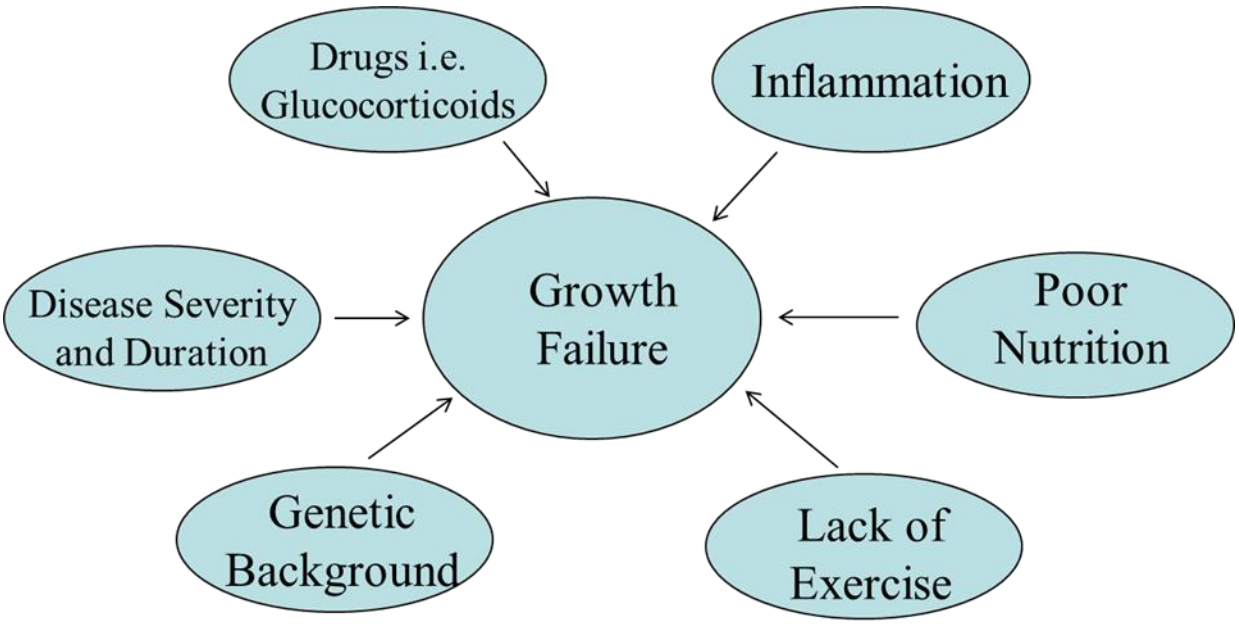

Figure 2.1. Factors impacting growth in children with JIA
(adapted from (Marcovecchio et al., 2012)

Nutritional impairment, while not the sole cause, can contribute to reduced growth, osteoporosis, anemia and suboptimal body composition in children with JIA (Cleary et al., 2004). Delayed or retarded growth in children with JIA has been established and is hypothesized to be related to disease activity (Liem & Rosenberg, 2003). In 1990, when Bacon et al. investigated the relationship between growth and dietary intake in children with JRA, it was found that one-third of the children studied were at or below the tenth percentile in height for age (Bacon et al., 1990). Overall, meal caloric and nutrient intakes were found to be adequate when compared to the Recommend Daily Allowance (RDA) of the National Academy of Science. No correlation was found linking dietary intake to growth percentiles. Biochemical measurements of dietary intake showed abnormalities amongst the subtypes of JRA and discrepancies between dietary intake and blood values (low plasma levels of vitamins A and C, albumin, prealbumin, retinol binding protein and zinc; and increased concentrations of copper and glutathione peroxidase activity). It was hypothesized that this was due to altered absorption, requirement or that there was increased utilization due to chronic inflammation (Bacon et al., 1990). Mixed results on the impact of JIA on body weight have been reported with both evidence of under and over nutrition available (Caetano et al., 2009; Cleary et al., 2004; Dey, Jahan, Yadav, Bhagwani,

& Sachdev, 2014; Markula-Patjas et al., 2014). These mixed results are most likely due to an individual's response to the disease activity through either reduced intake, increased requirement and/or physical activity.

Growth failure has been estimated to occur in 11-41% of patients with JIA (Gaspari, Marcovecchio, Breda, & Chiarelli, 2011). Growth failure occurs most often in children with systemic and polyarticular JIA and although less severe, it also presents in children with oligoarticular JIA (Marcovecchio et al., 2012). A study by Liem et al. in which the height for age z-score of 67 children with JRA had been followed for at least five years (most until their 18th year), found a delay in height in some children (Liem & Rosenberg, 2003). Additionally, children with RF-positive polyarticular and systemic JRA had more significant growth retardation than children with other subtypes of JRA, and in some cases, they did not recover the lost height. Rooney et al. reported that the degree of growth retardation is associated with the duration of the disease activity (longer duration of disease activity reduces growth potential) and that disease remission can improve growth (Rooney, Davies, Reeve, Preece, & Ansell, 2000). It was concluded that normal height can be achieved through catch-up growth after 2-3 years of disease remission unless the epiphyseal growth nuclei had already fused.

The relationship between disease activity and growth failure is described by Gaspari et al. (Gaspari et al., 2011). Pro-inflammatory cytokines such as IL-6, IL-1β and TNFα may act locally or systemically through altering growth hormone secretion (IL-6) or by directly affecting the grow plate chondrocytes of long bones (IL-1β and TNFα). Okumus et al. compared height standard deviation scores, bone mineral density and biochemical measures in children with JIA to healthy age and gender matched controls (Okumus, Erguven, Deveci, Yilmaz, & Okumus, 2008). Children with JIA were found to be shorter than controls and have lower bone mineral density. Steroid use was shown to have a negative effect on growth and children with JIA had significantly lower IGF1 than controls.

As new medications and treatments become available, growth outcomes may improve. While there is currently no standard nutrition therapy or specific diet for children with JIA nutrition does play a role in disease management. Some issues that may arise include the management of energy intake, increased requirements of key nutrients due to pro-inflammatory cytokines, secondary deficiencies caused by pharmacological treatments, food texture modifications and meeting general requirements for growth and development. Current

recommendations are the same as they are for all children and specific recommendations are made based on individual outcomes and requirements (Dinardo et al., 1991). This has not stopped parents of children with JIA from using unconventional nutritional therapies with one study finding that 70% of children were receiving some form of alternative nutritional therapy (Southwood, Malleson, Roberts-thomson, & Mahy, 1990). These therapies include mega doses of vitamins, fish oil supplementation, dietary restrictions and herbal remedies.

2.4. Vitamin D

Vitamin D is a pro-hormone that can be considered a "conditional dietary essential vitamin". To maintain adequate vitamin D levels humans require adequate UVB radiation exposure for cutaneous synthesis and/or adequate dietary intake. (Chung et al., 2009; Holick, 2007). Vitamin D status is impacted by both environmental and genetic factors and plays a role in inflammation (Cutolo et al., 2011). It has multiple roles including those in pathways related to the pathogenesis and progression of JIA. However further studies that explore vitamin D status and JIA development and activity are required to confirm a direct relationship between vitamin D and JIA

2.4.1. Physiology of Vitamin D

The most well-known function of vitamin D is regulating calcium and phosphorous concentrations in the blood by promoting their absorption from foods and supplements in the small intestine and by promoting re-absorption of calcium from the kidneys (Lips, 2006). Without vitamin D, humans would only be able to absorb 10-15% of dietary calcium and approximately 60% of phosphorous through passive transport (Institute of Medicine, 2011). In the presence of vitamin D, the efficiency of calcium and phosphorous absorption is increased to about 30-40% and 80%, respectively, through active transport processes (Holick, 2007). This supports bone formation and mineralization and is required to develop and maintain optimal peak bone mass. The role that vitamin D plays is not limited to the regulation of calcium. Vitamin D is involved in maintaining immune function, regulating cell growth and differentiation, neuromuscular function and suppressing inflammation (Hayes, Nashold, Spach, & Pedersen, 2003; Institute of Medicine, 2011). Vitamin D receptors (VDRs) are expressed in most tissues

and cell types throughout the body including bone, kidneys, lungs pancreas, brain, and muscle highlighting that vitamin D has a role outside of calcemic regulation (Yongji Wang, Zhu, & DeLuca, 2012).

2.4.1.1. Vitamin D Acquisition

Vitamin D can be acquired from limited sources in the Canadian diet, typically from fortified milk and margarine (see Table 2.8 for selected dietary sources of vitamin D) or through endogenous synthesis, which is triggered by ultraviolet beta (UVB) radiation from the sun (Holick, 1995; Holick, 2002; Holick, 2007).

Table 2.8. Selected foods containing vitamin D

Food	µg per serving*	IU per serving*
Cod liver oil, 1 Tablespoon	32.0	1,280
Salmon Atlantic, baked, 100 g	6.8	272
Salmon Sockeye, canned/bone, 100g	18.6	744
Mackerel, cooked, 100 g	2.6	104
Tuna fish, canned in oil, 171 g	2.1	84
Milk, non-fat, reduced fat, and whole, vitamin D fortified, 250 mL	2.6	104
Margarine, fortified, 15 mL	1.9	76
Orange juice, fortified, 250 mL	2.5	100
Egg, 1 large whole (vitamin D is found in egg yolk)	1.0	40
Liver, beef, cooked, 75 g	0.9	36

* Health Canada, Canadian Nutrient File, 2015

There are two dietary sterols that can form precursors for vitamin D. One form is found in fungi and protozoa and is called ergosterol. It can be activated through irradiation by UVB to form ergocalciferol (also known as vitamin D_2) (Institute of Medicine, 2011). The other sterol, cholecalciferol, is mammalian derived and is found in

sources such as fish or liver. Both forms, when ingested, are incorporated into the chylomicron fraction and transported to the liver. Endogenous synthesis of vitamin D is possible through 7-dehydroxycholesterol, which is found throughout both the dermis and epidermis; when it is exposed to a specific wavelength of UVB light (290-315 nm) it forms previtamin D_3 (precalciferol) (Lips, 2006). Within one day, precalciferol is thermalised to cholecalciferol (vitamin D_3). It diffuses from the skin to the blood stream where is it transported to the target tissue (usually the liver) by α-2globulin vitamin D-binding protein (Holick, 2007). A dose response study by Heaney et al. providing vitamin D_3 or vitamin D_2 found vitamin D_3 to be 13% more potent in raising and maintaining serum 25(OH)D concentrations and that it produced a 2- to 3-fold greater storage of vitamin D than an equimolar dose of vitamin D_2 (Heaney, Recker, Grote, Horst, & Armas, 2011).

Fortification of fluid milk and margarine is mandatory in Canada; regulations are administered through the Canadian Food and Drug Regulations. Fluid milk "shall contain added vitamin D in such an amount that a reasonable daily intake of the milk contains not less than 300 IU and not more than 400 IU of vitamin D" (35–45 IU vitamin D per 100 mL) and margarine, 530 IU/100g, respectively (Calvo & Whiting, 2013). Fortified plant-based beverages must contain vitamin D (either vitamin D_2 or D_3) in an amount equivalent to fluid milk. Foods such as yogurt, orange juice, and some meal replacement items are fortified in Canada; the amount of fortification permitted depends on the intended use of the product (Calvo, Whiting, & Barton, 2004; Calvo & Whiting, 2013).

Most people meet at least some of their vitamin D requirements through sunlight. However, there are a number of factors that can affect the endogenous synthesis of vitamin D from UVB radiation. Skin pigmentation is one of the factors influencing UVB absorption. The more melanin there is in the skin to compete for UVB photons, the fewer UVB photons available for endogenous synthesis of vitamin D (Kimlin et al., 2007). Wearing a sunscreen with an SPF greater than 8 eliminates endogenous synthesis by absorbing, reflecting or scattering UV radiation (Holick, 1995). The solar zenith angle which is a combination of the time of day, time of year and latitude impacts the strength of UV radiation as it forces the UV radiation to travel through a greater portion of the earth's atmosphere to reach its destination (Tsiaras & Weinstock, 2011). Above a level of 33° latitude UVB radiation is not strong enough for the cutaneous

synthesis of vitamin D all year long (Wacker & Holick, 2013). In populated Canada (latitude of 42° to 53° North) between October to March/ April UVB radiation is not strong enough to elicit endogenous synthesis of vitamin D (Webb, Kline, & Holick, 1988). Thus, during this time of the year there is increased risk for vitamin D deficiency. This is in part due to reduced UVB and is attributed to the zenith angle, which is in turn related to the shortening of days during the winter months (Webb et al., 1988). Vitamin D synthesis cannot occur if the sunlight passes through clothing or windows (Holick, 1995). Complete cloud cover including that caused by severe pollution can reduce UVB strength (Agarwal et al., 2002; Parisi, Turnbull, & Downs, 2012). These factors are important to consider since vitamin D deficiencies have been detected worldwide due to cultural or religious clothing habits (Holick, 1995), skin pigmentation (Kimlin et al., 2007), an increase in sedentary lifestyles that is associated with less exposure to UVB, and pollution (Holick, 1994; Holick, 2002). Body composition may also impact vitamin D status as vitamin D is readily taken up by adipose tissue. Obese individuals have lower serum 25(OH)D concentrations than normal weight individuals (Tsiaras & Weinstock, 2011). A study with the goal of characterizing the dose-response relationship of vitamin D supplementation and serum 25-Hydroxyvitamin D (25(OH)D) concentration in a large sample of healthy volunteers, and to quantify the dose-response relationship for different BMIs and for absolute body weight found that BMI was a better determinant of 25(OH)D concentrations than absolute weight. When compared to normal weight subjects, obese and overweight adult subjects have serum 25(OH)D concentrations that are on average 19.8 nmol/L and 8.0 nmol/L lower, respectively ($p<0.001$) (Ekwaru, Zwicker, Holick, Giovannucci, & Veugelers, 2014). The correlation between body fatness and vitamin D status has also been found in children (Wakayo, Belachew, Vatanparast, & Whiting, 2015). Overweight children were found to have lower 25(OH)D concentrations than non-overweight children, based on classification of both BMI-for-age and triceps skinfold-for-age percentile (Wakayo et al., 2015).

Regardless of the source of vitamin D, both dietary and endogenously acquired forms are only biologically active following further metabolism through hydroxylation in the liver at the 25th carbon to produce 25(OH)D which is the major circulating form of vitamin D. Subsequent hydroxylation is performed in the kidney at the first carbon yielding the metabolically active form 1,25-dihydroxyvitamin D ($1,25(OH)_2D$) (van den Berg, 1997). Although the kidneys are the major site of $1,25(OH)_2D$ production, some

other tissues (bone, placenta, macrophages, T-lymphocytes, dendric cells, several cancer cells, prostate, keratinocytes and the parathyroid gland) are capable of performing a small amount of this hydroxylation (Gröber, Spitz, Reichrath, Kisters, & Holick, 2013). The circulating serum concentration of 25(OH)D is commonly used to indicate vitamin D status as it reflects both dietary and endogenous sources. Since the half-life of 25(OH)D is 2-3 weeks, it is considered to be the most reliable measure of a person's cumulative vitamin D status (Holick, 2003). The half-life of the active form of vitamin D, $1,25(OH)_2D_3$, is only 4-6 hours making it a difficult and unrealistic measure of vitamin D status (Holick, 2003).

2.4.1.2. Functions of Vitamin D at the Cellular Level

Vitamin D status continues to be a public health concern due to its role in the prevention of rickets, osteomalacia, and osteoporosis. Vitamin D regulates a number of noncalcemic functions as well. Vitamin D is involved in the regulation of cell differentiation and proliferation, hormone secretion, stimulation of insulin secretion, modulation of immune function and inhibition of renin production (Hayes et al., 2003; Wacker & Holick, 2013). The VDR is involved in the majority of vitamin D's biological actions (Uitterlinden, 2004). These actions are mediated with $1,25(OH)_2D$ acting as a ligand, through the binding of VDR-RXR (Retinoid X Receptor) to the vitamin D response element of target genes (Kato, 2000).

The main function of vitamin D is to maintain adequate blood calcium levels. This is done through interacting with receptors in the small intestine. 1,25-dihydroxyvitamin D enhances absorption of intestinal calcium and dietary phosphorous through increasing the efficiency of their absorption (American Society for Bone and Mineral Research [ASBMR], 2005). If there is inadequate dietary calcium available to meet requirements, $1,25(OH)_2D$ and parathyroid hormone mobilizes monocytic stem cells in the bone marrow to become mature osteoclasts. Interaction with the VDR-RXR in osteoclasts ultimately leads to the dissolving of the bone mineral matrix in order to restore blood calcium, signal transduction and neuromuscular activity (ASBMR, 2005). When calcium status is adequate, vitamin D also stimulates bone formation by enhancing bone matrix synthesis and mineralization (Seino, Ishizuka, Shima, & Tanaka, 1993).

Cells in the immune system have the potential to synthesize $1,25(OH)_2D$ and elicit an autocrine or paracrine response from immune cells that express VDRs (Hewison, 2012). Cells involved in innate and adaptive immune responses such as macrophages, dendritic cells, T cells and B cells express the VDR, and CYP27B1 and, therefore, can both produce and respond to $1,25(OH)_2D$ (Adorini & Penna, 2008). Similar to the role of vitamin D in calcium homeostasis, $1,25(OH)_2D$ binding to the VDR results in a heterodimerization of VDR with the RXR in the nucleus of the cell (Holick, 2012). Then the complex binds to the VDRE. Depending on the target gene, either co-activators or co-repressors are attracted to the complex to induce or repress gene transcription (Baeke, Takiishi, Korf, Gysemans, & Mathieu, 2010). In most cells in the immune system vitamin D works to suppress the immune response (Cutolo et al., 2011).

2.4.1.3. Interpreting Vitamin D Status

While there is no cure for the disease, treatments and therapies are used to manage the disease and improve the quality of life**.** Treatment of JIA is based on pharmacotherapy, physiotherapy, occupational therapy, and psychosocial support (Prakken et al., 2011). In 2011 the American College of Rheumatology (ACR) published recommendations for the treatment of JIA which provided guidance for the use of therapeutic agents (medications) (Beukelman et al., 2011). The ACR did not use the ILAR sub-types in providing their recommendations. Instead, they grouped children into five distinct "treatment groups". These groups are 1) History of arthritis of 4 or fewer joints, 2) History of arthritis of 5 or more joints, 3) Active sacroiliac arthritis, 4) Systemic arthritis with active systemic features (and without active arthritis) and 5) Systemic arthritis with active arthritis (and without active systemic features). For each treatment group there is a therapeutic treatment pathway recommended, a description of the features of poor prognosis and methods to classify disease activity level (Beukelman et al., 2011). In 2013 updated recommendations were published by the ACR for the treatment of systemic JIA and for screening children receiving biologics for tuberculosis (Ringold et al., 2013).
Most patients are first treated with bon-steroidal anti-Inflammatory drugs (NSAIDs). However, early treatment with disease-modifying anti-rheumatic drugs (DMARDS) for certain JIA subsets is required to improve long-term outcomes. (Borchers et al., 2006). Another treatment option occasionally used is corticosteroids, either oral or provided by intra-articular injection (Gowdie & Tse, 2012). DMARDs help prevent irreversible damage and reduce long-term exposure to

medications with harmful side effects such as corticosteroids. (Gowdie & Tse, 2012). Biologic agents are a group of medications that blocks the body's inflammation response. They are used mostly in treating systemic and polyarticular JIA. They are occasionally used in the treatment of enthesitis-related arthritis and chronic uveitis. While biologic therapies are effective in treating JIA patients who do not respond to standard therapies use can be limited in some jurisdictions because of their high costs (Gowdie & Tse, 2012). Each medication has its own side effect profile that can impact quality of life and nutritional status such as gastro-esophageal reflux, nausea, stomach ulcers, elevated blood glucose, increased appetite and risk of infection as shown in Table 2.5. These factors can lead to an imbalance of energy and nutrients and subsequently affect growth and development in children with JIA.

Table 2.9. Serum 25-hydroxyvitamin D (25(OH) D) concentrations and health

Vitamin D status	**Range Units (nmol/L)**	**(ng/mL)**	**Health Implications**
Vitamin D deficient	<30 nmol/L	<12 ng/mL	Associated with vitamin D deficiency, leading to rickets in infants and children and osteomalacia in adults
Subclinical deficiency	30–50 nmol/L	12–20 ng/mL	Generally considered inadequate for bone and overall health in healthy individuals
Adequate	>50 nmol/L	20 ng/mL	Generally considered adequate for bone and overall health in healthy individuals
Toxicity	>125 nmol/L	>50 ng/mL	Emerging evidence links potential adverse effects, related to hypercalcemia, to such high levels, particularly >150 nmol/L (>60 ng/mL)

Adapted from (Institute of Medicine, 2011)

The Endocrine Society has published clinical practice guideline as well for patients at risk of vitamin D deficiency (Holick et al., 2011). They have included recommendations for a number of at-risk populations including: "obese children and adults and children and adults on anticonvulsant medications, glucocorticoids, antifungals such as ketoconazole, and medications for Acquired Immune Deficiency Syndrome be given at least two to three times more vitamin D for their age group to satisfy their body's vitamin D requirement" (Holick et al., 2011) (see Table 2.10). They suggest a daily intake value and tolerable upper limit levels, based on age and clinical circumstances (Holick et al., 2011). They also have daily intake values that are 2-3 times higher for obese patients (Holick et al., 2011). The Canadian Paediatric Society published vitamin D supplementation recommendations for Canadian mothers and infants in 2007; these were reaffirmed in 2013 (Godel & Society, 2013). They recommend, "Total vitamin D intake from all sources for the premature infant should be 200 IU/kg/day to a maximum of 400 IU/day. Subsequent vitamin D dosage should be 400 IU/day for all infants during the first year, with an increase to 800 IU/day from all sources between October and April north of the 55th parallel (approximate latitude of Edmonton) and between the 40^{th} and 55^{th} parallel in individuals with risk factors for vitamin D deficiency other than latitude alone (Godel & Society, 2007)." The Canadian Paediatric Society also has different definitions of 25(OH)D status than the IOM with 25(OH)D <25 nmol/L defined as deficient status, 25-75 nmol/L insufficient, optimal vitamin D status as between 75-225 nmol/L, pharmacological as > 255 nmol/L and potentially toxic as >500 nmol/L 25(OH)D (Godel & Society, 2013). The Canadian Cancer Society and Osteoporosis Canada (Hanley, Cranney, Jones, Whiting, & Leslie, 2010) have also published guidelines for vitamin D intake their guidelines were published for adults but not for children and adolescents. For the duration of this review all 25(OH)D concentrations and dietary intake values will be compared to and discussed in the context of the IOM recommendations.

Table 2.10. Dietary Reference Intakes (DRIs) values and daily allowance for vitamin D

	Institute of Medicine Recommendations			Endocrine Society's Recommendations	
Age	**Estimated Average Requirement (EAR) IU per day**	**Recommended Dietary Allowance (RDA) IU per day**	**Tolerable Upper Intake Level (UL) IU per day**	**Daily Allowance (IU/day)**	**Tolerable Upper Intake Level (UL) per day (IU/day)**
0-6 months	400 (10 μg) *	-	1000 (25 μg)	400-1000 (10-25 μg)	2000 (50 μg)
7-12 months	400 (10 μg) *	-	1500 (38 μg)	400-1000 (10-25 μg)	2000 (50 μg)
1-3 years	400 (10 μg)	600 (15 μg)	2500 (63 μg)	600-1000 (15-25 μg)	4000 (100 μg)
4-8 years	400 (10 μg)	600 (15 μg)	3000 (75 μg)	600-1000 (15-25 μg)	4000 (100 μg)
9-13 years	400 IU (10 μg)	600 IU (15 μg)	4000 IU (100 μg)	1500-2000 (38-50 μg)	4000(100 μg)
14-18 years	400 IU (10 μg)	600 IU (15 μg)	4000 IU (100 μg)	1500-2000 (38-50 μg)	4000 (100 μg)

* Adequate intake per day

Adapted from (Holick et al., 2011; Institute of Medicine, 2011)

Serum 25OHD levels increase in response to increased vitamin D intake in a non-linear manner (Institute of Medicine, 2011). A randomized, double-blind placebo-controlled trial by Cashman et al. (2008) was conducted in 238 men and women aged 20–40 years old who received supplemental doses of 0, 200, 400, or 600 IU/d of vitamin D_3 throughout the winter. It was found that, vitamin D intakes (combination of food and prescribed supplement) required to maintain serum 25(OH)D concentrations of 37.5, 50, and 80 nmol/L in 97.5% of the sample were 796, 1120, and 1644 IU, respectively (Cashman et al., 2008). A meta-regression conducted by Chung et al. (2009), showed that serum concentrations are increased by an average of 1.95 nmol/L for each 40IU per day supplementation. Supplementation with ergocalciferol instead of cholecalciferol resulted in smaller increases. A relationship between increasing doses of vitamin D_3 and increasing 25(OH)D concentrations was present in both adults and children and the dose-response relationships differed depending on study participants' serum 25(OH)D status at baseline with a difference in effect found among participants who had a vitamin D status ≤40 vs. >40 nmol/L, and duration of supplementation ≤3 vs. >3 months (Chung et al., 2009). The analysis contained four studies in children and adolescents and they summarised that the impact of supplementation in this group was increased 25(OH)D concentrations in a dose-dependent manner, ranging from 8 nmol/L (200 IU D3/day), 16.5 (with 600 IU D3/day) to 60 nmol/L (2000 IU of vitamin D_3/day) (Chung et al., 2009).

Vitamin D deficiency is a concern throughout the lifespan. In infants, it can lead to rickets which is characterized by abnormal growth of bones resulting in bowed legs, outward-bowed chest, and rachitic rosary on the ribs (Wharton, 2003). In adults, it manifests as osteomalacia where the symptoms include bending of the spine and bowing of the legs. It can also lead to muscle weakness and bone pain, which can go unnoticed during the initial stages of the deficiency. Long-term vitamin D deficiency, even that which does not lead to rickets or osteomalacia, can over time play a role in adult bone loss (Heaney, 2003). This reduction in bone mass in older persons is known as osteoporosis and is characterized as bones becoming porous and fragile (Heaney, 2003).

Vitamin D toxicity is rare and is most likely linked to high intakes of supplements or cod liver oil. It is unlikely to occur from intake of food or sun exposure. The symptoms of vitamin D toxicity include nausea, vomiting, poor appetite, constipation, weakness, and weight loss and, elevated blood concentrations of calcium that cause

mental confusion (ASBMR, 2005). High blood concentrations of calcium also can cause heart rhythm abnormalities and eventually calcification of soft tissues such as the renal tubules of kidney (Institute of Medicine, 2011).

2.5. Vitamin D Status in Children and Adolescents

2.5.1. Vitamin D Status of Children and Adolescents World Wide

Vitamin D deficiency is not only an issue in Canada but worldwide. It has been estimated that one billion people worldwide have vitamin D deficiency or insufficiency (Holick, 2007). A systematic review of vitamin D status of populations worldwide found that 88% of 25(OH)D values had a mean value below 75 nmol/l, 37% had mean values below 50 nmol/l and 7% had mean values below 25 nmol/l (Hilger et al., 2014). Vitamin D status was significantly higher in North America than in Europe or the Middle East and Africa. While no age-related differences were found in Europe and North America, in the Asia/Pacific region, children and adolescents were found to have significantly lower 25(OH)D values than adults and elderly. Children and adolescents from the Middle East/Africa have significantly higher 25(OH)D values than adult and the elderly populations (Hilger et al., 2014). Higler et al. speculate that the contrast between the two regions could be due to the amount of time spent outdoors by the varying age groups of both cultures. This systematic review found no sex-related differences for any of the populations studied. In the United Kingdom, sub-groups of the population have been found to be at increased risk of vitamin D deficiency, particularly South Asian ethnic minority (Prentice, 2013).

Traditional diet and clothing customs of South Asia have impacted vitamin D status. Rickets cases has been reported particularly among children from families of South Asian, African, Afro-Caribbean or Middle-Eastern origin children commonly affected are reported to be dark-skinned, to have had limited opportunities for skin UVB exposure, born to mothers who had poor vitamin D status during pregnancy, and were exclusively breastfed for an extended period and weaned onto poor diets (Prentice, 2013). This trend has also been found in Canada, where a national survey found that newcomer immigrant and refugee children particularly girls were more likely to be vitamin D deficient or insufficient compared to non-immigrant children (Vatanparast, Nisbet, & Gushulak, 2013). Countries closer to the equator also report vitamin D deficiency within their populations; this is thought to be due to an increase in sedentary lifestyle

as well as the religious or cultural requirement to remain fully covered (Balasubramanian et al., 2003; Challa et al., 2005; Goswami et al., 2000; Shaikh & Alpert, 2004). In Qatar, a country located in Southwest Asia at a latitude of approximately 25°, 25(OH)D status was measured in healthy children under 16 years of age (Bener, Al-Ali, & Hoffmann, 2009). The study revealed that vitamin D deficiency was most prevalent among children ages 11-16 (61.6%). Vitamin D deficiency increased with age, reduced intake of vitamin D and family history of vitamin D deficiency. Vitamin D deficiency was more prevalent in children with lower body mass indexes, lack of sunlight exposure, and children with reduced physical and outdoor activity (Bener et al., 2009).

2.5.2. Vitamin D Status of Canadian Children and Adolescents

To highlight vitamin D status in Canadians, the Canadian Health Measures Survey Cycle 1 (CHMS) measured 25(OH)D status and vitamin D intake of a representative sample of Canadians aged 6-79 years of age (Whiting, Langlois, Vatanparast, & Greene-Finestone, 2011). One quarter of Canadians did not meet the RDA for vitamin D intake and had 25(OH)D concentrations below the optimal level. Specifically, for children ages 6-11 years and 12-19 years, 14.1% and 26.3% of children had a vitamin D status below optimal concentrations (75 nmol/L). In both age groups a statistically significant ($p < 0.05$) percentage of males had lower vitamin D status than females. Overall vitamin D supplement users were found to have higher vitamin D concentrations than non-supplement users and non-white Canadians were at greater risk of not meeting the DRI's for vitamin D intake. A cross-sectional study investigating thevitamin D status of preschoolers attending licenced daycares in Montreal found that 88% of children ages 2-5 years had 25(OH)D concentrations above 50 nmol/L even though the 95% of children were not meeting the EAR for vitamin D (Hayek et al., 2013). Additionally, a cross-sectional study of preschoolers recruited from pediatric and family medicine primary care practices in Toronto (called TARGet Kids!) found, only 7% of children ages 1-6 had vitamin D concentrations below 50 nmol/l (Lee et al., 2014). Another cross-sectional study investigating vitamin D status of children in Newfoundland 35.5% of children 0-14 years of age were found to have a 25(OH)D value below 50 nmol/L. The effect of season on 25(OH)D status was also noticed with vitamin D insufficiency being more common in winter (Newhook et al., 2009). A systematic review of research related to the diets of Canadian Indigenous school-aged (6–18

years) which included 24 studies of cross-sectional design 6 of which investigated vitamin D intake concluded that vitamin D intake (along with a number of other key nutrients) was inadequate in this population (Gates, Skinner, & Gates, 2014). Ward et al. highlights that vitamin D deficient rickets is still present amongst Canadian children (2007). An annual incidence rate of 2.9 cases of rickets per 100, 000 children per year was documented between July 1, 2002, and June 30, 2004. The mean age of the children diagnosed with rickets was 1.4 years of age (Ward, Gaboury, Ladhani, & Zlotkin, 2007). Many of these infants were of First Nations ethnicity and were not receiving vitamin D supplements. Among newly arrived refugee children to Calgary, Canada aged 0-19 years 42% had 25(OH)D concentrations below 50 nmol/L (Aucoin, Weaver, & Thomas, 2013). The highest 25(OH)D mean concentrations were in children aged 0 to 5 years. Mean 25(OH)D values for male and female refugee children were statistically similar in all age groups with the exception of those aged 12- to 19-years, in which female refugees had lower mean values ($p = .01$). The TARGet Kids! Study found an association between non-Western immigration compared to immigration from western countries and lower serum 25(OH)D status in early childhood. The study suggested that the difference was due to reduced vitamin D supplementation practices in this group and suggested that supplementation was an opportunity for intervention (Omand et al., 2013).

2.6. Vitamin D in Immune and Inflammatory Pathways

Recently, the roles of vitamin D metabolism and signaling for both innate immune (antimicrobial activity and antigen presentation) and adaptive immune (T and B lymphocyte function) responses have been investigated (Christakos et al., 2013). The mechanism by which vitamin D impacts the immune system and inflammatory pathways is through the active form 1,25(OH)D (Cutolo et al., 2011). 1,25(OH)D can then induce a response in VDR to bind to the promoter region in a number of immune cells and promote transcriptional regulation (Christakos et al., 2013). Activated T- and B-lymphocytes, monocytes, and macrophages, as well as dendric cells, have VDRs the vitamin D-activating enzyme CYP27B1 and the enzymes capable of hydroxylating 25(OH)D to $1,25(OH)_2D$ (Gröber et al., 2013).

In relation to the innate immune system $1,25(OH)_2D$ either locally or systematically produced inhibits the maturation of dendritic cells and increases

macrophage differentiation (Gröber et al., 2013; Pludowski et al., 2013). 1,25$(OH)_2$D can modulate production of Th1 and Th2 cells and reduce the secretion of a proinflammatory cytokine such as TNFα (Gröber et al., 2013; Wöbke, Sorg, & Steinhilber, 2014). In general, vitamin D's role in immune function is to regulate inflammation when activate and up-regulate the production of cells that reduce inflammatory reactions (Cutolo et al., 2011). Reich et al. assessed the anti-inflammatory effects of 25$(OH)D_3$ doses on blood cells. When the cells were pre-incubated with ≥30 ng/ml 25$(OH)D_3$ (75 nmol/L), a significant inhibition of lipopolysaccharide-induced IL-6 mRNA expression was observed (Reich, Fedorak, Madsen, & Kroeker, 2014). This was not observed when cells were cultured with a 25(OH)D dose of 15 ng/ml (37.5 nmol/L). Providing >75 nmol/L 25(OH)D achieved a similar degree of suppression as providing 0.1 nmol/L of active 1,25(OH)D. When > 75 nmol/L of 25(OH)D was incubated with blood cells and lipopolysaccaride-induced TNF-α mRNA expression measured a similar effect as with IL-6 expression was observed (Reich et al., 2014).

2.7. Vitamin D and relationship to Juvenile Idiopathic Arthritis

A number of studies have demonstrated that patients with a autoimmune disease have lower levels of vitamin D (Nisar, Masood, Cookson, Sansome, & Ostör, 2013). The mechanism by which this occurs is believed to be through vitamin D non-calciotropic functions in the immune system and inflammation pathways (Nisar et al., 2013). Vitamin D may play a role in the prevention and pathogenesis of JIA by enhancing macrophage production of antimicrobial peptides, and suppressing proinflammatory properties of dendritic cells, and antigen presenting cells that stimulate immune responses, prevent autoimmunity and link adaptive immune response (Von Scheven & Burnham, 2011).

In adult patients with rheumatoid arthritis, low serum 25(OH)D concentrations are associated with increased disease activity (Sabbagh, Markland, & Vatanparast, 2013); however, little is known about children with juvenile arthritis. A meta-analysis, which included only three studies of the currently available data on JIA and vitamin D, reported the prevalence of vitamin D insufficiency in this population to be 82% (Nisar et al., 2013). There was, however, no comment as to if the reduced vitamin D was caused by reduced dietary intake, medication side

effect, or increased utilization due to the disease status. Additionally, details as to the disease outcome and date of diagnosis were not reported (Nisar et al., 2013). A study comparing JIA patients and healthy controls in Brazil found no statistically significant difference between the two groups. However, both controls and JIA patients had a high frequency of vitamin D insufficiency and deficiency (Munekata, Terreri, Peracchi, & Len, 2013). A number of studies conducted in the 1990's where vitamin D status in JRA patients was measured with the purpose of studying the relationship of vitamin D to bone mineralization, found no difference in 25(OH)D concentrations between JIA patients and controls (Falcini, Ermini, & Bagnoli, 1998; Hillman, Cassidy, Johnson, Lee, & Allen, 1994; Pepmueller, Cassidy, Allen, & Hillman, 1996). In these studies, however, many children were being actively treated for their JRA and the season of measurement was not reported, therefore, the relationship and impact of vitamin D on JRA could not be established.

The optimal level of vitamin D for patients with JIA has yet to be determined. A study designed to investigate the relationship of vitamin D status to JIA by Pelajo et al. (2012) found that children with JIA were more likely to be vitamin D deficient than healthy controls (n=154) (Pelajo et al., 2012). This study was conducted in children with established ongoing JIA, and a portion of the participants were taking medication that could potentially modify the disease outcome. This study found that when the entire sample was considered (n=154), there was no relation between serum 25(OH)D concentrations and disease activity. A cross-sectional study conducted which included 152 patients with JIA at the University of Florence found significantly reduced 25(OH)D status in JIA patients compared with 188 age-and-sex-matched controls (Stagi et al., 2014). Active disease or frequent disease relapse significantly reduced vitamin D status compared with patients with no active disease or frequent disease flare-ups. The authors questioned whether JIA patients with more severe disease require higher supplementation of vitamin D to maintain normal 25(OH) D concentrations. Vitamin D status was also found to be worse in winter (Stagi et al., 2014). Another cross-sectional study exploring the association between 25(OH)D and disease activity in children with JIA was conducted in Turkey (Çomak, Doğan, Uslu-gökçeoğlu, Akbaş, & Özdem, 2014). Most patients (72.3%) had 25(OH)D < 50 nmol/L, and a significant negative association between serum 25(OH)D concentrations and disease activity (p=0.01, r=-0,37). Disease activity was measured using the JADAS-27; mean JADAS-27 score was significantly higher in patients with 25(OH)D levels <37.5 nmol/L than in

patients with 25(OH)D levels >37.5 nmol/L ($p = 0.003$). This was not found in a study by Wang et al. who used the ACR Pediatric 30, CRP and ERS as measures of disease activity (Wang et al. 2015). There was a significant difference between JIA and control 25(OH)D concentrations with JIA patients having lower 25(OH)D status, but no statistically significant correlation between 25(OH)D and JIA subtypes. The relationship between vitamin D status and disease activity in children with JIA is still unclear. Studying newly diagnosed patients who are treatment naive will help discover this relationship as there will be fewer confounders that are associated with patients who have had the disease for varying degrees of time (medication, lifestyle modifications, and disease duration).

Thus far, all studies that have been conducted have included patients who were being treated for JIA many of whom were receiving corticosteroids as shown in Table 2.11. Many of the articles published before 2013 were described by Nissar et.al (2013) and the studies were conducted with the purpose of investigating the relationship between juvenile arthritis (JIA, JCA or JRA) and bone health. Of the 30 studies summarized as a scoping review in Chapter 3, six studies had mean 25(OH)D status above 75 nmol/L, the majority (15 studies) had mean 25(OH)D concentrations between 50-75 nmol/L and 9 had mean values below 50 nmol/L. Of the 11 studies that published mean 25(OH)D statuses of healthy control groups, 6 had mean values statistically significantly greater than the population with juvenile arthritis, 3 had concentrations that were statistically similar, and two studies had mean 25(OH)D values that were statistically significantly below. Mean 25(OH)D status of most of the studies was below optimal for children with juvenile arthritis and required further investigation into the dietary intake of vitamin D, lifestyle factors, disease activity and genetic polymorphisms to tease out the relationship between JIA and 25(OH)D status.

2.7.1. Vitamin D Receptors and Juvenile Idiopathic Arthritis

While the VDR is principally involved in calcium regulation, the receptor also regulates a variety of other metabolic pathways, including a number of pathways involved in both innate and adaptive immunity, suggesting that vitamin D plays a role in the pathogenesis of autoimmune diseases (Falcini et al., 2013; Ramagopalan et al., 2010). Specific polymorphisms in the VDR gene may be associated with different biologic responses to vitamin D. Certain polymorphisms (genetic variants) are associated with susceptibility to some diseases. By determining if a polymorphism occurs more frequently in those with JIA, we will be able to target preventative care towards those individuals. It has been suggested that the rs11568820 polymorphism of the VDR gene may impact transcriptional activity (Falcini et al., 2013). Specifically, the GG genotype and G allele were found to be more represented in Italian patients with JIA and were hypothesized to lead to a reduction in VRD activity and subsequent decrease in response to vitamin D. No study has investigated this relationship in a Canadian population. Recently the idea of investigating epistasis (gene-gene interactions) amongst genes in the inflammatory and vitamin D pathway and how their interactions contribute to JIA risk was explored by Ellis et al. (2015). This is the first study to explore this interaction, and the authors suspect that through exploring these interactions there is the opportunity to account for the missing heritability that has been observed with complex diseases with genetic components (Ellis et al., 2015). Their work found evidence of epistasis amongst PTPN2 and genes of the vitamin D pathway, including the vitamin D binding protein gene and VDR gene in contributing to the risk of JIA (Ellis et al., 2015).

2.8. Summary

Thus far, we know that there is a role for vitamin D in the inflammatory pathways, a high prevalence of 25(OH)D insufficiency among children with JIA, and an established link of vitamin D with other autoimmune diseases. We do not, however, know the optimal vitamin D status for children with JIA, whether reduced vitamin D is caused by increased utilization or reduced vitamin D status in children with JIA, the impact of vitamin D in disease activity or the role of VDR polymorphisms with JIA. Larger, long-term studies of new-onset JIA are required

to explore the association. The relationship between vitamin D status and JIA over time in newly diagnosed individuals in Canada or abroad has yet to be investigated. Investigating the genetic and environmental role that vitamin D plays in the prevention and control of JIA in the same children will help to tease out the multifaceted role played by vitamin D in this disease. Being able to suggest specific targets for vitamin D status as a potential adjunct therapy in the treatment of JIA and understanding how genetic variants increase the risk of disease development will enhance the quality of life of patients and their families.

CHAPTER 3

SCOPING REVIEW

Following the general literature review (Chapter 2), as indicated in page 43, the state of knowledge and gaps in research on the role of vitamin D in JIA were comprehensively evaluated in this scoping review.

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**format and structure of the original manuscript has been revised to provide a better flow within the thesis and be consistent with the format of previous chapters*

3.1. Introduction

Arthritis is among the most common chronic diseases in children. JIA is the current nomenclature applied to denote a group of clinically distinguishable subsets that share chronic, childhood-onset arthritis of unknown cause as a unifying feature. The etiologies of JIA are unknown and the pathogeneses unclear but are likely multifactorial. Among epidemiologic studies there is substantial variability in the frequencies with which JIA and its respective subtypes are reported to occur; chronic arthritis prevalence rates range from 0.07 to 4.01/1000 children and annual incidences from 0.008 to 0.226/1000 children (Manners & Bower, 2002). Putative explanations for the disparities in reported juvenile arthritis prevalence rates include, as examples, differences in diagnostic criteria applied (specifically, JIA or the earlier JRA (Brewer, Bass, Baum, Cassidy, Fink, Jacobs, Hanson, Levinson, Schaller, 1977) or JCA (Woods, 1978) classification systems) and in case ascertainment methods.

While methodologic inconsistencies among JIA epidemiologic studies might account for perceived prevalence differences, actual differences might occur as a consequence of genetic, ethnic, environmental, and lifestyle influences. Vitamin D status is potentially governed by these same factors; VDR genotype, ethnically-related skin tone and clothing, environmental variations in exposure to ultraviolet B radiation relating to the latitude of residence and season, and vitamin D nutritional intake are factors that modulate vitamin D concentrations.

As an immune and inflammatory mediator, vitamin D is implicated in the pathogenesis of autoimmune diseases including, as examples, multiple sclerosis, type 1 diabetes, rheumatoid arthritis, Crohn's disease, and chronic childhood arthritis (Agmon-Levin, Theodor, Segal, & Shoenfeld, 2013; Cutolo et al., 2011; Von Scheven & Burnham, 2011). Cells involved in innate and adaptive immune responses such as macrophages, dendritic cells, T cells, and B cells express enzymes required to activate and respond to vitamin D (Adorini & Penna, 2008; Hewison, 2012; Holick, 2012). Cytochrome p450 27B1 (CYP27B1) is the enzyme required to synthesize 1,25-dihydroxyvitamin D (1,25(OH)2D), the active form of vitamin D, from circulating 25(OH)D. The actions of 1,25(OH)2D are mediated by its binding to the VDR, a nuclear transcription factor. VDR then binds to the Vitamin D Response Element (VDRE), a genetic sequence located in the promotor region of genes regulated by vitamin D. (Adorini & Penna, 2008; Hewison, 2012; Holick, 2012). Vitamin D tends to suppress the immune response (Cutolo et al., 2011). Consequently, low vitamin D concentrations are associated with an increase in pro-inflammatory mediators and more active disease (Baeke et al., 2010; Cutolo et al., 2011) consistent, for example, with the observation that low serum 25(OH)D is associated with increased disease activity in rheumatoid arthritis (Sabbagh et al., 2013).

