An Exploration of the Associations Between Gut and Serum Immunoglobulin A in Infancy and Asthma in Childhood

By

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Abstract

Introduction

Early immune maturation and gut microbial composition have a clear impact on the development of asthma and atopy in children. There is a large body of evidence on the association between immunoglobulin A (IgA), asthma, and other atopic diseases. Low secretory Immunoglobulin A (sIgA) (mucosal) levels in infancy have been associated with the development of asthma and atopic disease in childhood. As well, absence of serum IgA is associated with increased risk for asthma. Serum IgA levels have also been shown to be increased in those with food sensitization, despite the levels being normal for their age. In this thesis, we determined if lower levels of the primary gut mucosal immunoglobulin (sIgA) during infancy were associated with the development of asthma and/or wheeze in a large prospective, normal birth cohort. In another cohort from a health administrative database, we determined associations between serum IgA during in relationship to Emergency Department (ED) visits for asthma and/or wheeze (AW) in childhood.

Objectives

This thesis aims to determine the relationships between fecal secretory immunoglobulin A and childhood AW (Study 1) and serum IgA and childhood

emergency department visits for AW (Study 2). The objective of study 1 was to determine whether infants with low fecal sIgA (vs normal-high) levels in the first few months of life have increased risk for development AW. Study 2 was developed to determine if low serum IgA children is a useful biomarker for future ED visits for AW.

Methods

In study 1, 951 infants from the CHILD study sites, Vancouver, Edmonton and Winnipeg were included based on availability of stool samples. A 3-category variable was used: breastfed (any fecal sIgA level), formula fed with low sIgA levels (lowest tertile) and formula fed with normal-high fecal sIgA levels (highest 2 tertiles). Logistic regression models determined the association (Odds Ratio, OR) between low or normal to high fecal sIgA levels in non-breastfed infants and child AW in comparison to breastfed infants, adjusting for confounding factors identified based on a directed acyclic graph to determine the effect of fecal sIgA levels on childhood AW. In study 2, anonymized administrative health data of 9,938 children who had serum IgA levels assessed when they were <=3 years of age between April 1, 2013 and June 30, 2018 was obtained for analysis from Alberta Health Services (AHS) (Alberta, Canada). Multiple logistic regression models determined the association (Odds Ratio, OR) between normal to high serum IgA (top two tertiles compared to the lowest tertile) and child ED visits for AW adjusting for covariates identified by directed acyclic graph.

Results

In study 1, when compared to breastfed infants, formula fed infants with low fecal sIgA levels had 2.20 times the odds of having a diagnosis of asthma in the first three years of life (OR: 2.13; 95%CI: 1.03, 4.43) when controlling for confounding factors. Formula fed infants with normal to high fecal sIgA were at increased risk for atopic AW at age 1-3 years (OR: 5.45; 95%CI: 1.69, 17.31) compared to their breastfeed counterparts. In study 2, when compared to infants with low serum IgA levels, infants with normal-high serum IgA levels (ages 1-2) had an adjusted OR of having an ED visit for AW of 1.21 (95%CI: 1.00, 1.46), controlling for confounding factors. Those with normal-high levels (from 2-3 years) also had significantly increased odds of atopic AW (adjusted OR: 1.79 (95%CI: 1.03, 3.09) when compared to those without.

Conclusion

Low levels of infant produced fecal sIgA was associated with increased odds of asthma, whereas normal to high levels were associated with increased odds of atopic AW in comparison to breastfed infants. Normal-high levels of serum IgA in the first 3 years of life appear to be associated with ED visits for AW and atopic AW from 1 until age 3. Further studies are needed to define the relationships between, sIgA, serum IgA, and asthma and atopic sensitization which may provide new insight into the

development of respiratory disease and atopic illness in childhood. Overall, both serum IgA and secretory IgA may be important biomarkers to aid in early identification and treatment of those prone to develop atopic diseases like asthma.

Dedication

I dedicate the thesis to my parents and siblings. You have all supported me endlessly with your love and care and I could not have done any of this without you.

Preface

This thesis is an original work by Aaron Peter van der Leek. The thesis was written in accordance to the guidelines set by the Faculty of Graduate Studies and Research at the University of Alberta. Part of this thesis received research ethics from the University of Alberta Health Research Ethics Board – Health Panel (#Pro00083778). Renewal approval obtained (MS2_#Pro00083778).