Reports of relationships between vitamin D and chronic childhood arthritis are derived from studies having different methodologic approaches, originating from multiple geographic regions, and comprising demographically disparate populations. Since the last analysis of these reports in 2013 (Nisar et al., 2013) the number of studies reporting 25(OH)D concentrations in children with chronic arthritis have increased from 14 to 38. An updated, systematic analysis of pertinent literature should help to further refine understanding of the relationships between vitamin D and juvenile arthritis, contribute to optimizing management of vitamin D status in children with arthritis, and clarify vitamin D's potential role in mediating disease pathogenesis.

Scoping reviews are methodologic approaches for thoroughly distilling and synthesizing information derived from different studies having varied designs. The purposes of scoping reviews are to not only capture key concepts that can guide care but also to recognize knowledge gaps that can inspire future research priorities (Daudt, van Mossel, & Scott, 2013)

Although nomenclature applied to chronic childhood arthritis classification systems has changed over the years, JIA is the current terminology. For clarity, this review will hereafter use the term JIA to encompass the forms of chronic childhood arthritis also included in the JCA and

JRA classification systems. However, in this review, when quoting the literature, we use the terminology for chronic childhood arthritis (JCA, JRA, or JIA) that was applicable at the time the cited reference was published (Arksey & Malley, 2005).

Here we report the results of a vitamin D- JIA scoping review that summarizes, synthesizes, evaluates, and interprets pertinent evidence from the literature to address the following research questions: 1) What is the relationship between vitamin D status and the occurrence of JIA? 2) What is the relationship between vitamin D status and childhood arthritis activity? 3) What is the relationship between vitamin D status in JIA and medication use? 4) What is the relationship between vitamin D status and geographic and demographic characteristics in children with JIA?

3.2. Methodology

To ensure a comprehensive literature scan, this scoping review applied the iterative methodological framework described by Arksey and O'Malley, with refinements by Levac *et al*. and Colquhoun *et al*. (Arksey & Malley, 2005; Colquhoun et al., 2014; Levac, Colquhoun, & O'Brien, 2010). Five biomedical literature search engines were accessed, in the following sequence: Medline (using Ovid), Embase (using Ovid), Cumulative Index to Nursing and Allied Health Literature (CINHAL), Web of Science, and Scopus. Reference lists within each of the publications retrieved from the web-based searches were scanned to ensure that no relevant citations were missed. Medical Subject Heading (MeSH) terms used for retrieval were "vitamin D" and "juvenile arthritis". The search term "juvenile arthritis" was general enough to capture citations that referred to JCA, JRA and JIA classification terms (Table 3.1). Search results, which included published articles, letters, and abstracts from conference proceedings, were collated and duplicates of articles removed. Publication titles and abstracts were then screened for relevance to the subject of vitamin D in children with idiopathic chronic arthritis as defined by JIA, JCA, or JRA classification criteria (Brewer, Bass, Baum, Cassidy, Fink, Jacobs, Hanson, Levinson, Schaller, 1977; Petty et al., 2004; Woods, 1978). The full texts of relevant articles were then reviewed.

Inclusion criteria for the review were 1) study conducted in humans, 2) 25(OH)D concentrations reported, 3) participants having a diagnosis of JCA, JRA, or JIA, and 4) JCA, JRA, JIA diagnosis without an associated coexistent autoimmune disease. Exclusion criteria

were 1) study conducted in animals, 2) the presence of an associated autoimmune disease 3) pregnant or lactating subjects, 4) 25(OH)D concentrations not reported), and 5) review articles. The process applied to identify eligible articles for the review is shown in Figure 3.1 (Moher, Liberati, Tetzlaff, & Altman, 2009). Articles in any language were eligible; however, no non-English language articles without at least an English abstract were found. From all relevant articles retrieved the following information was extracted: juvenile arthritis classification system (JCA, JRA, or JIA); sample size; sex ratio; patient age (at baseline or time of study if the study was cross-sectional); geographic location (country, city, and latitude and longitude), year of study; 25(OH)D concentration; study conclusion; and, if applicable, control group sample size, characteristics, and 25(OH)D concentration. The latitudes and longitudes reported were that of the city where the study was conducted; if unavailable, the province, state or region's center latitude was used and, as a last resort, the country's central latitude was used. 25(OH)D status by season of measurement was not reported in any of the articles found and therefore could not be considered.

Geographic Information Systems (GIS) mapping was performed using ArcGIS version 10.4 to visualize studies by juvenile arthritis classification, 25(OH)D status, location, and latitude. For all reported studies, an additional map comparing the difference in 25(OH)D concentration between those reporting active versus inactive disease was made.

Defining Vitamin D Status.

Vitamin D deficiency is defined as a serum 25(OH)D concentration less than 30 nmol/L, a 25(OH)D concentration between 30-50 nmol/L is considered insufficient, greater than 50 nmol/L is considered sufficient and a 25(OH)D status greater than 125 nmol/L is considered at risk of adverse effects (Institute of Medicine, 2010). These values are based on the IOM review of published research focused on determining the optimal vitamin D concentration for maximal calcium absorption, prevention of rickets, reduction of fracture risk and prevention of osteomalacia in healthy populations (Institute of Medicine, 2010). In the most recent review of vitamin D requirements, the IOM concluded that there was inadequate information to make intake recommendations in relation to other biologic roles of vitamin D (Institute of Medicine, 2010). The current RDA of vitamin D are 400 IU from 0 to 12 months of age and 600 IU per day from 1 to 18 years of age (Institute of Medicine, 2010).

The Endocrine Society has published clinical practice guidelines for patients at risk of vitamin D deficiency (Holick et al., 2011). The Society recommends that at-risk populations, including "obese children and adults and children and adults on anticonvulsant medications, glucocorticoids, antifungals such as ketoconazole, and medications for acquired immune deficiency syndrome be given at least two to three times more vitamin D for their age group to satisfy their body's vitamin D requirement" (Holick et al., 2011). The optimal 25(OH)D concentration suggested by the Endocrine Society is 75nmol/L. To meet this concentration it is recommended that 400-1000 IU be given between 0 to 12 months of age, 600 - 1000 IU per day from 1 to 8 years of age, and 1500-2000 IU for children between ages 9-18 years (Holick et al., 2011). These recommendations, however, are not specific for children with chronic arthritis.

3.3. Results of Scoping Review and Discussion

Considerations when evaluating the role of vitamin D in JIA include vitamin D requirements for this population and the role that vitamin D plays in disease activity. Using the specified MeSH search terms (vitamin D and childhood arthritis), 386 reports (full-text articles, conference abstracts, and letters to the editor) were identified. Thirty-eight studies met the inclusion criteria and are the subject of this review (Table 3.2). One meta-analysis reported cumulative 25(OH)D concentrations from fourteen studies comprising children with JIA, JCA, and JRA and other rheumatic conditions; this meta-analysis was not included in our scoping review but is referenced in the discussion (Nisar et al., 2013). This present review summarizes accumulated evidence on vitamin D and chronic childhood arthritis by disease activity and latitude. Additionally, this study provides new information about differences in 25(OH)D status between healthy controls and children with JIA.

3.3.1. Vitamin D Status in Relation to Chronic Childhood Arthritis Classification

Twenty-one of the 38 studies (55.3%) reported 25(OH)D status for patients with JIA (Alhomaidah, Alsagheir, & Al-mayouf, 2016; Bouaddi et al., 2014; Çomak et al., 2014; Dağdeviren-çakır, Arvas, Barut, Gür, & Kasapçopur, 2016; de Sousa Studart et al., 2015; Dey et al., 2014; Goralczyk, Konstantynowicz, Abramowicz, Dobrenko, & Babinska-Malec, 2015; Lien et al., 2005; Markula-patjas et al., 2012; Miettinen, Kinnunen, Harjutsalo, Reinert-Hartwall, Lamberg-Allardt, Toumilehto, Saila, 2013; Munekata et al., 2013; Nisar, Cookson, Masood,

Sansome, 2013; Peixoto, Teixeira, Lucas, Costa, Costa, 2013; Pelajo et al., 2012; Rosiles, Salazar, Velazquez, Ruiz, 2015; Siamopoulou et al., 2001; Stagi et al., 2014; Szymanska-Kaluza, Biernacka-Zielinska, Stanczyk, 2013; Tang, 2016; Valta, Lahdenne, Jalanko, & Aalto, 2007a), eight (21.1%) for patients with JRA (Bianchi et al., 1990; Falcini et al., 1998; Henderson et al., 1997; Hillman et al., 1994; Pepmueller et al., 1996; Reed, Haugen, Pachman, & Langman, 1991; Reed, Haugen, Pachman, & Langman, 1993; Stark, Davis, Janicke, Mackner, Hommel, Bean, Lovell, Heubi, 2006) and five (13.2%) for JCA patients (Elsasser et al., 1982; Johansson; Portinsson; Akesson; Svantesson; Ockerman; Akesson, 1986; Reeve et al., 1993; Rooney, Davies, Reeve, Preece, Ansell, 2000; Tzoufi, Siamopoulou-Mavridou, Challa, & Lapatsanis, 1994). Additionally, there were four studies that included patients with juvenile arthritis and other rheumatic diseases (Hillman et al., 2008; McNally et al., 2012; Reed, Haugen, Pachman, & Langman, 1990; Warady, Lindsley, Robinson, 1994). As the JRA classification system tended to be applied in North America and the JCA classification in Europe, there was a corresponding hemispheric-specific division in the geographic region from which JRA and JCA articles originated. Studies originated from 17 countries at latitudes ranging from 3°S to 61°N (Table 3.2). There were no eligible studies found that reported data below a latitude of 39°N prior to the introduction of the ILAR JIA disease classification systemic and no eligible JRA studies above 42°N.

The 2013, systematic literature review of 19 childhood arthritis studies reported vitamin D status (14 reporting 25(OH)D and 11 reporting 1,25(OH)D) suggested that at that time there was no clear link between vitamin D status and children with chronic arthritis (Nisar et al., 2013). The review also contained a meta-analysis comprising three studies that reported the prevalence of vitamin D insufficiency to be 82% in JIA (Nisar et al., 2013). Only three studies reported in the meta-analysis were conducted using ILAR JIA criteria (Nisar et al., 2013).

3.3.2. Comparison of Study Design

Seventeen studies used a cross-sectional design (n=17; 44.7%) (Alhomaidah et al., 2016; Bouaddi et al., 2014; Çomak et al., 2014; Elsasser et al., 1982; Goralczyk et al., 2015; Henderson et al., 1997; Markula-patjas et al., 2012; McNally, Matheson, & Rosenberg, 2009; Miettinen, Kinnunen, Harjutsalo, Reinert-Hartwall, Lamberg-Allardt, Toumilehto, Saila, 2013; Nisar, Cookson , Masood, Sansome, 2013; Peixoto, Teixeira, Lucas, Costa, Costa, 2013; Pelajo, Lopez-

Benitez, & Miller, 2012; Reed et al., 1993; Reed et al., 1990; Reeve et al., 1993; Tang, 2016; Valta, Lahdenne, Jalanko, & Aalto, 2007b) , 16 (42.1%) a case-control design (Bianchi et al., 1990; Dağdeviren-çakır et al., 2016; de Sousa Studart et al., 2015; Dey et al., 2014; Falcini et al., 1998; Hillman et al., 1994; Lien et al., 2005; Munekata et al., 2013; Pepmueller et al., 1996; Rooney, Davies, Reeve, Preece, Ansell, 2000; Rosiles, Salazar, Velazquez, Ruiz, 2015; Stagi et al., 2014; Szymanska-Kaluza, Biernacka-Zielinska, Stanczyk, 2013; Tzoufi et al., 1994; Ying Wang et al., 2015) and the remainder (5; 13.2%) were randomized controlled trials (Hillman et al., 2008; Reed et al., 1991; Siamopoulou et al., 2001; Stark, Davis, Janicke, Mackner, Hommel, Bean, Lovell, Heubi, 2006; Warady, Lindsley, Robinson, 1994). The primary objective of the majority of studies was to investigate the relationship between juvenile arthritis and bone health (n=20; 52.6%). In all studies where sex distribution was reported, there were significantly more female participants than males, an observation consistent with the overall preponderance of females in JIA (Borchers et al., 2006). With the exception of one study (Nisar, Cookson, Masood, Sansome, 2013), the age range of the participants was 0-21 years.

3.3.3. 25(OH)D status

Of the 38 studies reviewed, six (15.8%) had mean 25(OH)D concentrations above 75 nmol/L (Alhomaidah et al., 2016; Henderson et al., 1997; Hillman et al., 2008; Reed et al., 1993; Reeve et al., 1993; Stark, Davis, Janicke, Mackner, Hommel, Bean, Lovell, Heubi, 2006). Seventeen studies (44.7%) had mean 25(OH)D concentrations between 50-75 nmol/L (Bouaddi et al., 2014; de Sousa Studart et al., 2015; Falcini et al., 1998; Hillman et al., 1994; Markula-patjas et al., 2012; McNally et al., 2009; Miettinen, Kinnunen, Harjutsalo, Reinert-Hartwall, Lamberg-Allardt. Toumilehto, Saila, 2013; Munekata et al., 2013; Peixoto, Teixeira, Lucas, Costa, Costa, 2013; Pelajo et al., 2012; Reed et al., 1991; Rosiles, Salazar, Velazquez, Ruiz, 2015; Siamopoulou et al., 2001; Stagi et al., 2014; Tang, 2016; Warady, Lindsley, Robinson, 1994) and 15 (39.5%) had values below 50 nmol/L (Bianchi et al., 1990; Çomak et al., 2014; Dağdeviren-çakır et al., 2016; Dey et al., 2014; Elsasser et al., 1982; Goralczyk et al., 2015; Lien et al., 2005; Nisar, Cookson, Masood, Sansome, 2013; Pepmueller et al., 1996; Reed et al., 1990; Rooney, Davies, Reeve, Preece, Ansell, 2000; Szymanska-Kaluza, Biernacka-Zielinska, Stanczyk, 2013; Tzoufi et al., 1994; Valta et al., 2007a; Ying Wang et al., 2015). Of those 15 studies, 10 reported the mean 25(OH)D value, the median value reported for one study and a

range or cutoff was provided for four studies). Of the 14 studies (36.8%) that published mean 25(OH)D status of healthy control groups, nine (64.3%) had mean control values significantly greater than the population with childhood arthritis (Bianchi et al., 1990; Dağdeviren-çakır et al., 2016; de Sousa Studart et al., 2015; Dey et al., 2014; Pepmueller et al., 1996; Rooney, Davies, Reeve, Preece, Ansell, 2000; Stagi et al., 2014; Tzoufi et al., 1994), three (21.4%) had concentrations that were statistically similar (Munekata et al., 2013; Rosiles, Salazar, Velazquez, Ruiz , 2015; Ying Wang et al., 2015), and two studies (14.3%) had mean 25(OH)D values significantly below the juvenile arthritis comparison groups (Falcini et al., 1998; Hillman et al., 1994). One study compared 25(OH)D concentrations in children with JIA to hospitalized children and found no statistically significant difference between the two groups (Szymanska-Kaluza, Biernacka-Zielinska , Stanczyk, 2013). Mean 25(OH)D status of most studies (32 of the 38; 84.2%) was below the optimal concentration of 75 nmol/L in children with arthritis (Holick et al., 2011).

3.3.4. Geography in Relation to 25(OH)D Status in Chronic Childhood Arthritis

Vitamin D status in children with JIA appears to follow a north-south gradient (Figure 3.2). While this could be due to the diagnostic resources of the countries reporting values, the gradient does appear to be present in Europe where access to care and diagnostic resources are similar. Interestingly, the relationship between reduced vitamin D status and increased disease activity also appears to be present and follow a north-south gradient (Figure 3). More studies are required to confirm this relationship worldwide, especially in locations around the equator as well as in the southern hemisphere where thus far only two studies have taken place (de Sousa Studart et al., 2015; Munekata et al., 2013).

The major source of vitamin D for most people is endogenous vitamin D synthesis induced by sunlight exposure (Holick, 2016). Above 33° latitude UVB radiation is not intense enough for the cutaneous synthesis of vitamin D all year long (Tsiaras & Weinstock, 2011; Wacker & Holick, 2013). At latitudes 42° and 53º North, between October to April, UVB radiation is not intense enough to elicit endogenous vitamin D synthesis (Webb et al., 1988) thus potentiating the risk of vitamin D deficiency, (Tsiaras & Weinstock, 2011).

The prevalence of JIA, as well as the dominating subtype, varies with latitude (Manners & Bower, 2002). As illustrated in Figure 3.2, seven reviewed studies (18.4%) were conducted in

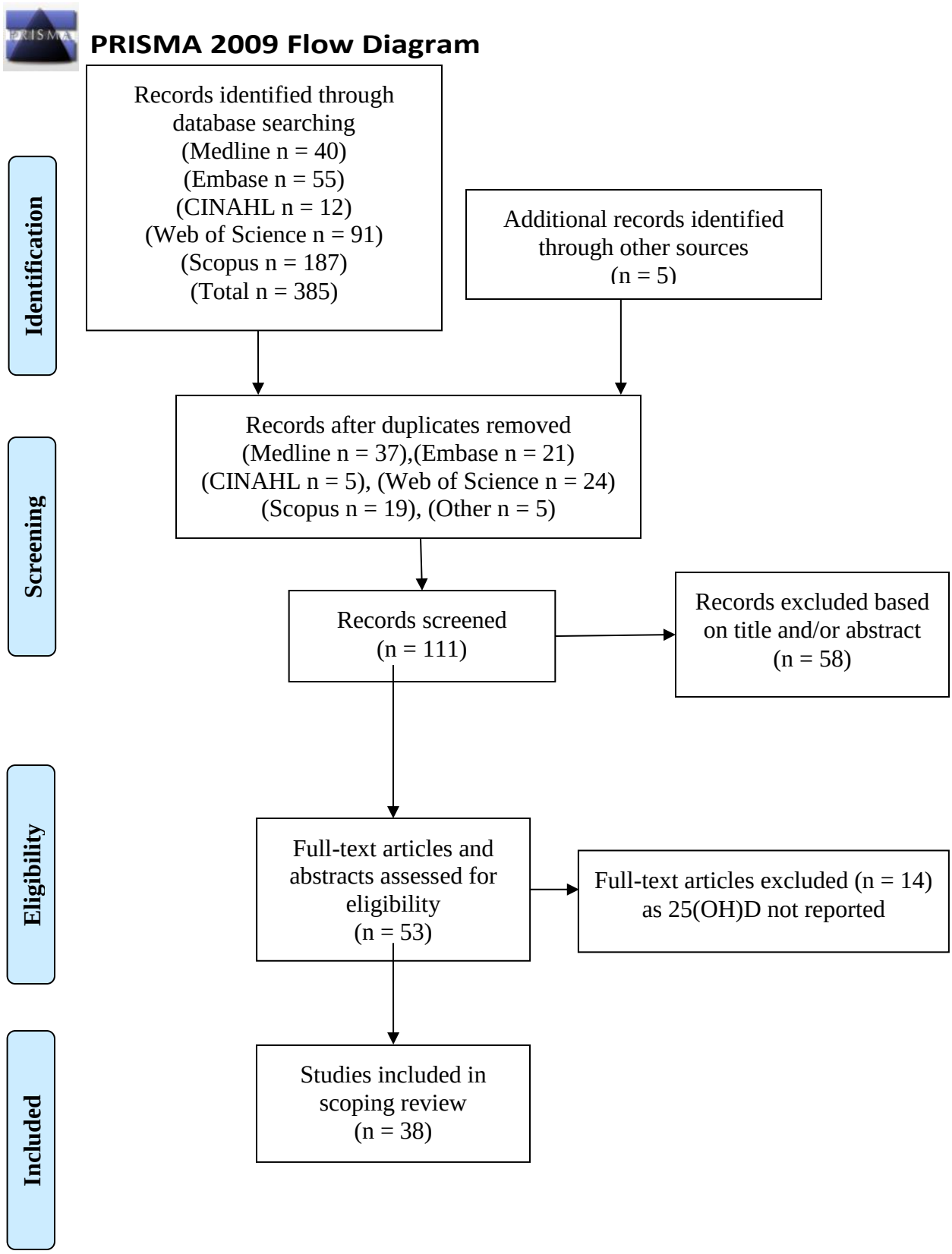
populations residing at latitudes at or below 33°(Alhomaidah et al., 2016; de Sousa Studart et al., 2015; Dey et al., 2014; Munekata et al., 2013; Rosiles, Salazar, Velazquez, Ruiz, 2015; Tang, 2016; Ying Wang et al., 2015), 19 studies (50.0%) were conducted between 33-50° (Bianchi et al., 1990; Bouaddi et al., 2014; Çomak et al., 2014; Dağdeviren-çakır et al., 2016; Falcini et al., 1998; Henderson et al., 1997; Hillman et al., 1994; Hillman et al., 2008; Peixoto, Teixeira, Lucas, Costa, Costa, 2013; Pelajo et al., 2012; Pepmueller et al., 1996; Reed et al., 1991; Reed et al., 1993; Reed et al., 1990; Siamopoulou et al., 2001; Stagi et al., 2014; Stark, Davis, Janicke, Mackner, Hommel, Bean, Lovell, Heubi, 2006; Tzoufi et al., 1994; Warady, Lindsley, Robinson, 1994), and 12 at a latitude above 50° (Elsasser et al., 1982; Goralczyk et al., 2015; Lien et al., 2005; Markula-patjas et al., 2012; McNally et al., 2009; Miettinen, Kinnunen, Harjutsalo, Reinert-Hartwall, Lamberg-Allardt, Toumilehto, Saila, 2013; Nisar, Cookson, Masood, Sansome, 2013; Reeve et al., 1993; Rooney, Davies, Reeve, Preece, Ansell, 2000; Szymanska-Kaluza, Biernacka-Zielinska, Stanczyk, 2013; Valta et al., 2007a). For those below 33°, 1 study (14.3%) reported a mean 25(OH)D concentration >75 nmol/L, 4 (57%) reported a concentration between 50-75 nmol/L, and two (29%) reported values less than 50 nmol/L. For the studies that took place between 33-45° latitude, four studies (21.1%) reported a 25(OH)D concentration >75 nmol/L (21%), nine (47.4%) reported a concentration between 50-75 nmol/L(47%) and six (31.6%) reported values less than 50 nmol/L. From the studies that took place above 45° latitude, one study (8.3%) reported a 25(OH)D concentration >75 nmol/L, four (21.1%) reported a concentration between 50-75 nmol/L (33%) and seven (36.8%) reported values less than 50 nmol/L (39%).

3.3.5. Current Chronic Childhood Arthritis Diagnostic Criteria

Of the 21 studies that applied the current chronic childhood arthritis criteria used by ILAR to diagnose children with JIA, only one study reported mean 25(OH)D concentrations above 75 nmol/L (15%) (Alhomaidah et al., 2016). The majority of the studies (n=11; 52.4%) reported mean concentrations between 50-75 (52%) nmol/L (Bouaddi et al., 2014; de Sousa Studart et al., 2015; Hernández Rosiles, Duarte Salazar, Maldonado Velazquez, Rivas Ruiz, & Clark, 2017; Markula-patjas et al., 2012; Miettinen, Kinnunen, Harjutsalo, Reinert-Hartwall, Lamberg-Allardt, Toumilehto, Saila, 2013; Munekata et al., 2013; Peixoto, Teixeira, Lucas, Costa, Costa, 2013; Pelajo et al., 2012; Siamopoulou et al., 2001; Stagi et al., 2014; Tang, 2016),

and the remaining studies (n=9; 42.9%) reported a mean concentration below 50 nmol/L (43%) (Çomak et al., 2014; Dağdeviren-çakır et al., 2016; Dey et al., 2014; Goralczyk et al., 2015; Lien et al., 2005; Nisar, Cookson, Masood, Sansome, 2013; Szymanska-Kaluza, Biernacka-Zielinska, Stanczyk, 2013; Valta et al., 2007a; Ying Wang et al., 2015). As latitude increased, the percentage of studies that reported mean 25(OH)D in the 50-75 nmol/L range decreased.

Figure 3.1. PRISMA flow diagram. Articles identified from the retrieved publications reference lists are identified as "other" in the flow diagram.



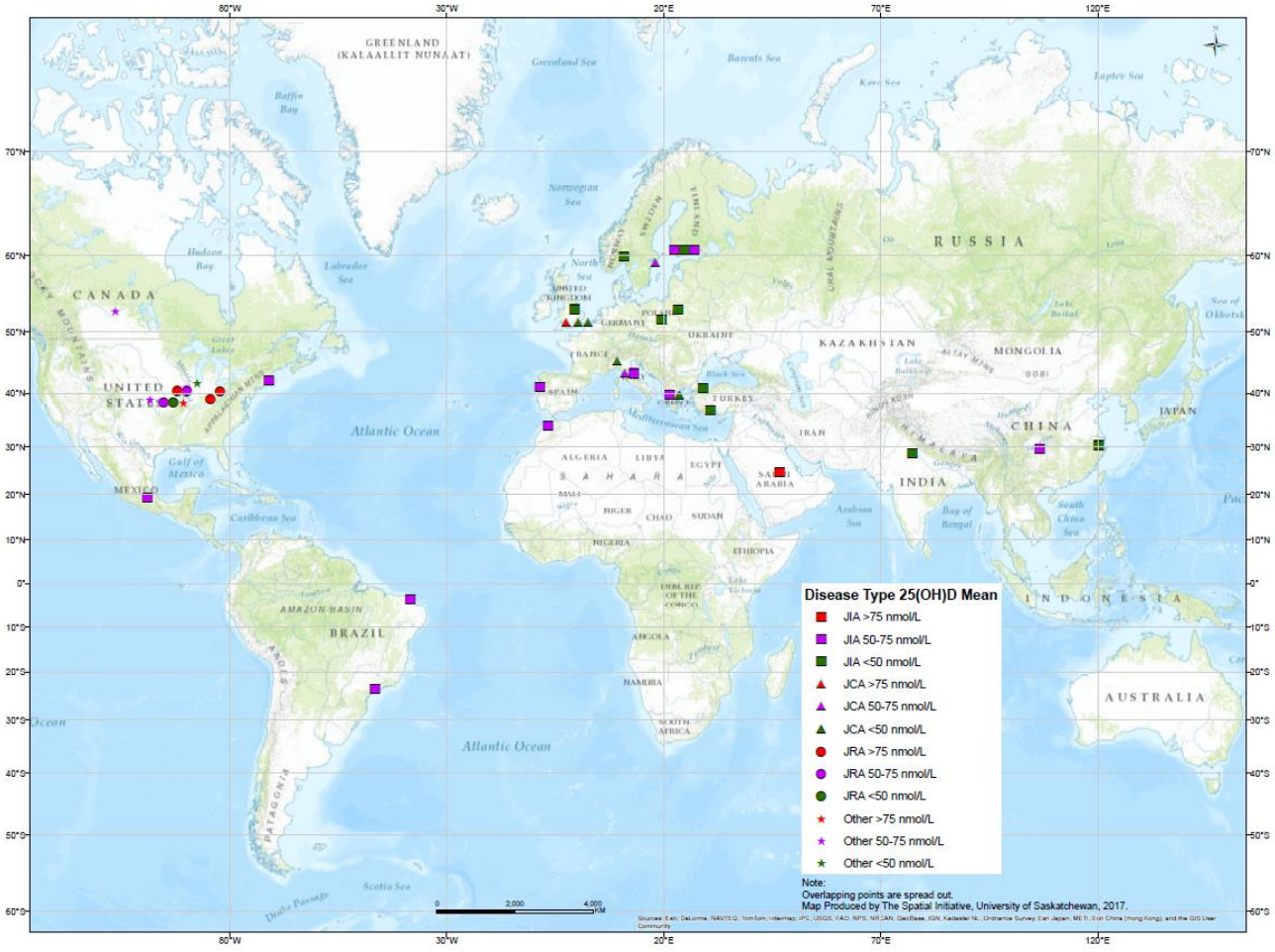


Figure 3.2. Disease type by 25(OH)D group

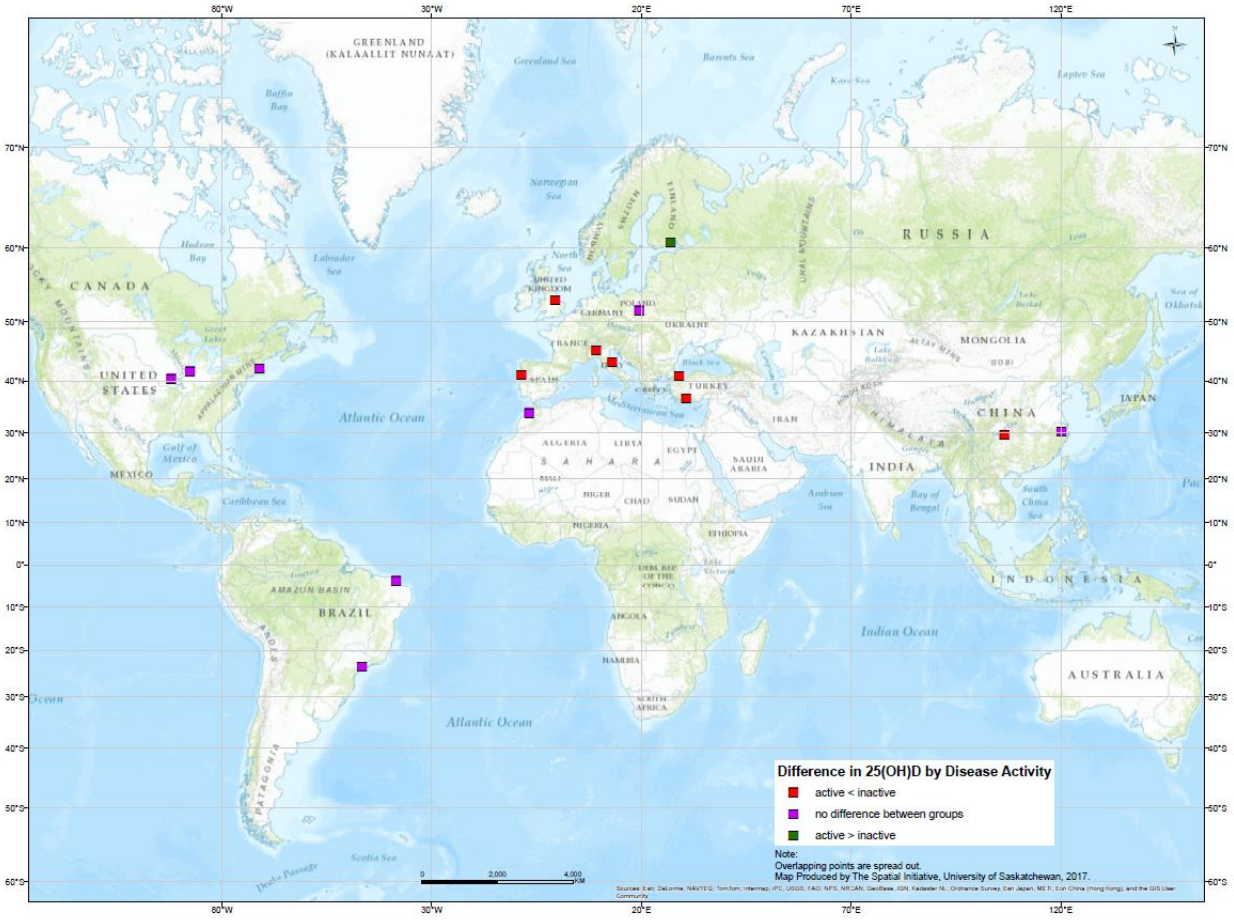


Figure 3.3. Difference in 25(OH)D concentration in active vs inactive chronic childhood arthritis patients

3.3.6. Vitamin D and Disease Activity

No single measure has been established as an accurate indicator of childhood arthritis disease activity. While C-reactive protein and Erythrocyte Sedimentation Rate are indicators of inflammation, they alone do not fully reflect overall disease activity. In the studies reviewed, a variety of validated composite scores were used to measure function or disease activity, including the CHAQ, JADAS-27 and ACR Peds 30) (Duffy, 2007; Ringold & Wallace, 2007; Ringold et al., 2013).

Fifteen of the 38 studies comprising this present review (39.5%) evaluated the relationship between vitamin D and disease activity (Figure 3.3). Seven studies (18.4%) reported that patients with active disease or those with elevated inflammatory biomarkers had lower 25(OH)D concentrations than those patients who were in remission or who had less disease activity (Bianchi et al., 1990; Çomak et al., 2014; Nisar, Cookson, Masood, Sansome, 2013; Peixoto, Teixeira, Lucas, Costa, Costa, 2013; Stagi et al., 2014; Tang, 2016; Tzoufi et al., 1994). One study (26.3%) showed the opposite relationship; those with active disease had higher vitamin D concentrations than those with inactive disease (Miettinen, Kinnunen, Harjutsalo, Reinert-Hartwall, Lamberg-Allardt, Toumilehto, Saila, 2013). Of the seven studies (18.5%) that reported no relationship between 25(OH)D and disease activity (Bouaddi et al., 2014; Dağdeviren-çakır et al., 2016; de Sousa Studart et al., 2015; Munekata et al., 2013; Pelajo et al., 2012; Reed et al., 1993; Reed et al., 1990) one found a relationship between 25(OH)D concentrations and disease activity in the univariate but not the multivariate analysis (Bouaddi et al., 2014). Except for one study conducted in Turkey (Dağdeviren-çakır et al., 2016), all other studies conducted in Europe that explored disease activity reported a negative association between vitamin D and disease activity.

Long-term cohort studies can further clarify the relationship between vitamin D concentration and disease duration or frequency of relapse. Such an association was explored in a cross-sectional study which found significantly reduced 25(OH)D status in JIA patients (n=152) compared with 188 age-and-sex-matched controls (Stagi et al., 2014). Active disease or frequent relapse was associated with reduced vitamin D status compared to patients with no active disease or frequent flare-ups. The authors questioned whether JIA patients with more severe disease require higher supplementation of vitamin D to maintain normal 25(OH) D concentrations. As latitude increases, more studies report a difference in vitamin D status

between patients with active versus inactive disease, in comparison to lower latitudes as illustrated in Figure 3.3.

To date, the evidence to support a relationship between vitamin D and disease activity with autoimmune diseases in humans is correlative and not causative (Dankers, Colin, van Hamburg, & Lubberts, 2017). Long-term, adequately powered randomized studies, which control for confounding variables (sun exposure, season, and vitamin D intake), are required to confirm a causative relationship between vitamin D and disease activity.

3.3.7. Potential Requirements of Vitamin D Intake

Vitamin D intake was only measured in seven studies (Dey et al., 2014; Henderson et al., 1997; Lien et al., 2005; Markula-patjas et al., 2012; Pepmueller et al., 1996; Stagi et al., 2014; Valta et al., 2007a). All of these studies reported a mean or median vitamin D intake that was less than the EAR of 400 IU per day set by the IOM (Institute of Medicine, 2010). This is the amount of vitamin D that is expected to be sufficient for 50% of the population (Institute of Medicine, 2010). This indicator is used to evaluate the prevalence of inadequacy at the population level. The recommendation at the individual level is the RDA that ranges from 400-600 IU based on age groups. Two studies reported intake of vitamin D supplements by study participants but neither had a mean 25(OH)D that reached the optimal concentration (Henderson et al., 1997; Valta et al., 2007a). Three studies reported intake of both children with JIA and healthy controls (Dey et al., 2014; Lien et al., 2005; Stagi et al., 2014). Vitamin D intake and status was similar for two JIA patient groups (Lien et al., 2005; Stagi et al., 2014), and lower intake resulted in lower vitamin D status in the third group (Dey et al., 2014). In comparison to the control groups, Lien et al. found similar intake and 25(OH)D status between those with JIA and controls (Lien et al., 2005). Stagi et al. reported similar intake of vitamin D and higher 25(OH)D in controls, and in the study reported by Dey et al. the intake of control participants was two times higher than the participants with JIA and the control group had higher 25(OH)D concentrations (Dey et al., 2014; Stagi et al., 2014).

It has been theorized that there may be an increased utilization of vitamin D during active inflammation, possibly caused by \the presence of vitamin D receptor polymorphisms in patients with autoimmune diseases (Cutolo, Otsa, Uprus, Paolino, & Seriolo, 2007; Cutolo et al., 2011). Additional studies investigating vitamin D intake from all sources (both food and supplements)

are required to determine if children with chronic arthritis require additional vitamin D to maintain serum concentrations in comparison to healthy children. Understanding this relationship will be important in the use of vitamin D as a potential adjunct therapy. Additionally, improved understanding of vitamin D needs in children with chronic arthritis will help to clarify the role of vitamin D in the underlying disease processes so that therapies that target specific vitamin D responsive immune pathways can be developed. By exploring the factors that influence vitamin D status (genetics, environment, and nutrition), we will be better able to discern an association between vitamin D and JIA.

Two articles have discussed vitamin D requirements for children with rheumatic conditions not in the context of corticosteroids. In 2011, von Scheven and Burnham suggested that in the absence of specific guidelines for children with rheumatic conditions that the American Academy of Pediatrics guidelines of 400 IU per day be used as a suggested minimum dosing regimen (Von Scheven & Burnham, 2011). The authors cautioned that providing large doses of vitamin D can result in providing "too much of a good thing" and that studies comparing children with rheumatic diseases to healthy children are required. The second article was published by Vojinovic and Cimaz and recommends that the guidelines set out by the Endocrinology Society for patients receiving corticosteroids be followed for all children with rheumatic diseases (Vojinovic & Cimaz, 2015). This would result in a dose of 2-3 times the current recommendation and would be approximately 2000 IU/day. This dose is still below the IOM's tolerable upper limit for all children over the age of one (2500 IU) (Institute of Medicine, 2010).

3.3.8. Vitamin D and Medication Interactions in JIA Patients

All but one study (Szymanska-Kaluza, Biernacka-Zielinska, Stanczyk, 2013), have been conducted with children already being treated for arthritis, many receiving corticosteroids, which could impact 25(OH)D concentration and inflammatory status. Corticosteroids promote the breakdown of both 25(OH)D and 1,25(OH)D and also counteract effects of vitamin D on bone formation (Gröber & Kisters, 2012; Gröber et al., 2013). These patients also had varying disease duration. A study of newly diagnosed individuals, however, did not compare patients with JIA to healthy controls but to hospitalized children (Szymanska-Kaluza, Biernacka-Zielinska, Stanczyk,

2013) . Comparing children with JIA to healthy controls allows for discerning biologic differences that could inform treatment targets.

The lowest mean 25(OH)D concentration, 22 nmol/L (n=35) was reported from India. These patients were found to be consuming significantly less vitamin D and had less sun exposure compared to healthy controls (Dey et al., 2014). The highest 25(OH)D, 140.8 nmol/L (n=17), concentrations were reported in Finland, in a population receiving Prednisone (Reeve et al., 1993). The authors hypothesized that the reason their patients' 25(OH)D concentrations were so high was that their previous research had found low concentrations of 25(OH)D and they were encouraging their patients to consume vitamin D fortified foods and to spend time in the sun (Reeve et al., 1993). A survey of steroid-related osteoporosis, prevention and treatment practices of pediatric rheumatologists in North America was conducted by Soybilgic et al. in 2014 (Soybilgic, Tesher, Wagner-Weiner, & Onel, 2014). They found that the majority of pediatric rheumatologists are recommending vitamin D for patients who were on long-term corticosteroids (Soybilgic et al., 2014). The role of vitamin D in mediating bone health, especially in relation to corticosteroids, has been established (Gröber & Kisters, 2012). Both short and long-term corticosteroid intake even at small doses impact bone health in patients with autoimmune diseases (Gröber et al., 2013). The role and an appropriate amount of vitamin D intake or 25(OH)D target for inflammation or disease activity have yet to be established.

3.3.9. Additional Research Directions

Considering ethnicity when comparing incidence and prevalence amongst populations would be useful in understanding if the regional differences observed are due to environmental or genetic factors or a combination of the two. Evidence from a multiethnic cohort study of 1082 children at The Hospital for Sick Children, Toronto, Canada investigated the influence of ethnicity on the risk of developing JIA (Saurenmann et al., 2007). When the diversity of the study population was compared to that of the general Toronto region population, there was an overrepresentation of patients of European and Indigenous descent and an underrepresentation of patients of Black, Asian, or Indian subcontinent ethnicity in their cohort. European descent was significantly associated with an increased risk of developing JIA, including all subtypes except RF-positive polyarthritis JIA. Exploring the environmental and genetic factors that may contribute to JIA risk in the same individual will help to clarify these findings.

While this review focused on vitamin D status in children with JIA, vitamin D may be involved in both disease development and subsequent disease activity status. Exploring elements of vitamin D status that may have a role in disease development such as early life and gestational vitamin D status as well as genes in the vitamin D pathway will help to clarify the role of vitamin D.

Season of birth has been suggested to have an impact on the risk of developing a number of autoimmune diseases such as multiple sclerosis, type 1 diabetes and celiac disease (Y Berkun et al., 2015). A recent study investigating month of birth and risk of JIA found a difference in the pattern of the birth month for children with JIA compared to that of the general population (Y Berkun et al., 2015). Children with JIA were more likely to be born between November to March, with the birth month for the general population peaking in the summer months. The study by Carlens et al. also investigated the relationship between season of birth and the risk of developing JIA and found no increased risk (Carlens et al., 2009). Season of birth may be a marker for vitamin D status *in utero* with children born in the non-vitamin D synthesizing periods being exposed to less vitamin D during their time *in utero* than those who are born during the vitamin D synthesizing seasons.

The CLARITY explored the use of nutritional supplements during pregnancy and the risk of developing JIA (Ellis et al., 2012). The use of vitamin D and fish oil during pregnancy in case mothers was not significantly different from controls following covariate adjustments (Ellis et al., 2012). A case-cohort investigation from Denmark, comparing 25(OH)D status in children diagnosed with either oligoarticular or polyarticular JIA using dried blood spot samples that were collected at birth did not find any association between 25(OH)D status at birth and risk of developing JIA (Thorsen et al., 2016). Concentrations of 25(OH)D fluctuated significantly by season of birth and year of birth (calendar year). There was no follow up to determine if 25(OH)D status or season during the first few months of life impacted risk of JIA or whether other subtypes of JIA were impacted by season of birth of 25(OH)D status at birth.

Certain VDR gene polymorphisms may be associated with different biologic response to vitamin D. The rs11568820 polymorphism of the VDR gene specifically the GG genotype, have been suggested to be more represented in patients with JIA compared to healthy controls who more often have the GA genotype (Falcini et al., 2013). Recently the idea of investigating epistasis (gene-gene interactions) amongst genes in the inflammatory and vitamin D pathway and

how their interactions contribute to JIA risk was explored by Ellis et al. (Ellis et al., 2015). This is the first study to explore this interaction, and the authors suspect that through exploring these interactions there is the opportunity to account for the missing heritability that has been observed with complex diseases with genetic components (Ellis et al., 2015). Their work found evidence of epistasis amongst tyrosine-protein phosphatase non-receptor type 2 (PTPN2) gene and the vitamin D binding protein gene in contributing to the risk of JIA (Ellis et al., 2015). The role of genes in the vitamin D pathway on both disease development and disease activity are still in the early stages of investigation. Also how they impact the biological response involving vitamin D and inflammation remains unclear. Investigating genetic, nutritional and environmental factors that influence vitamin D in JIA could help inform ways in which vitamin D status influences the occurrence and activity of JIA. Understanding if genetic variants increase the risk of disease development will help tailor vitamin D management in individual patients, contribute to improving control of disease activity, and improve outcomes.

A north-south gradient of incidence and a mechanism for the suppression of inflammation in relation to 25(OH)D status has been suggested. However, no study has summarized the current evidence of chronic childhood arthritis diagnosis by 25(OH)D status in relation to latitude and disease activity. This first step is important for the development of future studies leading to the exploration of potential optimal target concentrations of vitamin D for the reduction of inflammation in children with chronic arthritis.

3.3.10. Limitations

This scoping review has limitations due to the limited amount of comparative data. Season of measurement and JIA subtype could not be considered due to a lack of reporting in most reviewed articles. With the exception of one study, all studies reviewed used various unreported types of medication in patients who had had JIA for varying durations. These variables can make it difficult to interpret the relationship between vitamin D and disease activity in relation to both inflammation status and risk of relapse. Of the studies that investigated the relationship between vitamin D and function or disease activity, various measures were used. The most common included the CHAQ, JADAS-27 and ARC Peds 30. The JADAS-27 and ACRS Peds 30 both include active joint counts in their scoring which confounds comparisons of disease activity between patients with different JIA that are defined by numbers of joints

involved. subtypes (Alessandro Consolaro et al., 2012; Giannini et al., 1997; Petty et al., 2004). Many studies included multiple subtypes of JIA measured by the same disease activity score that included an active joint count. This review was unable to explore the relationship between vitamin D status and ethnicity, vitamin D receptor genes or other genes that influence vitamin D metabolism.

3.4. Conclusion

This is the first scoping review to summarize research relating to vitamin D and JIA in the context of vitamin D status, latitude, disease activity. It is also the first to map the results according to geography. Thirty-two studies (84.2%) reported a mean 25(OH)D concentration below 75nmol/L or the optimal value. This suggests that whether due to inadequate intake or increased utilization the majority of children with juvenile arthritis do not have optimal 25(OH)D status as defined by the Endocrine Society (Holick et al., 2011). The optimal concentration of 25(OH)D and the corresponding dietary requirements for patients with chronic childhood arthritis has yet to be determined. Further, the relationship between vitamin D status and disease activity in children with JIA is still unclear. Studying newly diagnosed patients who are treatment naive for longer periods of time would help characterize this relationship as there would be fewer confounders associated with patients who have had the disease for varying durations (medication, lifestyle modifications, and disease duration). Thus far, we know that there is a role for vitamin D in the inflammatory pathways, a high prevalence of 25(OH)D insufficiency among children with JIA, and an established link of vitamin D with other autoimmune diseases. We do not, however, know the optimal vitamin D status for children with JIA, whether reduced vitamin D is caused by increased utilization or reduced vitamin D status in children with JIA, the impact of vitamin D in disease activity or the role of VDR polymorphisms with JIA. Larger, long-term studies of new-onset JIA are required to explore the association. The relationship between vitamin D status and JIA over time in newly diagnosed individuals has yet to be investigated. Investigating the genetic and environmental role that vitamin D plays in the prevention and control of JIA in the same children will help to tease out the multifaceted role played by vitamin D in this disease. Being able to suggest specific targets for vitamin D status as a potential adjunct therapy in the treatment of JIA and understanding how genetic variants

increase the risk of disease development will enhance the quality of life of patients and their families.

After reviewing the literature and conducting the scoping review, regarding the association of vitamin D and JIA, we aimed at focusing on the above-identified gap and addressing the aforementioned objectives for the Canadians population in Objectives 1-3. In the next chapter, we demonstrate the general methodology that was used for Studies 1-3 of this thesis.

3.5. Acknowledgments

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Appendix

Table 3.1. Comparison of classification systems of chronic childhood arthritis*

	American College of Rheumatology 1977 (Brewer, Bass, Baum, Cassidy, Fink, Jacobs, Hanson, Levinson, Schaller, 1977)	European League Against Rheumatism 1978 (Woods, 1978)	International League Against Rheumatism 1994 and 2001(Petty et al., 2004)
Classification Title	Juvenile Rheumatoid Arthritis	Juvenile Chronic Arthritis	Juvenile Idiopathic Arthritis
Symptom duration	Minimum 6 weeks	Minimum 3 month	Minimum 6 weeks
Subtypes	Systemic	Systemic	Systemic
	Polyarticular	Polyarticular	Polyarthritis RF negative
		JRA (RF positive Polyarticular)	Polyarthritis RF positive
	Pauciarticular	Pauciarticular	Oligoarthritis
			Persistent
			Extended
		Juvenile psoriatic	Psoriatic arthritis
		Juvenile ankylosing spondylitis	Enthesitis-related arthritis
		Arthritis associated with inflammatory bowel disease	
			Undifferentiated arthritis

*Prior to 1997, two chronic childhood arthritis classification systems were used. The American College of Rheumatology (ACR) (Brewer, Bass, Baum, Cassidy, Fink, Jacobs, Hanson, Levinson, Schaller, 1977) classification criteria referred to chronic childhood arthritis as Juvenile Rheumatoid Arthritis (JRA) and the European League Against Rheumatism (EULAR) applied the term Juvenile

Chronic Arthritis (JCA) (Woods, 1978). Differences between the two classification systems hindered exchange and comparison of data between the two systems (Borchers et al., 2006). To reconcile differences between ACR and EULAR criteria, the International League Against Rheumatism (ILAR) JIA criteria were introduced. This table provides a comparison of diagnostic criteria (Petty et al., 2004). The ILAR classification system defines JIA as all forms of inflammation of one or more joints beginning in children younger than age16 years (Petty et al., 2004). JIA is further classified into seven categories based on inclusion and exclusion criteria according to features present within the first six months of disease**.** The seventh category includes those who do not fit into one category, meet criteria for more than one category, or have exclusion criteria that preclude assigning a category.

Appendix

Table 3.2. Summary of current literature of 25(OH)D status and chronic childhood arthritis

Study Location and Reference	Disease	Sample size (number female)	Age (years) Mean ± SD or range	25(OH)D (nmol/L) Mean ± SD or range	Results relating to vitamin D	Control Group Results	Vitamin D Intake
Study Design: Meta-Analysis							
Meta-Analysis Nisar et al. 2013 (Nisar et al., 2013)	JRA JCA & JIA	n=529	0-18	61.4	Mean of 14 studies 61.4 nmol/L (Range 28.7-139.8) prevalence reported from 3 studies 82% insufficient.		
Study Design: Randomized Controlled Trial							
Cincinnati, Ohio USA Stark et al. 2006 40°N (Stark, Davis, Mackner, Hommel, Bean, Lovell, Heubi, 2006)	JRA	n=49	4-10 y	79.9 ± 25.0 (39.9–142.3)	Behaviour intervention to increase calcium intake successful.		
Ioannina, Greece Siampoulou et al. 2001	JIA	n=10 (6F)	13.1 ± 2.5	53.9 ± 8.5	All patients were vitamin D replete 25(OH)D >17.5 nmol/L, most were		

39°N (Siamopoulou et al., 2001)					measured between February to May.	
Missouri, USA Hillman et al. 2008 37°N (Hillman et al., 2008)	Children with arthritis	n=18	3-15	82.1 ± 38.7	Supplemental vitamin D improved status, but supplemental vitamin D or calcium did not improve bone mass.	
Kansas, USA Warady et al. 1994 39°N (Warady, Lindsley, Robinson, 1994)	Rheumatic disease (6 with JRA)	n=10 (7F) 6 JRA	13.(10.9-18.0)	70.1±21.2	Children with rheumatic disease would benefit from receiving calcium and vitamin D supplements.	
Illinois, USA Reed et al. 1991 40°N (Reed et al., 1991)	JRA	n=13 (12F)	5-18	70.0 ± 40	Vitamin D may help prevent bone loss in children with active disease.	
Study Design: Case-Control						
Istanbul, Turkey Dagdeviren-	JIA	Active disease: n= 64 (41) Remission: 53(35)	Active disease: 9.7 ± 4.3	Active disease: 46.5 ± 23.0	Vitamin D concentrations in children with JIA were significantly	Healthy control n=100 66.8 ± 26.6 nmol/L.

Cakir et al. 2016 41°N (Dağdeviren -çakır et al., 2016)			Remissio n: 9.8 ± 4.3	Remission: 47.3 ± 27.5	lower than healthy children. Of those who were measured while in remission there was no difference in 25(OH)D concentrations.	
Mexico City, Mexico Hernandez Rosiles et al. 2015 19°N (Rosiles, Salaza , Velazquez, Ruiz., 2015)	JIA	n= 37 (27)	12.5 ± 3.1	55.0 ± 13.9	No difference between children with JIA and controls.	Healthy controls n=79 59.0 ± 7.7.
Fortaleza, Brazil De Sousa-Studart et al. 2015 3°S (de Sousa Studart et al., 2015)	JIA	n=51 (31 F)	13.4 ±4	55.4 ± 25.0	25(OH)D similar for disease activity status, JIA category, and arthritis severity measure.	Age sex-matched controls 25(OH)D, 75.9 ± 14.0 nmol/L.
Hangzhou, China (article in Chinese, English	JIA	n=53	Not reported	Median 42.6	A significant difference between JIA and control 25(OH)D $p<0.01$. No correlation	Control n=106 25(OH)D 49.9.

Abstract) Wang et al. 2015 30°N (Wang et al., 2015)					between 25(OH)D and JIA subtypes, ACR pediatric 30, CRP or ESR.		
Florence, Italy Stagi et al. (2014) 43°N (Stagi et al., 2014)	JIA	n=152 (115F)	16. ± 7.4	54.4 ± 20.5	JIA had reduced 25(OH)D and higher PTH compared to controls. Active disease or frequent flare-ups resulted in lower vitamin D than non-active and no frequent flare-ups.	Control group 25(OH)D 74.4 ± 28.0 nmol/L p<0.005.	Intake JIA 164 ± 84 IU/day control 160 ± 72 IU/day.
New Delhi, India Dey et al. 2014 28°N (Dey et al., 2014)	JIA	n=35	3-16	22.0 ± 18.0	Decreased dietary intake of vitamin D and calcium, decreased weight bearing physical activity and sunlight exposure were the major factors for low BMD. Duration of disease 2.30 ±1.91 yrs.	Age sex-matched controls 25(OH)D 37.9 ± 10.0 nmol/L significant difference.	Intake JIA 123±53.6 (50-207) control 309±62.38 (213-387) IU/day.
Lodz, Poland Szyamanska	JIA	n= 50 (40)	9.4 ± 5.52	43.4 ± 21.1	Vitamin D deficiency is common in this population. No	Control n=28 Age, gender matched, hospitalized	

-Kaluza et al 2013 51°N (Szymanska -Kaluza, Biernacka-Zielinska, Stanczyk, 2013)					correlation between disease activity, type of JIA or metabolites of vitamin D.	children 43.4 ± 40.7 nmol/L.	
Sao Paulo, Brazil Munekata et al. 2013 23°S (Munekata et al., 2013)	JIA-polyarticular	n=30 (23F)	14 (4-20)	64.1 ± 21.6	High frequency of 25(OH)D deficiency in both control and JIA groups; no difference between the two. No association of 25(OH)D with disease activity.	Control group age-sex matched (n=30). 16 non-Caucasian; mean disease duration 5y (1-12) control 2 5(OH)D 67.2 ± 19.0 nmol/L.	
Oslo, Norway Lien et al. 2005 59°N (Lien et al., 2005)	JIA	n=108	6 to 18	49.7 ± 16.5	No difference in 25(OH)D between control and JIA groups.	Control n=108 25(OH)D 50.4 ± 8.1 nmol/L D	Intake JIA 164 ± 84 IU control 160 ± 72 IU
London, UK Rooney et al. 2000 51°N (Rooney, Davies, Reeve,	JCA	n=34 (23F)	9.2 (4.6-13.6)	Estimated from graph 45 (14.5-62.5)	Vitamin D status was significantly lower in JCA patients than age-matched controls before treatment, Steroid-treated	Control group 25(OH)D estimate 75 nmol/L.	

Preece, Ansell, 2000)					children have low vitamin D. All but three children received corticosteroids.		
Florence, Italy Falcini et al. 1998 43°N (Falcini et al., 1998)	JCA	n=47 (34)	15 months-12 years (7.13±4.1)	61.4 ± 20.5	The lower serum concentrations of osteocalcin in active disease support the hypothesis that both bone formation and resorption are reduced in JRA	Controls n=47 25(OH)D 56.7±21.5 nmol/L.	
Missouri, USA Pepmueller et al. 1996 37°N (Pepmueller et al., 1996)	JRA	n=41	4-18.5	45.7 ± 23.5	Suggest an association between decreased bone mineralization in JRA and low bone formation that is related to disease severity.	Control n=62 65.5 ± 23.5 nmol/L significant difference.	Vitamin D intake in JRA 464 ± 262 IU Intake of controls not reported.
Ioannina, Greece Tzoufi et al. 1994 39°N (Tzoufi et al., 1994)	JCA	n=35 (14)	8.8 ± 4.1	39.8 ± 20.5	Disease activity of JCA appears to be associated with lower vitamin D.	Mean disease duration 3.4 years Control n=15 25(OH)D 68.1 ± 15.5 nmol/L control group taking corticosteroids	

						n=4 25(OH)D 51.4 ± 24.5 nmol/L.
Missouri, USA Hillman et al. (1994) 37°N (Hillman et al., 1994)	JRA	n=44 (28)	11.8 ± 3.8	66.6 ± 26.7	Lower bone mineral content and bone biomarkers in JRA patients that controls but higher vitamin D in JRA.	N= 37 controls 25(OH)D 53.2±18.7 nmol/L.
Milano, Italy Bianchi et al. 1990 45°N (Bianchi et al., 1990)	JIA	n=36 (64%)	9.96 (5-17)	45.9 * Reported from Nissar et al Review	Suggests severe JRA has an influence on bone mass possibly mediated by a decrease in active vitamin D metabolites.	Study duration one year, controls only measured at baseline 25(OH)D 92 ± 17.5 nmol/L N=20
Huddinge, Sweden Johansson et al. 1986 59°N (Johansson; Portinsson; Akesson; Svantesson; Ockerman; Akesson, 1986)	JCA	26 (all female)	11-16	63.2 ± 36.4	Statistically lower than controls, however no evidence of deficiency.	Healthy controls n=28 76.2 ± 28.0 nmol/L
Study Design: Cross Sectional						
Chongqing, China Tang	JIA	n=76 (36)	8.49 ± 3.09	52.8 ± 15.3 nmol/L	JIA patients have reduced serum	

& Mingyue Conference Abstract 2016 29°N (Tang, 2016)					25(OH)D3, particularly those with active disease or/and using glucocorticoid.
Riyahad, Saudi Arabia Alhomaidah et al. 2016 24°N (Alhomaida et al., 2016)	JIA	n=22 (13)	12.4	14 > 75 nmol/L 8 <75 nmol/L	Vitamin D insufficiency is frequent in children with JIA.
Bialystok, Poland (abstract only) Goralczyk et al. 2015 53°N (Goralczyk et al., 2015)	JIA	n=189 (113)	3-17.7	40.6 ± 23.5	67% 25(OH)D <50nmol/L. Obese children had significantly reduced 25(OH)D compared to normal weight peers. Negative relationship between MTX use and 25(OH)D.
Oporto, Portugal Peixoto et al. 2013 Conference Abstract 41°N	JIA	n=40 (31)	22.3 (4-63)	10 > 75 nmol/L, 19 between 50-75 nmol/L, 11 <20 nmol/L	Prevalence of vitamin D deficiency/insufficiency among JIA patients is very high.

(Peixoto, Teixeira, Lucas, Costa, Costa, 2013)						
Antalya, Turkey Comak et al. 2014 36°N (Çomak et al., 2014)	JIA	n=47 (29)	9.3 ± 3.9	44.2 ± 29.0	Only 27.7% patients had 25(OH)D >50 nmol/L. There was a significant negative correlation between vitamin D concentration and disease activity (p=0.01, r=-0,37).	
Salé, Morocco Bouaddi et al. (2014) 34°N (Bouaddi et al. 2014)	JIA	n=40 (18)	11 ± 4.23	55.4 ± 27.2	25(OH)D <75nmol/L in 75% of sample. Poly arthritis and oligoarthritis 25(OH)D status negatively associated with disease activity in univariate but not multivariate analysis.	Median disease duration two years.
Helinski, Finland Miettinen et al. 2013	JIA	n=136	1-18	M: 63.9±18.0 F: 62.9± 20.0	Suggest that JIA subtype may be associated with 25(OH)D	

Letter to the Editor 60°N (Miettinen, Kinnunen, Harjutsalo, Reinert-Hartwall, Lamberg-Allardt, Toumilehto, Saila, 2013)					concentration in female patients. Seasonal difference with female patients.
Cambridge UK Nisar et al. 2013 Conference Abstract 52°N (Nisar, Cookson, Masood, Sansome, 2013)	JIA	n=37 (31)	0-10 (n=13), 11-20 (n=12) and >21 years (n=12)	49.6 nmol/L (range 13.2-112.0 nmol/L).	Half of patients with JIA have low Vitamin D levels which are inversely-related to disease activity and disease duration.
Boston, USA Pelajo et al. 2012 42°N (Pelajo et al., 2012)	JIA	n=154 (61%)	10.6	72.9 ± 23.0	13% deficient, 42% insufficient. Age, ethnicity, season, BMI associated with 25(OH)D but not vitamin D deficiency. No association with whole sample; small negative

					association for new onset JIA; mean time since onset 28 months.	
Helinski, Finland Markula-Patjas et al. 2012 60°N (Markula-patjas et al., 2012)	JIA	n=50 (41)	14.8 (7.0-18.7)	53 nmol/L (20-95 nmol/L)	62% sufficient, 24% insufficient and 14% deficient.	52% taking vitamin D supplement % of DRI median and IQR 187 (57,331).
Saskatchewan, Canada McNally et al. 2009 52°N (McNally et al., 2009)	Pediatric arthralgia	n=730 25(OH)D n=73	<18	59.9	Significantly more reported fall and winter as season of onset – more referrals from northern SK 40%<50 nmol/L 42% 50-75 nmol/L Association between psychological stress, school absenteeism vitamin D insufficiency and arthralgia.	
Helinski, Finland Valta et al. 2007	JIA Glucocorticoid treated	n=62 (43)	Median 11.8 (4.6-17.9	Median 49 nmol/L 16 (23%) ≤ 37.5 nmol/L	Osteoporosis is a concern in glucocorticoid	32% prescribed 400-800 IU vitamin D daily

60°N (Valta et al., 2007b)					treated children with JIA.	Mean intake 316 IU (range 44-1204 IU).
Ohio, USA Henderson et al. 1997 40°N (Henderson et al., 1997)	JRA	n=48 (37)	8.1 ± 1.9	89.4 ± 28.7	Serum 1,25-dihydroxyvitamin D concentrations were able to accurately segregate 79.6% of the JRA subjects into either the low or normal BMD groups.	%RDA 87.6 ± 52.7.
Illinois, USA Reed et al. 1993 40°N (Reed et al., 1993)	JRA	n=27 (23)	2.9-16	84.9 ± 11.0	No difference in vitamin D status between active and inactive groups. Children with JRA who have improvement in their disease activity have an improvement in BMD heralded by an increase in serum osteocalcin values 4-87 months from disease onset.	
Harrow, UK Reeve et al. 1993 51°N	JCA-treated with glucocorticoids	Prednisone n=17 Deflazacort n=17	Prednisone 10.6 ±3.7 Deflazaco	Prednisone 140.8Deflazacort 115.6	25(OH)D was surprisingly high, there was no difference between	

(Reeve et al., 1993)			rt 10.3 ±3.9		the two groups p=0.8.
Chicago, Illinois Reed et al. 1990 40°N (Reed et al., 1990)	Chronic Rheumatic Disease	n=113 (82) JRA n= 83	1.5 to 21	Range of groups 44.9 ± 15.0 to 54.9 ± 22.5	No difference between those with active and inactive disease.
London, UK Elsasser et al. 1982 51°N (Elsasser et al., 1982)	JCA	n=63 serum 25(OH)D n=29	Not reported	24.5 nmol/L (9>25 nmol/L, 20<25 nmol/L)	There was a marginally significant correlation between TBD and 25(OH)D concentrations (r=0.37. P<0.05). Only nine children had acceptable vitamin D status.

CHAPTER 4

METHODOLOGY

In this chapter, the methodology related to the studies included in this thesis is demonstrated. Specific details to each study's methodology are available in the corresponding Study's chapter.

The overarching goal of this research was to understand how vitamin D affects disease activity in children who are suffering from JIA. The specific objectives of this research were to: (1) Evaluate and compare vitamin D status and anthropometric characteristics between healthy children/adolescents and patients with JIA. (2) Determine vitamin D status and its association with disease activity and outcomes in children with JIA. (3) Identify potential association of the vitamin D pathway gene polymorphisms and JIA. It was hypothesized that vitamin D has an impact on disease activity in children who are suffering from JIA. The study tested the following specific hypotheses: (1) Children and adolescents with JIA have lower vitamin D status and growth parameters (height, weight, waist circumference, BMI) than healthy children and adolescents. (2) Vitamin D status is negatively associated with inflammatory markers and disease activity among JIA cases. (3) There are specific polymorphisms of vitamin D pathway genes associated with JIA.

4.1. Research Plan

To address the aforementioned objectives, I used data from the Biologically Based Outcome Predictors (BBOP) in Juvenile Idiopathic Arthritis study.

Biologically Based Outcome Predictors (BBOP) in Juvenile Idiopathic Arthritis

The Biologically-based Outcome Predictors (BBOP) in JIA study is a multi-centre cross-Canada cohort study with the goal of identifying clinical and biological factors that help predict disease outcomes in JIA to help guide disease management and improve outcomes earlier. Additionally, BBOP aspired to demonstrate how environmental and modifiable risk factors interact with genetic and inflammatory processes to impact disease outcomes.

BBOP is a prospective cohort study with data collected between December 2007 and December 2012. One hundred and eighty-six patients aged (1-17 years old) with new onset JIA

were recruited from multiple centres across Canada (British Columbia Children's Hospital, University of British Columbia, Vancouver, BC; Alberta Children's Hospital, Calgary, University of Alberta, AB; Stollery Children's Hospital, Edmonton, University of Alberta, AB; Royal University Hospital, Saskatoon, University of Saskatchewan, SK; Children's Hospital, Winnipeg, University of Manitoba, MB; The Hospital for Sick Children, University of Toronto, Toronto, ON; The Montreal Children's Hospital, McGill University, Montreal, QC; Centre Hospitalier Universitaire de Sherbrooke, Sherbrooke, QC; Centre Hospitalier de l'universite Laval, Laval, QC; IWK Health Centre, Dalhousie University, Halifax, NS; Janeway Children's Health and Rehabilitation Centre, Memorial University, St. John's, NL).

Enrollment criteria included meeting the International League of Associations for Rheumatology (ILAR) classification criteria (Petty et al., 2004), as determined by a pediatric rheumatologist BBOP team member, being within six months of disease onset and having received no medications with the exception of nonsteroidal anti-inflammatories or methotrexate as a treatment for their condition. Informed consent was received from participant's parents as well as consent or assent from participants where appropriate. Additional consent forms allowing for storage of blood samples and DNA for future studies were also collected. Recruitment was from participants in each of seven JIA subtypes (systemic, oligoarthritis, rheumatoid-factor-positive polyarthritis, rheumatoid-factor-negative polyarthritis, enthesitis-related arthritis, psoriatic arthritis, undifferentiated arthritis). The aim was to recruit a representative sample for each subtype. To achieve this, only participants with polyarthritis or systemic JIA (the least prevalent subtypes) were eligible during the first six months of enrollment after which any JIA subtype was eligible. The study was approved by the research ethics boards at each participating site.

Overview of Data Collection and Methodologies

Environmental and clinical data were collected and blood samples were obtained. Follow-up took place every 6 months for 2 years. Surveys, physical examination, and anthropometric measurements occurred every 6 months for 2 years. Blood samples were collected at baseline and 6 months and a saliva sample for DNA extraction took place at 6 months. See Table 4.1 for BBOP data collected of relevance to this thesis and Table 4.2 for timeline of sample collection of relevance to this thesis. Biological samples collected discussed

in this thesis included plasma for 25(OH)D, inflammatory markers and cytokine analysis as well as saliva for DNA analysis.

Demographic Data

At enrollment, a patient identification number and a diagnostic category were assigned (following RF test results determined by the respective centres' service laboratories) and the following enrollment information documented: Birth date, sex, ethnic groups, date of JIA symptom onset, and primary household income.

Anthropometric Assessments

Standing and sitting heights and weight were recorded at enrollment and semi-annually thereafter to 24 months. Height was measured using a wall stadiometer and recorded to the nearest 0.1 centimeter. Weight was measured twice on an electronic scale and recorded to the nearest 0.1 kg. Duplicate measures were averaged. Z-scores for height, weight and BMI were calculated. We defined underweight as BMI <5 percentile, overweight risk as >85 to <95 percentile, and overweight as > 95 percentile.

Indicators of Disease Activity and Outcome Measures:

Cytokine Selection

Forty-six cytokines were analyzed for the BBOP study. We chose to focus on the cytokines listed below because they are most commonly listed in the literature as having been explored in participants with JIA or other autoimmune diseases and are associated with vitamin D (Cutolo et al., 2011; Dankers et al., 2017; Hayes et al., 2015).

Pro-Inflammatory Cytokines

From blood samples collected at baseline and at six months, inflammatory mediators IL-2, IL-17, IL-1α,IL-1rα, IL-1β, IFNɤ, IL-1β, IL-6, IL-8, and TNFα were measured

Anti-Inflammatory Cytokines

Anti-Inflammatory cytokines IL-4 and IL-10 were measured.

Measures of Inflammation

CRP and ESR were measured at all time points as measures of inflammation.

Objective and self-reported measures of disease outcomes

Physician global assessment of disease activity, and the CHAQ-JIA, were measured as indicators of function at all time points. Pain was assessed using the CHAQ question how much pain do you think your child has had because of his/her illness in the past week? .The patient or parent-completed Juvenile Quality of Life Questionnaire (JAQQ) was used to assess quality of life at all time points and the Hassle score was measured at baseline 12 months and 24 months.

Clinical disease remission

Clinical disease remission was defined using the criteria proposed by Wallace et al. (Wallace et al., 2004) defined as inactive disease, meaning no active joints, absence of fever, rash, serositis, splenomegaly or generalized lymphadenopathy attributable to JIA, normal ESR and CRP concentration, and physician global assessment of no disease activity, either for at least 6 months if on medication or for 12 months if off medications (Wallace et al., 2004). Patients were classified as having active disease (not meeting all of the criteria and designated lowest level for statistical analysis) remission on medication (designated intermediate level in statistical coding) and remission off medication (designated highest level in statistical coding).

Vitamin D status

In order to assess vitamin D intake over the last month, the frequency of milk intake (always, never/sometimes) and vitamin D containing supplement use (always/sometimes, never) were collected for analysis. Medications prescribed were assessed at every time-point and categorized by medication class (NSAID, steroid, DMARD or biologic) as certain medications 9steroids) can impact vitamin D concentrations. Season of measurement was also noted as it reflects the potential for vitamin D synthesis through UVB radiation.

A method to standardize, compare and combine inflammatory biomarkers developed by Tabung et al. was used (Tabung et al., 2015). Z-scores of the log-transformed CRP, IL-6, and TNFα concentrations were then added together to create the overall pro-inflammatory biomarker score. The same method was applied to create an anti-inflammatory score where the anti-inflammatory cytokines IL-4 and IL-10 were standardized then combined. Since CRP is used in

defining remission, we used these scores to include other measures of inflammation that are not included in the definition of remission.

Laboratory-Based Assays

Sample Collections and Handling:

At enrollment and at 6 months, blood was collected in P100 Blood Collection and Preservation vacutainer tubes (BD Biosciences, San Jose, CA). Immediately after collection the sample was shipped to the Medical and Related Sciences Centre (MaRS, Toronto, ON) and plasma recovered and stored at -80^{o}C in accord with previously described protocols (Matheson, Duong, Rosenberg, & Yeung, 2008). At the 6-month visit a saliva sample was collectedusing the OrageneTM collection systems (DNAgenotek). Saliva samples were courier shipped unrefrigerated to The Centre for Applied Genomics (TCAG), Hospital for Sick Children, DNA extracted and used for SNP genotyping according to standard protocols.

All visits

Total white blood cell, neutrophil, lymphocyte, and platelet counts; hemoglobin concentrations; ESR and CRP concentrations were measured in clinical service laboratories affiliated with each of the participating sites.

Cytokine Protein Expression

Certain soluble inflammatory mediators (cytokines) were assessed in plasma by multiplex bead-based assays (Fluorokine® Multi Analyte Profiling, Multiplex human cytokine panel A {R&D Systems} using a Luminex analyzer(Luminex Corp.)

Vitamin D Assay

Vitamin D assays were undertaken as previously described (McNally, et al. 2009). Blood samples were collected from a peripheral vein into P100 tubes (BD Biosciences, San Jose, CA) and lasma separated by centrifugation and then stored at -80^{0}C. Samples were batch assayed at the end the study using a competitive binding enzyme-linked immunoassay (EIA; Immunodiagnostic Systems Ltd, Montreal, Quebec, Canada) (Wilkinson, Llewelyn, Toossi, Patel et al., 2000) in accord with manufacturers instructions. Information available from DEQAS (Vitamin D External Quality Assessment Scheme) indicates that enzyme immunoassay

is one of two most commonly used methods to measure 25 25(OH)D (Carter, Carter, Gunter, Jones et al., 2004). Our quality assurance studies confirmed that plasma 25(OH)D concentrations obtained from EIA showed positive correlation with 25(OH)D measured by high performance liquid chromatography (HPLC) (slope=1.17, intercept= -8.8, r^2=0.92). These results are consistent with other reports comparing EIA and radioimmunoassay to HPLC (Carter, Carter, Jones & Berry, 2004; Roth, Schmidt-Gayk, Weber & Niederau, 2008). EIA measures both vitamin D2 (75%) and vitamin D3 (100%) and has intra-assay and inter-assay coefficients of variation of <8% and <10% respectively. Three quality control samples were included in each assay plate and samples and controls (from DEQAS) were assayed in duplicate. The absorbance was read at 450 nm using an ELx800 micro-plate reader (Bio-Tek Instruments, Winooski, VT). 25(OH)D levels were categorized as deficient (<50 nmol/L), insufficient (> 50 nmol/L <75 nmol/L), and sufficient (>75 nmol/L) in accord with guidelines (Canadian Paediatric Society, 2007).

Genotyping

Single nucleotide polymorphism (SNP) gene testing was done using Human 12-sample Immuno BeadChip 11419691 B (Illumina®). The data were analyzed using Genome Studio 2011.1. Testing was done at the Genetic Analysis Facility, The Centre for Applied Genomics, The Hospital for Sick Children, Toronto.

Table 4.1. Data collection of relevance to the proposed study

Category	**Variables to be used in Data Analysis**	**Method of Collection**
Demographic	Sex, age, ethnicity, season of birth	Baseline Questionnaire
Socioeconomic Status	Family income, parent's education	Socioeconomic Status Questionnaire
Anthropometry	Height, weight, waist circumference	Direct measurements at the clinic
Indicators of Disease Status	Biochemical markers of inflammation, CHAQ, JAQQ, Hassles Scale	Markers of inflammation were measured in serum. CHAQ, JAQQ, and Hassles Scale measured through questionnaires
Vitamin D Status	Milk intake, vitamin D supplement use, serum 25(OH)D, Season, VDR polymorphism	Food Frequency Questionnaire, serum 25(OH)D status measured through enzyme immunosorbent assay, and VDR obtained through saliva sample using GoldenGate® 96-sample Sentrix® Array Matrices and a customized 1536-SNP panel (Illumina Inc.).
Additional Information	Disease subtype, medication, family history of disease, physical activity,	All variables measured through questionnaire

Table 4.2. BBOP timeline of relevant data collection

	0 mo.	6 mo.	12 mo.	18 mo.	24 mo.
Sociodemographic questionnaire	**X**				
Saliva for DNA analysis		**X**			
25(OH)D and cytokines	**X**	**X**			
Establish remission or active disease	**X**	**X**	**X**	**X**	**X**
Measures of inflammation	**X**	**X**	**X**	**X**	**X**
Season of measurement	**X**	**X**	**X**	**X**	**X**
Medication use	**X**	**X**	**X**	**X**	**X**
Milk intake and supplement use (FFQ)	**X**	**X**	**X**	**X**	**X**

25(OH)D 25-hydroxyvitamin D, DNA deoxyribonucleic acid, FFQ Food Frequency Questionnaire, Mo. Month

4.1.1. Methodology for Objective 1

Objective 1: Evaluate vitamin D status and growth parameters in children and adolescents with JIA and compare with those of healthy children and adolescents.

To address Objective 1, we analyzed vitamin D status and anthropometric measurements in JIA patients enrolled in BBOP and compared them with a nationally representative age-matched sample of Canadian healthy children using CHMS data (Cycles 1, 2007-09, 2009-11).

Canadian Health Measures Survey

CHMS is a Canadian cross-sectional nationally representative health survey that runs in bi-yearly cycles. Healthy children who participated in this survey will serve as a control group. We will be comparing the baseline data collected in the BBOP study to healthy age-matched children from the CHMS Cycles 1 and 2 based on 25(OH)D status, anthropometric measures (weight, height and waist circumference, BMI), intake of vitamin D and calcium, socioeconomic status, physical activity and season of birth. Data from CHMS Cycles 1 (2007-2009) and

2 (2009-2011) were combined. There are approximately 4,500 children between the ages of 3-19 sampled for the 2 cycles of CHMS which ran at the same time as data collection for the BBOP study. In BBOP study the distribution of JIA patients has the approximate rate of 3:1 F to M, while in CHMS this rate is 1:1. To be able to properly consider this distribution at the population level we performed additional age- and sex-specific analyses. Further, considering the complex survey design in CHMS, we used weight variables and the combined bootstrap files as instructed by Statistics Canada to obtain estimates and their variation at the population level.

Data cleaning and management

Data manipulation, cleaning, grouping and creating the variables of interest was done in SPSS Version 22 (IBM, 2014). Existing variables of interest that are included in both CHMS and BBOP are as follows: age; sex; ethnicity; socioeconomic status; physical activity; weight; height; waist circumference; vitamin D intake, and 25(OH)D status. See Chapter 7 for age, sex and ethnicity of BBOP participants. We used 25(OH)D concentrations to estimate the prevalence of vitamin D deficiency, insufficiency, and sufficiency in both BBOP and CHMS data. After excluding children with chronic conditions in CHMS combined data, we created an age group equivalent to the BBOP children's age group.

Data analysis

The prevalence of vitamin D deficiency, vitamin D intake, and growth parameters respectively, were determined across demographic and socioeconomic factors via frequencies and cross-tabulation. The difference in estimates of children between BBOP and CHMS is considered significant at an alpha of 0.05 if the point estimates do not lie within the estimated confidence intervals for parameters from the other populations. This approach allows us to compare variables of interest at population levels. Analyses at this section addressed the hypothesis whether children and adolescents with JIA have lower vitamin D status and growth parameters (height, weight, waist circumference, BMI) than healthy children and adolescents.

4.1.2. Methodology for Objective 2

Objective: Examine the association between plasma levels of 25(OH)D and disease activity and outcomes as determined by clinical and biomarker profiles in JIA.

Using the BBOP data collected every 6 months for 2 years characterized the relationships between both vitamin D intake and vitamin D status with disease outcome over time in children with JIA. In order to assess vitamin D intake over the last month, food frequency questionnaires focusing on the intake of vitamin D and calcium-containing foods have been collected for analysis. Medication and supplement use were assessed at every time-point. Indicators of disease activity data are available as assessed by ACR core set of disease activity variables which include the following: number of joints with active arthritis, number of joints with limited movement, physician global assessment of disease activity, acute phase reactants (ESR and CRP), and the Child Health Assessment Questionnaire – JIA (CHAQ-JIA) (Giannini et al., 1997). Measures of inflammatory mediators (cytokines), and 25-hydroxyvitamin D are available at enrollment and 6 months post-enrollment. See Table 4.3 for biochemical measurements conducted. The biochemical markers of inflammation as well as the surveys to measure disease activity were investigated to determine if there is a relationship between vitamin D status and markers of inflammation, and disease activity.

Descriptive statistics (means and standard deviations for continuous variables and percentages for categorical variables) were summarized the data at five time points. The descriptive analysis done using SPSS Version 22 (IBM 2014). For inference, Multi-Level Mixed-Effects (MLM) Modeling with manuel backwards selection with patients set as a random effect factor (individual patient change by time/visit number), was utilized. The repeated measures across time points had unstructured covariance structure. Criteria for variable elimination was any variables that were statistically insignificant (p-value > 0.05), the one making the smallest contribution as determined by p-value was dropped. After each variable was removed, the likelihood ratio test was performed to ensure that removing the parameter/ predicting variable from the model does not significantly reduce the fit of the model. If removing the statistcally non-significant variable from the model reduced the fit then it was left in the model. Missing data was assumed to be missing at random and initial exploration of missing variables by time point showed no pattern of dropout by age, sex, disease subtype, ethnicity or income. The goal was, to determine if there is association between factors associated with vitamin D status and markers of inflammation /disease outcomes in the presence of potential covariates, such as socioeconomic status, sex, age, ethnicity. STATA 15 (StataCorp LP 2015)

was used for analysis. Analyses of this section will address whether vitamin D status is negatively associated with inflammatory markers and disease activity among JIA cases.

Table 4.3. Biochemical markers

Marker	Abbreviation
Interferon gamma	IFNγ
The interleukin-1 receptor antagonist	IL-1Rα
Interleukin-1a	IL-1α
Interleukin-1b	IL-1β
Interleukin-2	IL-2
Interleukin-3	IL-3
Interleukin-4	IL-4,
Interleukin-5	IL-5
Interleukin-6	IL-6
Interleukin-7	IL-7
Interleukin-8	IL-8
Interleukin 10	IL-10
Interleukin-13	IL-13
Interleukin-15	IL-15
Interleukin-17	IL-17
Tumor necrosis factor	TNFa
Hemoglobin	HGB
Total neutrophil count	ANC
Total lymphocyte count	TLC
Platelet count	PLT
White blood cell count	WBC
Erythrocyte sedimentation rate	ESR
C-reactive protein rate	CRP
25-Hydroxyvitamin D	25(OH)D

4.1.3. Methodology for Objective 3

Objective 3: Evaluate association between vitamin D pathway gene polymorphisms, vitamin D levels, and JIA disease activity.

At the second visit, a saliva sample was collected for genotyping. DNA was extracted to analyze VDR polymorphisms and the genotype data were analyzed to determine if there is a higher proportion of certain polymorphisms of VDR's in children with JIA.

Genotyping was performed using GoldenGate® 96-sample Sentrix® Array Matrices and a customized 1536-SNP panel (Illumina Inc.) at the Centre for Applied Genomics, Toronto, Canada.

4.1.3.1. Genetic Analysis

Step 1: Genome-Wide Association Studies (GWAS)

For the BBOP study single nucleotide polymorphism (SNP) gene testing was done using: Human 12-sample Immuno BeadChip 11419691 B (Illumina®). Genome information (.bed, .fam, .bam files (https://www.ensembl.org/info/website/upload/bed.html)) was provided by the Genetic Analysis Facility, The Centre for Applied Genomics, The Hospital for Sick Children, Toronto.

In addition to BBOP, two other genetic datasets were used. 1) The "*Better Outcomes for Children: GWAS from Cincinnati Children's Hospital Medical Center (CCHMC) - eMERGE Phase II*" dataset (dbGaP Study Accession: phs000494.v1.p1). This dataset includes genotyping data from six cohorts. Cohort D of the dataset comprises Caucasian patients with JIA. The dataset includes 814 JIA cases and 658 controls of self-reported non-Hispanic European American (EA) ancestry (Thompson et al., 2012). 2) The National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) healthy control genetic dataset were merged to build a case/control dataset, where BBOP samples were used as cases and the NIDDK Dataset used as controls. GWAS analysis was performed using PLINK version 1.9 (http://pngu.mgh.harvard.edu/purcell/plink/) (Purcell, Neale, Todd-Brown, Thomas et al., 2007) according to the protocol specified by Anderson et al. (Anderson et al., 2011). This approach was also used to analyze the data from cohort D of the Cincinnati dataset. After applying quality

control for these two datasets by following the procedure suggested by Anderson et al. (Anderson et al., 2011), there was a small overlap between SNPs in these datasets. This was mainly because NIDDK and BBOP used two different platforms with a small overlap between their results; therefore, we avoided extracting control samples from NIDDK dataset and conducting a case-control study.

A subset of markers was assembled based on the following criteria:

- Markers suspected to be involved in JIA extracted from the literature
- Markers associated with the vitamin D pathway

Markers extracted from the analysis of the Cincinnati cohort D The genotype data were extracted for the candidate SNPs for all samples, in the BBOP dataset, that passed the quality control procedure (Anderson et al., 2011). These SNPs later were used for regression analysis.

GWAS results are presented in Table 4.4.

4.1.3.2. Statistical Analysis

Step 2: Descriptive statistics

Descriptive statistics (means and standard deviations for continuous variables and percentages for categorical variables) were used to summarize the data at five time points of measurements (CHAPTER 7 Table 7.2).

Step 3: Genetic predictors of disease activity

Bivariate analysis was performed to explore what individual genes identified through GWAS as well as genes known to be in the vitamin D pathway (Table 4.4) may predict disease activity. Any genes that were identified to have a $p<0.05$ were included in the full MLM Modeling building process. A linear as opposed to a non-linear model was chosen because the parameters to be estimated were either linear or could be log-transformed to become linear. Specifically, MLM was chosen because it provided individual-specific effects (as opposed to the population average effect). The repeated measures across time points had unstructured covariance structure. Given that the goal was to describe the potential impact of vitamin D on patient disease activity this method was considered appropriate.

Step 4: Gene-Gene interactions (epistasis)

Next, multivariate analysis of epistasis of genes identified through GWAS and those in the vitamin D pathway was performed. Those with a p-value <0.01 were included in the full model building process. Variables that were significant in the previous steps were included in the full MLM building process.

Step 5: Environmental, genetic and interactions that predict disease activity

Analysis of environmental, treatment and lifestyle-related factors as well as detailed methodology for that objective are presented in Chapter 7. Linear regressions were performed to identify gene-environment interactions that significantly (p-value <0.05) impacted disease activity measures to be included in the final MLM. Significant variables from linear regressions performed in Chapter 7, as well as those genes identified through GWAS, vitamin D pathway genes, gene-gene interactions were included in the final analysis to identify what environmental and genetic factors impact disease activity.

Step 6: Multi-Level Mixed-Effects (MLM) Modeling

MLM Modeling and manuel backwards methodology with patients set as random variable (individual patient change by time/visit number), was utilized. The repeated measures across the time points had unstructured covariance structure. Criteria for variable elimination was any variables that were statistically not significant, the one making the smallest contribution as determined by p-value was dropped. After each variable was removed the likelihood ratio test was performed to ensure that removing the parameter/ predicting variable from the model does not signifigantly reduce the fit of the model. If removing the statistcally non-significant variable from the model reduced the fit then it was left in the model. Missing data was assumed to be missing at random and initial exploration of missing variables by time point showed no pattern of dropout by age, sex, disease subtype, ethnicity or income. Intreactions with a p-value > 0.01 were not selected for the final model to adjust for the increased false discovery rate of multiple comparisons. The goal was to determine if there is association between genes potentially related to the vitamin D pathway and markers of inflammation /disease outcomes in the presence of potential covariates, such as socioeconomic status, sex, age, and ethnicity. STATA 15 (StataCorp LP 2015) was used for analysis.

Table 4.4. Genes and SNPs explored for interactions

Vitamin D Pathway Genes	Single Nucleotide Polymorphism	Genes Identified Through GWAS*	Single Nucleotide Polymorphism
VDR		NOTCH4	
	rs4516035		rs2071286
	rs11568820		rs415929
	rs1544410	C6orf10	rs6907322
	rs1540339	HLA-DQA1	rs9272219
	rs3890733	LEP	rs2071045
	rs4760648	IGFBP4	
	rs731236		rs584438
	rs7975232		rs584828
	rs2248098	GPS1	rs9916764
	rs10783219		
	rs2238136		
GC	rs7041/rs4588		
CYP24A1			
	rs4809959		
	rs2248359		
CYP1R1			
	rs17116978		
	rs1035798		

*GWAS Genome Wide Association Study, SNPs Single Nucleotide Polymorphisms

CHAPTER 5

OBJECTIVE 1

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Vitamin D levels are not decreased in Canadian children with new onset juvenile idiopathic arthritis compared to healthy children

To address objective 1 of this thesis I evaluated vitamin D status and growth parameters in children and adolescents with JIA and compare with those of healthy children and adolescents.

**Prepared for submission, format and structure of the original manuscript has been revised to provide a better flow within the thesis and be consistent with the format of previous chapters*

Note: Additional findings relating to anthropometric values that are not included in this manuscript are presented in Chapter 6.

Abstract

Objective. The objective of this study was to compare vitamin D levels in children with Juvenile Idiopathic Arthritis (JIA) to healthy children.

Methods. Data from a Canadian cohort of children with new onset JIA were compared to Canadian Health Measures Survey (CHMS) data. Comparisons were made with respect to data from healthy children comparing 25-hydroxy vitamin D (25(OH)D) concentrations, measures of inflammation, vitamin D supplement use, milk intake, and season of birth.