This thesis is comprised of 4 separate sections:

Chapter 1: consists of a literature review. The review is followed by an outline of the overall purpose, objectives, hypotheses, and sample size calculation for the studies.

Chapter 2: presents the first research study on fecal secretory IgA and asthma in a large, prospective normal birth cohort.

Chapter 3: presents the second research study on serum IgA and emergency department visits for asthma in a retrospective administrative health database cohort.

Chapter 4: presents general discussion and conclusions based on these works. This chapter also covers limitations, strengths, bias assessment and a summary of the significance of these findings for families and in clinical practice.

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List of Abbreviations

AW	Asthma and/or Wheeze
BMI	Body Mass Index
CCI	Canadian Classification of Health Interventions
CHILD	Canadian Healthy Infant Longitudinal Development Study
DAG	Directed Acyclic Graph
ED	Emergency Department
FTT	Failure to Thrive
ICD-10CA	International Statistical Classification of Diseases and Related Health Problems, Tenth Revision, Canada
IgA	Immunoglobulin A
IgD	Immunoglobulin D
IgE	Immunoglobulin E
IgG	Immunoglobulin G
IgM	Immunoglobulin M
RSV	Respiratory Syncytial Virus
MALT	Mucosal Associated Lymphoid Tissue
NARCS	National Ambulatory Care Reporting System
OR	Odds Ratio
PC	Practitioner claims
pIgR	Polymeric Immunoglobulin Receptor
SC	Secretory Component
sIgA	Secretory Immunoglobulin A
ULI	Unifying linking identifier
UVs	Unexpected Healthcare Visits

1.0 Chapter 1: Introduction

Chapter 1 provides a literature review on the relationship between asthma and Immunoglobulin A (IgA), first focusing on the burden of asthma and why it is a key area of study. The chapter then focuses on the factors we understand to play a role in the development of asthma, with a particular interest on the role of IgA, the microbiome and other factors. The final sections of this chapter outline the role of IgA in mucosal immunity, gaps of understanding about the role/association between IgA and asthma development, and introduce how this thesis fills some of those gaps.

1.1 The Burden of Asthma

Atopic disease is an umbrella term that classifies a group of diseases (asthma, atopic dermatitis, allergic rhinitis and food allergy) linked by a shared underlying problem with the immune system. The main connection between these diseases is atopy, which is the development of immunoglobulin E directed against allergens. The prevalence of asthma and other atopic diseases place a huge economical and physical burden on the world's population—particularly in western societies [1]. Globally, there are estimated to be 300 million cases of asthma and nearly 100 deaths per day are attributed to asthma [1,2]. As well as impacting morbidity and mortality, asthma and allergy present a significant financial burden to health care. It is estimated that annual asthma-related health care costs for Canadian provinces vary between $46 million (British Columbia) to $141 million (Ontario) per year. Other atopic diseases have been reported to pose a similar economic burden [1,2]. These intricately linked diseases significantly reduce quality of life for patients and their families, as well as present a large financial cost through missed work and school [2–4]. As such, it is a key area of research to understand the factors associated with development of asthma.

1.2 Asthma Phenotypes and Trajectories in Children

This thesis focuses primarily on childhood asthma, specifically until the end of age five. Although a diagnosis of asthma before age five has proved difficult, in the last decade, researchers and clinicians have become adept in diagnosing asthma at an early age and characterizing the various asthma phenotypes and trajectories that predict either short-term wheezing or long-term persistent asthma. In a seminal study by Henderson et al., (2008), 6 major trajectories of early childhood wheezing were characterized [5]. These trajectories include transient early, prolonged early, intermediate, late onset, persistent and never/infrequent which predict chronic and persistent asthma into adulthood to varying degrees. As noted in an editorial by Sears et al., (2015), there are major shifts towards an increase of prevalence and probability of wheezing in intermediate and late phenotypes, which is driven by atopic sensitization at 3 and 4 years, respectively [6]. It is these phenotypes in particular which are characterized by a greater proportion of atopic sensitization and severity of symptoms that are less likely to go into remission in adulthood. By contrast, individuals labelled as (non-atopic) transient early and prolonged early phenotypes are less likely to have persistent disease into adulthood. Many studies report on the prevalence of childhood asthma and wheezing in relation to various demographic and biological factors. Longitudinal studies report that wheezing that starts in early life and persists past 6 years of age generally persists into adulthood. However, it is important to keep the various phenotypes of trajectories in mind as we try to understand underlying mechanisms of disease and work to predict outcomes and manage or prevent persistent asthma [7].