Results. The mean 25(OH)D blood level was significantly higher in JIA patients (79 ± 3.1 nmol/L) than in healthy controls (68 ± 1.8 nmol/L $p < 0.05$) and JIA patients more often used vitamin D supplements (50% vs. 7% $p < 0.05$). The prevalence of 25(OH)D deficiency (<30

nmol/L) was 6% for both groups. Children with JIA with 25(OH)D deficiency or insufficiency (<50 nmol/L) had higher CRP levels. Children with JIA were more likely to be born in the fall and winter compared to healthy children.

Conclusion. Contrary to the expectations, we found that vitamin D levels in Canadian children with JIA were not lower than healthy children; an observation that might relate to the frequent use of vitamin D supplements in the JIA population. A preponderance of JIA patients born in seasons with reduced endogenous vitamin D production could implicate low vitamin D during gestation and early life as a factor influencing JIA pathogenesis. Among children with JIA, a low CRP concentrations, indicating less inflammatory disease activity, are associated with a 25(OH)D concentration above 50 nmol/L.

5.1. Introduction

Juvenile Idiopathic Arthritis is one of the most frequent chronic childhood diseases. Reported JIA prevalence rates range from 0.07 to 4.01/1000 children and annual incidence rates from 0.008 to 0.226/1000 (Manners & Bower, 2002). The occurrence and outcomes of JIA are likely influenced by multiple factors including genetic (Cobb, Hinks, & Thomson, 2014; Ellis et al., 2015), sociodemographic (Chiaroni-Clarke, Munro, & Ellis, 2016), lifestyle (Carlens et al., 2009) and environment (Ellis et al., 2010). However, the precise cause and pathogenesis of JIA are unknown.

Low vitamin D has been reported in a number of autoimmune diseases including multiple sclerosis, type 1 diabetes, Crohn's disease and JIA (Berkun et al., 2015; Cutolo et al., 2011; Nisar M., Cookson P., Masood F., Sansome A., 2013; Nisar et al., 2013; Shapira, Agmon-Levin, & Shoenfeld, 2010; Von Scheven & Burnham, 2011). In adults with rheumatoid arthritis, low 25(OH)D is associated with increased disease activity (Sabbagh et al., 2013). Vitamin D is known to exert an immunosuppressive effect by suppressing the synthesis of pro-inflammatory cytokines and preventing prolonged inflammatory responses (Cutolo et al., 2011).

Although a meta-analysis reported the prevalence of vitamin D insufficiency in JIA as 82% (Nisar et al., 2013), it is unclear if this was a consequence of reduced dietary intake, season of measurement, a medication effect, or increased utilization and/or decreased absorption in the context of inflammation. In contrast, higher concentrations of vitamin D have been observed

with more active arthritis in certain JIA categories when patients are receiving vitamin D supplementation while receiving glucocorticoids to treat active disease (Miettinen, Kinnunen, Harjutsalo, Reinert-Hartwall, Lamberg-Allardt, Toumilehto, Saila, 2013).

Vitamin D status is categorized by the IOM based on serum 25(OH)D concentrations and classified as : (i) deficient <30 nmol/L; (ii) insufficient >30 and <50 nmol/L; or (iii) sufficient ≥50 nmol/L (Institute of Medicine, 2010). In contrast, the Endocrine Society (ES) proposed that the optimal serum 25(OH)D concentration is ≥ 75 nmol/L and recommended vitamin D supplementation dose for at-risk populations that is twice as high as the IOM recommendations (Holick et al., 2011). The discrepancies in IOM and ES recommendations, both of which are made in the context of bone health, reflect current uncertainty as to the optimal 25(OH)D concentration; no targets specific for children with JIA are available.

The aim of the present study was to further investigate the associations of vitamin D status in JIA patients compared to healthy children. We hypothesized that Vitamin D levels would be decreased in children with JIA and that children with higher levels of inflammation would have further reduced vitamin D levels.

5.2. Subjects and Methods

5.2.1. Data Sources

Participants from the Biologically Based Outcome Predictors (BBOP) Study were compared with a sample of overall healthy Canadian children and matched by age and sex, taken from CHMS Cycles 1 (2007–09) and 2 (2009–11) in a case-control study design To be able to keep the integrity of the population-based data in CHMS and avoid selection bias, we used the same 1:1 ratio of female to male as exist based on the complex survey design in CHMS. JIA impacts more females than males (Ravelli, 2007) although the reason for sex disparity in JIA is not well-understood. We analyzed the data in both sexes together and separately to explore if the relationship of disease development and sex was associated with vitamin D status.

BBOP is a prospective inception cohort study that enrolled 186 new-onset JIA patients aged 1–16 years from 11 Canadian pediatric rheumatology centers. Enrolment criteria included consenting participants who: (i) met ILAR JIA classification criteria (Petty et al., 2004); (ii) were diagnosed within six months of disease onset; and (iii) had not been treated with DMARDs or biologically-based anti-cytokine therapies (biologics). The cohort comprised participants from

each of the seven JIA categories. The study aimed to recruit a representative sample from each category. To achieve this, only participants with polyarthritis or systemic JIA (the least prevalent categories) were eligible during the first six months of enrollment after which any JIA category was eligible. Patients and/or legal patients legal guardians provided written informed consent for study participation and publication of summarized results. The study was approved by the research ethics boards at each participating site (Research Ethics Board approval #07-86).

CHMS is a Canadian cross-sectional health survey that runs in bi-yearly cycles (Giroux, 2007). Data from CHMS Cycles 1 and 2 were combined for children aged 6–16 years. For 3 to 5 year olds data were taken from Cycle 2 (Statistics Canada, 2014). Healthy children who were reported as having no chronic disease served as the comparison group (Tremblay & Connor Gorber, 2007).

For participants in the BBOP study, plasma 25(OH)D was measured from a blood sample obtained at enrollment using a competitive binding enzyme-linked immunoassay (EIA Immunodiagnostic Systems) as previously described (McNally et al., 2008). Total white blood cell, neutrophil, lymphocyte, and platelet counts; hemoglobin; and CRP were measured in clinical service laboratories affiliated with each of the participating sites.

For participants in the CHMS study plasma 25(OH)D was measured using the LIAISON 25(OH)D TOTAL assay (Diasorin, Ltd,) (Sarafin, Durazo-Arvizu, Tian, & Phinney, 2015). Further, the original 25(OH)D values in CHMS Cycles 1 and 2 were then standardized to the internationally recognized reference measurements as described elsewhere (Sarafin et al., 2015). In a study evaluating the accuracy of 25(OH)D assessment methods in comparison with liquid chromatography–tandem mass spectrometry, each approach used in BBOP and in CHMS had similar concordance with the reference measure (Roth, Schmidt-Gayk, Weber, & Niederau, 2008).

Comparison of groups.

We compared baseline BBOP data from children aged 3–16 years with healthy children from CHMS Cycles 1 and 2 assessed at the same time as the BBOP study. In BBOP, distribution of JIA patients had the approximate ratio of 2:1 female to male, while in CHMS sex distribution was 1:1.

Data analysis.

We performed age group and sex-specific analyses by weighting variables and the combined bootstrap files from the two survey cycles to obtain generalizable results at a population level (Giroux, 2007). Since children aged 3–5 years were only included in CHMS Cycle 2, this age group was analyzed separately.

Variables of interest included age, sex, ethnicity, household income, date of birth, date of blood collection, laboratory measures mentioned above, use of supplements containing vitamin D, milk intake, and 25(OH)D status. We used 25(OH)D concentrations to determine the IOM-defined prevalence of vitamin D deficiency, insufficiency, and sufficiency. Self-identified ethnicity categories were White, Non-white and off-reserve Indigenous. Season of birth and season of 25(OH)D measurement were based on the seasonal equinoxes. The period between April to October, when cutaneous synthesis of vitamin D is most efficient, was designated the synthesizing period and November to March was the non-synthesizing period (Wacker et al., 2013). Milk intake and vitamin D supplement use were assessed using a food frequency questionnaire.

Differences between BBOP and CHMS cohorts were considered significant at alpha= 0.05 if point estimates (mean values) did not lie within the estimated 95% confidence intervals for parameters from the respectively compared population (Dixon et al., 2018). Data manipulation, cleaning, grouping and selecting variables of interest were done in SPSS Version 22 (IBM, 2014). Stat/Transfer Version 10 (Circle Systems, Inc) was then used to transfer data to STATA version 14 (StataCorp) for analysis including cross-tabulation, weighting and bootstrapping to obtain nationally representative population level estimates.

5.3. Results

To be consistent with the CHMS age ranges, BBOP participants comprised 164 children with JIA aged 3–16 years (3–5-year olds n=28; 6 to 16-year olds n= 136). Those outside of this range were excluded. Characteristics of children with JIA and the CHMS healthy population representative of ~4.2 million Canadian children free of chronic disease are shown in Table 5.1.

Socio-Demographic Characteristics.

More females were in the JIA cohort (70 ± 4.0%) than the healthy control group (50 ± 1%). The proportion of Indigenous children with JIA (10 ± 2.8 %) was greater than the healthy population (3.4 ± 7.1 %); ($p<0.05$). There was no difference between populations in those who identified as White and Non-white, or in household income.

Vitamin D.

Mean 25(OH)D concentration was significantly higher in children with JIA than in the healthy control children (79 ± 3.1 nmol/L vs 68 ± 1.8 nmol/L). Children with JIA used vitamin D-containing supplements significantly more than healthy controls (50% vs 7%) (Table 5.2). Children with JIA who took supplements had significantly higher 25(OH) D concentrations than healthy control children who both did and did not take supplements (Table 5.2). The proportion of children with 25(OH)D >100 nmol/L was significantly greater in children with JIA than healthy controls (18 ± 3.5% vs. 10 ± 1.2%). The prevalence of 25(OH)D deficiency (<30 nmol/L) was 6% for both populations. Healthy control children reported "consuming milk everyday" significantly more often than children with JIA (Table 5.2). Children with JIA who drank milk everyday had a 25(OH)D concentration of 86.28 ± 4.97 nmol/L, significantly higher when compared to those who occasionally or never drank milk who had a 25(OH)D concentration of 73 ± 5.2 nmol/L.

Vitamin D and Seasonality.

Significantly more children with JIA compared to healthy control children were born in the fall and winter (Table 5.2). More children with JIA were born in February or December than healthy children (Table 5.2). Males with JIA were significantly more likely to be born in the winter (males, JIA: 41 ± 8% [27 – 57] vs. healthy: 25 ± 2% [21 – 28]) and females with JIA were more likely to be born in the fall (females JIA: 31 ± 5 [22 – 41] vs. healthy: 21 ± 2% [18 – 24]). Vitamin D concentrations were highest during the synthesizing period and were significantly lower for healthy control children in the non-synthesizing period (Table 2). Children ages 6–8 years old with JIA were significantly more likely to be born in the non-synthesizing period (JIA: 74 ± 9% [54 - 87] vs. healthy: 45 ± 3% [38 - 52]).

Vitamin D and Ethnicity.

Children with JIA who identified as White had significantly higher 25(OH)D concentrations (83.3 ± 4.1 nmol/L) compared to Non-white (71.5 ± 6.1 nmol/L). Children with JIA who identified as White and Non-white had higher 25(OH)D concentrations than their healthy peers. Indigenous children with JIA had similar vitamin D concentrations as their healthy Indigenous peers (Table 2). Children aged 9-13 years with JIA had the greatest proportion with a 25(OH)D concentration > 100 nmol/L and the highest 25(OH)D concentration in the non-synthesizing period. Children aged 9-13 years with JIA who identified as White had the highest 25(OH)D status (91.9 ± 7.0 nmol/L). Children who identified as Non-white in the 6 to 8-year and 9 to 13-year age groups with JIA had higher 25(OH)D concentrations (6 to 8 year olds 87.0 ± 8.6 nmol/L [70.0 – 104.0], 9 to 13 year olds 79.0 ± 7.4 [64.3 - 93.7]) than healthy children (6 to 8 year olds 62.2 ± 3.9 nmol/L [54.2 - 70.1] 9 to 13 year olds 52.8 ± 4.9 [42.8 - 62.9]). Children who identified as Indigenous and had JIA in the 14 to 16 year age group had the lowest 25(OH)D concentrations (46.3 nmol/L) but not significantly different from healthy Indigenous children.

Vitamin D and Other Laboratory Tests.

There was a significant difference in all laboratory test results including blood cell counts and measures reflective of inflammation, between healthy children and those with JIA, with the exception of lymphocyte counts (Table 5.3). All markers were higher in children with JIA with the exception of hemoglobin concentrations, which were lower. In JIA, those who had a 25(OH)D concentration < 50 nmol/L had significantly higher CRP concentrations than the other groups (Table 3). There were no differences by age or sex.

Vitamin D in 3 to 5-year olds.

Fewer 3 to 5-year old children with JIA were reported to be choosing milk every day and a greater proportion were taking a vitamin D containing supplement every day or sometimes (50% vs. 32%, $p<0.05$). Three to five-year old children with JIA who were given supplements had higher 25(OH)D than children who were not taking supplements (taking supplements JIA 121.8 ± 14.0 nmol/L vs not JIA 88.8 ± 12.2 nmol/L, $p<0.05$).

Vitamin D and JIA Categories.

The numbers of patients in each JIA category are shown in Table 4. Children with psoriatic arthritis had the lowest mean 25(OH)D concentration at 65.3 nmol/L (Table 5.4). This was significantly lower than in children diagnosed with enthesitis-related arthritis and oligoarthritis who had 25(OH)D concentrations of 95.0 and 85.0 nmol/L respectively. Over 60% of children with undifferentiated arthritis were born in the winter. This was significantly different from the proportion born in the summer and fall; no children with undifferentiated arthritis were born in the spring (Table 5.4). Children diagnosed with systemic arthritis were significantly more likely to be born in the winter. Significantly more children with rheumatoid factor negative polyarthritis were born in the fall than the winter or spring with no statistical difference in the proportion for those born in the summer compared to other seasons.

5.4. Discussion

This is the first study, to our knowledge, that compares multiple factors associated with vitamin D status in newly diagnosed JIA patients and healthy children at a national level. Contrary to our expectation, vitamin D levels in Canadian children with JIA were not decreased when compared to levels in healthy Canadian children. This might be explained by the high use of vitamin D supplements in children with JIA (Institute of Medicine, 2010); approximately half of newly diagnosed children were taking a vitamin D supplement which is higher than in the healthy population. 25(OH)D status was higher for JIA patients, and 18 nmol/L higher than the mean reported in the meta-analysis by Nisar et al. (Nisar, Cookson, Masood, Sansome, 2013).

25(OH)D levels were significantly different based on self-identified ethnicity and health status. In contrast to other studies (Dağdeviren-çakır et al., 2016; de Sousa Studart et al., 2015; Nisar et al., 2013; Szymanska-Kaluza, Biernacka-Zielinska, Stanczyk, 2013) BBOP participants had significantly higher 25(OH)D levels than healthy children. Those who identified as White in both groups had the highest 25(OH)D concentrations. There was a significant negative difference in CRP concentrations in children with JIA based on 25(OH)D concentration below or above the IOM defined adequate level of 50 nmol/L (Table 3) (Institute of Medicine, 2010). Additionally, compared to healthy children, children with JIA are more likely to be born in the fall or winter seasons.

In our study the prevalence of 25(OH)D deficiency (<30 nmol/L) was 6% for both populations. The higher rate of supplement use in children with JIA might be explained by a higher likelihood of supplement use in sick children (Bailey, Gahche, Thomas, & Dwyer, 2013; Dwyer et al., 2013). In a population-based study, the decision to use supplements was the caregivers' and in only 15% of cases at the physician's recommendation (Bailey et al., 2013). Prior to diagnosis parents may be providing supplements to treat the undiagnosed symptoms as was reported in the study by Bailey et al. (Bailey et al., 2013); however, information relating to the reasons for administering vitamin D supplements and dosage information were not collected in our study.

There was a greater proportion of children of Indigenous descent in the BBOP cohort compared with the control cohort because the CHMS did not recruit as far north as did the BBOP study nor did it collect information from Indigenous reserve communities. Additionally, there may be a higher proportion of JIA cases in the Indigenous population than in other populations (Jarvis & Cleland, 2003). Contrary to White or Non-White JIA children, children with JIA who identified as Indigenous had significantly lower mean 25(OH)D concentrations compared to healthy Indigenous children. As having a 25(OH)D level above 50 nmol/L appears to be associated with a lower CRP, children with JIA of Indigenous descent may require a targeted vitamin D intervention to contribute to modulating inflammation.

We found children with JIA are more often born in the fall and winter seasons. Season of birth has been suggested to have an impact on the risk of developing a number of autoimmune diseases (Berkun et al., 2015). In a previous report children with JIA were more likely to be born between November to March compared to birth months for the general population peaking in the summer (Berkun et al., 2015). Carlens et al. also investigated this relationship but found no increased risk relating to season (Carlens et al., 2009). Maternal vitamin D status, which can be affected by season, may alter fetal vitamin D status or have detrimental consequences in the child during the first few months after birth (Çuhacı-Çakır & Demirel, 2015). Investigation into maternal vitamin D status and behaviors such as sun exposure, supplement use and intake of fortified foods during each gestational trimester could help to understand if, and when maternal vitamin D status influences the occurrence of JIA.

Children with JIA had higher CRP levels and higher white blood cell, neutrophil and platelet counts than healthy children and lower hemoglobin concentrations, a profile suggestive

of active inflammation. Children with JIA who had a 25(OH)D <50 nmol/L had higher CRP levels than children with higher 25(OH)D concentrations. Consistent with our finding, an association between 25(OH)D concentrations and CRP concentrations was found by Stagi et al. (Stagi et al., 2014). Vitamin D has been suggested to play a role in the pathogenesis of JIA because vitamin D has immunosuppressive effects (Cutolo et al., 2011). Vitamin D enhances macrophage production of antimicrobial peptides, and suppresses proinflammatory properties of dendritic cells and antigen presenting cells (Von Scheven & Burnham, 2011). Immune system cells that express vitamin D receptors have the potential to synthesize 1,25-dihydroxycholecalciferol ($1,25(OH)_2D$) and elicit an autocrine or paracrine response (Hewison, 2012). In most cells in the immune system vitamin D suppresses the immune response (Cutolo et al., 2011). Therefore, low vitamin D concentrations would be associated with inflammation (Cutolo et al., 2011). The role of vitamin D in other autoimmune disorders has been established (Holick, 2005). Our observations might suggest that adequate 25(OH)D reduces inflammation in children with JIA. However, further studies are needed to determine if vitamin D status influences disease pathogenesis.

Disease Categories.

The meta-analysis by Nisar et al. summarizes the research into juvenile chronic arthritis and juvenile rheumatoid arthritis and 25(OH)D (Nisar et al., 2013). Three studies have explored 25(OH)D concentrations by JIA category (de Sousa Studart et al., 2015; Stagi et al., 2014; Szymańska-Kałuża, Biernacka-Zielińska, Stańczyk, & Smolewska, 2013). The two studies with n=50 reported no difference among categories. (de Sousa Studart et al., 2015; Szymańska-Kałuża et al., 2013). The study with n= 152 found children with systemic JIA had lower 25(OH)D concentrations than children with enthesitis-related arthritis, oligoarticular arthritis, and polyarticular arthritis (Stagi et al., 2014). Our results show that although children with systemic JIA had the second lowest 25(OH)D concentration it was not significantly lower than in other categories. The one other study that has explored season of birth by JIA category found rhythmicity (cyclic occurrence) in the enthesitis-related arthritis category with peaks in August and December (Berkun et al., 2015).

Limitations.

Data reported here were derived from two separate studies with differences in collection methodology including 25(OH)D measurement assays although earlier studies have shown that results from these different methodologies yield comparable results (Roth et al. 2008). Additionally, the 3-Epi-25-hydroxycholecalciferol epimer of vitamin D was not measured. This epimer is less bioactive than other vitamin D metabolites (Aghajafari Field, Rabi, Kaplan, et al. 2016). Enrolment criteria included being within 6 months of disease onset; therefore, some patients may have already changed their behavior because of their disease or the advice of a health care practitioner. Additionally, exact time since disease onset was not included in the analysis. Skin pigmentation influences baseline 25(OH)D and endogenous synthesis (Mangin, Sinha, & Fincher, 2014) but was not directly measured in our study.

5.5. Conclusion

Results of this study show that mean vitamin D concentrations were in the optimal range JIA population and that higher concentrations of vitamin D in JIA compared to healthy controls is associated with more frequent use of vitamin D supplements (Holick et al., 2011). Our results show that a preponderance of JIA patients are born in seasons in which endogenous vitamin D synthesis is low raising the consideration that low vitamin D during the perinatal period could be a factor influencing JIA occurrence. Having a 25(OH)D level above 50 nmol/L is associated with a lower CRP, reflective of less disease activity. However, more research is required to fully assess the potential influence of vitamin D on JIA occurrence, pathogenesis and outcomes.

5.6 Acknowledgments

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Table 5.1. Sociodemographic information of healthy children in comparison to children with JIA

		Overall 6- to 16-year olds		3- to 5-year olds	
		Healthy Mean ± SE (95% CI)	JIA Mean ± SE (95% CI)	Healthy Mean ± SE (95% CI)	JIA Mean ± SE (95% CI)
Age (years)		12 ± 0.1 (11–12)	12 ± 0.3 (11–12)	4 ±0.1 (3.8–4.2)	4 ±0.2 (3.6–4.3)
Sex (%)	Male	**50 ± 1 (48–53)**	**30 ± 4 (23–38)***	46 ± 3 (38–53)	39 ± 9 (22 – 59)
	Female	**50 ± 1 (47–52)**	**70 ± 4 (62–77)***	54 ± 3 (47–62)	61 ± 9 (41–78)
Annual Household Income (%)	< $49,999	11 ± 1.5 (7.5–14)	9.4 ± 2.9 (5.1–17)	4 ± 1 (2–6)	14 ± 7 (4–37)
	$50,000 – 74,999	14 ± 1.4 (11–17)	14 ± 3.4 (9–22)	9 ± 2 (5–14)	32 ± 10 (15–55)
	$75,000 – 99,999	41 ± 1.7 (37–44)	38 ± 4.7 (29–47)	41 ± 3 (34–48)	27 ± 10 (12–51)
	> $100,000	35 ± 2.4 (30–40)	39 ± 4.8 (30–48)	46 ± 4 (38–55)	27 ± 10 (12–51)
Self-Identified Ethnicity (%)	White	74 ± 4.4 (65–83)	71 ± 4.1 (62–78)	77 ± 3 (70–84)	81 ± 8 (60–92)
	Non-White	23 ± 4.8 (13–33)	19 ± 3.5 (13–26)	20 ± 3 (12–27)	8 ± 5 (2–28)
	Indigenous	**3 ± 0.7 (2–5)**	**10 ± 3 (6–17)***	**3 ± 2 (2–8)**	**12 ± 6 (3–32)***

*Bold font represent statistically significant ($p<0.05$) differences between healthy children and those with JIA. Significance established when the point estimates did not lie within the estimated 95% confidence intervals for parameters from the respectively compared population

Table 5.2. 25(OH)D concentration and vitamin D-related variables in healthy children and children with JIA

		6- to 16-year olds		3- to 5-year olds	
		Healthy Mean ± SE (95% CI)	JIA Mean ± SE (95% CI)	Healthy Mean ± SE (95% CI)	JIA Mean ± SE (95% CI)
25(OH)D nmol/L		**68 ± 1.8 (65–72)**	**79 ± 3.1 (73–85) ***	78 ± 1.2 (75–80)	101 ± 8.5 (83–118)
Proportion by Vitamin D Cut-off Category	< 30 nmol/L	6 ± 1.6 (2–9)	6 ± 2.1 (3–12)	<50 nmol/L	<50 nmol/L
	30 - 50 nmol/L	17 ± 1.4 (15–20)	10 ± 2.7 (6–17)	8 ± 3 (2–14)	5 ± 5 (1–31)
	> 50 – 75 nmol/L	42 ± 1.5 (39–45)	37 ± 4.4 (29–46)	39 ± 4 (31–48)	19 ± 9 (7–44)
	> 75-100 nmol/L	25 ± 1.6 (22–28)	29 ± 4.1 (22–38)	42 ± 3 (35–49)	38 ± 11 (19–62)
	> 100 nmol/L	**10 ± 1.2 (8–12)**	**18 ± 3.5 (12 –26) ***	**11 ± 2 (7–14)**	**38 ± 11 (19–62) ***
Milk Consumption (%)	Everyday	**81 ± 1.3 (78–83)**	**68 ± 4.5 (59–76) ***	**95 ± 2 (90–100)**	**77 ± 9 (54–91) ***
	Never/sometimes	**19 ± 1.3 (17–22)**	**32 ± 4.5 (24–41) ***	**5 ± 2 (0–10)**	**23 ± 9 (9–46) ***
Vitamin D use (%)	Never	**93 ± 0.9 (91 – 94)**	**50 ± 5 (40 – 59) ***	68 ± 3 (62–74)	50 ± 11 (29–71)
	Everyday/sometimes	**7 ± 0.9 (6 – 9)**	**50 ± 5 (41 – 60) ***	32 ± 3 (26–38)	50 ± 11 (29–71)
Season of Birth (%)	Winter	**24 ± 1.2 (21–26)**	**33 ± 4 (26–41) ***	21 ± 4 (13–30)	29 ± 9 (14–49)
	Spring	**27 ± 1.2 (25–30)**	**19 ± 3.4 (13–27) ***	29 ± 4 (20–37)	21 ± 8 (9–42)
	Summer	**27 ± 1.5 (24–30)**	**19 ± 3.4 (13–27) ***	24 ± 3 (17–31)	25 ± 8 (12–45)

	Fall	**22 ± 1.2 (20–25)**	**29 ± 3.9 (22–37) ***	26 ± 3 (19–33)	25 ± 8 (12–45)
Born in synthesizing vs. non-synthesizing season (%)	Non-synthesizing period	48 ± 2 (45–52)	52 ± 4 (44–61)	50 ± 3 (43–57)	57 ± 10 (38–75)
	Synthesizing	52 ± 2 (48–55)	48 ± 4 (39–56)	50 ± 3 (43–57)	43 ± 10 (25–62)
25(OH)D by supplement containing vitamin D use (nmol/L)	Never Sometimes	**68.1 ± 1.8 (64.3 – 71.9)**	**78.1 ± 6.3 (65.6– 90.5) ***	74.6 ± 1.8 (70.7 – 78.4)	88.8 ± 12.2 (63.0–114.6)
	Everyday	**71.5 ± 1.8 (67.9 – 75.1)**	**84.8 ± 4.4 (76.2 – 93.4) ***	**84.0 ± 2.8 (77.9–90.0)**	**121.8 ± 14.04 (92.1–151.4) ***
25(OH)D by self-identified ethnicity (nmol/L)	White	**73.1 ± 1.2 (70.5–75.7)**	**83.3 ± 4.1 (75.2–91.3) ***	77.7 ± 1.9 (73.6–81.9)	102.6 ± 9.0 (83.9–121.4)
	Non-white	**53.6 ± 3.8 (45.6–61.5)**	**71.5 ± 6.1 (59.4–83.7) ***	76.0 ± 4.0 (67.4–84.7)	Inadequate sample size
	Indigenous	62.5 ± 3.9 (54.5–70.5)	56.2 ± 7.3 (41.6–70.7)	**87.4± 13.9 (57.3–117.5)[1]**	**63.0 ± 10.1 (41.9–84.1) ***
25(OH)D by season (nmol/L)	Winter	**55.2 ± 3.5 (48.0–62.5)**	**80.7 ± 6.0 (68.8–92.6)***	**67.3 ± 3.1 (60.6–74.0)**	**100.5 ± 12.1 (75.3–125.7) ***
	Spring	69.8 ± 2.5 (64.7–75.0)	74.6 ± 5.9 (62.9–86.2)	75.1 ± 2.4 (70.0–80.2)	86.0± 13.2(58.6–113.4)
	Summer	76.2 ± 2.9 (70. 3–82.0)	86.7 ± 7.5 (71.8–101.4)	**76.5 ± 3.2 (69.5–83.4)**	**157.5 ± 22.5 (110.6–204.4) ***
	Fall	69.0 ± 1. 5 (66.0–72.0)	74.5 ± 4.7 (65.2–83.9)	81.2 ± 2.1 (76.7–85.7)	90.3 ± 20.1 (48.4–132.1)
25(OH)D synthesizing period (nmol/L)	Non-synthesizing period	**64.2 ± 2.5 (59.1–69.3)**	**76.2± 3.9 (68.4–83.9)***	77.2 ± 1.6 (73.6–80.7)	90.9 ± 10.6(68.8–112.9)
	Synthesizing	73.0 ± 1.9 (69.2–76.9)	82.6 ± 5.0 (72.8–92.5)	**78.9 ± 1.8 (75.0–82.7)**	**116.3 ± 13.2 (88.7–143.8) ***
25(OH)D by milk intake (nmol/L)	Always	**70.3 ± 1.8 (66.7–74.0)**	**86.3 ± 5.0 (76.4–96.2)***	78.1 ± 1.1 (75.8–80.4)	115.5 ± 26.1 (60.4–170.6)
	Never/sometimes	**60.0 ± 2.3 (55.2–64.7)**	**73.9 ± 5.2 (63.7–84.1)***	**68.7 ± 4.8 (58.3–79.1)**	**100.0 ± 10.6 (77.7–122.3) ***

[1] includes those who did not report their ethnicity

* **Bold** font represent statistically significant ($p<0.05$) differences between healthy children and those with JIA. Significance established when point estimates did not lie within the estimated 95% confidence intervals for parameters from the respectively compared population

Table 5.3. The comparison of measures of inflammation between healthy children and children

		6- to 16-years old		3- to 5-years old	
		Healthy Mean ± SE (95% CI)	JIA Mean ± SE (95% CI)	Healthy Mean ± SE (95% CI)	JIA Mean ± SE (95% CI)
25(OH)D nmol/L		**68.1 ± 1.8 (65.3–72.4)**	**79.2 ± 3.1 (73.4–85.2)***	**77.8 ± 1.2 (75.2–80.4)**	**100.5 ± 8.5 (83.3 –117.6)***
White Blood Cell Count (10^9/L)		**6.7 ± 0.01 (6.5–6.8)**	**8.7 ± 0.04 (7.9–9.5)***	**7.5 ± 0.2 (7.0–7.9)**	**11.0± 1.1 (8.9–13.1)***
Platelet Count (10^9/L)		**260.2 ± 2.0 (260.2–270.5)**	**360.4 ± 10.1 (340.6–380.8)***	**306.3 ± 2.7 (300.4–312.1)**	**429.9 ± 30.7 (368.2–491.7)***
Lymphocytes (10^9/L)		2.3 ± 0.02 (2.2–2.3)	2.3 ± 0.1 (2.1–2.4)	3.1 ± 0.1 (2.9–3.4)	3.7 ± 0.3 (3.0 –4.4)
Neutrophils (10^9/L)		**3.6 ± 0.1 (3.5–3.7)**	**5.6 ± 0.39 (4.8–6.3)***	**3.4 ± 0.1(3.2–3.7)**	**6.1 ± 1.0 (4.0–8.2)***
Hemoglobin (g/L)		**140.2 ± 0.5 (140.0–140.4)**	**120.1 ± 1.2 (119.8–120.3)***	**126.3 ± 0.6 (125.0–127.6)**	**113.9 ± 2.7 (108.4–119.3) ***
CRP (mg/L)		**1.3 ± 0.1 (1.1–1.5)**	**19.2 ± 2.6 (14.7–24.5)***	**1.4 ± 0.47 (0.36–2.4)**	**18.7± 6.7 (5.1 – 32.2)***
CRP by 25(OH)D cutoffs	<50 nmol/L	**1.4 ± 0.2 (0.9–1.8)**	**32.2 ±11.8 (8.8–55.6)*[a]**	0.9 ± 0.5 (-0.3–2.1)	none
	<75 nmol/L	**1.2 ± 0.1 (0.9–1.4)**	**19.1 ± 4.7 (9.7–28.5)*[b]**	0.6 ± 0.2 (0.3–0.9)	15.0 ± 14.7 (-16.1–46.1)
	<100 nmol/L	**1.4 ± 0.2 (1.0–1.9)**	**13.7 ±2.9 (7.8–19.5)*[b]**	1.5 ± 0.5 (0.5–2.6)	36.2 ± 16.8 (0.5–71.9)
	>100 nmol/L	**1.6 ± 0.3 (1.0–2.3)**	**15.0 ± 5.0 (6.0–25.9)*[b]**	4.0 ± 2.0 (-0.3–8.4)	17.7 ± 12.2 (-8.1–43.5)

* **Bold** font represent statistically significant ($p<0.05$) differences between healthy children and those with JIA. Significance established when the point estimates do not lie within the estimated 95% confidence intervals for parameters from the respectively compared population. Differing letter superscripts represent statistically significant differences ($p<0.05$) within group for children with JIA by CRP concentrations by 25(OH)D cutoff values.

** Cut-off categories based on the Institute of Medicine categories (Institute of Medicine, 2010) of deficient < 30 nmol/L, insufficient 30-50 nmol/L, and sufficient >50nmol/L as well as the Endocrinology Society's definition of optimal >75 nmol/L(Holick et al., 2011). 25(OH)D > 100 represents a greater risk of adverse events with higher 25(OH)D concentrations (Institute of Medicine, 2010).

Table 5.4. Vitamin D status of children with JIA in different seasons across JIA categories

JIA Category	Systemic	Oligoarthritis	Rheumatoid-factor-positive polyarthritis	Rheumatoid-factor-negative polyarthritis	Enthesitis-related arthritis	Psoriatic arthritis	Undifferentiated arthritis
n=	25	36	15	56	13	11	8
25(OH)D nmol/L Mean ± SE (95% CI All seasons	75.4 ± 5.6 (64.3 – 86.4)*	85.6±5.6 (74.5 – 96.6) †	84.2 ± 16.5 (51.6 - 116.8)	82.8 ± 5.0 (72.9 – 92.8) §	95.0 ±10.8 (73.7–116.2) *§‡	65.4 ± 10.0 (45.6 – 85.2) †§	85.0 ± 13.6 (58.1– 92.8) ‡
Season of Birth							
Winter (%) Mean ± SE (95% CI	52.0 ± 10.2 (32.6– 70.8) **	27.8 ± 7.6 (15.4 – 44.8)	33.3 ± 12.6 (14.0 – 60.5)	19.6 ± 5.4(11.1 – 32.3) **	30.8 ± 13.3 (11.5 – 60.5)	45.5 ± 15.8 (19.2 – 74.5)	62.5 ± 18.3 (26.3 – 88.6) **
Spring (%) Mean ± SE (95% CI	12.0 ± 6.6 (3.8 – 32.0)	19.4 ± 6.7 (9.4 – 35.9)	20.0 ± 10.7 (6.3 – 48.3)	19.6 ± 5.4 (11.1 – 32.3) ††	38.5 ± 14.0 (16.2 – 66.9)	27.3 ± 14. (8.5 – 60.4)	No observations
Summer (%) Mean ± SE (95% CI	12.0 ± 6.6 (3.8 – 32.0)	27.8 ± 7.6 (15.4 – – 44.8)	26.7 ± 11.8 (9.9 – 54.5)	25.0 ± 5.8 (15.3 – 38.1) ††	No observations	9.1 ± 9.1 (1.1 – 46.7)	12.5 ± 12.5 (1.5 – 57.7)
Fall (%) Mean ± SE (95% CI	24.0 ± 8.7 (10.9 – 44.8)	25.0 ± 7.3 (13.4 – 41.9)	20.0 ± 10.7 (6.3 – 48.3)	35.7 ± 6.5 (24.2 – 49.2) **	30.7 ± 13.3 (11.5 – 60.5)	18.2 ± 12.2 (4.2 – 52.9)	25.0 ± 16.4 (5.6 – 65.1)

*†‡§Same superscript symbols denote statistically significant differences ($p<0.05$) for 25(OH)D concentrations by disease category

**†† Denotes statistically significant different by season within disease category.

Significance established when the point estimates did not lie within the estimated 95% confidence intervals for parameters from the respectively compared population

CHAPTER 6

OBJECTIVE 1

Evaluation of Nutritional Status through Anthropometric Measurements

6.1. Introduction

Nutrition and Juvenile Idiopathic Arthritis (JIA)

Children with JIA often experience nutrition-related concerns, such as protein-energy malnutrition, micronutrient deficiencies and growth abnormalities (Dinardo, Dwyer, Goldberg, & Holland, 1991). While it is not the sole cause, nutritional impairment can contribute to reduced growth, osteoporosis, anemia and suboptimal body composition in children with JIA (Cleary et al., 2004). As new medications and treatments have become available, it is important to monitor and update the expected growth outcomes of children with JIA. Currently, no standard recommendations for nutrition therapy or specific diet for children with JIA exists, however nutrition does play a role in disease treatment. Current nutrition recommendations are the same as they are for all children and specific recommendations are made based on individual projected outcomes and requirements (Dinardo, Dwyer, Goldberg, & Holland, 1991).

Waist circumference is a measure of central adiposity and body composition. Children with JIA weigh less than healthy children; they may have lower muscle mass and greater central adiposity (Jednacz, & Rutkowska-Sak, 2014, Sousa et al., 2006). Few longitudinal studies comparing JIA patients to healthy controls exist. Central obesity during childhood increases the risk of cardio metabolic risk in young adulthood (Barbour-Tuck, Erlandson, Muhajarine, Foulds, & Baxter-Jones, 2018). Concern over increased cardiovascular risk in patients with rheumatoid arthritis and impaired nutritional status in children with JIA, warrant the further research to explore cardio metabolic risk factors in children wit JIA (Jednacz, & Rutkowska-Sak, 2014).

Vitamin D and JIA

Nutrition in children with JIA may be improving as more effective treatments for the underlying disease become available. The correlation between body fatness and vitamin D status has been found in children (Wakayo et al., 2015). Overweight children were found to have lower 25(OH)D concentrations than non-overweight children based on the classification of both BMI-

for-age and triceps skinfold-for-age percentile (Wakayo et al., 2015). Thus, overweight and obese children may require additional vitamin D.

Rooney et al. reported that the degree of growth retardation is associated with the duration of the disease activity (longer duration of disease activity reduces growth potential) in children with JCA, and that disease remission can improve growth (Rooney, Davies, Reeve, Preece, Ansell, 2000). It was concluded that normal height can be achieved through catch-up growth after 2-3 years of disease remission unless the epiphyseal growth plate had already fused. Vitamin D may play a role in suppressing inflammation (Cutolo et al., 2011) therefore establishing appropriate target concentrations for children with JIA considering their body composition may suppress inflammation and assist in optimizing growth.

Endocrine Society has published clinical practice guideline as well for patients at risk of vitamin D deficiency. They have included recommendations for a number of at-risk populations, including: “obese children and adults and children and adults on anticonvulsant medications, glucocorticoids, antifungals such as ketoconazole, and medications for AIDS be given at least two to three times more vitamin D for their age group to satisfy their body's vitamin D requirement” (Holick et al., 2011)

To date and to our knowledge, no study has investigated the relationship between vitamin D status, growth outcomes and JIA from the time of the first presentation. This research compares at a population level whether there are significant differences in growth between healthy children and those recently diagnosed with JIA. We compared the vitamin D status and growth outcomes for children and adolescents with JIA to a healthy age-matched population of children and adolescents. The objectives of this research were to evaluate vitamin D status and growth in newly diagnosed children with JIA compared to healthy children.

6.2. Methodology

Data from the BBOP study A (n=136) and the CHMS were used. BBOP data from children, aged 3-16 years with new onset JIA, were compared to age-matched healthy children from CHMS cycle 1 and 2 after weighting and bootstrapping to be able to generalize data at population level. As 3 to 5 years olds only participated in cycle 2 of CHMS they were analyzed separately from the 6-16 year olds so that the appropriate bootstrap variables could be applied. 25(OH)D concentrations, height, weight, waist circumference, BMI with appropriate z-scores

(age and sex specific) and maturation offset were calculated. Means and 95% confidence intervals were calculated to determine significance. Anthropometric z-scores and percentiles were calculated using the Center for Disease Control reference data (Fryar, Gu, & Ogden, 2012), and maturation offset to explore the impact of puberty on growth and disease development were calculated using the equation by Mirwald et al. (Mirwald, Baxter-Jones, Bailey, & Beunen, 2002).

6.3. Results

Growth parameters

The proportion of children in each BMI class did not differ between the two groups, and there was no difference in BMI z-score as shown in Table 6.1 (JIA 0.14 ± 0.10, healthy 0.24 ± -2.40). Weight and height were higher in healthy children both as measured and based on weight and height for age z-scores (weight z-score JIA 0.06 ± 0.11, healthy 0.37 ± 0.03, height JIA -0.11 ± 0.10, healthy 0.28 ± 0.03). There were a greater proportion of children with JIA who had a waist circumference above the 90th percentile for their age and sex (9.0 ± 3%) than healthy children (4.1 ±1%). No difference was found in the proportion of children by maturity offset classification. There was a significantly greater proportion of children ages 14-16 years with JIA who were underweight compared to healthy children (JIA 13 ± 5%, healthy 3 ± 1%).

25(OH)D by body composition.

There was a significant difference in vitamin D concentrations for children based on weight category with normal and underweight children from both groups having higher vitamin D than the children in the overweight and obese categories from both groups. While 25(OH)D concentrations were lower in children who had a waist circumference >90th percentile the results were not significant.

3 to 5 year olds

Children with JIA had statistically significantly higher BMI that healthy children. Other than that, they did not differ. Waist circumference was not measured on 3 to 5 year olds in CHMS and therefore could not be compared.

6.4. Discussion

This is the first study in Canada to investigate the relationship between vitamin D status, growth outcomes and JIA from the time of the first presentation and also the first to make comparisons at a population level to children who are free of chronic disease. In comparison to children free of chronic disease, children with JIA are more likely to be shorter and weight less. 7% of children 6-16 years old from our study were underweight based on BMI compared to 11-41% of patients with JIA reported by Gaspari et al. (Gaspari et al., 2011). Growth failure is reported to occur most often in children with systemic and polyarticular JIA and, although less severe, also in children with oligoarticular JIA (Marcovecchio et al., 2012).

Body composition may also impact vitamin D status as vitamin D is readily taken up by adipose tissue. Obese individuals have lower serum 25(OH)D concentrations than normal weight individuals (Tsiaras & Weinstock, 2011). A study with the goal of characterizing the dose-response relationship of vitamin D supplementation and 25(OH)D concentration was conducted in a large sample of healthy volunteers. Specifically the aim was to quantify the dose-response relationship for different BMIs and for absolute body weight. The authors found that BMI was a better determinant of 25(OH)D concentrations than absolute weight. When compared to subjects with a BMI in the healthy range, obese and overweight adults have serum 25(OH)D concentrations that are on average 19.8 nmol/L and 8.0 nmol/L lower than normal weight adults, respectively ($P<0.001$) (Ekwaru et al., 2014). The correlation between body fatness and vitamin D status has also been found in children (Wakayo et al., 2015). Overweight children were found to have lower 25(OH)D concentrations than non-overweight children, based on classification of both BMI-for-age and tricepskinfold-for-age percentile (Wakayo et al., 2015).

While the proportion of children in each BMI class did not differ between the two groups (healthy children and children with JIA), weight and height z-scores were not the same, with z-scores being higher in healthy children. A greater proportion of children in the JIA group were above the 90^{th} percentile for age and sex-adjusted waist circumference. As waist circumference is an indicator of central adiposity and body composition, these results suggests that while children with JIA weigh less than healthy children they may have lower muscle mass. These results highlight that at disease onset children with JIA already differ from healthy children in terms of growth characteristics and that discussion and monitoring of body composition and growth outcomes should take place from diagnosis onward.

Other studies have reported mixed results on the impact of JIA on body weight with both evidence of under and over nutrition available (Caetano et al., 2009; Cleary et al., 2004; Dey et al., 2014; Markula-Patjas et al., 2014). These mixed results are most likely due to an individual's response to the disease activity either through reduced intake, reduced physical activity, and/or increased caloric requirement. In 1990, when Bacon et al. investigated the relationship between growth and dietary intake in children with JRA, it was found that one-third of the children studied were at or below the tenth percentile in height for age (Bacon et al., 1990). Overall, meal caloric and nutrient intakes were found to be adequate when compared to the RDA of the National Academy of Science. Biochemical measurements showed abnormalities amongst the subtypes of JRA and discrepancies between dietary intake and blood values. It was hypothesized that this was due to increased energy utilization due to chronic inflammation (Bacon et al., 1990).

6.5. Conclusion

Children with JIA are shorter and weigh less than healthy children weigh; they are at increased risk of having a waist circumference above the 90^{th} percentile. Vitamin D, which was adequate in the JIA population, is not likely to be a factor accounting for impeded growth. Further research into strategies to minimize inflammation and optimize nutritional status outcomes such as growth and adiposity are required to establish early interventions.

Table 6.1. Anthropometric measures and adjusted anthropometric measures

		Overall 6-16		3-5	
		Healthy	JIA	Healthy	JIA
		Mean ± SE (95% CI)	Mean ± SE (95% CI)	Mean ± SE (95% CI)	Mean ± SE (95% CI)
Weight (kg)		47 ± 0.63 (46-48)	**43± 1.6 (40 - 46)**	33.5 ± 15.6 (-0.15-67.15)	17.2 ± 0.76 (15.7-18.8)
Height (cm)		150 ± 0.48 (150-150)	**146 ± 1.6 (143 -149)**	122.8±14.5 (91.6-154.1)	102.6 ± 1.5 (99.6-105.6)
Waist Circumference (cm)		67 ± 0.44 (66-68)	68 ± 1.7 (65 - 71)	75.5±16.2(40.5-110.4)	55.3 ± 1.8 (51.5-60.0)
Body Mass Index		20 ± 0.16 (19.9-20.3)	19 ± 0 .40 (18.45 - 20.05)	17.7±1.4 (14.7-20.6)	16.2 ± 0.39 (15.4-17.0)
Waist Circumference (%)	< 90th percentile	96.4 ± 0.70 (94-97)	**91.1 ± 3 (83-96.1)**	Not available	77 ± 12 (43-94)
	$\geq$ 90th percentile	4.1 ± 0.70 (02.7-5.6)	**9.0 ± 3 (4.5-17)**	Not available	23 ± 1.2 (6-57)
Weight Category (%)	Under weight	3.8 ± 0.8 (2.2-5.5)	6.9 ± 2.2 (3.6-13)	3-5 combined normal and under weight	
	Normal Weight	71 ± 1.6 (68-74)	72 ± 3.9 (64-79)	75±4(67-83)	88 ± 7 (66-96)
	Overweight	14 ± 1.2 (12-17)	11 ± 2.7 (6.4-17)	18±3(10-25)	13 ± 7 (4-34)
	Obese	11 ± 0.94 (8.8-13)	10 ± 2.6 (5.9-17)	7±2(3-12)	none
Maturation offset	Before puberty	49 ± 1 (47 - 51)	41 ± 4 (33 - 50)	N/A	N/A
	During puberty	21 ± 1 (18 - 23)	19 ± 3 (13 - 26)	N/A	N/A
	After	30 ± 1 (28 - 32)	40 ± 4 (32 - 49)	N/A	N/A
Weight for age z-score		0.37 ± 0.03 (0.30-0.43)	**0.06 ± 0.11 (-0.15- 0 .27)**	0.37 ± 0.12 (0.12-0.63)	0.19 ± 0.11 (-0.05-0.43)
Height for age z-score		0.28 ± 0.03 (0.21-0.34)	**-0.11 ± 0.10 (-0.31-0.08)**	1.26 ± 0.92 (-0.73-3.25)	-0.23 ± 0.11 (-0.47-0.01)
BMI for age z-score		0.24 ± 0.02 (0.20 - 0.28)	0.14 ± 0.10 (-0.06-0.35)	0.25 ± -2.02 (0.13-0.37)	**0.50 ± 0.10 (0.27- 0.72)**

*Bold font represent statistically significant ($p<0.05$) differences between healthy children and those with JIA. Significance established when the point estimates did not lie within the estimated 95% confidence intervals for parameters from the respectively compared population

Table 6.2. 25(OH)D concentration and vitamin D-related variables in healthy children and children with JIA

		Overall 6-16		3-5	
		Healthy	JIA	Healthy	JIA
25(OH)D nmol/L		68.0 ± 1.8 (65.2-72.4)	**79.1 ± 3.1 (73.2-85.1)**	77.8±1.2(75.2-80.4)	**100.5 ± 8.5 (83.3 -117.8)**
Vitamin D by waist circumference nmol/L	<90%	68.6±1.8(64.8-72.4)	**79.0±3.9(71.4-86.7)**	Not available	
	>90%	62.1±2.9(56.2-68.0)	61.4±10.2(41.0-8.7)		
Vitamin D by BMI group nmol/L	Under and normal weight	73.5±4.9(63.4-83.7)	83.3±3.4(76.6-90.1)	79.3±1.6(75.9-82.8)	**103.2 ± 11.3(79.5-126.8)**
	Overweight	62.9±1.7(59.4-66.3)	**51.9±6.0(40.1-68.8)**	73.1±4.9(62.4-83.7)	73.7 ± 9.4(53.9-93.4
	Obese	62.5±2.8(56.8-68.2)[b]	59.3±6.7(45.9-72.6)	72.8±4.5(63.0-82.5)	**90.3 ± 19.4(49.7-131.0)**

*Bold font represent statistically significant ($p<0.05$) differences between healthy children and those with JIA.

Differing superscript letters denote statistically significant differences ($p<0.05$) for 25(OH)D concentrations by waist circumference category or BMI category. Significance established when the point estimates did not lie within the estimated 95% confidence intervals for parameters from the respectively compared population

CHAPTER 7

OBJECTIVE 2

Vitamin D is associated with markers of disease activity among children with Juvenile Idiopathic Arthritis

To address objective 2 of this thesis, I examined the association between plasma levels of 25(OH)D and disease activity and outcomes as determined by clinical and biomarker profiles in JIA.

Abstract

Introduction: While the role of vitamin D in other autoimmune disorders has been established, less is known about its association with Juvenile Idiopathic Arthritis (JIA). Low vitamin D levels could be associated with inflammation. We aimed to determine associations between vitamin D and inflammatory markers in newly diagnosed JIA patients.

Methods: Data from the Biologically-based Outcome Predictors (BBOP) in JIA prospective multi-centre study was used (n=186). Environmental and clinical data were collected every 6 months for 2 years and blood samples were obtained at baseline (treatment-naive) and 6 months (receiving treatment). Longitudinal analysis then explored whether 25(OH)D and related factors could predict disease activity in BBOP children.

Results: No difference was found in 25- hydroxy vitamin D (25(OH)D) concentrations between baseline and 6 months (84.5 ± 37.7 vs. 84.4 ± 43.7 nmol/L). Overall, 36% of children achieved remission on continuing medications; 25% had sustained remission after discontinuing medication. Increased 25(OH)D or its associated factors predicted lower ESR, CRP and cytokine concentrations. Remission was predicted by increasing 25(OH)D concentrations at the 6 month time point.

Conclusion: Serum 25(OH)D concentrations and factors that influence 25(OH)D status such as season and daily milk consumption are associated with measures of disease activity. 25(OH)D predicted remission suggesting that it may play a role in suppressing inflammatory-related factors of JIA outcomes.

7.1. Introduction

Vitamin D has been implicated in the pathogenesis of certain autoimmune diseases (Holick, 2012). The mechanism by which this occurs is believed to be through vitamin D's effect on non-calciotropic immune and inflammatory pathway functions (Trochoutsou, Kloukina, Samitas, & Xanthou, 2015). Cytokines mediate the inflammatory response in humans; during inflammation, pro-inflammatory cytokine concentrations increase and anti-inflammatory cytokines decrease (Armon, 2014).

The association of vitamin D with cytokine mediators of inflammation have not been explored in children with JIA. Pelajo et al. (2012) found that children with JIA were more likely to be vitamin D deficient than healthy controls (Pelajo, Lopez-Benitez, & Miller, 2011). A 2013 meta-analysis, of vitamin D in JIA reported the prevalence of vitamin D insufficiency in this population to be 82%. There was, however, no information available to elucidate if the reduced vitamin D concentrations were a consequence of reduced dietary intake, medication effect, or increased utilization due to the disease status. Additionally, there was no information reporting the relationship between vitamin D status and disease courses and outcomes (Nisar et al., 2013).

The objectives of this present study were to examine the association between plasma concentrations of 25-hydroxyvitamin D (25(OH)D) and vitamin D associated factors (as examples, vitamin D intake, the season of measurement, and the use of corticosteroids) and relationships with disease activity outcomes. Outcomes were determined by composite scores of disease activity, disease remission, and biomarker profiles in a JIA inception cohort of over a two-year period. This is the first cohort study to explore the association of vitamin D with disease outcomes in newly diagnosed children with JIA.

7.2. Methods

We used data from the Biologically Based Outcome Predictors (BBOP) in Juvenile Idiopathic Arthritis study, a prospective, inception cohort study. Recruitment of 186 participants aged 1-17 years with new-onset JIA were recruited from 11 Canadian pediatric rheumatology centers (Appendix A). Enrollment criteria included conforming to ILAR classification criteria (Petty et al., 2004), being within six months of disease onset, having not been treated previously

with DMARDs or biologically-based anti-cytokine therapies (biologics), and providing consent to be enrolled in the study. To achieve a reasonable number of participants in each of the seven JIA categories rather to achieve a typical JIA category distribution, only participants with polyarthritis or systemic JIA, the least prevalent categories, were eligible during the first six months of the enrollment period; after six months and until the end of the two-year enrollment period participants with any JIA category were eligible. Each participating institution provided ethics approval.

7.2.1. Data and Sample Collection

Demographic and clinical data were collected at enrollment and every six months for two years. Clinical data included variables required for the American College of Rheumatology pediatric core set of measures of disease activity (Giannini et al., 1997). These variables include the physician global assessment (PGA) of disease activity, acute phase reactants (ESR and/or CRP), and the CHAQ-JIA (Giannini et al., 1997). Inactive disease (remission on or off medication) was defined by criteria proposed by Wallace et al. (Wallace et al., 2004). Additionally, data was collected using the Juvenile Arthritis Quality of Life Questionnaire (JAQQ). Participant pain status was assessed using the CHAQ question "how much pain do you think your child has had because of his/her illness in the past week?".

Laboratory-Based Assays

Sample Collections and Handling:

From plasma samples collected at baseline and at six months, 25(OH)D, pro- and anti-inflammatory mediators IL-2, IL-1α, IL-1rα, IL-1β, IFN-ɤ, IL-6, IL-8, IL-17, TNFα, IL-4, and IL-10 were measured (Matheson, Duong, Rosenberg, & Yeung, 2008). ESR and CRP measures were derived from results generated by the clinical service laboratories in each of the respective participating centers and reported for all time points. Table 7.1 shows the inflammatory biomarker measurements analyzed.

Cytokine Measurements:

At enrollment and at six months, blood was collected in P100 Blood Collection and Preservation tubes (BD Biosciences, San Jose, CA) (Matheson, Duong, Rosenberg, & Yeung, 2008). Inflammatory cytokines were measured in plasma by multiplex bead-based assays

(Fluorokine® Multi-Analyte Profiling, Multiplex human cytokine panel A {R&D Systems}using a Luminex analyzer (Luminex Corp.) at the Toronto University Health Network Microarray Centre (www.mircoarray.ca). Forty-six cytokines were analyzed for the BBOP study. We chose to focus on the cytokines listed above because they are most commonly listed in the literature as having been explored in participants with JIA or other autoimmune diseases and have been studied in the context of vitamin D. (Cutolo et al., 2011; Dankers et al., 2017; Hayes et al., 2015).

Vitamin D Assay

25(OH)D was measured from a plasma sample obtained at enrollment and at six months after enrollment using a competitive binding enzyme-linked immunoassay (EIA Immunodiagnostic Systems) as previously described (Matheson et al., 2008). All biochemical measures were log-transformed, as they did not follow normal distribution.

To assess vitamin D intake over the month prior to blood sample collection, the frequency of milk intake (two categories every day, and never/sometimes) and vitamin D containing supplement use (two categories every day/sometimes, and never) were collected for analysis. Medications were documented at every time-point and categorized by medication class (non-steroidal anti-inflammatory [NSAID], corticosteroid [steroids], DMARD or biologic).

A method to standardize, compare and combine inflammatory biomarkers developed by Tabung et al. was used (Tabung et al., 2015). Z-scores of the log-transformed. CRP, IL-6, and TNFα concentrations were then added together to create the overall pro-inflammatory biomarker score. The same method was applied to create an anti-inflammatory score where the anti-inflammatory cytokines IL-4 and IL-10 were standardized then combined. Since CRP is used in defining remission, we used the pro and anti-inflammatory scores to include other measures of inflammation that are not included in the definition of remission.

Statistical Analysis

Descriptive statistics (means and standard deviations for continuous variables and percentages for categorical variables) were used to summarize the data at the five-time points of measurements. Multi-Level Mixed-Effects (MLM) Modeling and backwards methodology with patients set as a random effect factor (individual patient change by time/visit number), was

utilized to determine if there is association between factors associated with vitamin D status (milk intake, supplement intake, season) and markers of inflammation /disease outcomes in the presence of potential covariates, such as socioeconomic status, sex, age, ethnicity. The repeated measures across time points had unstructured covariance structure. STATA 15 (StataCorp LP 2015) was used for both the descriptive and inference statistics; alpha was set at $p<0.05$.

7.3. Results

The mean age of participants at recruitment was 9.34 ± 4.78 years. One-hundred and twenty-eight (69%) of the 186 patients recruited were female. Demographic data and clinical characteristics are presented in Table 7.2. Mean 25(OH)D concentrations did not change from baseline to 6 months (84.48 ± 37.54 nmol/L vs 84.38 ± 43.68nmol/L) (Table 7.1). Forty-five percent of JIA patients were vitamin D insufficient (< 75 nmol/L) (Holick et al., 2011). Measurements taken at all time points (baseline to 24 months) are presented in Table 7.3.

Baseline and six months

As most biochemical measurements including 25(OH)D, were analyzed at only baseline and at six months, separate models were made to include 25(OH)D concentrations.

Measures of inflammation

CRP concentrations decreased between the baseline visit and the visit at six months. The average CRP levels decrease when 25(OH)D increases if on a steroid (β=-0.95) (Appendix A Table 7.4); average CRP levels increase when 25(OH)D increased, if not on a steroid (β 0.04 = -0.95+0.99). Reporting consuming milk every day also predicted a reduction in average CRP (β= -0.52, p=0.028). Average between the baseline visit and the visit at six months (Table A.4). There was an interaction between milk consumption and supplement use (p=0.033). For children who drank milk everyday and did not take a supplement, the average ESR concentration decreased (β= -0.25). For those who did drink milk and did take a supplement, the average ESR concentrations increased (β 0.47= -0.25+0.72).With respect to ESR, there was an interaction between age and supplement intake (p=0.019). For those who did not take a supplement the average ESR concentration increased with age (β=0.02). For those who are taking a supplement the average ESR concentration decreased with age (β -0.06= 0.02+-0.08.

Pro-Inflammatory Cytokines

Follow up measurement at the six month time point was not associated with any of the inflammatory cytokines (Table A.5). The only predictive variables to impact IL-1α were being prescribed a biologic and milk intake. Those who had not received biologics had lower average concentrations of IL-1α than those who had been prescribed biologics (β= -0.88, p=0.012). Taking a supplement every day predicted an average positive association with IL-1α compared to children not taking a supplement (β= 0.31, p=0.03). Drinking milk every day predicted higher average concentrations of IL-1rα (β= 0.75, p=0.024). Increasing age predicted lower average IL-1rα concentrations (β= -0.09, p=0.099). Not being prescribed a biologic predicted lower average IL-1rα concentrations (β= -2.02, p <0.001). There was an interaction between steroid used and 25(OH)D concentrations (p=0.017); 25(OH)D predicted lower average IL-1rα concentrations in children prescribed a steroid (β= -1.44). Those not prescribed a steroid had higher average IL-1ra concentrations as their 25(OH)D increased (β 0.07= -1.44+ 1.51). Average IL-1β was higher in the summer compared to the winter (β=0.64, p=0.008). The average IL-2 concentrations decrease when 25(OH)D increases if on a steroid (β=-0.-1.31); average IL-2 concentrations increase when 25(OH)D increased, if not on a steroid (β 0.15 = -1.31+1.46). Taking a supplement every day predicted higher average IL-2 concentrations (β= 0.57, p=0.002). Average IL-6 concentrations were higher in the summer than winter (β= 0.54, p=0.013). There was an interaction between age and milk intake (p= 0.018). The average IL-6 concentrations increased when age increases if children reported not drinking milk every day (β=0.008); average IL-6 concentrations increased when age increased, if children reported drinking milk every day (β 1.19= 1.31+- 0.12). Average IL-8 concentrations were predicted to be higher in the spring (β= 1.25, p= <0.001) and summer (β= 2.25, p= <0.001) compared to the winter. With respect to IL-8, there was an interaction between age and milk intake (p= 0.002). The average IL-8 concentrations increased when age increases if children reported not drinking milk every day (β=0.08); average IL-6 concentrations decreased when age increased, if children reported drinking milk every day (β -0.09= 0.08 +-0.17). Average 25(OH)D plasma concentration outcomes decreased with age for children less than 11 and increased with age for children older that 11 (β -1.7= -1.87 + 0.17). With respect to IL-17, there was an interaction between milk and supplement intake (p= 0.001). The average IL-17 concentrations decreased when children

reported drinking milk every day and not taking a supplement. (β=0.-0.15); average IL-17 concentrations decreased further in children who reported not drinking milk and taking a supplement every day (β -1.6= -0.15+-1.45). Older children have lower average TNFα than younger children (β= -0.05, p=0.008). There was negative association between 25(OH)D concentrations and average INFγ concentrations (β= -1.20, p=0.008). Older children were predicted to have higher average IFNγ concentrations than younger children (β= 0.10, p=0.016). Children taking a supplement containing vitamin D had an average INFγ concentration that was lower than children who reported not taking a supplement (β= -0.51, p=0.007). With respect to the pro-inflammatory index, there was an interaction between steroid use and 25(OH)D (p=0.010). For those taking a steroid higher vitamin D levels resulted in lower average pro-inflammatory index values (β= -1.67); however, for those not taking a steroid having higher vitamin D concentrations resulted in higher inflammation than those taking a steroid with higher vitamin D concentrations (β 0.31=-1.67+1.98)

Anti-Inflammatory Cytokines

Significant main effects and significant interactions with associated main effects for anti-inflammatory cytokines are presented in (Table A.6). There was no change in average IL-4 between the two visits (β=0.23, p=0.078). With respect to Il-4 there was an interaction between age and 25(OH)D (p=0.034). For children less than 13 years of age having a higher 25(OH)D concentration lowers average IL-4 concentrations and after 13 years of age having higher 25(OH)D increases average IL-4 concentrations (β -1.53= -1.66 +0.13). Females were predicted to have higher average IL-4 than males (β= 0.94, p=0.013). Average IL-10 did not statistically change from baseline to the six-month follow up. There was seasonal variation in average IL-10 concentrations with spring (β= 0.41, p=0.036) and summer (β= 0.39, p=0.005) predicting higher average IL-10 concentrations than winter. Those who identified as non-white had higher average IL-10 concentrations than those who identified as white (β=0.065, p=0.04). Those in the highest income group (>$100,000) also had higher average IL-10 than the comparison group (<51,000((β= 0.63, p=0.036). As BMI percentile increased so did average IL-10 (β=0.01, p=0.044). There was an interaction between milk intake and supplement use (p=0.019).The average IL-10 concentrations increased when children reported drinking milk every day and not taking a supplement. (β=0.09); average IL-10 concentrations increased further in children who reported

not drinking milk and taking a supplement every day (β 0.73 = 0.09+0.64). There was no change in the average anti-inflammatory index between baseline and the six month follow up. The average anti-inflammatory index was predicted to be higher in the summer compared to the winter (β=0.44, p=0.029). With respect to the anti-inflammatory index, there was an interaction between age and 25(OH)D (p=0.033). For children less than 12 years of age having a higher 25(OH)D concentration lowers average anti-inflammatory index and after 13 years of age having higher 25(OH)D increases average anti-inflammatory index (β -1.62 = -1.76 +0.14).

Objective and Self-reported Measures of Disease Outcomes

PGA scores fell from the baseline to the six month visit (Table A.7). There was an association between higher 25(OH)D and a higher average PGA score (β=0.55, p=0.044) Those in the middle-income category had a lower average PGA score than those in the lowest income category (β= -0.91, p=0.007).

All visits (baseline through 24 months)

Laboratory measures of inflammation

Over the five study visits CRP decreased (Table A.8) (β= -0.34, p= <0.001) Children who reported drinking milk every day, had decreased average CRP concentrations (β= -0.44, p=0.007). Those who reported not taking a steroid had decreased average CRP concentrations (β=0.48, p=0.007). Children who reported drinking milk every day milk had lower average ESR than those who did sometimes or never (β= -0.49, p=0.006) (Table A.8). There was an interaction between supplement and age (p=0.033). The average ESR concentrations increased when age increases if children reported not taking a supplement (β=0.82); average ESR concentrations increased to a lesser degree when age increased, if children reported taking a supplement every day (β 0.75 = 0.082 +-0.07).

Objective and self-reported measures of disease outcomes

Objective and self-reported measures of disease outcomes over the five study visits are presented in Table A.9. Average CHAQ scores decreased from visit one through five (β= -0.43, p= <0.001). Children who reported drinking milk every day had on average less disability and discomfort than those who sometimes or never did (β= -0.25, p=0.014). Average JAQQ score decreased over the five study visits (β= 0-0.19, p= 0.001. Higher CRP predicted a higher average

JAQQ score over the five visits (β=0.26, p= <0.001). Increasing age predicted a higher JAQQ score(β= 0.06, p= 0.004). With respect to the JAQQ scores, the relationship between not taking an NSAID and a reduction in average JAQQ score remained (β= -0.4, p=0.018). Average pain reduced with time (β=-1.54, p= <0.001). The average pain score decreased in the spring than in the winter (β= -1.25, p=0.014). Younger children also reported less average pain than older children (β= 0.13, p=0.028) With respect to the pain score, there was an interaction between milk intake and supplement use (p=0.021). On average pain decreased when children reported drinking milk every day and not taking a supplement. (β=-0.37); the average pain score increased in children who reported drinking milk and taking a supplement every day (β= -0.37+1.92). PGA values decreased on average with time (β= -0.74, p=<0.001). The only predictive variables for the model were time and ethnicity. Those who identified as non-white (β= 0.56, p=0.016) and those who self-identified as Indigenous (β= 0.72, p=0.008) had a higher PGA than those who identified as white.

Remission

When using CRP in the model 25(OH)D did not predict remission on or off medications (Table A.10). 25(OH)D predicted remission while a higher pro-inflammatory score predicted active disease (β= 0.016, p=0.019) (Table A.11). Vitamin D associated factors did not predict remission in the model for all five time points (Table A.12).

7.4. Discussion

This prospective inception cohort study is the first to investigate the relationship between vitamin D status and JIA from the time of first presentation. Mean 25(OH)D status in Canadian children with JIA appears to meet the Endocrine Society optimal concentrations at both baseline and after six months of disease (Holick et al., 2011). 25(OH)D and factors that impact vitamin D status impact biological markers of disease activity. Vitamin D status was shown to predict disease remission when pro- and anti-inflammatory cytokine scores were included in the model suggesting that 25(OH)D and associated factors are associated with a reduction in important measures of inflammation, a reflection of disease activity.

We found that higher 25(OH)D concentrations and factors associated with improving vitamin D status predicted a reduction in measures of inflammation. Thus far all previous studies but one have been conducted with participants who were being treated for JIA, many of whom were receiving corticosteroids (Szymańska-Kałuża et al., 2013). This cross-sectional study compared children newly diagnosed with JIA to children hospitalized due to circulatory system functional disorders. The groups of children had statistically similar mean 25(OH)D concentrations of 17.35ng/mL which is lower than the optimal 30ng/mL suggested by the endocrine society (Holick et al., 2011). No correlation was found between disease activity, JIA type or vitamin D metabolite (25(OH)D or 1,25(OH)D. In our recent scoping review, which summarized the available literature reporting 25(OH)D concentrations in children with JIA, we found that 15 of the 38 studies (39.5%) considered the relationship between vitamin D and disease activity (Finch, Rosenberg, & Vatanparast, 2018). Seven studies (46.7%) reported that patients with active disease or those with elevated inflammatory biomarkers had lower 25(OH)D concentrations than those patients who were in remission or who had less disease activity (Finch et al., 2018).

The impact of 25(OH)D on cytokines has been studied at the cellular level and in other disease models (Cutolo et al., 2011; Gubatan et al., 2018; Han, Forno, Boutaoui, Canino, & Celedón, 2018; Pappa et al., 2014). This is the first study to explore the association of vitamin D with of inflammatory cytokines in children with JIA. Our results suggest that 25(OH)D and factors related to vitamin D status prevent inflammation in children with JIA. Further studies are required to confirm this relationship. The few studies that have explored the impact of vitamin D on cytokines in children have found higher vitamin D status is associated with a reduced inflammatory profile (Batmaz, Arikoglu, Tamer, Eskandari, & Kuyucu, 2018; Gubatan et al., 2018; Han et al., 2018; Pappa et al., 2014). One study exploring the relationship of vitamin D with cytokine concentrations in children with inflammatory bowel disease found a lower incidence of inflammatory markers and cytokines in children receiving higher doses of vitamin D_2 (Pappa et al., 2014). In children with ulcerative colitis who were in remission higher vitamin D concentrations correlated with a higher anti-inflammatory ratio of IL-4+ IL-10/ IL-17+TNFα and IL-4+ IL-10/ IL-6+TNFα suggesting that vitamin D may be protective against relapse (Gubatan et al., 2018). Vitamin D insufficiency modified the effect of IL-5 and IL-13 on IgE by increasing serum production through the potential upregulating Th2 response in children with

asthma (Han et al., 2018). Seasonal variations in vitamin D status were also shown to impact cytokine concentrations in children with asthma (Batmaz et al., 2018). Like our study, higher vitamin D concentrations were associated with lower IL-4. They also found that concentrations of IL-10 were significantly lower in the winter than the fall and summer (Batmaz et al., 2018). Identifying an association of vitamin D with cytokines that mediate inflammation is an important step in understanding the underlying mechanism and clinical implications of vitamin D in inflammatory diseases such as JIA.

There is no single, universally accepted tool to assess JIA outcomes (van Mater, Williams, Coeytaux, Sanders, & Kemper, 2012). We used a variety of tools to assess the potential for vitamin D status, together with other metrics, to predict JIA outcomes. Plasma 25(OH)D concentrations did not predict a reduction in composite outcome scores explored in our study. There was a negative relationship between 25(OH)D and PGA. Bouaddi et al. found a significant association between a reduction in Disease Activity Score-28 (DAS-28) and increasing 25(OH)D as well as the same relationship between the Patient Global Assessment and 25(OH)D (Bouaddi et al., 2014). Similar to our study, de Sousa et al. found no association between 25(OH)D and CHAQ. We did, however, find an association between drinking milk every day and predicting a reduction in CHAQ (de Sousa Studart et al., 2015). A further understanding of vitamin D's role in the inflammatory pathway will help to establish the appropriate evaluation score to assess its impact on disease activity.

The most commonly used composite score for disease activity improvement measurement is the ARC Pediatric 30 (Giannini et al., 1997). A limitation of the ACR Pediatric 30 Criteria is that it does not quantify the disease activity of an individual in a way that allows for comparison between individuals or JIA categories (Giannini et al., 1997). Due to this limitation, we decided to use the remission criteria established by Wallace et al. instead (Wallace et al., 2004). Plasma 25(OH)D and associated factors did not predict remission in the model that included CRP. The only factors that did were medication use and a reduction in CRP, which are part of the remission definition. However, 25(OH)D did predict remission when CRP was replaced by the pro- and anti-inflammatory scores in the model. CRP is a general indicator of inflammation and possibly does not fully describe the factors associated with remission. Currently, no specific indicators or full mechanistic understanding of disease activity or specific

indicators exists. This is the first time that 25(OH)D has been explored in the prediction of remission and further studies are required to confirm this association.

Limitations

Some limitations of this study include that there was no individual measurement of sun exposure or sunscreen use. We did include the season of measurement in our model as a consideration of UVB exposure. Milk intake was measured by food frequency questionnaire as a measure of vitamin D intake instead of through a 24-hour recall; however, milk is the most common source of vitamin D for this age group (Whiting et al., 2011). While an estimated 20% of newly diagnosed children with JIA in Canada were recruited to the study, this was not enough to explore 25(OH)D by JIA categories with our models. This cohort study also did not have a control group for comparison to healthy children; however, we did compare baseline data for age and sex-matched children older than three years who are free of chronic disease from the Canadian Health Measures Survey in a previous publication (Finch, Rezaei, Whiting, Rosenberg, & Vatanparast, 2017). 25(OH)D, pro- and anti-inflammatory cytokines were only measured at two time points and not throughout the entire study. Additionally, we tried to explore if factors associated with vitamin D could predict disease activity measured by JIA category, using all seven categories, then by inflamed joint count > four and $\leq$ four. However, there was an inadequate sample size for predictions even through bivariate analysis.

7.5. Conclusion

Serum 25(OH)D concentrations and factors that influence 25(OH)D status such as season and daily milk consumption are associated with measures of disease activity. 25(OH)D predicted remission suggesting that it may play a role in suppressing inflammatory-related factors of JIA outcomes. Being able to suggest specific targets for vitamin D status as a potential adjunct therapy or specific actions to reduce inflammation in the treatment of JIA will enhance the quality of life of patients and their families. Intervention studies exploring 25(OH)D and factors associated with vitamin D status will help in the development of actionable/ feasible recommendations for health care professionals and families.

Table 7.1. Biomarker measures collected at baseline and 6 month follow up

Outcome	Baseline (mean ± SD)	6 month (mean ± SD)
Interleukin-1a pg/mL	133.88 ± 708.62	121.81 ± 691.33
Interleukin-1b pg/mL	134.71 ± 432.51	171.06 ± 478.01
Interleukin-1ra pg/mL	1462.73 ± 6495.19	2074.51 ± 7892.50
Interleukin-2 pg/mL	421.64 ± 5191.54	41.33 ± 265.40
Interleukin-6 pg/mL	76.66 ± 412.22	99.46 ± 695.01
Interleukin-17 pg/mL	143.91 ± 1290.05	44.20 ± 312.07
Interferon-γ IU/mL	250.11 ± 2174.51	83.50 ± 476.06
Tumor necrosis factor-α pg/mL	125.92 ± 1160.66	51.89 ± 231.82
Pro-Inflammatory Score†	0.48 ± 0.19	0.11 ± 0.24
Interleukin-4 pg/mL	126.99 ± 686.07	106.46 ± 620.41
Interleukin-10 pg/mL	142.86 ± 1204.55	61.22 ± 363.62
Anti-Inflammatory Score†	0.24 ± 0.18	0.35 ± 0.19
25(OH)D nmol/L	84.48 ± 37.54	84.38 ± 43.68
Proportion by 25(OH)D Cut-off Category*, (% ± SD)		
< 30 nmol/L	3.68 ± 1.48	6.38 ± 2.07
30-50 nmol/L	7.36 ± 2.05	11.35 ± 2.68
50-75 nmol/L	33.74 ± 3.71	25.53 ± 3.69
75-100 nmol/L	33.74 ± 3.71	31.91 ± 3.94
>100 nmol/L	21.47 ± 3.23	24.82 ± 3.65

25(OH)D (25-hydroxyvitamin D), SD (standard deviation), % (percent)

*Cut-off categories based on the Institute of Medicine categories (Institute of Medicine, 2010) of deficient < 30 nmol/L, insufficient 30-50 nmol/L, and sufficient>50nmol/L as well as the Endocrinology Society's definition of optimal >75 nmol/L (Holick et al., 2011). 25(OH)D > 100 represents a greater risk of adverse events with higher 25(OH)D concentrations (Institute of Medicine, 2010)

† A method of standardization to compare and combine biochemical markers was developed by Tabung et al. (Tabung et al., 2015). The standardized values were combined to create the overall pro-inflammatory biomarker score (C-Reactive Protein + Tumor Necrosis Factor –α + Interleukin (IL)- 6). The same method of standardization was used to calculate an anti-inflammatory score to combine the concentrations of cytokines IL-4 and IL-10.

Table 7.2. Sociodemographic information of BBOP participants

Sociodemographic measure	(% ± SD)
Sex, % (N)	
Male	31.18 (58)
Female	68.82 (128)
Family Income, (% ± SD)	
< $50,000/ year	27.21 ± 3.68
$50,000- $100,000/year	36.73 ± 3.99
>$100,000/year	36.05 ± 3.97
Ethnicity, (% ± SD)	
White	71.01 ± 3.50
Non-White	17.75 ± 2.95
Indigenous	11.24 ± 2.44
Age (mean ± SD)	9.34 ± 4.78
Age Category, (% ± SD)	
0-2 years old	11.83 ± 2.37
3-5 years old	30.11 ± 3.37
6-8 years old	14.52 ± 2.59
9-13 years old	30.11 ± 3.37
14-16 years old	28.49 ± 3.32
Smoker in home, (% ± SD)	
No %	67.53 ± 3.79
Yes %	32.47 ± 3.79
JIA subtype, (% ± SD)	
Psoriatic arthritis	6.45 ± 1.81
Rheumatoid-factor-negative polyarthritis	34.41 ± 3.49
Rheumatoid-factor- positive polyarthritis	9.14 ± 2.12
Systemic	14.52 ± 2.59
Oligoarthritis	24.19 ± 3.15
Undifferentiated	4.30 ± 1.49
Enthesitis-related	6.99 ± 1.87

Table 7.3. Variables measured at all-time points

			Baseline (mean and SD) or (% and SD)	6 month follow up	12 month follow up	16 month follow up	24 month follow up
Biomarkers							
	C-Reactive Protein (mean ± SD)		18.40 ± 27.64[§]	7.89 ± 18.89[†]	5.28 ± 9.14	6.05 ± 13.08	2.23 ± 3.06[†]
	Erythrocyte Sedimentation Rate (mean ± SD)		29.35 ± 26.58[§]	16.68 ± 18.77	14.31 ± 15.74	12.39 ± 13.27	10.45 ± 8.65
Objective and Self-reported Assessments							
	Physician Global Assessment (mean ± SD)		3.75 ± 2.20[§]	1.51 ± 1.67[†‡]	1.15 ± 1.75	0.91 ± 1.60[†]	0.71 ± 1.54[‡]
	Child Health Assessment Questionnaire (CHAQ) (mean ± SD)		0.82 ± 0.71[§]	0.43 ± 0.47	0.40 ± 0.50	0.34 ± 0.49	0.41 ± 0.51
	Pain assessment (mean ± SD)		4.12 ± 2.78[§]	2.54 ± 2.72	2.56 ± 2.68	1.75 ± 2.36	1.94 ± 2.89
	Juvenile Arthritis Quality of Life Questionnaire (JAQQ) (mean ± SD)		3.19 ± 1.60[§]	2.62 ± 1.26	2.32 ± 1.25	2.29 ± 1.39	2.11 ± 1.30
	Milk intake (% ± SD)						
		Every day	71.81 ± 3.70	75.97 ± 3.78	76.19 ± 4.18	78.26 ± 4.32	72.06 ± 5.48
	Taking a vitamin D containing supplement (%± SD)	Never/sometimes Every day/ sometimes	45.95 ± 4.75	47.52 ± 4.99	43.75 ± 4.71	43.48 ± 4.64	49.54 ± 4.81
	Season of measure (% ± SD)						
		Winter	22.58 ± 3.07	21.30 ± 3.16	19.72 ± 3.35	22.13 ± 3.77	
		Spring	19.89 ± 2.93	34.32 ± 3.66	22.54 ± 3.52	31.15 ± 4.21	
		Summer	27.42 ± 3.28	23.08 ± 3.25	22.54 ± 3.52	24.59 ± 3.91	
		Fall	30.11 ± 3.37	21.30 ± 3.16	35.21 ± 4.02	22.13 ± 3.77	

	BMI percentiles (mean ± SD)	54.22 ± 2.35	60.60 ± 2.44	56.96 ± 2.76	54.46 ± 2.79	54.24 ± 3.45
Prescribed Medications						
	NSAID (% ± SD)	28.50 ± 3.31[§§]	32.54 ± 3.61	45.07 ± 4.19[†]	56.91 ± 4.48	60.22 ± 5.10[†]
	DMAR (% ± SD)	25.27 ± 3.19[§]	49.70 ± 3.86	50.00 ± 4.21	52.85 ± 4.52	49.46 ± 5.21
	Steroids (% ± SD)	26.88 ± 3.26[§§]	24.26 ± 3.31	8.45 ± 2.34	11.38 ± 2.88	6.52 ± 2.5
	Biologics (% ± SD)	32.26 ± 1.30[§]	8.28 ± 2.13[†]	14.79 ± 2.99[‡]	19.51 ± 3.59[†‡]	20.43 ± 4.20
Remission Category*						
	Active disease (% ± SD)	96.13 ± 1.44 [§§]	68.29 ± 3.64	52.21 ± 4.3	40.34 ± 4.52	39.08 ± 5.26
	With medication (% ± SD)	3.31 ± 1.33 [§]	23.78 ± 3.33[†]	32.35 ± 4.03	39.50 ± 4.50[†]	35.63 ± 5.16
	Without medication (% ± SD)	0.55 ± 0.55 [§]	7.93 ± 2.12 [ᵂ]	15.44 ± 3.11	20.17 ± 3.69	25.29 ± 4.69

Continuous variables were analyzed using linear regression and Tukeys post-hoc test. Categorical variable were analyzed using χ^2 and linear comparison post estimation command for post-hoc testing of significant variables.

* Remission category defined by criteria proposed by Wallace et al. (Wallace et al., 2004).

SD (standard deviation), % (percent), BMI (Body Mass Index), NSAID (non-steroidal anti-inflammatory), Biologics (biologically-based anti-cytokine therapies), Steroids (corticosteroid), DMARD (disease-modifying anti-rheumatic drug)

[§] Superscript indicates statistically significant difference ($p<0.05$) in baseline value from other time points.

[§§] Superscript indicates baseline and 6-month follow up values statistically similar ($p>0.05$) with statistically significant difference from other time points.

[†‡] Same superscripts indicate statistically significant difference by time point ($p<0.05$).

[ᵂ] Superscript indicates statistically significant difference ($p<0.05$) in value from other time points.

CHAPTER 8

OBJECTIVE 3

Vitamin D pathway-related genes relate to the association between 25(OH)D and disease activity in children with juvenile idiopathic arthritis

To address objective 3 of this thesis, I evaluated the association between vitamin D pathway gene polymorphisms, vitamin D levels, and JIA disease activity.

Abstract

Background: Both genetic and environmental factors influence Juvenile Idiopathic Arthritis (JIA) development. Factors that influence vitamin D status and their interactions might be associated with vitamin D effects on immune and inflammatory responses in children with JIA.
Objective: To evaluate the association between vitamin D pathway gene polymorphisms, vitamin D levels, and JIA disease activity.

Methods: We analyzed data from the Biologically-Based Outcome Predictors (BBOP) Study, a prospective multi-center study of newly diagnosed Canadian children with JIA (n=186). Blood samples were obtained at baseline and 6 months follow up, 25(OH)D, CRP, ESR, Interleukin (IL)-2, IL-4, IL-1ra, IL-10, IL-6, and TNFα were measured. Vitamin D related factors (milk intake, season, supplement and steroid use) and clinical data defining remission (no active arthritis, fever, uveitis, normal ESR and CRP, best possible physician's global assessment score) were collected every 6 months for 2 years. Genome-Wide Association Studies (GWAS) techniques were applied to identify frequent gene polymorphisms of potential relevance to the vitamin D pathway in JIA. Longitudinal analysis explored whether 25(OH)D and related factors could predict disease activity in BBOP children. Significant variables from linear regressions, genes identified through GWAS, vitamin D pathway genes and gene-gene interactions were selected for further analysis.
Results: GWAS-identified genes were NOTCH4, c6orf9, HLA-DQA1, LEP, IGFBP4, and GPS1. When genes were not included in the model there was an inverse association of 25(OH)D to disease activity. Genes, when included, modified the association between 25(OH)D and indicators of disease activity; only certain genotypes maintained the inverse association of

25(OH)D and disease activity markers. Drinking milk every day predicted a reduction in indicators of disease activity as measured by CRP, ESR, and IL-6.
Conclusions: A negative association of 25(OH)D and inflammation is influenced by vitamin D-related genetic polymorphisms. Milk intake, as a source of dietary vitamin D, is associated with suppression of inflammation in children with JIA.

8.1. Introduction

As with other immune-mediated inflammatory diseases, the pathogenies of JIA is believed to be a consequence of interaction of environmental factors with disease susceptibility genes (Gowdie, 2012). Vitamin D has been suggested to play a role in JIA development at both the environmental (season, sun exposure, dietary intake, medication use) and genetic (vitamin D pathway gene polymorphisms) levels (Berkun et al., 2015; Berkun & Padeh, 2010; Ellis et al., 2010; Ellis et al., 2015; Falcini et al., 2013). Vitamin D is also being explored for its potential to suppress inflammation in children with active JIA (Stagi et al., 2014). Low levels of vitamin D are implicated as an environmental factor influencing JIA occurrence and course; children with JIA tend to have suboptimal vitamin D levels and low vitamin D levels in JIA are associated with more active disease (Finch et al., 2018). It is vitamin D's influence on gene expression, including expression of genes governing immune and inflammatory processes, which may best explain vitamin D's putative role in the pathogenesis of diseases such as JIA (Cutolo et al., 2011; Ellis et al., 2015).

The active form of vitamin D, 1, 25 hydroxyvitamin D (1,250D), influences expression of hundreds of genes involved in an array of biologic systems including bone mineralization and immune function. (Fuleihan et al., 2015; Holick, 2012). The action of 1,25OHD is mediated by its binding to VDR in bone, muscle, immune, intestinal, cardiac and endocrine cells (Haussler et al., 1998; Souberbielle et al., 2010). The 1,25(OH)D-liganded VDR dimerizes with nuclear RXR. The VDR-RXR heterodimer binds to the VDRE of target DNA sequences to turn gene transcription on or off (Jolliffe, Walton, Griffiths, & Martineau, 2016). The influence of 1,25(OH)D on gene expression and associated cell functions can be modulated by factors that determine the body's vitamin D levels including, as examples, exposure to ultra-violet B radiation, dietary intake of vitamin D , use of vitamin D supplements, and certain medications that affect vitamin D absorption and metabolism. In addition, vitamin D's effect on gene

expression is influenced by SNPs and epigenetic alteration of genes involved in the vitamin D pathway (Knight, Wong, Cole, Lee, & Parra, 2017; Saccone, Asani, & Bornman, 2015).

Polymorphisms of the rs11568820 SNP (specifically the GG genotype and G alleles), located in the promotor region of the VDR gene, are more frequent in patients with JIA and are hypothesized to be associated with reduction in VDR activity and a related blunting of response to vitamin D (Falcini et al., 2013). The idea of investigating epistasis (gene-gene interactions) amongst genes in the inflammatory and vitamin D pathways in the context of JIA was explored by Ellis et al. (2015). They reported various interacting SNPs involving the PTPN2 gene with the vitamin D binding protein gene contirbuted to JIA risk (Ellis et al., 2015). This suggests that the role of vitamin D may be through its interaction with the inflammatory pathway.

Vitamin D's benefits as adjunctive therapy to favorably affect disease activity has been explored in a variety of immune and inflammatory-mediated disease including, as examples, rheumatoid arthritis, diabetes, asthma, and inflammatory bowel disease (Hu et al., 2018; Ishikawa et al., 2017; Jeffery, Raza, & Hewison, 2015; Kerley, Elnazir, Faul, & Cormican, 2015; Tabatabaeizadeh, Tafazoli, Ferns, Avan, & Ghayour-Mobarhan, 2018). However, to our knowledge, there are no reports of vitamin D's influence on disease outcomes in the context of genes governing the vitamin D pathway. The objectives of this study were to investiage vitmain D pathway gene polymorphisms in JIA and ascertain elements of the vitamin D pathway that predict disease activity outcomes in children with JIA.

8.2. Methods

8.2.1. Data and Sample Collection

We analyzed data from the Biologically-Based Outcome Predictors (BBOP) in Juvenile Idiopathic Arthritis study, a prospective multi-center study of newly diagnosed Canadian children with JIA (n=186, aged 1-16 years old). Enrollment criteria included meeting the ILAR classification criteria for JIA (Petty et al., 2004), being within six months of disease onset, having not been treated previously with DMARDs or biologically-based anti-cytokine therapies (biologics), and consenting to be enrolled in the study. Each participating institution provided ethics approval.

Demographic and clinical data were collected from December 2007 to December 2012 at enrollment and at 6, 12, 18, and 24 months post-enrollment. Clinical data was used to define

remission and included variables required for the American College of Rheumatology pediatric core set of measures of disease activity (Giannini et al., 1997) including PGA of disease activity, acute phase reactants ESR and/or CRP, and the CHAQ-JIA (Giannini et al., 1997). Inactive disease (remission) was established using criteria defined by Wallace et al. (Wallace et al., 2004) and categorized as remission on medication or remission off medication.