1.3 Early Life Factors and the Development of Asthma

As noted in these asthma phenotype and trajectory papers, traditionally recognized risk factors cannot fully explain the shifting trends of incidence and prevalence of childhood asthma and atopy in the past few decades. New causation theories are required to explain these trends. The impact of early exposures on disease development, known as Developmental Origins of Health and Disease, include various pre- and post-natal factors such as gestational age, mode of delivery and breastfeeding that have been shown to effect the development of atopic disease [8]. These effects are mediated through multiple epigenetic mechanisms; for example, nutrition and the microbiome influence the promotion of atopic pathways in susceptible individuals [8]. In particular, emerging evidence highlights the significant impacts the human microbiome has on the development of early immune tolerance, affecting future development of asthma and atopy [9]. For proper development, fecal-oral and vaginal-oral transmission ('seeding') of the microbiome at birth during delivery, and further development of a healthy microbiome via breastfeeding, are critical in the establishment of a 'healthy gut' microbiota [10]. A healthy microbiome is linked to disease prevention in issues ranging from depression and atopic disease to obesity [11].

One of the major determinants of a healthy microbiome is mucosal immune system function. Immunoglobulin A (IgA) is the major mediator of humoral mucosal immunity and is particularly important in development and regulation of the microbiome [12]. In particular, differential binding patterns of mucosal IgA to microbiota in early life are associated with gut microbial dysbiosis and later development of atopic diseases [13]. This thesis will investigate the associations

between development of fecal secretory and serum IgA levels early in life, and existence of preschool asthma and wheeze.

1.4 Mucosal Immunity, Immunoglobulin A, Asthma and the Microbiome

An understanding of the ontogeny, or normal development, of the mucosal immune system is critical to understand the relationship between IgA and asthma. This section will focus on the role of IgA in development of mucosal immunity.

1.4.1 Basic Mucosal Immunity

The mucosal immune system of humans is a series of lymphoid-associated structures at mucosal surfaces throughout the body, including the breast, and gastrointestinal, respiratory and urogenital tracts [14,15]. The extensive immune protection at mucosal surfaces is mediated by mucosal associated lymphoid tissues (MALT), which form vast interconnected networks by the elaborately regulated, selective localization of cells and molecules activated at one mucosal site and seeded to other sites throughout the body [15]. At these mucosal sites, there is a predominance of dimeric IgA (bound by a J-chain) secreting plasma cells. Secretion of this molecule onto mucosal surfaces is facilitated by binding of dimeric IgA to polymeric immunoglobulin receptor protein (pIgR) which also forms the stabilizing secretory component (SC), forming secretory IgA (sIgA) when cleaved and secreted into the mucosal surfaces. SC helps stabilize sIgA from breakdown by proteases. Secretory Immunoglobulin M (sIgM), which is also polymeric and bound to SC, is secreted by a similar process. As well, small numbers of IgM, Immunoglobulin G (IgG) and

Immunoglobulin D (IgD) and rare Immunoglobulin E (IgE) secreting plasma cells are found at mucosal sites [16]. IgG and IgD are found early in mucosal immune maturation, and are also compensatory antibodies in individuals with IgA-deficiency, but they are not bound to SC or J-chains [16].

There are two IgA subclasses (IgA1 and IgA2) which vary in proportion at different mucosal sites. IgA2 is predominant in the gastrointestinal tract, whereas IgA1 is predominant in the salivary glands and nasal lymphoid tissues. Response to protein antigens at the mucosal sites are predominantly IgA1, whereas IgA2 subclass antibodies are primarily produced in response to polysaccharide antigens [17]. Induction of an IgA mediated immune response to antigens and microbes is thought to occur largely via antigen sampling through M-cells in Peyer's patches, and through both T-cell independent and dependent mechanisms resulting in terminally differentiated IgA+ plasma cells, which yield antibodies of varying affinities [18]. All the IgA antibodies and immune cells present at mucosal surfaces serve three main functions: a first line of defense from infection by viruses and microbial agents, prevention of systemic immune response to commensal microbiota and food antigens, and regulating the immune responses to pathogens [19].