Markers of inflammation ESR and CRP were generated by the clinical service laboratories in each of the respective participating centers and reported for all time points. Blood was collected in P100 tubes (BD Biosciences, San Jose, CA) obtained at baseline and six and the plasma stored at -80^{o}C until assayed for levels of 25(OH)D as previously described (McNally et al., 2008), and the following cytokines: IL-2, IL-4, IL-6, IL-10, and TNFα (Eng et al., 2014) (cytokine analysis is described in Chapter 4). Biomarker variables were log transformed for statistical analysis because they did not follow normal distribution. Forty-six cytokines were analyzed for the BBOP study. We chose to focus on the cytokines listed above because they are most commonly listed in the literature as having been explored in participants with JIA or other autoimmune diseases and are associated with vitamin D (Cutolo et al., 2011; Dankers et al., 2017; Hayes et al., 2015). We used a method of standardization developed by Tabung et al. to compare inflammatory biomarker concentrations (Tabung et al., 2015). This method takes the log-transformed biomarker concentration then computing a z-score for each inflammatory biomarker. Tabung et al. combined (added together) the standardized values for CRP, IL-6, and TNFα to create a pro-inflammatory index score (Tabung et al., 2015). Using the same method of standardization, we created an anti-inflammatory score that combined the anti-inflammatory cytokines IL-4 and IL-10.

CRP, ESR, vitamin D-related factors (milk consumption, season of measurement, vitamin D supplementation, and steroid use) and clinical data to define remission were recorded every six months for two years. To assess vitamin D intake over the last month, information on the frequency of milk intake (every day, never/sometimes) and vitamin D containing supplement use (every day/sometimes, never) were collected. Medication use was assessed at every time-point and categorized by medication class including NSAID, steroid, disease-DMARD, or biologic agents. Demographic data included age, sex, family income and self-identified ethnicity.

At the second visit, a saliva sample was collected for genotyping. DNA was extracted to analyze VDR polymorphisms, and the genotype data analyzed to determine VDR

polymorphisms. For the BBOP study single nucleotide polymorphism (SNP) gene testing was done using Human 12-sample Immuno BeadChip 11419691 B (Illumina®). The data were analyzed using Genome Studio 2011.1. Genetic testing was done at the Genetic Analysis Facility, The Centre for Applied Genomics, The Hospital for Sick Children, Toronto.

Genome-Wide Association Studies (GWAS) were applied to identify SNPs of potential relevance to the vitamin D pathway in JIA. GWAS was performed by the University of Saskatchewan Bioinformatics Lab. In addition to BBOP, two other genetic datasets were used for GWAS. 1) The "*Better Outcomes for Children: GWAS from Cincinnati Children's Hospital Medical Center (CCHMC) - eMERGE Phase II*" dataset (dbGaP Study Accession: phs000494.v1.p1). This dataset includes genotyping data from six cohorts. Cohort D of the dataset comprises Caucasian patients with JIA. The dataset includes 814 JIA cases and 658 controls of self-reported non-Hispanic European American (EA) ancestry (Thompson et al., 2012). 2) The National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) healthy control genetic dataset were merged to build a case/control dataset, where BBOP samples were used as cases and control samples from the NIDDK Dataset were used as controls. PLINK: Whole genome data analysis toolset software version v1.90b4.4 was used for the analysis (http://pngu.mgh.harvard.edu/purcell/plink/) (Purcell, Neale, Todd-Brown, Thomas et al., 2007). This approach was used to replicate the results achieved from the analysis of the cohort D of the Cincinnati dataset. After applying quality control for these two datasets by following the procedure suggested by Anderson et al. (Anderson et al., 2011), there was a small overlap between SNPs in these datasets. This was mainly because NIDDK and BBOP used two different platforms with a small overlap between their results; therefore, we avoided extracting control samples from NIDDK dataset and conducting a case-control study.

A subset of markers was assembled based on the following criteria:

- Markers suspected to be involved in JIA extracted from the literature
- Markers associated with the vitamin D pathway

Markers extracted from the analysis of the Cincinnati cohort D, the genotype data were extracted for the candidate SNPs for all samples, in the BBOP dataset, that passed the quality control procedure. The step-by-step protocol described by Anderson et al. was followed to perform the GWAS analysis (Anderson et al., 2011). These SNPs later were used for regression analysis.

Genetic predictors of disease activity

Univariate analysis was performed to explore what individual genes identified through GWAS as well as genes from the vitamin D pathway (Table 8.1) predict disease activity. Any genes identified to have a $p<0.05$ were included in the full model building process. Next, analysis of epistasis of genes identified through GWAS and those in the vitamin D pathway was performed. Those with a p-value <0.01 were included in the full model building process. Variables that were significant in the previous steps were included in the full model building process.

Environmental, genetic and interactions that predict disease activity

Linear regressions identified significant gene-environment interactions associated with disease activity measures for inclusion in the final model. Significant variables from linear regressions performed in Chapter 7, as well as those genes identified through GWAS, vitamin D pathway genes, gene-gene interactions were included in the final analysis to identify what environmental and genetic factors associate with disease activity.

Statistical Analysis

Descriptive statistics (means and standard deviations for continuous variables and percentages for categorical variables) were used to summarize the data at five time-points. Multi-Level Mixed-Effects (MLM) Modeling and backwards methodology with patients set as a random effect factor (individual patient change by time/visit number), was utilized to determine if there is an association between genes potentially related to the vitamin D pathway and markers of inflammation /disease outcomes in the presence of potential covariates, such as socioeconomic status, sex, age, ethnicity. The repeated measures across time points had unstructured covariance structure. Intreactions with a p-value > 0.01 were not selected for the final model to adjust for the increased false discovery rate of multiple comparisons. All vitamin D pathway gene*GWAS identified gene interaction and environment*gene interaction were explored in the model however only significant interactions are presented in the tables. STATA 15 (StataCorp LP 2015) was used for analysis.

8.3. Results

8.3.1. Genome-Wide Association Studies (GWAS)

Genes identified through GWAS were NOTCH4, C6orf10, HLA-DQA1, LEP, IGFBP4, GPS1. SNPs and allele frequencies are shown in Table 8.1. Environmental factors influencing vitamin D as well as biochemical and remission variables are summarized in Chapter 7.3. Multivariate analysis was performed to explore the epistasis of differing polymorphisms of genes identified through GWAS and those in the vitamin D pathway found to predict significant differences in disease activity measures. All SNPs in Table 8.1 predicted a variation in disease activity with the exception of those for LEP rs 2071045 and VDR rs4516035. Additionally, the VDR SNP rs3890733 was only significant in the genetics models and not in the gene-environment models.

Analysis of both genetic and environmental factors to predict disease activity in children with JIA reveals that overall the interaction between differing SPN allelic variations in vitamin D pathway genes, IGFBP4, HLA-DQA1 or GPS1 and environmental factors associated with vitamin D predict differences in disease activity. While the other GWAS genes predicted gene-gene interactions, they did not predict significant gene-environment interactions. Interaction between 25(OH)D and VDR rs731236, VDR rs7975232, GC, GSP1 or HLA-DQA1 was present in a number of inflammatory models (ESR, IL-1α, IL-2, IL-6) as well as the model that predicted remission.

8.3.2. Genetic Predictors of Disease Activity

Biochemical measures of inflammation

Differences in the prediction of CRP concentrations based on allelic variation were found in the following genetic SNPs and genetic interactions ([variable name] *[variable name] represents interaction term): VDR rs4760648, CYP24A1 rs4809959, C6orf10 rs6907322, HLA-DQA1 rs9272219, IGFBP4 rs584438, VDR rs4760648* IGFBP4 rs584438, CYP24A1 rs4809959 * IGFBP4 rs584438 (Table 8.2). CRP was also predicted to decrease over the five study visits. In comparison to their reference alleles, differences in the prediction of ESR concentrations were ovserved in the following genetic SNPs and genetic interactions: VDR rs4760648, C6orf10 rs6907322, HLA-DQA1 rs9272219, IGFBP4 rs584438, VDR rs4760648*

IGFBP4 rs584438, and CYP24A1 rs4809959 * IGFBP4 rs584438 (Table 8.3). ESR was also predicted to decrease over the five study visits.

Pro-Inflammatory Cytokines

In comparison to their reference alleles, differences in the prediction of IL-1ra concentrations were found in the following genetic SNPs and genetic interactions: VDR rs11568820, VDR rs4760648, IGFBP4 rs584828, NOTCH4 rs415929, VDR rs4760648 * NOTCH4 rs415929, VDR rs11568820* IGFBP4 rs584828, VDR rs2238136* NOTCH4 rs2071286 (Table 8.4). IL-1ra was not predicted to change over time. Differences in the prediction of IL-2 concentrations based on allelic variations were seen in the following genetic SNPs and genetic interactions: VDR rs1540339, VDR rs3890733, rs731236, rs7975232, CYP24A1 rs2248359, VDR rs1540339 * NOTCH4 rs415929, VDR rs3890733 * IGFBP4 rs584828, VDR rs7975232 * GPS1 rs9916764, CYP24A1 rs4809959* HLA-DQA1 rs9272219, CYP24A1 rs2248359* IGFBP4 rs584438 (Table 8.5). IL-2 concentration did not change over time. Predicted IL-6 concentrations differed based on allelic variations in the following genetic SNPs and genetic interactions: IL-6 VDR rs1544410, rs4760648, rs2238136, GC rs7041/rs4588, IGFBP4 rs584438, GPS1 rs9916764, VDR rs1544410 * GPS1 rs9916764, VDR rs4760648* GPS1 rs9916764, VDR rs2238136* IGFBP4 rs584438. IL-6 was predicted to decrease from baseline to the first follow-up visit (Table 8.6). Differences in the prediction of TNF-α concentrations based on allelic variations were found in the following genetic SNPs and genetic interactions: VDR rs731236, VDR rs2238136, CYP24A1 rs4809959, IGFBP4 rs584438, CYP24A1 rs4809959* IGFBP4 rs584828. There was no statistically significant difference in TNF-α concentration between the two visits (Table 8.7). In comparison to their reference alleles, differences in the prediction of the Pro-Inflammatory Index Score were observed in the following genetic SNPs and genetic interactions: VDR rs10783219, CYP24A1 rs4809959, IGFBP4 rs584828, GPS1 rs9916764, VDR rs1544410 * GPS1 rs9916764, VDR rs10783219* GPS1 rs9916764 (Table 8.8). This score significantly decreased between baseline and the six-month follow-up visit.

Anti- Inflammatory Cytokines

Predicted IL-4 concentrations differed based on allelic variations in the following genetic SNPs and genetic interactions: VDR rs2238136, IGFBP4 rs584828, VDR rs1544410 * GPS1 rs9916764 (Table 8.9). There was no statistically significant difference in IL-4 concentrations between the two visits. There was differences in the prediction of IL-10 concentrations based on allelic variations in the following genetic SNPs and genetic interactions: IGFBP4 rs584828, VDR rs7975232* GPS1 rs9916764 (Table 8.10). IL-10 did not change over time. In comparison to their reference alleles differences in the prediction of the Anti-Inflammatory Index Score (IL-10+IL-4) were found in the following genetic SNPs and genetic interactions: VDR rs731236, VDR rs7975232, VRD rs2248098, VDR rs2238136, GC rs7041/rs4588, CYP24A1 rs4809959, HLA-DQA1 rs9272219, IGFBP4 rs584438, IGFBP4 rs584828, VDR rs11568820 * GPS1 rs9916764, VDR rs731236* GPS1 rs9916764,
VDR rs2238136 * NOTCH4 rs2071286, CYP24A1 rs4809959* HLA-DQA1 rs9272219, CYP24A1 rs4809959* IGFBP4 rs584438 (Table 8.11). This score did not change between the baseline and the six-month follow-up visit.

Remission

In comparison to their reference alleles, differences in the prediction of achieving remission were seen in the following genetic SNPs and genetic interactions: VDR rs10783219, CYP24A1 rs2248359, CYP24A1 rs2248359 * NOTCH4 rs415929 (Table 8.12). Achieving remission was also predicted to increase over the five study visits.

8.3.3. Genetic and Environmental Predictors of Disease Activity

Baseline and six-month follow up (first follow-up)

Biochemical measures of inflammation

CRP concentrations decreased from baseline to the six-month follow-up (Table 8.13). Children who were not prescribed a steroid were predicted to have lower CRP than those that were prescribed. Differences in the prediction of CRP concentrations based on allelic variation were found in the following genetic SNPs and genetic interactions: VDR rs4760648, VDR rs10783219, NOTCH4 rs415929, VDR rs4760648 * NOTCH4 rs415929. The following gene-environment interactions were associated with differences in CRP concentrations: Season* VDR rs1544410, Milk Intake*VDR rs1544410, and Milk Intake* VDR rs7975232. ESR decreased

from baseline by the first follow-up. Significant environmental variables included 25(OH)D, Milk Intake and Season. Significant environmental interaction occurred between Milk Intake and supplement use. Differences in the prediction of ESR concentrations based on allelic variation were seen in the following genetic SNPs and genetic interactions: VDR rs4760648, C6orf10 rs6907322 HLA-DQA1 rs9272219, IGFBP4 rs584438, VDR rs4760648* IGFBP4 rs584438 (Table 8.14). The following gene-environment interactions were associated with differences in ESR concentrations: 25(OH)D* GPS1 rs9916764 and Season*CYP24A1 rs4809959.

Pro-Inflammatory Cytokines

There was no difference in IL-1ra between baseline and the first follow-up (Table 8.15). Significant environmental variables included 25(OH)D, age (years), use of a steroid or biologic agent. There was a significant interaction between 25(OH)D and steroid use. Genes and gene-gene interactions of significance include VDR rs4760648, VDR rs1544410, VDR rs7975232, VDR rs2248098, VDR rs731236, GC rs7041/rs4588, CYP24A1 rs2248359, IGFBP4 rs584828, NOTCH4 rs2071286, NOTCH4 rs415929, VDR rs4760648 * NOTCH4 rs415929, VDR rs11568820*IGFBP4 rs584828. Gene-environment interactions that were significant included 25(OH)D*VDR rs7975232, 25(OH)D*GC rs7041/rs4588, 25(OH)D*VDR rs2248098, Season* VDR rs1544410, Season* VDR rs731236, and Season*VDR rs2238136.

There was no significant reduction in IL-2 from baseline to the six-month follow-up (Table 8.16). 25(OH)D, Milk Intake, Vitamin D containing supplement use and Season predicted variations in IL-2 concentrations. The genetic factors that predicted differing IL-2 concentrations were: VDR rs731236, VDR rs7975232, VDR rs11568820, CYP24A1 rs4809959, CYP24A1 rs2248359, GC rs7041/rs4588, IGFBP4 rs584438, IGFBP4 rs584828, VDR rs1540339 * NOTCH4 rs415929, VDR rs7975232 * GPS1 rs9916764, CYP24A1 rs4809959* HLA-DQA1 rs9272219, CYP24A1 rs2248359* IGFBP4 rs584438. Gene-environment interactions that significantly predicted varying IL-2 were: 25(OH)D *VDR rs731236, 25(OH)D*GC rs7041/rs4588, Season* VDR rs1544410, Season* VDR rs7975232, Milk Intake *VDR rs11568820 Taking a supplement containing vitamin D * VDR rs4760648, and taking a supplement containing vitamin D* CYP24A1 rs2248359.

IL-6 concentrations did not significantly change over time (Table 8.17). The environmental variables that predicted a change in IL-6 included: 25(OH)D, age, Milk Intake

and Season. The following genes and genetic interactions were significant: GC rs7041/rs4588, GC rs7041/rs4588 * GPS1 rs9916764. The following gene-environment interactions were significant: 25(OH)D*GPS1 rs9916764, Season* VDR rs1544410, Season* VDR rs7975232, Season* CYP24A1 rs2248359, Milk Intake*VDR rs1544410, Milk Intake * VDR rs7975232

TNF-α did not change over time (Table 8.18). There was an association with age (years), and Season, as well as the following genetic factors: VDR rs731236, VDR rs11568820, CYP24A1 rs4809959, IGFBP4 rs584438, IGFBP4 rs584828, CYP24A1 rs4809959* IGFBP4 rs584828, The genetic and environmental interactions that predicted differences in TNF-α were: Milk Intake *VDR rs11568820, and Season*VDR rs2238136.

There was a decrease in the pro-inflammatory index score between the baseline and six-month follow-up visits (Table 8.19). 25(OH)D concentrations, Milk Intake, taking a supplement containing vitamin and Season predicted variations in the pro-inflammatory index score. The following genes and genetic interactions were significant: VDR rs1544410, VDR rs10783219, GPS1 rs9916764, IGFBP4 rs584438.The following gene-environment interactions predicted variations in the pro-inflammatory index concentrations: 25(OH)D* GPS1 rs9916764, Season* VDR rs7975232, Season* VDR CYP24A1 rs2248359, Season* IGFBP4 rs584438, Milk Intake * VDR rs7975232, and taking a supplement containing vitamin D * GC rs7041/rs4588.

Anti-Inflammatory cytokines

There was an association with age, sex, and Milk Intake that predicted differenced in IL-4 concentrations (Table 8.20). In addition, the following genes and genetic interactions were significant: VDR rs1544410, VDR rs2238136, IGFBP4 rs584828, GPS1 rs9916764, VDR rs1544410 * GPS1 rs9916764. The following gene-environment interactions were significant: Season* VDR rs1544410, Season* GC rs7041/rs4588, Milk Intake * VDR rs7975232. The variables that predicted variations in IL-10 were: taking a supplement containing vitamin D, Season, VDR rs11568820, CYP24A1 rs2248359, IGFBP4 rs584828, Season* VDR rs731236, Season* VDR rs7975232, taking a supplement containing vitamin D * VDR rs11568820 (Table 8.21). IL-10 also decreased from the baseline to the first follow-up. Age, Milk Intake, and Season predicted variations in the anti-inflammatory index score. The following genes and genetic interactions were significant: VDR rs731236 VDR rs7975232, VRD rs2248098, VDR rs2238136, GC rs7041/rs4588, CYP24A1 rs4809959, IGFBP4 rs584828, IGFBP4 rs584828,

GPS1 rs9916764, The following gene-environment interactions predicted variations in the anti-inflammatory index concentrations: VDR rs11568820* GPS1 rs9916764, VDR rs731236 * GPS1 rs9916764, VDR rs2238136* NOTCH4 rs2071286, CYP24A1 rs4809959* HLA-DQA1 rs9272219, CYP24A1 rs4809959* IGFBP4 rs584438 (Table 8.22). The following gene-environment interactions were significant: Season* VDR rs7975232, Milk Intake * VDR rs11568820, Milk Intake * VDR rs7975232.

Remission

At the six-month follow-up, a significant number of patients had achieved remission (Table 8.23). The following genes and genetic interactions were significant: CYP1R1 rs17116978, NOTCH4 rs415929, HLA-DQA1 rs9272219, GPS1 rs9916764, CYP1R1 rs17116978* NOTCH4 rs415929. The following gene-environment interactions were significant: 25(OH)D*HLA-DQA1 rs9272219, and 25(OH)D*GPS1 rs9916764.

<u>All time points: Baseline to 24-month follow-up</u>

Biochemical measures of inflammation

CRP concentrations decreased over the two-year follow-up period (Table 8.24). Significant environmental variables included milk intake, using a steroid and having a smoker in the home. The following genes and genetic interactions were significant: VDR rs4760648 VDR rs10783219, VDR rs4760648*NOTCH4 rs415929, as well as the gene-environmental interaction of Season* VDR rs1544410. ESR decreased through the follow-up visits (Table 8.25). Significant environmental variables included age, taking a supplement containing vitamin D, and Season. Significant environmental interaction occurred between Milk Intake and supplement use. There was also a significant interaction of Season*CYP24A1 rs4809959.

Remission

There was an increase in patients achieving remission over the two-year follow-up period (Table 8.26). Significant environmental variables included CRP, taking a supplement containing vitamin D, being prescribed an NSAID or biologic. The following genes and genetic interactions were significant: NOTCH4 rs415929, CYP1R1 rs17116978* NOTCH4 rs415929.

8.4. Discussion

This is the first study to explore the association of gene, environment and biochemical factors in the vitamin D pathway on disease activity and outcomes in children with JIA. None of the genes identified through GWAS were direct vitamin D pathway genes. Vitamin D pathway genes and their interactions with GWAS identified genes did predict differing disease outcomes based on the gene polymorphisms. When the environmental and biochemical information factors were added to the statistical models, disease outcomes were modulated by genetic polymorphism, milk intake, and 25(OH)D along with key GWAS gene-environment interactions and gene-biochemical interactions.

No direct vitamin D pathway genes were identified through GWAS in this study, suggesting that the impact of vitamin D in autoimmune disease is through the impact on 25(OH)D or the interaction of vitamin D pathway genes with genes identified to be associated with JIA. There is evidence of over 100 genes targeted by 1,25(OH)D in immune cells (Maruotti & Cantatore, 2010). Understanding the contribution of genes to disease development, treatment response and outcome is an important step in the development of personalized medicine and furthering our comprehension of molecular pathways (Hersh & Prahalad, 2015). Our GWAS found variations in the polymorphisms of HLA-DQA1 between healthy controls and children with JIA. This gene has been confirmed to be associated with JIA (Chiaroni-Clarke et al., 2014; Hersh & Prahalad, 2015). The other GWAS genes identified in Table 8.1 will require further study to validate the association with JIA development.

Except for the LEP gene, all other genes identified by GWAS were associated with differences in selected disease activity measures. Additionally, SNPs of VDR, GC, and CYP24A1 were also associated with differing disease activity status in both models including only genes and gene-environment. Vitamin D pathway genes have been found both singly and collectively to be risk factors for other autoimmune diseases (Jolliffe, Walton, Griffiths, & Martineau, 2016; Maruotti & Cantatore, 2010). Interactions between vitamin D pathway genes and PTPN2 (rs2542151) a gene confirmed in the heritability of JIA has been explored (Ellis et al., 2015). However, this is the first time that this concept has been explored in relation to disease outcomes. The identified interactions will need to be confirmed in a validation cohort.

Analysis of modifiable and environmental risk factor without the inclusion of genetic data was presented and discussed in Chapter 7. We found an interaction between 25(OH)D and

two GWAS identified genes and between 25(OH)D and genes in the vitamin D pathway over the six-month period during which cytokine data was collected. This is the first time that this has been explored in JIA. Young et al. found a combined role of vitamin D status and CYP24A1 in the transition to Systematic Lupus Erythematosus (SLE) (Young et al., 2016). Where the association between the development of SLE and 25(OH)D was modified for each minor allele of CYP24A1 rs4809959, increased 25(OH)D concentrations decreased the risk of developing SLE in participants who have family member already diagnosed with SLE. While we did not find the same association with CYP24A1 rs4809959 as found in the study on SLE, we did find the association to be present with another vitamin D pathway gene, the VDR polymorphisms of rs7975232 and rs731236 presented such association.

Milk intake was also shown to interact with genetic polymorphisms and correlate with disease outcomes. Milk, with mandatory fortification in Canada, is the most common source of vitamin D for this age group (Whiting et al., 2011) and suggests an actionable way to improve vitamin D concentrations in children with JIA. A systematic review of clinical trials exploring dairy product intake and inflammation demonstrated that dairy products might have anti-inflammatory properties in humans (Bordoni et al., 2017). Our study suggests that genetic factors may modify this relationship.

This is the first study to explore the association of the vitamin D pathway (environmental, biochemical and genetic factors) and JIA over time in newly diagnosed individuals. Investigating the genetic and environmental role, that vitamin D plays in the prevention and control of JIA in the same children helps to tease out the multifaceted role played by vitamin D in this disease.

Limitations

While this study has many strengths, it has limitations. Limitations include the lack of measurement of seasonal sun exposure or sunscreen use. This could explain why we did not see the same pattern for the Season of measurement and genetic variables as we did for milk intake. This cohort study did not have a control group for comparison to healthy children. To mitigate the impact of this we used additional available data for GWAS analysis by exploring two additional datasets. 25(OH)D, pro- and anti-inflammatory cytokines were only measured at two time-points and not throughout the entire two years of the study. Another limitation is that we did not control for the familywise error rate and multiple comparisons across the statistical

analysis using the Bonferoni correction. Instead, we chose to include only interactions with a p-value < 0.01 to adjust for the increased false discovery rate of multiple comparisons. To explore the association of vitamin D in the later time points we used surrogate measures such as Season, milk intake and supplement. Our sample size was not large enough to explore 25(OH)D by JIA categories with our models. We explored factors associated with vitamin D that could predict disease activity measured by JIA category, using all seven categories, then by inflamed joint count > four and < four. However, there was an inadequate sample size to perform the analysis using MLM even when the participants were divided into two groups for bivariate analysis. Investigation of differing environmental and genetic factors impacting individual JIA categories will require large multi-country studies.

8.5. Conclusion

Genes identified through GWAS (HLA-DQA1, and GPS1) and VRD polymorphisms (rs7975232 and rs731236) modified the association between 25(OH)D and indicators of disease activity; only certain genotypes maintained the negative association of 25(OH)D and disease activity markers. Disease activity measures were also modified by the interaction of milk consumption and VDR polymorphisms (rs1544410, rs7975232 and rs11568820) where drinking milk in children with certain SNPs of these genes predicted a reduction in indicators of disease activity as measured by CRP, ESR, and IL-6. Our findings indicate a negative association of 25(OH)D and inflammation was modified by vitamin D-related genetic polymorphisms. Milk intake as a source of dietary vitamin D, modified by VDR polymorphisms SNPs, was associated with suppression of inflammatory markers in children with JIA. Understanding how genetic variants interacting with environmental and biochemical factors increase the risk of disease development or alter disease outcomes will enhance the quality of life of patients and their families.

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Table 8.1. The allele frequency of genes found through Genome-Wide Association Studies (GWAS) and vitamin D pathway genes

Gene	Single Nucleotide Polymorphism	Allele Frequency (%)		
Vitamin D Pathway Genes				
VDR				
	rs4516035	CC (20.2)	CT (39.3)	TT (40.5)
	rs11568820	AA (6.5)	AG (31.1)	GG (62.5)
	rs1544410	AA (14.9)	AG (49.4)	GG (35.7)
	rs1540339	AA (10.7)	AG (43.5)	GG (45.8)
	rs3890733	TT (8.9)	TC (38.7)	CC (52.4)
	rs4760648	TT (23.2)	TC (50.0)	CC (26.8)
	rs731236	CC (13.7)	CT (50.0)	TT (36.3)
	rs7975232	CC (21.7)	CA (51.2)	AA (27.1)
	rs2248098	TT (21.0)	TC (52.7)	CC (26.3)
	rs10783219	TT (14.9)	TA (42.9)	AA (42.3)
	rs2238136	AA (5.4)	AG (32.9)	GG (61.7)
GC	rs7041/rs4588	TT (19.2)	TG (46.7)	GG (34.1)
CYP24A1				
	rs4809959	TT (22.8)	TG (53.9)	GG (23.3)
	rs2248359	TT (11.9)	TC (49.4)	CC38.7
CYP1R1				
	rs17116978	CC (0.6)	CT (7.7)	TT (91.7)
	rs1035798	TT (8.9)	TC (44.6)	CC (46.4)
Genes Identified through GWAS				
NOTCH4				
	rs2071286	AA (7.8)	AG (41.0)	GG (51.2)
	rs415929	GG (13.7)	GA (46.6)	AA (40.0)
C6orf10	rs6907322	AA (3.6)	AG (46.7)	GG (50.0)
HLA-DQA1	rs9272219	TT (19.6)	TG (42.3)	GG (38.1)
LEP	rs2071045	CC (7.7)	CT (37.50	TT (54.8)
IGFBP4				
	rs584438	TT (20.2)	TG (48.8)	GG (31.0)
	rs584828	AA (20.4)	AG (50.30	GG (29.3)

GPS1	rs9916764	GG (10.7)	GT (44.6)	TT (44.6)

Genetic variables and their interactions

Biochemical measures of inflammation

Table 8.2. SNPs allelic variations and interactions that predict differing C-Reactive Protein concentrations

Variable	C-Reactive Protein Coefficient (95% Confidence Interval)	p-value
VDR rs4760648, reference value (TT)		
TC	0.76 (0.0 , 1.6)	0.062
CC	1.61 (0.5 , 2.8)	0.006
VDR rs7975232, reference value (CC)		
CA	-0.57 (-0.9 , -0.2)	0.002
AA	-0.64 (-1.0 , -0.2)	0.002
VDR rs10783219, reference value (TT)		
TA	-0.38 (-0.8 , 0.1)	0.087
AA	-0.74 (-1.2 , -0.3)	0.001
NOTCH4 rs415929, reference value (GG)		
GA	1.01 (0.2 , 1.8)	0.01
AA	1.77 (1.0 , 2.5)	< 0.001
VDR rs4760648 * NOTCH4 rs415929, reference value (TT/GG)		
TC/AA	-1.25 (-2.2 , -0.3)	0.012
CC/GA	-1.49 (-2.8 , -0.2)	0.026
CC/AA	-2.24 (-3.5 , -1.0)	0.001
Follow ups	-0.39 (-0.5 , -0.3)	p < 0.001
cons	2.22 (1.4 , 3.0)	p < 0.001
Random Effects		Standard Error
Participant	0.38 (0.2 , 0.8)	0.132
Residual	1.12 (1.0 , 1.2)	0.0578

SNP (Single Nucleotide Polymorphism),
[variable name] *[variable name] represents interaction term

Table 8.3. SNPs allelic variations and interactions that predict differing Erythrocyte Sedimentation Rates

Variable Fixed Effects	Erythrocyte Sedimentation Rate Coefficient (95% Confidence Interval)	p-value
VDR rs4760648, reference value (TT)		
TC	-1.2 (-1.8 , -0.5)	0.001
CC	-1.0 (-1.8 , -0.2)	0.019
CYP24A1 rs4809959 TT		
	-0.1 (-0.9 , 0.6)	0.743
C6orf10 rs6907322, reference value (AA)		
AG	-0.6 (-1.4 , 0.2)	0.114
GG	-0.9 (-1.7 , -0.1)	0.03
HLA-DQA1 rs9272219, reference value (TT)		
TG	-0.4 (-0.8 , -0.1)	0.024
GG	-0.2 (-0.6 , 0.2)	0.265
IGFBP4 rs584438, reference value (TT)		
TG	-0.6 (-1.3 , 0.2)	0.133
GG	-1.4 (-2.2 , -0.6)	0.001
VDR rs4760648* IGFBP4 rs584438, reference value (TT/TT)		
TC/GG	1.3 (0.4 , 2.2)	0.003
CC/GG	1.4 (0.3 , 2.4)	0.01
CYP24A1 rs4809959 * IGFBP4 rs584438, reference value (GG/TT)		
TT/GG	1.5 (0.4 , 2.6)	0.008
Follow ups	-0.3 (-0.3 , -0.2)	< 0.001
Constant	4.7 (3.7 , 5.8)	< 0.001
Random Effects		Standard Error
Participant	0.6 (0.5 , 0.7)	0.100
Residual	0.8 (0.8 , 0.9)	< 0.001

SNP (Single Nucleotide Polymorphism)
[variable name] *[variable name] represents interaction term

Pro-Inflammatory Cytokines

Table 8.4. SNPs allelic variations and interactions that predict differing Interleukin-1ra concentrations

Variable	Interleukin-1ra	
Fixed Effects	Coefficient (95% Confidence Interval)	p-value
VDR rs2238136, reference value (AA)		
AG	-2.28 (-6.6 , 2.0)	0.298
GG	-1.69 (-3.6 , 0.2)	0.082
VDR rs11568820, reference value (AA)		
AG	-3.28 (-6.4 , -0.2)	0.036
GG	-2.05 (-4.5 , 0.5)	0.108
VDR rs4760648, reference value (TT)		
TC	-2.37 (-4.3 , -0.4)	0.017
IGFBP4 rs584828, reference value (AA)		
AG	-5.51 (-8.7 , -2.3)	0.001
GG	-3.23 (-6.4 , -0.1)	0.046
NOTCH4 rs415929, reference value (GG)		
GA	-1.89 (-3.7 , 0.0)	0.045
AA	-1.81 (-3.7 , 0.1)	0.067
NOTCH4 rs2071286, reference value (AA)		
AG	-2.59 (-5.4 , 0.2)	0.072
GG	0.81 (-0.8 , 2.4)	0.316
VDR rs4760648 * NOTCH4 rs415929, reference value (TT/GG)		
TC/GA	2.30 (0.0 , 4.6)	0.046
TC/AA	2.96 (0.6 , 5.3)	0.013
VDR rs11568820* IGFBP4 rs584828, reference value (AA/AA)		
AG/AG	6.08 (2.3 , 9.9)	0.002
AG/GG	4.76 (0.9 , 8.6)	0.016
GG/AG	4.34 (1.0 , 7.7)	0.01
VDR rs2238136* NOTCH4 rs2071286, reference value (AA/AA)		
GG/AG	3.15 (0.5 , 5.8)	0.02
Follow ups	0.08 (-0.2 , 0.4)	0.621
Constant	10.22 (6.5 , 13.9)	0.00
Random Effects		Standard Error
Participant	1.42 (0.1 , 1.2)	1.717
Residual	1.23 (0.1 , 1.1)	1.415

SNP (Single Nucleotide Polymorphism)
[variable name] *[variable name] represents interaction term

Table 8.5. SNPs allelic variations and interactions that predict differing Interleukin-2 concentrations

Variable	Interleukin-2 Coefficient (95% Confidence Interval)	p-value
Fixed Effects		
VDR rs1540339, reference value (AA)		
AG	2.89 (0.9 , 4.9)	0.005
GG	1.21 (-0.2 , 2.6)	0.086
VDR rs3890733, reference value (TT)		
TC	-1.45 (-3.8 , 0.9)	0.223
CC	-2.79 (-5.0 , -0.5)	0.015
VDR rs731236, reference value (CC)		
CT	-1.30 (-2.3 , -0.3)	0.010
TT	-1.38 (-2.6 , -0.2)	0.022
VDR rs7975232, reference value (CC)		
CA	-1.31 (-3.5 , 0.9)	0.250
AA	-1.68 (-3.2 , -0.2)	0.029
CYP24A1 rs4809959, reference value (TT)		
TG	0.05 (-1.5 , 1.6)	0.950
GG	0.33 (-1.4 , 2.0)	0.709
CYP24A1 rs2248359, reference value (TT)		
TC	3.54 (1.9 , 5.2)	< 0.001
CC	3.87 (2.1 , 5.6)	< 0.001
NOTCH4 rs415929, reference value (GG)		
GA	0.38 (-1.8 , 2.5)	0.733
AA	0.43 (-0.7 , 1.5)	0.443
HLA-DQA1 rs9272219, reference value (TT)		
TG	0.60 (-0.9 , 2.1)	0.445
GG	-0.18 (-1.5 , 1.2)	0.793
IGFBP4 rs584438, reference value (TT)		
TG	0.62 (-3.0 , 4.2)	0.735
GG	-0.39 (-3.3 , 2.5)	0.792
IGFBP4 rs584828, reference value (AA)		
AG	-1.77 (-4.6 , 1.1)	0.223
GG	(omitted)	
GPS1 rs9916764, reference value (GG)		
GT	-0.98 (-2.9 , 0.9)	0.313
TT	-0.48 (-1.8 , 0.9)	0.483
VDR rs1540339 * NOTCH4 rs415929, reference value (AA/GG)		
AG/GA	-2.86 (-5.4 , -0.4)	0.025
VDR rs3890733 * IGFBP4 rs584828, reference value (TT/AA)		
CC/AG	2.96 (0.3 , 5.6)	0.027
VDR rs7975232 * GPS1 rs9916764, reference value (CC/GG)		
AA/GT	1.96 (0.2 , 3.7)	0.027

CYP24A1 rs4809959* HLA-DQA1 rs9272219, reference value (TT/GG)		
GG/TT	2.21 (0.1 , 4.3)	0.036
CYP24A1 rs2248359* IGFBP4 rs584438, reference value (TT/TT)		
TC/TG	-3.34 (-5.5 , -1.2)	0.002
TC/GG	-2.56 (-4.9 , -0.2)	0.030
CC/TG	-3.00 (-5.1 , -0.9)	0.005
CC/GG	-3.15 (-5.3 , -1.0)	0.005
Follow ups	0.01 (-0.2 , 0.2)	0.920
Constant	2.33 (-1.0 , 5.7)	0.175
Random Effects		Standard Error
Participant	1.05 (0.9 , 1.3)	0.092
Residual	0.67 (0.6 , 0.8)	0.052

SNP (Single Nucleotide Polymorphism)
[variable name] *[variable name] represents interaction term

Table 8.6. SNPs allelic variations and interactions that predict differing Interleukin-6 concentrations

Variable	Interleukin-6 Coefficient (95% Confidence Interval)	p-value
Fixed Effects		
VDR rs1544410, reference value (AA)		
AG	3.51 (0.9 , 6.2)	0.009
GG	4.55 (1.7 , 7.4)	0.002
VDR rs4760648, reference value (TT)		
TC	-4.31 (-6.7 , -1.9)	< 0.001
CC	-3.64 (-6.3 , -1.0)	0.007
VDR rs2238136, reference value (AA)		
AG	-2.19 (-3.8 , -0.6)	0.007
GG	-0.89 (-2.4 , 0.7)	0.260
GC rs7041/rs4588, reference value (TT)		
TG	3.37 (1.2 , 5.5)	0.002
GG	4.52 (2.1 , 7.0)	< 0.001
IGFBP4 rs584438, reference value (TT)		
TG	-1.40 (-3.5 , 0.7)	0.190
GG	-2.15 (-4.2 , -0.1)	0.036
GPS1 rs9916764, reference value (GG)		
GT	4.42 (1.7 , 7.1)	0.001
TT	3.48 (0.7 , 6.2)	0.014
VDR rs1544410 * GPS1 rs9916764, reference value (AA/GT)		
AG/GT	-5.17 (-8.0 , -2.4)	< 0.001
AG/TT	-2.85 (-5.6 , -0.1)	0.045
GG/GT	-5.91 (-8.9 , -2.9)	< 0.001

GG/TT	-3.69 (-6.7 , -0.7)	0.017
VDR rs4760648* GPS1 rs9916764, reference value (TT/GG)		
TC/GT	4.90 (2.4 , 7.4)	< 0.001
TC/TT	3.81 (1.3 , 6.3)	0.003
CC/GT	3.34 (0.5 , 6.1)	0.019
CC/TT	3.03 (0.2 , 5.8)	0.035
VDR rs2238136* IGFBP4 rs584438, reference value (AA/TT)		
AG/GG	3.16 (0.9 , 5.4)	0.006
GG/GG	2.15 (0.0 , 4.3)	0.052
GC rs7041/rs4588* GPS1 rs9916764, reference value (TT/GG)		
TG/GT	-3.42 (-5.7 , -1.1)	0.003
TG/TT	-3.92 (-6.3 , -1.6)	0.001
GG/GT	-5.14 (-7.7 , -2.6)	< 0.001
GG/TT	-5.19 (-7.8 , -2.6)	< 0.001
Follow ups	-0.29 (-0.6 , 0.0)	0.045
Constant	1.19 (-1.6 , 4.0)	0.411
Random Effects		Standard Error
Participant	0.84 (0.6 , 1.1)	0.115
Residual	1.10 (1.0 , 1.3)	0.074

SNP (Single Nucleotide Polymorphism)
[variable name] *[variable name] represents interaction term

Table 8.7. SNPs allelic variations and interactions that predict differing Tumor Necrosis Factor-α (TNF- α) concentrations

Variable	TNF-α Coefficient (95% Confidence Interval)	p-value
Fixed Effects		
VDR rs731236, reference value (CC)		
CT	-0.90 (-1.4 , -0.4)	< 0.001
TT	-1.04 (-1.6 , -0.5)	< 0.001
VDR rs2238136, reference value (AA)		
AG	-1.04 (-1.8 , -0.3)	0.007
GG	-0.60 (-1.3 , 0.1)	0.113
CYP24A1 rs4809959, reference value (TT)		
TG	-1.59 (-2.5 , -0.7)	< 0.001
GG	-1.19 (-2.1 , -0.3)	0.013
IGFBP4 rs584438, reference value (TT)		
TG	-0.65 (-2.3 , 1.0)	0.454
GG	-1.63 (-2.6 , -0.7)	0.001
IGFBP4 rs584828, reference value (AA)		
AG	-1.10 (-2.7 , 0.5)	0.191
CYP24A1 rs4809959* IGFBP4 rs584828, reference value (TT/AA)		
TG/AG	1.62 (0.5 , 2.7)	0.003

GG/GG	2.23 (0.9 , 3.6)	0.001
Follow ups	0.11 (-0.1 , 0.3)	0.200
Constant	5.59 (4.5 , 6.7)	< 0.001
Random Effects		Standard Error
Participant	0.87 (0.7 , 1.0)	0.070
Residual	0.67 (0.6 , 0.8)	0.042

SNP (Single Nucleotide Polymorphism)
[variable name] *[variable name] represents interaction term

Table 8.8. SNPs allelic variations and interactions that predict differing Pro-Inflammatory Index Score†

Variable	Pro-Inflammatory Index Coefficient (95% Confidence Interval)	p-value
Fixed Effects		
VDR rs1544410, reference value (AA)		
AG	1.11 (-1.9 , 4.1)	0.465
GG	2.31 (-2.4 , 7.0)	0.336
VDR rs10783219, reference value (TT)		
TA	4.21 (0.9 , 7.5)	0.014
AA	2.35 (-1.5 , 6.2)	0.227
CYP24A1 rs4809959, reference value (TT)		
TG	-1.18 (-2.0 , -0.4)	0.005
GG	-0.81 (-1.8 , 0.2)	0.099
IGFBP4 rs584828, reference value (AA)		
AG	-1.30 (-2.2 , -0.4)	0.003
GG	-0.29 (-1.2 , 0.6)	0.543
GPS1 rs9916764, reference value (GG)		
GT	5.24 (1.0 , 9.5)	0.015
TT	2.67 (-1.5 , 6.9)	0.214
VDR rs1544410 * GPS1 rs9916764, reference value (AA/GG)		
AG/GT	-4.06 (-7.3 , -0.8)	0.015
AG/TT	-0.99 (-4.2 , 2.2)	0.546
GG/GT	-4.60 (-9.5 , 0.3)	0.067
GG/TT	-1.58 (-6.4 , 3.3)	0.523
VDR rs10783219* GPS1 rs9916764, reference value (AA/GG)		
TA/GT	-4.54 (-8.2 , -0.9)	0.015
TA/TT	-4.89 (-8.4 , -1.3)	0.007
Follow ups	-0.66 (-1.1 , -0.2)	0.006
Constant	0.44 (-3.7 , 4.6)	0.833
Random Effects		Standard Error
Participant	1.18 (0.9 , 1.6)	0.187

Residual	1.36 (1.1 , 1.6)	0.132

SNP (Single Nucleotide Polymorphism)

† A method of standardization to compare and combine biochemical markers was developed by Tabung et al. (Tabung et al., 2015). The standardized values were combined to create the overall pro-inflammatory biomarker score (C-Reactive Protein + Tumor Necrosis Factor –α + Interleukin (IL)- 6). The same method of standardization was used to calculate an anti-inflammatory score to combine the concentrations of cytokines IL-4 and IL-10.