1.4.2 Serum IgA vs Secretory IgA in Health

This project focuses on the role of serum and secretory immunoglobulin A--in relation to asthma and respiratory illness in childhood. As outlined above, sIgA (the secreted form) is critical to development of the microbiome, infection prevention, and the development of asthma. In contrast, serum IgA has less well understood relationships to the microbiome and asthma. Some insight can be gained through

observation of patients with selective IgA deficiency with extremely low or undetectable serum IgA [20,21]. Serum (blood) IgA represents only approximately 7% of the total IgA present in the body, and serum levels do not correlate with secreted IgA levels [22,23]. Despite the poor correlation between serum IgA and sIgA, children with selective IgA deficiency (absence of serum IgA) have an increased risk of developing sinopulmonary and gastrointestinal infections, allergy, asthma and autoimmune diseases; presumably effects of lacking mucosal immunity associated with sIgA [24]. Although there is a well-reported increased risk, only a small number of selective IgA deficient individuals are symptomatic in comparison to those who have presence of IgA. Interestingly, this mismatch between serum and secretory IgA may partially account for the lack of symptoms in the majority of selective IgA deficiency individuals since these individuals may retain some levels of sIgA with absence of serum IgA. Other compensatory factors, such as increased secretory immunoglobulin M in patients with low sIgA may explain this phenomenon but, these relationships remain poorly understood [25].

Besides the research on IgA deficiency and asthma, further studies have shown that levels of serum IgA in those without an immunodeficiency also relate to asthma. Kim et al. (2015) showed that serum IgA levels were significantly related to sensitization to house dust mites and airway hyper responsiveness, two key defining factors in asthma exacerbation and diagnosis, respectively [26]. They demonstrated that high levels of serum IgA correlated with increased odds of house dust mite sensitization and also decreased odds of airway hyperresponsiveness, though this relationship was not significant in final models [26]. This research highlights the potential differential relationships between serum IgA and secretory IgA on asthma and atopic sensitization, since high fecal IgA has previously been associated with reduced risk for atopic disease [27].

1.4.3 Immunoglobulin A – A Key Factor in Gut Microbiota Development

As highlighted previously, sIgA has multifaceted roles in the mucosal immune system. Key roles include controlling inflammation and regulating the immune response to enteric and respiratory pathogens, commensal microflora and certain dietary antigens [18,28–31]. This regulation of microbes by sIgA is a critical factor in the development of a healthy microbiome [12,18].

Breastfeeding plays a well-established role in immune system development in infancy and one of the main components of breastmilk is sIgA [32]. sIgA in breastmilk is particularly important in the development of the early microbiome because it is the main source of sIgA during this key period in which the infant's immune system is programmed for tolerance to commensal microbes and antigens. Normally, infants are able to produce normal levels by sIgA at 6 months of age [33,34]. Delayed production of fecal IgA in infants is associated with increased risk for atopic disease [27].

Current evidence for the effects of pre- and post-natal influences on sIgA levels include: higher 3-month fecal sIgA concentrations with higher breastfeeding status; lower 3-month fecal sIgA levels in infants with maternal stress during and after pregnancy; and having greater colonization of *Clostridioides difficile (C. difficile)* in gut microbiota [35–37]. Persistently low levels of sIgA in infants may lead to the development of atopy, infection and asthma, mediated by effects of an aberrant microbiome composition as a result of loss of immune exclusion of typical pathogens, and decreased ability to maintain more beneficial bacteria [13,27,38,39]. Exposures that affect the interaction between gut sIgA and the microbiota may result in persistent altered gut microbial colonization, increasing risk for chronic diseases [12,13,36,38,40].

1.4.4 The Microbiome and Asthma

The microbiome provides a major link between sIgA and the development of asthma. The next section provides a broad overview of the microbiome and asthma, with a particular focus on the role of IgA in both phenomena.