[variable name] *[variable name] represents interaction term

Anti- Inflammatory Cytokines

Table 8.9. SNPs allelic variations and interactions that predict differing Interleukin-4 concentrations

Variable	Interleukin-4 Coefficient (95% Confidence Interval)	p-value
Fixed Effects		
VDR rs1544410, reference value (AA)		
AG	-1.82 (-4.1 , 0.4)	0.115
GG	2.01 (-0.8 , 4.8)	0.164
VDR rs2238136, reference value (AA)		
AG	-2.14 (-3.7 , -0.6)	0.007
GG	-0.99 (-2.5 , 0.5)	0.194
IGFBP4 rs584828, reference value (AA)		
AG	-1.11 (-1.9 , -0.3)	0.005
GG	-0.27 (-1.1 , 0.6)	0.516
GPS1 rs9916764, reference value (GG)		
GT	0.83 (-1.3 , 2.9)	0.443
TT	-1.63 (-3.8 , 0.5)	0.131
VDR rs1544410 * GPS1 rs9916764, reference value (AA/GT)		
AG/TT	3.05 (0.4 , 5.7)	0.024
GG/GT	-3.54 (-6.7 , -0.4)	0.028
Follow ups	0.13 (-0.1 , 0.4)	0.338
Constant	5.08 (2.7 , 7.4)	< 0.001
Random Effects		Standard Error
Participant	1.42 (1.2 , 1.7)	0.125
Residual	0.83 (0.7 , 1.0)	0.069

SNP (Single Nucleotide Polymorphism)
[variable name] *[variable name] represents interaction term

Table 8.10. SNPs allelic variations and interactions that predict differing Interleukin-10 concentrations

Variable	Interleukin-10 Coefficient (95% Confidence Interval)	p-value
Fixed Effects		
VDR rs7975232, reference value (CC)		
CA	-0.56 (-2.5 , 1.4)	0.582
AA	-0.08 (-1.8 , 1.6)	0.924
NOTCH4 rs415929, reference value (GG)		
GA	-1.07 (-2.4 , 0.2)	0.111
AA	0.76 (-0.6 , 2.1)	0.263
IGFBP4 rs584828, reference value (AA)		
AG	-0.97 (-1.6 , -0.4)	0.001
GG	-0.37 (-1.0 , 0.3)	0.247
GPS1 rs9916764, reference value (GG)		
GT	-1.18 (-2.7 , 0.4)	0.136
TT	-0.64 (-1.8 , 0.5)	0.267
VDR rs7975232* GPS1 rs9916764, reference value (CC/GG)		
AA/GT	2.20 (0.8 , 3.6)	0.003
AA/TT	(omitted)	
Follow ups	-0.16 (-0.3 , 0.0)	0.057
Constant	4.09 (2.4 , 5.7)	< 0.001
Random Effects		Standard Error
Participant	1.20 (0.1 , 1.0)	0.086
Residual	0.60 (0.0 , 0.5)	0.042

SNP (Single Nucleotide Polymorphism)
[variable name] *[variable name] represents interaction term

Table 8.11. SNPs allelic variations and interactions that predict a differing Anti-Inflammatory Index Score†

Variable	Anti-Inflammatory Index Coefficient (95% Confidence Interval)	p-value
Fixed Effects		
VDR rs11568820, reference value (AA)		
AG	0.41 (-2.6 , 3.5)	0.794
GG	2.86 (-0.5 , 6.2)	0.096
VDR rs731236, reference value (CC)		
CT	-4.88 (-7.4 , -2.4)	< 0.001
TT	-6.31 (-9.8 , -2.9)	< 0.001
VDR rs7975232, reference value (CC)		
CA	3.72 (1.9 , 5.6)	< 0.001

AA	2.96 (0.8 , 5.1)	0.008
VRD rs2248098, reference value (TT)		
TC	-3.81 (-5.6 , -2.0)	< 0.001
CC	-3.03 (-5.1 , -1.0)	0.003
VDR rs2238136, reference value (AA)		
AG	-7.52 (-10.7 , -4.4)	< 0.001
GG	-3.16 (-4.7 , -1.6)	< 0.001
GC rs7041/rs4588, reference value (TT)		
TG	-0.84 (-1.6 , -0.1)	0.026
GG	-0.72 (-1.6 , 0.1)	0.095
CYP24A1 rs4809959, reference value (TT)		
TG	-0.17 (-1.7 , 1.4)	0.835
GG	-2.57 (-4.5 , -0.6)	0.010
NOTCH4 rs2071286, reference value (AA)		
AG	-1.74 (-4.3 , 0.8)	0.183
GG	-0.47 (-1.7 , 0.8)	0.458
HLA-DQA1 rs9272219, reference value (TT)		
TG	1.92 (0.4 , 3.5)	0.016
GG	1.13 (-0.1 , 2.4)	0.070
IGFBP4 rs584438, reference value (TT)		
TG	-0.46 (-2.9 , 2.0)	0.714
GG	-2.52 (-3.9 , -1.2)	< 0.001
IGFBP4 rs584828, reference value (AA)		
AG	-2.68 (-4.8 , -0.6)	0.012
GG	(omitted)	
GPS1 rs9916764, reference value (GG)		
GT	0.68 (-1.9 , 3.3)	0.608
TT	-2.53 (-6.4 , 1.3)	0.199
VDR rs11568820 * GPS1 rs9916764, reference value (AA/GG)		
AA/GT	-4.01 (-7.6 , -0.4)	0.029
VDR rs731236* GPS1 rs9916764, reference value (CC/GG)		
CT/GT	3.29 (0.5 , 6.1)	0.022
CT/TT	5.33 (2.7 , 8.0)	< 0.001
TT/GT	4.38 (0.7 , 8.1)	0.019
TT/TT	6.05 (2.7 , 9.5)	< 0.001
VDR rs2238136 * NOTCH4 rs2071286, reference value (AA/AA)		
AG/AG	4.73 (1.1 , 8.3)	0.010
AG/GG	3.70 (1.0 , 6.4)	0.007
CYP24A1 rs4809959* HLA-DQA1 rs9272219, reference value (TT/TT)		
TG/TG	-3.30 (-5.3 , -1.3)	0.001
TG/GG	-2.19 (-3.9 , -0.5)	0.011
CYP24A1 rs4809959* IGFBP4 rs584438, reference value (TT/TT)		
TG/TG	2.85 (1.3 , 4.4)	< 0.001
TG/GG	1.91 (0.2 , 3.6)	0.028
GG/TG	3.15 (1.2 , 5.1)	0.001
GG/GG	4.94 (3.0 , 6.8)	< 0.001

Follow ups	0.11 (-0.1 , 0.3)	0.257
Constant	7.55 (4.1 , 11.0)	< 0.001
Random Effects		Standard Error
Participant	0.98 (0.8 , 1.2)	0.089
Residual	0.60 (0.5 , 0.7)	0.051

SNP (Single Nucleotide Polymorphism)

† A method of standardization to compare and combine biochemical markers was developed by Tabung et al. (Tabung et al., 2015). The standardized values were combined to create the overall pro-inflammatory biomarker score (C-Reactive Protein + Tumor Necrosis Factor –α + Interleukin (IL)- 6). The same method of standardization was used to calculate an anti-inflammatory score to combine the concentrations of cytokines IL-4 and IL-10.

[variable name] *[variable name] represents interaction term

Remission

Table 8.12. SNPs allelic variations and interactions that predict differing Remission Categories

Variable	Remission Category Coefficient (95% Confidence Interval)	p-value
Fixed Effects		
VDR rs10783219, reference value (TT)		
TA	0.22 (0.0 , 0.4)	0.029
AA	0.17 (0.0 , 0.4)	0.096
CYP24A1 rs2248359, reference value (TT)		
TC	0.26 (-0.3 , 0.8)	0.329
CC	0.62 (0.1 , 1.2)	0.021
NOTCH4 rs415929, reference GG		
GA	0.03 (-0.6 , 0.6)	0.936
AA	0.29 (-0.3 , 0.8)	0.300
CYP24A1 rs2248359 * NOTCH4 rs415929, reference value (TT/GG)		
CC/AA	-0.75 (-1.4 , -0.1)	0.021
Follow ups	0.22 (0.2 , 0.3)	< 0.001
Constant	-0.56 (-1.0 , -0.1)	0.016
Random Effects		Standard Error
Participant	0.30 (0.2 , 0.4)	0.031
Residual	0.51 (0.5 , 0.5)	0.017

SNP (Single Nucleotide Polymorphism)

[variable name] *[variable name] represents interaction term

Full Models, Environmental, Biochemical and Genetic variables as well as Interactions

Baseline and First follow up (6 Months)

Biochemical measures of inflammation

Table 8.13. Environmental, biochemical and genetic factors and their interactions that predict a differing C-Reactive Protein concentrations

Variable	C-Reactive Protein Coefficient (95% Confidence interval)	p-value
25(OH)D	0.10 (-0.3 , 0.5)	0.642
Season, reference value (Winter)		
Spring	1.30 (-0.1 , 2.7)	0.063
Summer	0.82 (-0.6 , 2.2)	0.255
Fall	0.32 (-0.7 , 1.3)	0.546
Steroid Prescribed, reference value (Yes)		
No	-0.96 (-1.4 , -0.5)	< 0.001
Milk Intake, reference value (Never/sometimes)		
Everyday	0.64 (-1.3 , 2.6)	0.514
VDR rs7975232, reference value (CC)		
CA	0.27 (-0.5 , 1.1)	0.508
AA	0.15 (-0.8 , 1.1)	0.763
VDR rs1544410, reference value (AA)		
AG	-0.17 (-1.3 , 0.9)	0.754
GG	-0.93 (-2.3 , 0.4)	0.18
IGFBP4 rs584438, reference value (TT)		
TG	0.15 (-0.3 , 0.6)	0.527
GG	0.44 (-0.1 , 1.0)	0.095
VDR rs4760648, reference value (TT)		
TC	4.54 (2.7 , 6.3)	< 0.001
CC	3.66 (1.9 , 5.4)	< 0.001
VDR rs10783219, reference value (TT)		
TA	0.06 (-0.5 , 0.6)	0.829
AA	-0.66 (-1.2 , -0.1)	0.023
NOTCH4 rs415929, reference value (GG)		
GA	-1.63 (-3.0 , -0.3)	0.018
AA	-1.98 (-3.3 , -0.7)	0.003
Season* VDR rs1544410, reference value (Fall*GG)		
Spring*GA	-1.56 (-2.6 , -0.5)	0.005
Milk Intake*VDR rs1544410, reference value (Every day*AA)		
Never/sometimes*AG	3.27 (1.5 , 5.0)	< 0.001
Never/sometimes*GG	2.65 (0.9 , 4.4)	0.003
Milk Intake* VDR rs7975232, reference value (Every day* CC)		
Never/sometimes*CA	-2.26 (-3.6 , -0.9)	0.001

VDR rs4760648 * NOTCH4 rs415929, reference value (CC/AA)		
TT/GA	3.82 (1.9 , 5.7)	< 0.001
TT/AA	4.29 (2.5 , 6.1)	< 0.001
TC/GG	-3.03 (-4.6 , -1.5)	< 0.001
First follow up	-0.68 (-1.1 , -0.3)	0.001
Constant	0.18 (-2.9 , 3.2) 0.909	0.909
Random Effects		Standard Error
Participant	< 0.001 (0.0 , 0.0)	4.78E-11
Residual	0.94 (0.8 , 1.1)	0.056821

25(OH)D (25-hydroxyvitamin D), Steroid (corticosteroid)
[variable name] *[variable name] represents interaction term

Table 8.14. Environmental, biochemical and genetic factors and their interactions that predict differing Erythrocyte Sedimentation Rates

Variable	Erythrocyte Sedimentation Rate Coefficient (95% Confidence interval)	p-value
25(OH)D	1.28 (0.3 , 2.3)	0.011
Age (years)	-0.04 (-0.1 , 0.0)	0.050
Milk Intake, reference value (Never/sometimes)		
Everyday	-0.50 (-1.0 , 0.0)	0.039
Taking a supplement containing vitamin D, reference value (Never)		
Every day/Sometimes	-0.48 (-1.1 , 0.1)	0.108
Milk Intake* Taking a supplement containing vitamin D		
Milk intake every day* supplement everyday/sometimes	0.76 (0.1 , 1.4)	0.029
Season, reference value (Winter)		
Spring	-0.25 (-0.7 , 0.2)	0.304
Summer	-0.58 (-1.1 , -0.1)	0.024
Fall	0.46 (-0.1 , 1.0)	0.104
VDR rs4760648, reference value (TT)		
TC	-1.24 (-2.0 , -0.5)	0.002
CC	-1.19 (-2.1 , -0.3)	0.008
CYP24A1 rs4809959, reference value (CC)		
TT	0.07 (-0.6 , 0.8)	0.845
C6orf10 rs6907322, reference value (AA)		
AG	-1.39 (-2.3 , -0.5)	0.002
GG	-1.58 (-2.5 , -0.7)	< 0.001
HLA-DQA1 rs9272219, reference value (TT)		
TG	-0.49 (-0.9 , -0.1)	0.021
GG	-0.38 (-0.8 , 0.0)	0.075
IGFBP4 rs584438, reference value (TT)		
TG	-0.66 (-1.4 , 0.1)	0.082
GG	-1.19 (-2.0 , -0.4)	0.003

GPS1 rs9916764, reference value (GG)		
GT	6.96 (2.2 , 11.7)	0.004
TT	2.54 (-2.2 , 7.2)	0.290
VDR rs4760648* IGFBP4 rs584438, reference value (TT/TT)		
TC/GG	1.66 (0.6 , 2.7)	0.001
CC/GG	1.52 (0.4 , 2.6)	0.007
25(OH)D* GPS1 rs9916764, reference value (25(OH)D*GG)		
25(OH)D* GT	-1.48 (-2.6 , -0.4)	0.008
Season*CYP24A1 rs4809959, reference value (Winter*CC)		
Spring*TT	1.15 (0.0 , 2.3)	0.043
First follow up	-0.59 (-0.8 , -0.3)	< 0.001
Constant	0.76 (-3.7 , 5.2)	0.737
Random Effects		Standard Error
Participant	0.51 (0.3 , 0.8)	0.118
Residual	0.73 (0.6 , 0.9)	0.073

25(OH)D (25-hydroxyvitamin D)
[variable name] *[variable name] represents interaction term

Pro Inflammatory Cytokines

Table 8.15. Environmental, biochemical and genetic variables and their interactions that predict differing Interleukin-1ra concentrations

Variable Fixed Effects	Interleukin-1ra Coefficient (95% Confidence Interval)	p-value
25(OH)D	3.44 (0.8 , 6.1) 0.012	0.012
Age (years)	-0.11 (-0.2 0.0)	0.006
Milk Intake, reference value (Never/sometimes)		
Everyday	0.44 (-0.2 1.1)	0.15
Season, reference value (Winter)		
Spring	-0.29 (-1.6 1.1)	0.672
Summer	-0.70 (-1.8 0.4)	0.198
Fall	0.53 (-0.9 2.0)	0.474
Steroid Prescribed, reference value (Yes)		
No	-7.60 (-13.2 -2.0)	0.007
Biologic Prescribed, reference value (Yes)		
No	-1.15 (-2.2 -0.1)	0.027
25(OH)D* Steroid Prescribed, reference value (25(OH)D*Yes)		
25(OH)D*No	1.73 (0.5 3.0)	0.008
VDR rs2238136, reference value (AA)		
AG	-0.19 (-2.8 2.4)	0.887
GG	2.12 (-0.4 4.6)	0.095
VDR rs11568820, reference value (AA)		
AG	-0.16 (-2.4 2.1)	0.889
GG	0.55 (-1.5 2.6)	0.590

VDR rs4760648, reference value (TT)		
TC	-2.81 (-5.6 0.0)	0.049
CC	-2.27 (-4.9 0.3)	0.086
VDR rs1544410, reference value (AA)		
AG	-8.39 (-14.4 -2.4)	0.006
GG	-4.70 (-11.0 1.6)	0.146
VDR rs7975232, reference value (CC)		
CA	-67.13 (-115.3 -18.9)	0.006
AA	-104.95 (-175.8 -34.1)	0.004
VDR rs10783219, reference value (TT)		
TA	0.16 (-0.8 1.1)	0.748
TT	0.49 (-0.6 1.6)	0.383
VDR rs2248098, reference value (TT)		
TC	68.21 (21.0 115.4)	0.005
CC	106.56 (36.6 176.5)	0.003
VDR rs731236, reference value (CC)		
CT	6.72 (0.9 12.5)	0.024
TT	3.27 (-2.7 9.3)	0.285
GC rs7041/rs4588, reference value (TT)		
TG	8.47 (2.4 14.6)	0.006
GG	5.13 (-1.9 12.1)	0.151
CYP24A1 rs2248359, reference value (TT)		
TC	16.78 (8.1 25.5)	< 0.001
CC	11.55 (2.1 21.0)	0.016
IGFBP4 rs584828, reference value (AA)		
AG	-1.64 (-2.5 -0.8)	< 0.001
GG	-0.40 (-1.4 0.6)	0.438
NOTCH4 rs2071286, reference value (AA)		
AG	2.15 (0.9 3.4)	0.001
GG	1.32 (0.1 2.5)	0.035
NOTCH4 rs415929, reference value (GG)		
GA	-2.14 (-4.1 -0.1)	0.037
AA	-0.82 (-2.9 1.3)	0.448
25(OH)D*VDR rs7975232, reference value (25(OH)D*CC)		
CA	15.30 (4.4 26.2)	0.005
AA	24.63 (8.1 41.2)	0.003
25(OH)D*GC rs7041/rs4588, reference value (25(OH)D*TT)		
25(OH)D*TG	-1.83 (-3.2 -0.4)	0.011
25(OH)D*VDR rs2248098, reference value (25(OH)D*TT)		
25(OH)D*TC	-15.51 (-26.3 -4.8)	< 0.001
Season* VDR rs1544410, reference value (Fall*GG)		
Spring*AG	7.41 (2.5 12.3)	0.003
Season* VDR rs731236, reference value (Fall*TT)		
Spring*CT	-7.48 (-12.4 -2.5)	0.003
Season*VDR rs2238136, reference value (Summer*GG)		
Spring*AG	4.62 (1.8 7.5)	0.002

Fall*AA	5.06 (2.2 7.9)	< 0.001
Winter*AG	2.60 (1.1 4.1)	0.001
Summer* AG	1.84 (0.4 3.3)	0.013
First follow up	-0.10 (-0.6 0.4)	0.689
Constant	-7.99 (-21.2 5.2)	0.235
Random Effects		Standard Error
Participant	0.69 (0.4 1.2)	0.195
Residual	1.12 (0.9 1.4)	0.111

25(OH)D (25-hydroxyvitamin D), Steroid (corticosteroid), Biologic (biologically-based anti-cytokine therapie)
[variable name] *[variable name] represents interaction term

Table 8.16. Environmental, biochemical and genetic factors and their interactions that predict differing Interleukin-2 concentrations

Variable	Interleukin-2 Coefficient (95% Confidence interval)	p-value
25(OH)D	3.12 (1.9 , 4.3)	< 0.001
Milk Intake, reference value (Never/sometimes)		
Every day	3.41 (2.1 , 4.7)	< 0.001
Taking a supplement containing vitamin D, reference value (Never)		
Every day/Sometimes	1.14 (0.2 , 2.1)	0.018
Season, reference value (Winter)		
Spring	-4.16 (-6.5 , -1.8)	0.001
Summer	-2.83 (-5.7 , 0.1)	0.055
Fall	-2.16 (-3.9 , -0.5)	0.013
VDR rs1540339, reference value (AA)		
AG	-0.03 (-1.4 , 1.3) 0.963	0.963
GG	0.31 (-1.0 , 1.6) 0.630	0.630
VDR rs4760648, reference value (TT)		
TC	-0.55 (-1.3 , 0.2)	0.148
CC	-0.75 (-1.7 , 0.2)	0.126
VDR rs731236, reference value (CC)		
CT	6.26 (1.1 , 11.5)	0.018
TT	5.96 (-0.2 , 12.1)	0.056
VDR rs7975232, reference value (CC)		
CA	0.17 (-1.4 , 1.7)	0.833
AA	-2.12 (-4.0 , -0.2)	0.030
VDR rs1544410, reference value (AA)		
AG	0.49 (-2.8 , 3.8)	0.775
GG	3.50 (-1.2 , 8.2)	0.142
VDR rs11568820, reference value (AA)		
AG	-1.52 (-2.8 , -0.2)	0.022
GG	-0.84 (-2.1 , 0.4)	0.192

CYP24A1 rs4809959, reference value (TT)		
TG	0.35 (-1.8 , 2.5)	0.749
GG	2.23 (0.5 , 4.0)	0.013
CYP24A1 rs2248359, reference value (TT)		
TC	3.03 (1.0 , 5.0)	0.003
CC	2.83 (1.0 , 4.6)	0.002
GC rs7041/rs4588, reference value (TT)		
TG	5.92 (1.2 , 10.6)	0.013
GG	8.38 (3.9 , 12.9)	< 0.001
NOTCH4 rs2071286, reference value (AA)		
AG	0.45 (-0.8 , 1.7)	0.470
GG	0.07 (-1.1 , 1.3)	0.913
HLA-DQA1 rs9272219, reference value (TT)		
TG	-0.41 (-1.9 , 1.1)	0.599
GG	1.04 (-0.6 , 2.7)	0.214
IGFBP4 rs584438, reference value (TT)		
TG	2.37 (-0.1 , 4.9)	0.061
GG	-2.30 (-3.5 , -1.1)	< 0.001
IGFBP4 rs584828, reference (AA)		
AG	-4.83 (-7.0 , -2.7)	< 0.001
GG	(omitted)	
GPS1 rs9916764, reference value (GG)		
GT	0.56 (-0.7 , 1.8)	0.373
TT	-0.47 (-1.8 , 0.9)	0.493
VDR rs1540339 * NOTCH4 rs415929, reference value (GG/AA)		
AG/GG	2.42 (0.6 , 4.224)	0.008
AG/GA	-2.03 (-3.3 , -0.769)	0.002
VDR rs7975232 * GPS1 rs9916764, reference value (AA/TT)		
CA/GT	-2.32 (-3.8 , -0.838)	0.002
CYP24A1 rs4809959* HLA-DQA1 rs9272219, reference value (GG/GT)		
TG/GG	2.03 (0.6 , 3.511)	0.007
25(OH)D *VDR rs731236, reference value (25(OH)D*CC)		
CT	-1.79 (-2.8 , -0.8)	0.001
TT	-2.47 (-3.5 , -1.4)	< 0.001
25(OH)D*GC rs7041/rs4588, reference value (25(OH)D*TT)		
TG	-1.53 (-2.6 , -0.5)	0.005
GG	-2.03 (-3.0 , -1.0)	< 0.001
Season* VDR rs1544410, reference value (Fall*GG)		
Winter*AG	-1.44 (-2.4 , -0.459)	0.004
Winter*GG	-1.94 (-3.4 , -0.441)	0.011
Spring*AA	2.62 (1.4 , 3.867)	< 0.001
Spring*AG	1.39 (0.5 , 2.258)	0.002
Season* VDR rs7975232, reference value (Fall*AA)		
Spring*CC	2.19 (1.1 , 3.243)	< 0.001
Spring*CA	1.15 (0.6 , 1.732)	< 0.001
Milk Intake *VDR rs11568820, reference value (Every day *AA)		

never/sometimes *AG	3.13 (1.6 , 4.665)	< 0.001
never/sometimes *GG	3.15 (1.8 , 4.510)	< 0.001
Taking a supplement containing vitamin D* CYP24A1 rs2248359, reference value (Every day/Sometimes*TC)		
Never*CC	2.04 (1.1 , 3.020)	< 0.001
First follow up	-0.15 (-0.3 , 0.0)	0.065
Constant	-12.47 (-18.6 , -6.3)	< 0.001
Random Effects		Standard Error
Participant	1.05 (0.9 , 1.2)	0.085
Residual	0.28 (0.2 , 0.3)	0.030

25(OH)D (25-hydroxyvitamin D)
[variable name] *[variable name] represents interaction term

Table 8.17. Environmental, biochemical and genetic variables and their interactions that predict differing Interleukin-6 concentrations

Variable	Interleukin-6 Coefficient (95% Confidence	p-value
25(OH)D	1.90 (0.7 , 3.1)	0.002
Age	-0.07 (-0.1 , 0.0)	0.027
Milk Intake, reference value (Never/sometimes)		
Every day	-0.78 (-3.0 , 1.4)	0.483
Season, reference value (Winter)		
Spring	-3.00 (-6.9 , 0.9)	0.128
Summer	-0.44 (-4.7 , 3.9)	0.842
Fall	-0.83 (-3.7 , 2.1)	0.574
VDR rs1544410, reference value (AA)		
AG	-0.38 (-1.7 , 1.0)	0.585
GG	1.77 (-0.4 , 3.9)	0.111
VDR rs7975232, reference value (CC)		
CA	1.72 (-0.1 , 3.5)	0.058
AA	1.15 (-0.9 , 3.2)	0.272
CYP24A1 rs2248359, reference value (TT)		
TC	0.96 (-0.3 , 2.2)	0.140
CC	0.52 (-0.8 , 1.8)	0.436
GC rs7041/rs4588, reference value (TT)		
TG	2.62 (-0.1 , 5.3)	0.056
GG	2.97 (0.4 , 5.5)	0.022
GPS1 rs9916764, reference value (GG)		
GT	3.82 (-2.4 , 10.1)	0.229
TT	4.66 (-1.6 , 10.9)	0.146
GC rs7041/rs4588 * GPS1 rs9916764, reference value (GG*TT)		
TT/GT	4.35 (1.7 , 7.042)	0.002

25(OH)D*GPS1 rs9916764, reference value (25(OH)D*GG)		
GT	-1.79 (-3.125 , -0.5)	0.009
TT	-1.76 (-3.091 , -0.4)	0.010
Season* VDR rs1544410, reference value (Fall*GG)		
Spring*AA	2.99 (1.1 , 4.919)	0.002
Season* CYP24A1 rs2248359, reference value (Fall*CC)		
Summer*TC	-2.04 (-2.9 , -1.132)	< 0.001
Milk Intake*VDR rs1544410, reference value (Every day* AA)		
Never/sometimes*AG	1.80 (0.4 , 3.215)	0.013
Milk Intake * VDR rs7975232, reference value (Every day* CC)		
never/sometimes *CA	-3.03 (-4.7 , -1.315)	0.001
First follow up	-0.22 (-0.5 , 0.0)	0.109
Constant	-5.29 (-11.3 , 0.7)	0.084
Random Effects		Standard Error
Participant	1.13 (0.943 , 1.361)	0.106
Residual	0.64 (0.528 , 0.769)	0.061

25(OH)D (25-hydroxyvitamin D)
[variable name] *[variable name] represents interaction term

Table 8.18. Environmental, biochemical and genetic variables as well as interactions that predict a differing Tumor Necrosis Factor (TNF)-α concentrations

Variable	TNF-α	
Fixed Effects	Coefficient (95% Confidence Interval)	p-value
Age	-0.06 (-0.1 , 0.0)	0.005
Milk Intake, reference value (never/sometimes)		
Every day	1.16 (0.0 , 2.3)	0.052
Season, reference value (Winter)		
Spring	1.51 (0.3 , 2.8)	0.018
Summer	-0.39 (-1.9 , 1.1)	0.613
Fall	1.55 (0.5 , 2.6)	0.004
VDR rs731236, reference value (CC)		
CT	-0.94 (-1.4 , -0.5)	< 0.001
TT	-0.90 (-1.4 , -0.4)	0.001
VDR rs2238136, reference value (AA)		
AG	-0.22 (-1.2 , 0.7)	0.657
GG	0.27 (-0.7 , 1.2)	0.573
VDR rs11568820, reference value (AA)		
AG	0.55 (-0.7 , 1.8)	0.388
GG	1.23 (0.1 , 2.4)	0.035
CYP24A1 rs4809959, reference value (TT)		
TG	-1.48 (-2.4 , -0.6)	0.001
GG	-1.16 (-2.1 , -0.2)	0.015

IGFBP4 rs584438, reference value (TT)		
TG	-0.84 (-2.4 , 0.8)	0.307
GG	-1.58 (-2.5 , -0.6)	0.001
IGFBP4 rs584828, reference value (AA)		
AG	-1.03 (-2.6 , 0.5)	0.186
CYP24A1 rs4809959* IGFBP4 rs584828, reference value (TT/AA)		
TG/AG	1.68 (0.6 , 2.736)	0.002
GG/GG	2.34 (1.0 , 3.633)	< 0.001
Season*VDR rs2238136, reference value (Winter*AA)		
Fall*AG	-1.64 (-2.8 , -0.440)	0.007
First follow up	0.15 (0.0 , 0.3)	0.112
Constant	4.06 (2.4 , 5.7)	< 0.001
Random Effects		Standard Error
Participant	0.80 (0.7 , 1.0)	0.072
Residual	0.57 (0.5 , 0.7)	0.045

[variable name] *[variable name] represents interaction term

Table 8.19. Environmental, biochemical and genetic variables as well as interactions that predict differing Pro-Inflammatory Index Scores†

Variable	Pro-Inflammatory Index Coefficient (95% Confidence	p-value
25(OH)D	4.54 (2.8 , 6.3)	< 0.001
Milk Intake, reference value (Never/sometimes)		
Every day	-2.00 (-3.6 , -0.4)	0.015
Taking a supplement containing vitamin D, reference value (Never)		
Every day/Sometimes	-1.92 (-3.8 , 0.0)	0.046
Season, reference value (Winter)		
Spring	6.31 (2.7 , 9.9)	0.001
Summer	7.75 (4.0 , 11.5)	< 0.001
Fall	4.00 (0.9 , 7.1)	0.012
VDR rs1544410, reference value (AA)		
AG	-3.18 (-4.7 , -1.6)	< 0.001
GG	-2.29 (-4.1 , -0.4)	0.015
VDR rs797523,2 reference value (CC)		
CA	0.71 (-1.0 , 2.4)	0.422
AA	-1.55 (-3.7 , 0.6)	0.155
VDR rs10783219, reference value (TT)		
TA	-1.20 (-2.4 , 0.0)	0.052
AA	-1.41 (-2.7 , -0.2)	0.027
GC rs7041/rs4588, reference value (TT)		
TG	0.96 (-0.5 , 2.4)	0.206
GG	0.79 (-0.8 , 2.4)	0.325

CYP24A1 rs4809959, reference value (TT)		
TG	1.19 (-0.6 , 2.9)	0.182
GG	0.93 (-0.9 , 2.8)	0.332
GPS1 rs9916764, reference value (GG)		
GT	17.83 (9.5 , 26.2)	< 0.001
TT	21.16 (12.2 , 30.1)	< 0.001
IGFBP4 rs584438, reference value (TT)		
TG	-2.02 (-3.4 , -0.6)	0.006
GG	-1.27 (-2.7 , 0.1)	0.078
25(OH)D* GPS1 rs9916764, reference value (25(OH)D*GG)		
GT	-4.20 (-6.1 , -2.3)	< 0.001
TT	-5.12 (-7.1 , -3.1)	< 0.001
Season* VDR rs7975232, reference value (Fall*AA)		
Winter*AA	4.01 (1.6 , 6.4)	0.001
Summer*CC	-3.71 (-6.3 , -1.1)	0.006
Summer*CA	-2.89 (-4.6 , -1.2)	0.001
Season* CYP24A1 rs2248359, reference value (Fall*CC)		
Spring*TC	-1.82 (-3.2 , -0.4)	0.011
Season* IGFBP4 rs584438, reference value (Fall*GG)		
Summer*TT	-3.31 (-5.2 , -1.4)	0.001
Milk Intake * VDR rs7975232, reference value (Every day *CC value		
never/sometimes *AA	-3.89 (-5.8 , -2.0)	< 0.001
Taking a supplement containing vitamin D * GC rs7041/rs4588, reference value (Every day/Sometimes*TT)		
Never*TG	-2.99 (-5.0 , -1.0)	0.004
First follow up	-0.72 (-1.3 , -0.2)	0.008
Constant	-14.58 (-22.9 , -6.3)	0.001
Random Effects		Standard Error
Participant	1.63 (1.3 , 2.0)	0.160
Residual	0.54 (0.4 , 0.8)	0.096

25(OH)D (25-hydroxyvitamin D)

† A method of standardization to compare and combine biochemical markers was developed by Tabung et al. (Tabung et al., 2015). The standardized values were combined to create the overall pro-inflammatory biomarker score (C-Reactive Protein + Tumor Necrosis Factor –α + Interleukin (IL)- 6). The same method of standardization was used to calculate an anti-inflammatory score to combine the concentrations of cytokines IL-4 and IL-10.

[variable name] *[variable name] represents interaction term

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Table 8.20. Environmental, biochemical and genetic variables as well as interactions that predict differing Interleukin-4 concentrations

Variable	Interleukin-4 Coefficient (95% Confidence	p-value
Age	-0.13 (-0.2 , -0.1)	< 0.001
Sex, reference value (Male)		
Female	0.90 (0.2 , 1.6)	0.009
Season, reference value (Winter)		
Spring	-1.91 (-4.136 , 0.3)	0.094
Summer	-1.73 (-4.324 , 0.9)	0.190
Fall	-1.52 (-3.563 , 0.5)	0.143
Milk Intake, reference value (Never/sometimes)		
Every day	-1.75 (-3.015 , -0.5)	0.007
VDR rs7975232, reference value (CC)		
CA	0.06 (-1.191 , 1.3)	0.928
AA	0.12 (-1.520 , 1.8)	0.890
VDR rs1544410, reference value (AA)		
AG	3.99 (0.8 , 7.1)	0.013
GG	3.51 (0.5 , 6.5)	0.021
VDR rs2238136, reference value (AA)		
AG	-1.67 (-3.1 , -0.3)	0.017
GG	-0.64 (-1.9 , 0.7)	0.332
GC rs7041/rs4588, reference value (TT)		
TG	-0.11 (-1.485 , 1.3)	0.880
GG	-0.49 (-1.9 , 0.9)	0.501
IGFBP4 rs584828, reference value (AA)		
AG	-0.83 (-1.5 , -0.1)	0.020
GG	-0.16 (-0.9 , 0.6)	0.669
GPS1 rs9916764, reference value (GG)		
GT	-2.94 (-5.2 , -0.7)	0.011
TT	-3.67 (-6.1 , -1.2)	0.003
VDR rs1544410 * GPS1 rs9916764, reference value (GG*TT)		
AG/GG	-5.85 (-8.6 , -3.102)	< 0.001
Season* VDR rs1544410, reference value (Fall*GG)		
Winter*GG	-2.54 (-4.4 , -0.656)	0.008
Summer*AA	2.81 (1.0 , 4.591)	0.002
Season* GC rs7041/rs4588, reference value (Fall*GG)		
Summer*TG	-1.66 (-2.9 , -0.446)	0.007
Milk Intake * VDR rs7975232, reference value (Every day *CC)		
never/sometimes *CA	-2.18 (-3.7 , -0.671)	0.005
never/sometimes *AA	-2.65 (-4.2 , -1.119)	0.001
First follow up	0.04 (-0.3 , 0.4)	0.811
Constant	8.42 (5.4 , 11.5)	< 0.001

Random Effects		Standard Error
Participant	1.07 (0.8 , 1.3)	0.124
Residual	0.75 (0.6 , 0.9)	0.082

[variable name] *[variable name] represents interaction term

Table 8.21. Environmental, biochemical and genetic factors and their interactions that predict differing Interleukin-10 concentrations

Variable	Interleukin-10 Coefficient (95% Confidence	p-value
Taking a supplement containing vitamin D, reference value (Never)		
Every day/Sometimes	-2.65 (-4.9 , -0.4)	0.021
Season, reference value (Winter)		
Spring	-2.87 (-5.4 , -0.4)	0.025
Summer	-3.18 (-6.0 , -0.4)	0.027
Fall	-1.65 (-3.5 , 0.2)	0.074
Annual Household Income, reference value (>$51,000)		
$51,000 – 99,999	0.41 (-0.2 , 1.0)	0.198
> $100,000	0.41 (-0.2 , 1.0)	0.183
VDR rs7975232, reference value (CC)		
CA	0.28 (-0.9 , 1.5) 0.7	0.655
AA	0.62 (-0.8 , 2.1) 0.4	0.401
VDR rs11568820, reference value (AA)		
AG	2.21 (0.4 , 4.0)	0.015
GG	2.72 (1.0 , 4.5)	0.002
VDR rs731236, reference value (CC)		
CT	0.52 (-0.7 , 1.7)	0.397
TT	1.34 (-0.3 , 3.0)	0.106
CYP24A1 rs2248359, reference value (TT)		
TC	0.95 (0.2 , 1.7)	0.018
CC	0.96 (0.2 , 1.8)	0.018
IGFBP4 rs584828, reference value (AA)		
AG	-1.07 (-1.7 , -0.5)	< 0.001
GG	-0.50 (-1.1 , 0.1)	0.126
Season* VDR rs731236, reference value (Fall*TT)		
Winter*CT	-1.75 (-3.1 , -0.4)	0.009
Winter*TT	-2.56 (-4.2 , -0.9)	0.002
Summer*CC	2.83 (1.1 , 4.5)	0.001
First follow up	-0.20 (-0.4 , 0.0)	0.044
Constant	2.99 (0.6 , 5.4)	0.013
Random Effects		Standard Error
Participant	1.10 (0.9 , 1.3)	0.088
Residual	0.44 (0.4 , 0.5)	0.038

[variable name] *[variable name] represents interaction term

Table 8.22. Environmental, biochemical and genetic factors and their interactions that predict differing Anti-Inflammatory Index Scores†

Variable	Anti-Inflammatory Index Coefficient (95% Confidence	p-value
Age	-0.08 (-0.1 , 0.0)	0.005
Milk Intake, reference never/sometimes		
Every day	1.16 (-0.9 , 3.2)	0.267
Season, reference value (Winter)		
Spring	2.07 (0.7 , 3.5)	0.004
Summer	2.89 (1.5 , 4.3)	< 0.001
Fall	1.94 (0.7 , 3.2)	0.002
VDR rs11568820, reference value (AA)		
AG	2.60 (-0.3 , 5.5)	0.077
GG	2.49 (-0.3 , 5.3)	0.078
VDR rs731236, reference value (CC)		
CT	-6.93 (-9.9 , -3.9)	< 0.001
TT	-7.85 (-10.7 , -5.0)	< 0.001
VDR rs7975232, reference value (CC)		
CA	5.71 (3.6 , 7.9)	< 0.001
AA	6.06 (3.0 , 9.1)	< 0.001
VRD rs2248098, reference value (TT)		
TC	-5.48 (-7.4 , -3.6)	< 0.001
CC	-6.52 (-9.2 , -3.9)	< 0.001
VDR rs2238136, reference value (AA)		
AG	-4.59 (-6.0 , -3.2)	< 0.001
GG	-3.63 (-5.0 , -2.3)	< 0.001
GC rs7041/rs4588, reference value (TT)		
TG	-0.95 (-1.6 , -0.3)	0.004
GG	-0.46 (-1.2 , 0.3)	0.218
CYP24A1 rs4809959, reference value (TT)		
TG	-5.14 (-7.3 , -3.0)	< 0.001
GG	-2.41 (-4.1 , -0.7)	0.005
NOTCH4 rs415929, reference value (GG)		
GA	0.30 (-0.8 , 1.4)	0.581
AA	0.18 (-0.9 , 1.3)	0.745
HLA-DQA1 rs9272219, reference value (TT)		
TG	-0.58 (-1.9 , 0.8)	0.398
GG	1.00 (-0.4 , 2.4)	0.148
IGFBP4 rs584828, reference value (AA)		
AG	3.35 (1.4 , 5.3)	0.001
GG	2.46 (1.3 , 3.6)	< 0.001
IGFBP4 rs584828, reference value (AA)		
AG	-2.70 (-4.5 , -0.9)	0.003
GG	(omitted)	

GPS1 rs9916764, reference value (GG)		
GT	2.33 (0.3 , 4.4)	0.028
TT	1.89 (-0.3 , 4.1)	0.090
VDR rs11568820* GPS1 rs9916764, reference value (GG/TT)		
AG/GT	1.65 (0.5 , 2.8)	0.005
VDR rs731236 * GPS1 rs9916764, reference value (TT/TT)		
CC/GT	-7.00 (-10.3 , -3.7)	< 0.001
CC/TT	-6.96 (-10.0 , -4.0)	< 0.001
CYP24A1 rs4809959* HLA-DQA1 rs9272219, reference value (GG/GG)		
TT/TG	2.68 (0.8 , 4.6)	0.006
CYP24A1 rs4809959* IGFBP4 rs584438, reference value (GG/GG)		
TT/TG	-3.46 (-5.1 , -1.8)	< 0.001
TT/GG	-4.97 (-6.7 , -3.3)	< 0.001
TG/TT	3.31 (1.9 , 4.7)	< 0.001
Season* VDR rs7975232, reference value (Fall*AA)		
Winter*AA	2.23 (0.7 , 3.7)	0.003
Milk Intake * VDR rs11568820, reference value (Every day *AA)		
never/sometimes *AG	1.55 (-0.3 , 3.4)	0.103
never/sometimes *GG	3.10 (1.2 , 5.0)	0.001
Milk Intake * VDR rs7975232, reference value (Every day *CC)		
never/sometimes *CA	-2.53 (-3.7 , -1.4)	< 0.001
never/sometimes *AA	-1.81 (-3.0 , -0.6)	0.004
First follow up	0.04 (-0.2 , 0.3)	0.768
Constant	6.37 (3.2 , 9.5)	< 0.001
Random Effects		Standard Error
Participant	0.69 (0.5 , 0.9)	0.089
Residual	0.53 (0.4 , 0.7)	0.059

† A method of standardization to compare and combine biochemical markers was developed by Tabung et al. (Tabung et al., 2015). The standardized values were combined to create the overall pro-inflammatory biomarker score (C-Reactive Protein + Tumor Necrosis Factor –α + Interleukin (IL)- 6). The same method of standardization was used to calculate an anti-inflammatory score to combine the concentrations of cytokines IL-4 and IL-10.
[variable name] *[variable name] represents interaction term

Remission

Table 8.23. Environmental, biochemical and genetic factors and their interactions that predict differing Remission Categories

Variable	Remission Category Coefficient (95% Confidence interval)	p-value
Fixed Effects		
C-Reactive Protein	-0.04 (-0.1 , 0.0)	0.094
25(OH)D	0.48 (0.0 , 0.9)	0.047
NSAID Prescribed, reference value (Yes)		
No	0.12 (0.0 , 0.2)	0.050
CYP1R1 rs17116978, reference value (CC)		
CT	0.35 (-0.1 , 0.8)	0.122
TT	0.43 (0.0 , 0.9)	0.046
NOTCH4 rs415929, reference value (GG)		
GA	-0.33 (-0.6 , -0.1)	0.016
AA	-0.25 (-0.5 , 0.0)	0.057
HLA-DQA1 rs9272219, reference value (TT)		
TG	-1.34 (-2.6 , -0.1)	0.040
GG	-1.23 (-2.5 , 0.1)	0.061
GPS1 rs9916764, reference GG		
GT	3.44 (1.5 , 5.4)	0.001
TT	2.56 (0.7 , 4.4)	0.007
25(OH)D*HLA-DQA1 rs9272219, reference value (25(OH)D*TT)		
25(OH)D*TG	0.31 (0.0 , 0.6)	0.038
25(OH)D*GPS1 rs9916764, reference value (25(OH)D*GG)		
25(OH)D*GT	-0.81 (-1.3 , -0.4)	< 0.001
25(OH)D*TT	-0.60 (-1.0 , -0.2)	0.006
First follow up	0.33 (0.2 , 0.4)	< 0.001
Constant	-2.43 (-4.5 , -0.3)	0.023
Random Effects		Standard Error
Participant	< 0.001 (0.0 , 0.0)	< 0.001
Residual	0.35 (0.3 , 0.4)	0.019

25(OH)D (25-hydroxyvitamin D)
[variable name] *[variable name] represents interaction term

Baseline through 24 months follow ups

Biochemical measures of inflammation

Table 8.24. Environmental and genetic factors and their interactions that predict differing C-Reactive Protein concentrations

Variable	C-Reactive Protein Coefficient (95% Confidence interval)	p-value
Milk Intake, reference value (never/sometimes)		
Every day	-0.39 (-0.7 , -0.1)	0.016
Steroid Prescribed, reference value (Yes)		
No	-0.68 (-1.0 , -0.3)	< 0.001
Season, reference value (Winter)		
Spring	0.43 (-0.7 , 1.6)	0.461
Summer	-0.62 (-1.8 , 0.5)	0.291
Fall	0.02 (-0.9 , 1.0)	0.969
Smoker in home, reference value (No)		
Yes	0.45 (0.1 , 0.7)	0.003
VDR rs1544410, reference value (AA)		
AG	0.45 (-0.4 , 1.3)	0.275
GG	0.54 (-0.3 , 1.3)	0.193
VDR rs4760648, reference value (TT)		
TC	1.84 (0.6 , 3.1)	0.003
CC	1.50 (0.4 , 2.6)	0.010
VDR rs10783219, reference value (TT)		
TA	-0.22 (-0.6 , 0.2)	0.292
AA	-0.63 (-1.0 , -0.2)	0.003
NOTCH4 rs415929, reference value (GG)		
GA	-0.42 (-1.4 , 0.5)	0.388
AA	-0.51 (-1.4 , 0.4)	0.274
VDR rs4760648 * NOTCH4 rs415929, reference value (CC/AA)		
TT/AA	2.16 (0.9 , 3.4)	0.001
Season* VDR rs1544410, reference value (Fall*GG)		
Spring*AG	-0.96 (-1.8 , -0.1)	0.025
Follow ups	-0.33 (-0.4 , -0.2)	< 0.001
Constant	2.02 (0.8 , 3.2)	0.001
Random Effects		Standard Error
Participant	< 0.001 (0.0 , 0.0)	< 0.001
Residual	1.06 (1.0 , 1.2)	0.048

[variable name] *[variable name] represents interaction term

Table 8.25. Environmental and genetic factors and their interactions that predict differing Erythrocyte Sedimentation Rates

Variable	Erythrocyte Sedimentation Rate Coefficient (95% Confidence interval)	p-value
Age (years)	-0.03 (-0.1 , 0.0)	0.043
Milk Intake, reference value (Never/sometimes)		
Every day	-0.34 (-0.7 , 0.0)	0.062
Taking a supplement containing vitamin D, reference Never		
Every day/Sometimes	0.37 (0.1 , 0.6)	0.006
Season, reference value (Winter)		
Spring	-0.44 (-1.4 , 0.5)	0.361
Summer	-0.51 (-1.5 , 0.5)	0.301
Fall	0.19 (-0.7 , 1.0)	0.666
Milk Intake* Taking a supplement containing vitamin D		
Never/sometimes drink milk*never take a supplement	-0.66 (-1.2 , -0.2)	0.010
VDR rs4760648, reference value (TT)		
TC	-1.14 (-2.2 , -0.1)	0.034
CC	-1.31 (-2.2 , -0.4)	0.003
CYP24A1 rs2248359, reference value (TT)		
TG	0.41 (-0.7 , 1.5)	0.481
GG	0.53 (-0.5 , 1.5)	0.301
C6orf10 rs6907322, reference value (AA)		
AG	-0.69 (-1.4 , 0.0)	0.067
GG	-0.82 (-1.6 , -0.1)	0.030
HLA-DQA1 rs9272219, reference value (TT)		
TG	-0.51 (-0.9 , -0.1)	0.006
GG	-0.29 (-0.7 , 0.1)	0.132
IGFBP4 rs584438, reference value (TT)		
TG	0.37 (-0.5 , 1.2)	0.379
GG	-0.06 (-0.9 , 0.8)	0.890
GPS1 rs9916764, reference value (GG)		
GT	0.65 (0.2 , 1.1)	0.005
TT	0.45 (0.0 , 0.9)	0.052
VDR rs4760648* IGFBP4 rs584438, reference value (TT/TT)		
TT/TG	-1.51 (-2.6 , -0.4)	0.005
TT/GG	-1.31 (-2.4 , -0.2)	0.018
CYP24A1 rs4809959* IGFBP4 rs584438, reference value (CC/GG)		
TT/GG	1.41 (0.3 , 2.5)	0.012
Season*CYP24A1 rs4809959, reference value (Winter *TC)		
Summer*TT	0.74 (0.2 , 1.3)	0.007
Fall*TT	0.81 (0.2 , 1.4)	0.008
Follow ups	-0.25 (-0.3 , -0.2)	< 0.001
Constant	4.72 (3.2 , 6.2)	< 0.001
Random Effects		Standard Error

Participant	0.52 (0.4 , 0.7)	0.078
Residual	0.75 (0.7 , 0.8)	0.042

[variable name] *[variable name] represents interaction term

Remission

Table 8.26. Environmental and genetic factors and their interactions that predict differing Remission Categories

Variable	Remission Category Coefficient (95% Confidence interval)	p-value
Fixed Effects		
C-Reactive Protein	-0.08 (-0.1 , 0.0)	0.001
Taking a supplement containing vitamin D, reference value (Never)		
Every day/Sometimes	0.13 (0.0 , 0.3)	0.044
NSAID Prescribed, reference value (Yes)		
No	0.32 (0.2 , 0.4)	< 0.001
Biologic Prescribed, reference value (Yes)		
No	0.28 (0.1 , 0.5)	0.003
CYP1R1 rs17116978, reference value (CC)		
CT	0.46 (-0.1 , 1.0)	0.103
TT	0.44 (-0.1 , 1.0)	0.090
NOTCH4 rs415929, reference value (GG)		
GA	-0.41 (-0.7 , -0.1)	0.010
AA	-0.30 (-0.6 , 0.0)	0.073
Follow ups	0.18 (0.1 , 0.2)	< 0.001
Constant	-0.50 (-1.0 , 0.0)	0.047
Random Effects		Standard Error
Participant	0.19 (0.1 , 0.3)	0.047
Residual	0.44 (0.4 , 0.5)	0.025

NSAID (non-steroidal anti-inflammatory drug), Biologic (biologically-based anti-cytokine therapie)

[variable name] *[variable name] represents interaction term

CHAPTER 9

GENERAL DISCUSSION

There is evidence that vitamin D may play a role in both the development and treatment of JIA (Berkun et al., 2015; Ellis et al., 2010; Pelajo et al., 2012; Stagi et al., 2014). The role of vitamin D has been suggested to have impact at both the environmental level (factors associated with 25(OH)D concentrations) (Ellis et al., 2010) and the genetic level (Ellis et al., 2015; Falcini et al., 2013). Therefore, understanding how vitamin D affects disease activity in children with JIA is important to study further. The Biologically Based Outcomes Predictors (BBOP) in JIA study provided mixed longitudinal data allowing for the evaluation of the long-term outcomes in children with JIA with the hope of developing better treatment care plans and evaluation method. My thesis explored the impact of vitamin D and related factors on disease activity measures in BBOP participants

In my thesis, I compared healthy children from the Canadian Health Measured Survey (CHMS) to children from the BBOP Study to address the objective of evaluating vitamin D status and comparing between healthy children/adolescents and patients with JIA (Study 1). Then, I explored how environmental factors associated with vitamin D impact biochemical and objective measured of disease activity to determine the association of factors that could be modified to impact vitamin D status and disease activity in JIA patients. (Study 2). In the next step, I investigated if genetic factors related to vitamin D are associated with disease activity outcomes. The goal was to identify potential associations of vitamin D pathway gene polymorphisms and JIA as well as explore their impact on disease activity (Study 3).

9.1. Scientific Contributions of Study 1

This is the first study to compare multiple factors associated with vitamin D status in newly diagnosed JIA patients (within 6 months of diagnosis) and healthy children at a national level. To date and to our knowledge, no study has investigated the relationship between vitamin D status and JIA from the time of the first presentation. This research allowed us to compare at a population level whether there are significant differences in factors associated with vitamin D and growth outcomes in healthy children and those with JIA. We further compared the vitamin D status and growth outcomes for children and adolescents with JIA to a healthy age-matched

population. Advancement in medications have been made since these outcomes were last documented and compared. In addition, factors associated with vitamin D and growth outcomes of children with newly diagnosed JIA have not been compared to a nationally representative population. Findings from this research will provide valuable information on vitamin D and growth status of children with JIA.

Vitamin D status appears to meet current cut-offs in the majority of children with JIA at disease onset. However, biochemical measures of inflammation are higher. Mean 25(OH)D status was significantly higher for JIA patients (79 ± 3.1 nmol/L vs. 68 ± 1.8 nmol/L). This is in contrast to other studies in which participants had lower 25(OH)D concentrations than healthy children (Dağdeviren-çakır et al., 2016; de Sousa Studart et al., 2015; Nisar et al., 2013; Szymanska-Kaluza J., Biernacka-Zielinska M., Stanczyk J., 2013). JIA patients use a supplement containing vitamin D more often (50% vs. 7% $p < 0.05$). Prevalence of 25(OH)D deficiency (25(OH)D < 30 nmol/L) was 6% for both groups. Children with JIA were more likely to be born in the fall and winter and healthy children were more likely to be born in the spring and summer. Similar findings were reported by Berkun et al., they found that children with JIA were more likely to be born between November to March compared to birth months for the general population peaking in the summer (Berkun et al., 2015). Children with JIA (at disease onset) differ from healthy children in terms of growth characteristics. The proportion in each BMI class did not vary by group. Weight and height z-scores were higher in healthy children. Daily milk intake was associated with a significantly higher 25(OH)D status for both groups.

In summary, children with JIA are shorter and weigh less than healthy children weigh. Vitamin D, which was adequate in the JIA population, is not likely to be a factor accounting for impeded growth. Season of birth may reflect vitamin D status in utero or during the first few months of life. Further research into what role vitamin D status plays in the development and progression of JIA is required.

9.2. Scientific Contributions of Study 2

Analyses in this section addressed whether vitamin D and its associated factors are negatively associated with inflammatory markers and disease activity among JIA cases.

We found that serum 25(OH)D concentrations and factors that influence 25(OH)D status such as season and daily milk consumption are associated with measures of disease activity.

Consistent with our finding, an association between 25(OH)D concentrations and CRP concentrations in JIA patients was found by Stagi et al (Stagi et al., 2014). Dairy products have also been shown to suppress inflammation in healthy people; however, this is the first time that they have been explored in the context of JIA (Bordoni et al., 2017; Stancliffe, Thorpe & Zemel, 2011). 25(OH)D predicted remission suggesting that it may play a role in a reduction in inflammatory-related factors of JIA outcomes. Milk, with mandatory fortification in Canada, is the most common source of vitamin D for this age group (Whiting et al., 2011) and suggests an actionable way to improve vitamin D concentrations in children with JIA. No researcher had explored milk intake and remission prior to our study and further research is required to develop specific recommendations around milk intake and this population. Being able to suggest specific targets for vitamin D status as a potential adjunct therapy or specific actions to reduce inflammation in the treatment of JIA will enhance the quality of life of patients and their families. Further studies exploring 25(OH)D and factors associated with vitamin D status will help in the development of actionable/ feasible recommendations for health care professionals and families.

Very recently (December 13, 2018), the results of a prospective multicenter cohort study of newly diagnosed (<12 months since diagnosis) children with JIA from Germany was published (Senger et al. 2018). Three hundred and sixty patients had 25(OH)D concentrations measured twice in the first 2 years of follow up. Disease activity measured using the clinical Juvenile Arthritis Disease Activity Score (c-JADAS)-10 which is a composite score that includes active joint count in its total. Children from the cohort were also compared to age, sex and month of blood collection matched children from the general population. They found that 44% of patients with JIA were vitamin D deficient (25(OH)D <20ng.mL) at the first measurement, and a quarter were deficient at both measurements (second measure not published). The research group speculated that vitamin D status might be changed by the established therapy or treatment. Therefore, they only used the first 25(OH)D measure in exploring the association between 25(OH)D and disease activity. Disease activity was found to be negatively correlated with 25(OH)D concentrations in their analysis.

The study by Senger et al. is the publication that most closely resembles my thesis study 2. However, there are a number of methodological differences that are important to note. As discussed in my thesis, I used a number of biochemical, objective and self-reported assessment

tools as measures of disease activity because there is no single measure of disease activity that has been deemed the gold standard, especially in the analysis of vitamin D and disease activity. Additionally, the measure that the authors use the c-JADAS-10 is a measure that includes joint count. Given that the number of joints with active arthritis is a part of the definition of the subtypes as well as a measurement of disease activity, the cut-offs are not appropriate for comparison between subtypes. I discuss in my thesis why I chose not to use this as a measure of disease activity when there are more than one JIA subtype included in a study. The research group included migration pattern that they, defined as mother or father not of German origins to represent skin pigmentation, and cultural habits that may influence UVB vitamin D production. The authors note that a limitation of their study was that they did not explore vitamin D intake from food or supplements. My thesis explored this association and found that both food and supplement intake of vitamin D are associated with disease activity. While this study chose to explore the role of vitamin D in disease activity using different methodological choices than what was explored in the BBOP study it does provide evidence towards the value of our study exploring the role of the vitamin D pathway in JIA disease development and disease progression.

9.4. Scientific Contributions of Study 3

Analyses of this section addressed whether there are specific polymorphisms of vitamin D pathway genes associated with JIA. GWAS-identified genes were NOTCH4, c6orf9, HLA-DQA1, LEP, IGFBP4, and GPS1. When genes were not included in the multi-level mixed-effects modeling, there was a negative association of 25(OH)D to disease activity. Genes, when included, modified the association between 25(OH)D and indicators of disease activity. The only other exploration of vitamin D genes and their impact on JIA reported by was Ellis et al. (Ellis et al., 2015). Epistasis of vitamin D pathway genes and a gene confirmed in the heritability of JIA (PTPN2 rs2542151) was shown to be associated with disease development (Ellis et al., 2015). Our study is the first to explore the concept of epistasis in relation to disease outcomes.

Genes identified through GWAS (HLA-DQA1, and GPS1) and VRD polymorphisms (rs7975232 and rs731236) modified the association between 25(OH)D and indicators of disease activity; only certain genotypes maintained the negative association of 25(OH)D and disease activity markers. Our study is the first to explore gene-environment and gene-biochemical interactions in children with JIA. Young et al. explored the association of vitamin D associated

factors and the transition to Systematic Lupus Erythematosus (SLE) (Young et al., 2016). A combined role of vitamin D status and CYP24A1 was associated with 25(OH)D and the transition to SLE was modified by the genetic polymorphism of the CYP24A1 gene. Disease activity measures were also modified by the interaction of milk consumption and VDR polymorphisms (rs1544410, rs7975232 and rs11568820) where drinking milk in children with certain SNPs of these genes predicted a reduction in indicators of disease activity as measured by CRP, ESR, and IL-6.

Our findings indicate a negative association of 25(OH)D and inflammation that influenced by vitamin D-related genetic polymorphisms. Milk intake, as a source of dietary vitamin D, is associated with suppression of inflammation in children with JIA.

This is the first time the potential influence of gene and environment in relation to vitamin D were analyzed together in association with JIA disease activity. Environmental, biochemical, and genetic factors including interactions of key genetic polymorphism of the vitamin D pathway predict disease activity in children with JIA.

9.5. Strengths

My study has several strengths. This prospective inception cohort study is the first to investigate the relationship between vitamin D status and JIA from the initial diagnosis and to measure disease outcomes over a 2-year period. It is the first time that environmental, behavioural and genetic factors associated with vitamin D have been explored in the same JIA patient. Also, the first time that interactions of vitamin D associated factors and genetic polymorphisms that may differ in children with JIA identified through GWAS have been considered.

To date and to our knowledge, no study has investigated the relationship between vitamin D status and JIA over time in newly diagnosed individuals. The ultimate goal of this research is to document the vitamin D status in a JIA population and to gain insight into the optimal doses to improve the diseases status. We explored the relationship of vitamin D status on markers of inflammation and measures of disease activity in children and adolescents with JIA. We explored a possible genetic association with vitamin D pathway gene polymorphisms and JIA. We further compared vitamin D status and growth outcomes for children and adolescents with JIA to a

healthy age-matched population of children and adolescents. Findings from this research provide valuable information on the role of vitamin D status in relation to disease activity and outcomes.

9.6. Limitations

The main limitation of my study was the sample size through the number of children who enrolled in the study recruitment period, which did not allow us to explore JIA type in some form of sub-group analysis. I explored JIA type using both the seven JIA categories defined by ILAR as well as by using inflamed joint count > four and ≤ four. However, sample was too small for predictions even through bivariate analysis. An additional limitation was that biochemical measurements including 25(OH)D measurements only took place at two time points. This meant that milk intake was used as a surrogate measure of dietary 25(OH)D. As mentioned in previous chapters, fortified milk is the primary dietary source of vitamin D for Canadians (Whiting et al., 2011). However, milk is not the only food that contains vitamin D; therefore, it is not an exact duplication of total vitamin D intake. Children were also within 6 months of diagnosis and not at the date of diagnosis. They (and their parents) may have modified their behaviour already due to their disease or disease diagnosis. Lastly, season was used as a proxy for sun exposure and UVB vitamin D synthesis. We are not aware if some participants traveled during the winter months or used sunscreen in the summer months. This cohort study also did not have a control group for comparison to healthy children. However, we overcame this limitation by the following actions: We compared baseline data for age- and sex-matched children older than three years who are free of chronic disease from the Canadian Health Measures Survey and also used The National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) healthy control genetic dataset and the *Better Outcomes for Children: GWAS from Cincinnati Children's Hospital Medical Center (CCHMC) - eMERGE Phase II*" dataset as genetic controls.

Serum 25(OH)D and markers of inflammation were only measured at two time points in BBOP, therefore we do not have biochemical data to correspond with the outcome of the disease beyond this time point. We did not directly measure the skin pigmentation to control for the amount of vitamin D endogenous synthesis. Therefore, we only used ethnicity as a descriptor term since skin pigmentation will influence baseline 25OHD values and endogenous synthesis.

Although this study was able to identify that patients with JIA differ from the healthy population in vitamin D status and vitamin D status relates to disease activity this study was not

designed to evaluate the optimal dose of vitamin D or 25(OH)D concentration for children with JIA.

9.7. Future Research

This study identified some key area for future research around factors associated with vitamin D and JIA. First questions around vitamin D and disease development can be further explored. We identified that children with JIA are more likely to be born in the fall and winter than healthy children. Future research into the intra-uterine environment and maternal behaviour surrounding vitamin D practices would help to clarify this. In addition, the role and prevalence of vitamin D gene polymorphisms and the interactions of genes associated with the vitamin D pathway will help in understanding the causes of JIA. Our study also identified key areas associated with vitamin D in the treatment of JIA as well as actions and behaviours of patients at diagnosis associated with vitamin D that may influence outcomes. Our models suggest an association between 25(OH)D and milk intake and a suppression of inflammation. This might be modified in patients with certain polymorphisms of vitamin D pathway genes and genes that have polymorphisms that are more frequent in children with JIA that interact with the vitamin D pathway. Exploring if there is a dose-dependent relationship between vitamin D intake and inflammation and if this is modified by certain genetic polymorphisms will require further research. Additionally, research into the impact of milk intake on inflammation in JIA would be beneficial. Children with JIA reported drinking milk everyday less than healthy children. If milk (the primary dietary source of vitamin D in children) were effective in suppression of inflammation, daily milk consumption to reduce inflammation would be a simple recommendation for clinicians to make.

9.8. Conclusion

25(OH)D was optimal in the JIA population and JIA patients used vitamin D supplements more often than healthy children. Children with JIA were more likely to be born in the fall and winter, while healthy children were more likely to be born in the spring and summer. Being born in seasons with reduced endogenous vitamin D could implicate low vitamin D during gestation and early life as a factor influencing JIA pathogenesis. Children with JIA are shorter and weigh less than healthy children weigh. Vitamin D, which was adequate in the JIA

population, is not likely to be a factor accounting for impeded growth. CRP and ESR concentrations decreased significantly over the 2 years. Increased 25(OH)D or its associated factors predicted lower ESR, CRP and cytokine levels suggesting that vitamin D may suppress inflammation in children with JIA. GWAS identified the following genetic components, NOTCH4, C6orf10, HLA-DQA1, LEP, IGFBP4, and GPS1. Interactions between frequent gene polymorphisms and factors associated with vitamin D including genes in the vitamin D pathway (VDR, GC, CYP24A1, and CYP1R1) significantly predicted disease activity-related outcomes. This is the first time gene and environment influences in relation to vitamin D were analyzed together in association with JIA disease activity. Environmental, biochemical, and genetic factors including their interactions predict disease activity in children with JIA.

Investigating the genetic and environmental role in a cohort of children with JIA over years, will help to understand the multifaceted role played of vitamin D in the prevention and management of JIA. Understanding how genetic variants increase the risk of disease development will help guide vitamin D management in individual patients and thus contribute to improving disease outcomes. The evidence discovered through this thesis suggests that the achievement of adequate vitamin D status using environmental factors associated with vitamin D (diet, supplement intake and limited UVB exposure) should be included in the comprehensive medical plan for children with JIA.

9.9. Implications for Practice

This thesis demonstrates that vitamin D is an important nutrient for children with JIA. We do not have specific recommendations for the optimal dose required for children with JIA. However, applying current daily dosing recommendations for healthy children to the JIA population would seem appropriate. Clinicians should establish vitamin D status in their patients and work towards achieving or maintaining at minimum adequate 25(OH)D concentrations (50 nmol/L) through the use of food, supplements and when appropriate safe sun exposure.

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Appendix

Appendix A

Tables and Additional Results Related to Chapter 7

Baseline and 6 months

Table A.4. Biochemical measures of inflammation multivariate linear regression analysis at baseline and 6 month follow up

Variable	C-Reactive Protein		Erythrocyte Sedimentation Rate	
	Coefficient (95% Confidence Interval)	p-value	Coefficient (95% Confidence Interval)	p-value
Fixed Effects				
25(OH)D	-0.95 (-1.8 , -0.1)	0.021	0.29 (-0.1, 0.7)	0.126
Milk Intake, reference value (Never/Sometimes)				
Every day	-0.52 (-1.0 , -0.1)	0.028	-0.25 (-0.7, 0.2)	0.263
Taking a supplement containing vitamin D, reference value (Never)				
Every day/Sometimes	0.07 (-0.3 , 0.5)	0.749	0.64 (-0.09 , 1.37)	0.086
Age	-	-	0.02 (-0.02, 0.1)	0.412
Steroid Prescribed, reference value (Yes)				
No	-5.22 (-9.4 , -1.0)	0.015	-	-
25(OH)D* Steroid prescribed, reference value (25(OH)D* Yes)				
25(OH)D∗No	0.99 (0.1 , 1.9)	0.041	-	-
Age*Taking a supplement containing vitamin D, reference value (Age*Never)				
Age*Every day/ Sometimes	-	-	-0.08 (-0.1, -0.01)	0.019
Milk Intake∗ Taking a supplement containing vitamin D, reference value (Never/Sometimes drink milk*Never take a supplement)				
Never/sometimes drink milk* supplement everyday/sometimes	-	-	0.72 (0.1, 1.4)	0.033
First follow up	-0.70 (-1.1 , -0.3)	0.001	-0.59 (-0.9, -0.3)	< 0.001

Constant	7.80 (4.2 , 11.4)	< 0.001	2.28 (0.5, 4.1)	0.014
Random Effects	Estimate	Standard Error	Estimate	Standard Error
Participant	0.55 (.2 , 1.2)	0.218	0.71 (0.5, 1.0)	0.106
Residual	1.01 (.8 , 1.3)	0.124	0.70 (.6, 0.9)	0.081

25(OH)D (25-hydroxyvitamin D), Steroid (corticosteroid)
[variable name] *[variable name] represents interaction term

Table A.5. Pro-Inflammatory cytokines multivariate linear regression analysis at baseline and 6 month follow up

Variable	Interleukin-1α		Interleukin-1r α		Interleukin-1β	
	Coefficient (95% Confidence Interval)	p-value	Coefficient (95% Confidence Interval)	p-value	Coefficient (95% Confidence Interval)	p-value
Fixed Effects						
25(OH)D	-	-	-1.44 (-2.5, -.36)	0.009		
Age	-	-	-0.09 (-0.2, 0.0)	0.009		
Season, reference value (Winter)						
Spring	-	-	-	-	-0.04 (-0.6, 0.5)	0.881
Summer	-	-	-	-	0.64 (0.2, 1.1)	0.008
Fall	-	-	-	-	-0.26 (-0.8, 0.3)	0.362
Taking a supplement containing vitamin D, reference value (Never)						
Every day/Sometimes	0.31 (0.03, 0.6)	0.030	-	-	-	-
Milk Intake, reference value (never/sometimes)						
Every day	-	-	0.75 (0.1, 1.4)	0.024	-	-
Biologic Prescribed, reference value (Yes)						
No	-0.88 (-1.6, -0.2)	0.012	-2.02 (-3.1, -1.0)	< 0.001	-	-
Steroid Prescribed, reference Yes						
No	-	-	-6.40 (-11.9, -0.9)	0.021	-	-
25(OH)D*Steroid prescribed, reference value (25(OH)D* Yes)						
25(OH)D* No	-	-	1.51 (0.3, 2.7)	0.017	-	-
First follow up	0.002 (-0.2, 0.2)	0.980	-0.15 (-0.6, 0.3)	0.512	0.27 (-0.05, 0.6)	0.097
Constant	3.73 (3.0, 4.5)	< 0.001	13.15 (8.1, 18.2)	< 0.001	2.64 (2.0, 3.3)	< 0.001

Random Effects		Standard Error		Standard Error		Standard Error
Participant	1.55 (1.4, 1.8)	0.102	1.22 (0.9, 1.8)	0.223	1.39 (1.2, 1.7)	0.129
Residual	0.65 (0.5,0.8)	0.055	1.56 (1.3, 1.9)	0.142	1.34 (1.2, 1.5)	0.084

	Interleukin-2		Interleukin-6		Interleukin-8	
Variable	Coefficient (95% Confidence Interval)	p-value	Coefficient (95% Confidence Interval)	p-value	Coefficient (95% Confidence Interval)	p-value
Fixed Effects						
25(OH)D	-1.31 (-2.4, -0.3)	0.016	-	-	-	-
Age	-	-	0.008 (-0.1, 0.1)	0.866	0.08 (-0.01, 0.2)	0.095
Taking a supplement containing vitamin D, reference value (Never)						
Every day/Sometimes	0.57 (0.2, 0.9)	0.002	-	-	-	-
Season, reference value (Winter)						
Spring	-	-	0.20 (-0.3, 0.7)	0.457	1.25 (0.7, 1.8)	< 0.001
Summer	-	-	0.54 (0.1, 1.0)	0.013	2.25 (1.7, 2.8)	< 0.001
Fall	-	-	0.20 (-0.3, 0.7)	0.452	0.43 (-0.2, 1.0)	0.158
Milk Intake, reference value (Never/sometimes)						
Every day	-	-	1.31 (0.2, 2.4)	0.018	1.75 (0.5, 3.0)	0.007
Steroid Prescribed, reference value (Yes)						
No	-6.07 (-11.6, -0.5)	0.032	-	-	-	-
25(OH)D* Steroid prescribed, reference value (25(OH)D* Yes)						
25(OH)D* No	1.46 (0.2, 2.7)	0.022	-	-	-	-
Age*Milk Intake, reference value (Age*Never/sometimes)						
Age*Every day	-	-	-0.12 (-0.2, 0.0)	0.018	-0.17 (-0.3, -0.1)	0.002
First follow up	0.17 (-0.1, 0.4)	0.192	-0.21 (-0.5, 0.1)	0.181	-0.38 (-0.8, 0.02)	0.062
Constant	6.92 (2.2 , 11.7)	0.004	2.31 (1.2, 3.4)	< 0.001	2.12 (0.8, 3.4)	0.001
Random Effects		Standard Error		Standard Error		Standard Error
Participant	1.64 (1.4, 1.9)	0.129	1.29 (1.1, 1.6)	0.134	1.02 (0.7,1.4)	0.169
Residual	0.65 (0.5, 0.8)	0.073	1.04 (0.9, 1.2)	0.089	1.42 (1.2, 1.6)	0.109

Variable	Interleukin-17		TNF-α		INF-ɣ	
	Coefficient (95% Confidence Interval)	p-value	Coefficient (95% Confidence Interval)	p-value	Coefficient (95% Confidence Interval)	p-value
Fixed Effects						
25(OH)D	-1.87 (-3.2, -0.6)	0.004	-	-	-1.20 (-2.1, -0.3)	0.008
Age	0.28 (-1.3, -0.2)	0.005	-0.05 (-0.08 , -0.01)	0.008	0.10 (0.02, 0.2)	0.016
Milk Intake, reference value (Never/sometimes)						
Every day	-0.15 (-0.8, 0.5)	0.619	-	-	0.19 (-0.1, 0.5)	0.170
Taking a supplement containing vitamin D, reference value (Never)						
Every day/Sometimes	0.24 (-0.2, 0.7)	0.300	-	-	-0.51 (-0.8, -0.1)	0.007
Age*25(OH)D	0.17 (0.04, 0.3)	0.009				
Milk Intake* Taking a supplement containing vitamin D, reference Never/sometimes drink milk*never take a supplement						
Never/sometimes drink milk* supplement everyday/sometimes	-1.45 (-2.3, -0.6)	0.001	-	-	-	-
First follow up	-0.09 (-0.4, 0.2)	0.549	0.12 (-.04, 0.3)	0.148	-0.13 (-0.3, 0.1)	0.203
Constant	10.73 (4.9, 16.6)	< 0.001	2.88 (2.5, 3.3)	< 0.001	8.43 (4.4, 12.5)	< 0.001
Random Effects		Standard Error		Standard Error		Standard Error
Participant	1.57 (1.3, 1.9)	0.132	1.01 (0.9, 1.2)	0.070	1.49 (1.3, 1.7)	0.099
Residual	0.70 (0.6, 0.9)	0.086	0.70 (0.6, 0.8)	0.040	0.62 (0.5, 0.7)	0.052

Variable	Pro-Inflammatory Index	
	Coefficient (95% Confidence Interval)	p-value
Fixed Effects		
25(OH)D	-1.67 (-3.0, -0.3)	0.016
Ethnicity, reference value (White)		
Non-White	0.59 (-0.4, 1.5)	0.232

Indigenous	-0.61 (-1.7, 0.5)	0.273
Milk Intake, reference value (Never/sometimes)		
Everyday	-0.50 (-1.2, 0.2)	0.155
Taking a supplement containing vitamin D, reference value (Never)		
Every day/Sometimes	-0.46 (-1.1, 0.1)	0.131
Steroid Prescribed, reference value (Yes)		
No	-8.32 (-14.9, -1.7)	0.014
25(OH)D* Steroid prescribed, reference value (25(OH)D* Yes)		
25(OH)D* No	1.98 (0.5, 3.5)	0.010
First follow up	-0.40 (-1.0, 0.2)	0.169
Constant	8.35 (2.3, 14.4)	0.007
Random Effects		Standard Error
Participant	1.01 (0.6, 1.8)	0.293
Residual	1.23 (0.9, 1.7)	0.212

25(OH)D (25-hydroxyvitamin D), Steroids (corticosteroid), Biologic (biologically-based anti-cytokine therapie)
[variable name] *[variable name] represents interaction term

Table A.6. Anti-Inflammatory cytokines multivariate linear regression analysis at baseline and 6 month follow up

Variable	Interleukin-4		Interleukin-10	
Fixed Effects	Coefficient (95% Confidence Interval)	p-value	Coefficient (95% Confidence Interval)	p-value
25(OH)D	-1.66 (-3.0, -0.4)	0.012	-	-
Age	-0.72 (-1.3, -0.2)	0.010	-	-
BMI percentile	-	-	0.01 (0.0002 , 0.01)	0.044
Season, reference value (Winter)				
Spring	-	-	0.41 (0.03, 0.8)	0.036
Summer	-	-	0.39 (0.1, 0.7)	0.005
Fall	-	-	0.37 (-0.04, 0.9)	0.076
Sex, reference value (Male)				
Female	0.94 (0.2, 1.7)	0.013	-	-
Taking a supplement containing vitamin D, reference value (Never)				
Every day/Sometimes	0.08 (-0.3, 0.4)	0.674	0.09 (-0.2, 0.4)	0.528
Ethnicity, reference value (White)				
Non-White	-	-	0.65 (0.03, 1.3)	0.040
Indigenous	-	-	0.39 (-0.3, 1.1)	0.283
Annual Household Income, reference value (>$51,000)				
$51,000 – 99,999	-	-	0.50 (-0.1, 1.1)	0.107
> $100,000	-	-	0.63 (0.04, 1.2)	0.036
Milk Intake, reference value (Never/sometimes)				
Everyday	-	-	0.27 (-0.1, 0.7)	0.171
Age*25(OH)D	0.13 (0.01 , 0.3)	0.034	-	-
Milk Intake* Taking a supplement containing vitamin D, reference value (Never/sometimes drink milk*never take a supplement)				
Never/sometimes drink milk* supplement everyday/sometimes	-	-	0.64 (0.1, 1.2)	0.019
First follow up	0.23 (-.03, 0.5)	0.078	-0.01 (-0.2, 0.2)	0.926
Constant	10.44 (4.6 , 16.3)	< 0.001	0.87 (0.1, 1.7)	0.029

Random Effects		Standard Error		Standard Error
Participant	1.68 (1.4,2.0)	0.132	1.04 (0.9, 1.2)	0.087
Residual	0.63 (0.5, 0.8)	0.067	0.43 (0.3 , 0.5)	0.049

Variable	Anti-Inflammatory Index	
	Coefficient (95% Confidence Interval)	p-value
Fixed Effects		
25(OH)D	-1.76 (-3.0, -0.6)	0.004
Age	-0.71 (-1.3, -0.2)	0.012
Season, reference value (Winter)		
Spring	-0.17 (-0.8, 0.4)	0.557
Summer	0.44 (0.0, 0.8)	0.029
Fall	-0.23 (-0.9, 0.4)	0.463
Milk Intake, reference value (Never/sometimes)		
Everyday	0.19 (-0.3, 0.7)	0.492
Taking a supplement containing vitamin D, reference value (Never)		
Every day/Sometimes	0.04 (-0.3, 0.4)	0.814
Age*25(OH)D	0.14 (0.0, 0.3)	0.033
First follow up	0.18 (-0.1, 0.4)	0.190
Constant	8.57 (3.1, 14.0)	0.002
Random Effects		Standard Error
Participant	1.75 (1.5, 2.1)	0.144
Residual	0 .51 (0.4, 0.7)	0.065

25(OH)D (25-hydroxyvitamin D)
[variable name] *[variable name] represents interaction term

Table A.7. Objective and self-reported measures of disease outcomes multivariate linear regression analysis at baseline and 6 months

	Child Health Assessment Questionnaire		Juvenile Arthritis Quality of Life Questionnaire		Pain Score	
Variable	Coefficient (95% Confidence Interval)	p-value	Coefficient (95% Confidence Interval)	p-value	Coefficient (95% Confidence Interval)	p-value
Fixed Effects						
Age	-	-	0.06 (0.03, 0.1)	0.001	0.11 (0.03, 0.2)	0.006
Milk Intake, reference value (Never/sometimes)						
Every day	-0.25 (-0.4, -0.1)	0.014	-	-	-0.04 (-1.1, 1.0)	0.937
Taking a supplement containing vitamin D, reference value (Never)						
Every day/Sometimes	0.05 (-0.1, 0.2)	0.578	-	-	-0.40 (-1.2, 0.4)	0.323
Smoker in home, reference value (No)						
Yes	0.25 (0.1, 0.4)	0.010	-	-	-	-
Annual Household Income, reference value (>$51,000)						
$51,000 – 99,999	-	-	-0.39 (-0.8 , 0.04)	0.077	-	-
> $100,000	-	-	-0.51 (-1.0, -0.1)	0.023	-	-
NSAID Prescribed, reference value (Yes)						
No	-	-	-0.36 (-0.6, -0.1)	0.005	-	-
Milk Intake* Taking a supplement containing vitamin D, reference value (Never/sometimes drink milk*never take a supplement)						
Never/sometimes drink milk* supplement everyday/sometimes	-	-			2.34 (0.7, 4.0)	0.005
First follow up	-0.43 (-0.6, -0.3)	< 0.001	-0.27 (-0.3, -0.2)	< 0.001	-1.64 (-2.3, -1.0)	< 0.001
Constant	1.38 (1.1, 1.7)	< 0.001	3.20 (2.7, 3.7)	< 0.001	4.92 (3.4, 6.4)	< 0.001
Random Effects		Standard Error		Standard Error		Standard Error
Participant	0.30 (0.2, 0.5)	0.082	0.86 (0.7, 1.0)	0.078	1.09 (0.5, 2.2)	0.386
Residual	0.51 (0.4, 0.6)	0.048	1.04 (1.0, 1.1)	0.042	2.29 (1.9, 2.7)	0.0199

Variable	Physician Global Assessment

	Coefficient (95% Confidence Interval)	p-value
Fixed Effects		
25(OH)D	0.55 (0.01, 1.1)	0.044
Age	-	-
Ethnicity, reference value (White)		
Non-White	-	-
Indigenous	-	-
Sex, reference value (Male)		
Female	-	-
Annual Household Income, reference value (>$51,000)		
$51,000 – 99,999	-0.91 (-1.6, -0.2)	0.007
> $100,000	-0.59 (-1.2, 0.0)	0.068
First follow up	-2.31 (2.8, -1.9)	< 0.001
Constant	4.27 (0.8, 6.7)	0.001
Random Effects		Standard Error
Participant	0.74 (0.4, 1.4)	0.252
Residual	1.72 (1.5, 2.0)	0.122

25(OH)D (25-hydroxyvitamin D), NSAID (non-steroidal anti-inflammatory)
[variable name] *[variable name] represents interaction term

All visits (baseline through 24 months)

Table A.8. Biochemical measures of inflammation multivariate linear regression analysis all visits

	C-reactive Protein		Erythrocyte Sedimentation Rate	
Variable	Coefficient (95% Confidence Interval)	p-value	Coefficient (95% Confidence Interval)	p-value
Fixed Effects				
Age	-	-	0.01 (-0.04, 0.05)	0.718
Smoker in home, reference value (No)				

Yes	0.38 (0.1, 0.7)	0.017	0.31 (-0.04, 0.8)	0.083
Milk Intake reference value (Never/sometimes)				
Every day	-0.44 (-0.8, -0.1)	0.007	-0.49 (-0.8, -0.1)	0.006
Steroid Prescribed, reference value (Yes)				
No	-0.48 (-0.8, -0.1)	0.007		
Taking a supplement containing vitamin D, reference value (Never)				
Every day/Sometimes	-	-	0.82 (0.1, 1.5)	0.019
Age*Taking a supplement containing vitamin D, reference value (Age*Never)				
Age*Every day/Sometimes	-	-	-0.07 (-0.1, -0.01)	0.033
Follow ups	-0.34 (-0.4, -0.2)	< 0.001	-0.66 (-0.9, -0.4)	< 0.001
Constant	2.83 (2.4, 3.3)	< 0.001	3.75 (3.1, 4.4)	< 0.001
Random Effects	Estimate	Standard Error		Standard Error
Participant	0.43 (0.3, 0.7)	0.118	0.69 (0.5, 0.9)	0.104
Residual	1.12 (1.0, 1.2)	0.060	0.73 (0.6, 0.9)	0.078

Steroids (corticosteroid)
[variable name] *[variable name] represents interaction term

Table A.9. Objective and self-reported measures of disease outcomes multivariate linear regression analysis all visits

	Child Health Assessment Questionnaire		Juvenile Arthritis Quality of Life Questionnaire		Pain Score	
Variable	Coefficient (95% Confidence Interval)	p-value	Coefficient (95% Confidence Interval)	p-value	Coefficient (95% Confidence Interval)	p-value
Fixed Effects						
Age	-	-	0.06 (0.02, 0.1)	0.004	0.13 (0.05, 0.2)	
C-Reactive Protein	-	-	0.26 (0.1, 0.4)	< 0.001	-	-
Season, reference value (Winter)						
Spring	-	-	-	-	-1.25 (-2.2, -0.3)	0.014
Summer	-	-	-	-	-0.21 (-1.1, 0.7)	0.661

Fall	-	-	-	-	-0.38 (-1.4, 0.6)	0.466
Milk Intake, reference value (Never/sometimes)						
Everyday	-0.25 (-0.4, -0.1)	0.014	-	-	-0.33 (-1.4, 0.7)	0.546
Taking a supplement containing vitamin D, reference value (Never)						
Every day/Sometimes	0.05 (-0.1, 0.2)	0.578	-	-	-0.37 (-1.2, 0.4)	0.360
Smoker in home, reference value (No)						
Yes	0.25 (0.1, 0.4)	0.010	-	-		
Sex, reference value (Male)						
Female	-	-	-	-	-0.81 (-1.6, 0.0)	0.053
NSAID Prescribed, reference value (Yes)						
No	-	-	-0.40 (-0.7, -0.1)	0.018	-	-
Milk Intake* Taking a supplement containing vitamin D, reference value (Never/sometimes drink milk*never take a supplement)						
Never/sometimes drink milk* supplement everyday/sometimes	-	-	-	-	1.92 (0.3, 3.6)	0.021
Follow ups	-0.43 (-0.6, -0.3)	< 0.001	-0.19 (-0.3, -0.1)	0.001	-1.54 (-2.2, -0.9)	< 0.001
Constant	1.38 (1.1, 1.7)	< 0.001	2.44 (1.9, 3.0)	< 0.001	5.87 (4.1, 7.7)	< 0.001
Random Effects		Standard Error		Standard Error		Standard Error
Participant	0.30 (0.2, 0.5)	0.082	0.81(0.1, 1.0)	0.102	1.05 (0.5, 2.1)	0.384
Residual	0.51 (0.4, 0.6)	0.048	1.12 (0.0 , 1.3)	0.062	2.24 (1.9, 2.7)	0.195

Variable	Physician Global Assessment	
	Coefficient (95% Confidence Interval)	p-value
Fixed Effects		
Age	-	-
Ethnicity, reference value (White)		
Non-White	0.56 (0.1, 1.0)	0.016
Indigenous	0.72 (0.2, 1.3)	0.008
Annual Household Income, reference value (<$51,000)		

$51,000 – 99,999	-	-
> $100,000	-	-
Follow ups	-0.74 (-0.8, -0.6)	< 0.001
Constant	3.56 (3.2, 3.9)	< 0.001
Random Effects		Standard Error
Participant	0.60 (0.4, 0.9)	0.129
Residual	1.77 (1.7, 1.9)	0.060

NSAID (non-steroidal anti-inflammatory)
[variable name] *[variable name] represents interaction term

Table A.10. Remission prediction multivariate linear regression analysis at baseline and 6 months

Variable	Coefficient (95% Confidence Interval)	p-value
Fixed Effects		
C-Reactive Protein	-0.08 (-0.1, 0.0)	< 0.001
NSAID Prescribed, reference value (Yes)		
No	0.31 (0.2, 0.4)	< 0.001
Biologic Prescribed, reference value (Yes)		
No	0.26 (0.1, 0.4)	0.002
First follow up	0.17 (0.1, 0.2)	< 0.001
Constant	-0.26 (-0.5, 0.0)	0.019
Random Effects		Standard Error
Participant	0.26 (0.2, 0.3)	0.036
Residual	0.45 (0.4, 0.5)	0.021

NSAID (non-steroidal anti-inflammatory), Biologic (biologically-based anti-cytokine therapie)

Table A.11. Remission prediction using the indexes instead of C-Reactive Protein multivariate linear regression analysis at baseline and 6 months

Variable	Coefficient (95% Confidence Interval)	p-value
Fixed Effects		
Pro-Inflammatory Index†	-0.05 (-0.1, -0.0)	0.006
Anti-Inflammatory Index†	0.04 (-0.0, 0.1)	0.070
25(OH)D	0.16 (0.0, 0.3)	0.019
First follow up	0.18 (0.1, 0.3)	0.002
Constant	-0.82 (-1.5, -0.2)	
Random Effects		Standard Error
Participant	0.13 (0.1, 0.3)	0.055
Residual	0.30 (0.2, 0.4)	0.028

25(OH)D (25-hydroxyvitamin D)

† A method of standardization to compare and combine biochemical markers was developed by Tabung et al. (Tabung et al., 2015). The standardized values were combined to create the overall pro-inflammatory biomarker score (C-Reactive Protein + Tumor Necrosis Factor –α + Interleukin (IL)-6). The same method of standardization was used to calculate an anti-inflammatory score to combine the concentrations of cytokines IL-4 and IL-10.

Table A.12. Remission prediction multivariate linear regression analysis all visits

Variable	Remission Classification	
	Coefficient (95% Confidence Interval)	p-value
Fixed Effects		
C-Reactive Protein	-0.08 (-0.1, 0.0)	< 0.001
NSAID Prescribed, reference value (Yes)		
No	0.31 (0.2, 0.4)	< 0.001
Biologic Prescribed, reference value (Yes)		
No	0.26 (0.1, 0.4)	0.002

Follow ups	0.17 (0.1, 0.2)	< 0.001
Constant	-0.26 (-0.5, 0.0)	0.019
Random Effects		Standard Error
Participant	0.26 (0.2, 0.3)	0.036
Residual	0.45 (0.4, 0.5)	0.021

NSAID (non-steroidal anti-inflammatory), Biologic (biologically-based anti-cytokine therapy)

Appendix B

Recruitment Centres Participating in BBOP Study

British Columbia Children's Hospital, University of British Columbia, Vancouver, BC

Alberta Children's Hospital, Calgary, University of Alberta, AB; Stollery Children's Hospital, Edmonton, University of Alberta, AB

Royal University Hospital, Saskatoon, University of Saskatchewan, SK

Children's Hospital, Winnipeg, University of Manitoba, MB

The Hospital for Sick Children, University of Toronto, Toronto, ON

The Montreal Children's Hospital, McGill University, Montreal, QC

Centre Hospitalier Universitaire de Sherbrooke, Sherbrooke, QC

Centre Hospitalier de l'universite Laval, Laval, QC;

IWK Health Centre, Dalhousie Univeristy, Halifax, NS

Janeway Children's Health and Rehabilitation Centre, Memorial University, St. John's, NL