In the past 5 years, the link between gut microbiota and asthma has become increasingly clear [41]. Although the gut microbiota is the most well-characterized microbiome, there are distinct microbial communities on the skin, nose, oral cavity, respiratory tract, stomach, intestines and vagina [42–48]. Community composition, relative abundance, and bacterial load vary significantly between locations in the body, but a few phyla have been characterized as the major colonizers in and on the body; these include: Firmicutes, Bacteroidetes, Proteobacteria, Actinobacteria, Fusobacteria, and Cyanobacteria [49].

The intestinal microbiome is the most extensively studied, with over 100,000 articles published in the last 10 years. In the gastrointestinal tract, Bacteroidetes represents the most abundant phylum, followed by Firmicutes [50]. Compared to the intestinal microbiome, the respiratory tract is one of the least colonized surfaces of the body. From the upper respiratory tract to the lower respiratory tract lies a gradient from high to low microbial presence [44,51,52]. Similar to the gut, the predominant phyla in the airways are the Firmicutes and Bacteroidetes [43–45]. It is important to note that in spite of the large number of microbiome studies, their outcomes should be taken with a grain of salt. By nature, microbiome studies are inherently biased based on sampling, culturing and sequencing techniques used to detect bacteria and estimate predominance; methodological hurdles are especially present in characterizing the relatively sparse microbiota of the respiratory tract [52]. New techniques to elucidate microbial ‘dark matter’ or microbes that are too low of an abundance to detect could

provide more useful information for determining true candidates for disease causative/associated microbes [53].

1.4.5 The Respiratory Microbiome and Asthma

Despite the inherent limitations in defining the respiratory microbiome, there is evidence to support the association between simultaneous and connected development of the intestinal and respiratory microbiome after birth [54]. As shown by Madan et al., (2012), a number of bacteria which first appear in the intestine are detected subsequently in the respiratory tract, which they hypothesize is due to microaspiration of intestinal microbes promoting the development of the airway microbiota [54]. As well, fluctuations in diet affect both the respiratory and intestinal microbiome [54,55]. Culture and sampling methods in children are challenging and there is discordance between upper and lower respiratory tract microbiota; however, when the data is combined between all sampling methods, general trends indicate that the intestinal and respiratory compartments are closely connected and that changes at one site have the potential to impact the other [43,54]. Furthermore, there is a notable difference between number and diversity in the airway microbial population between healthy subjects and those with asthma [9,45].

1.4.6 Respiratory and Intestinal Microbiomes and the Link to Asthma

There are strong shifts in overrepresentation of Proteobacteria and Firmicutes with diminished Bacteroidetes in lung microbiomes from those with asthma, compared to healthy controls [45]. These changes in the lungs are also comparable to changes in the gut microbiome in early infancy associated with development of asthma in childhood, and there is further evidence from cross-sectional studies on the microbial differences between those with asthma and healthy adults [10,56,57]. This points towards a link between in early microbiome dysbiosis and established dysbiosis in the respiratory and gastrointestinal tracts in the development of asthma [10,56,57].

Recurrent gut infection is a sign of microbial dysbiosis, which has been linked to increased risk for asthma, atopic disease, depression, and obesity [58–64]. Preliminary evidence suggests that probiotics may prevent infection and resultant atopic disease in "at-risk" groups, though these results are highly contentious [65]. This is in agreement with the hygiene hypothesis, in which typical early exposure to "beneficial" microbes may prevent gut dysbiosis through tolerance-inducing mechanisms and resultant harmonious balance between Th1 and Th2 T-helper cell subsets [66]. Previous research has also linked respiratory infections to chronic airway disease, though little is known about how viral and bacterial infections, which underly exacerbations of chronic lung disorders, can shape the microbiota or are caused by the various gut microbial compositions. Respiratory infections lead to an inflammatory state and this inflammation leads to temporary or permanent damage and possible reprogramming of immune responses. The difference between temporary damage in the average person with a cold, and remodeling or other changes in people with chronic lung disease like asthma and cystic fibrosis, may be mediated in part through the microbiota. Separately, or possibly additively, the microbiota contributes through

the development, maintenance and regulation of immune tolerance/immunity [67]. Evidence points to a strong link between respiratory infections and distinct respiratory microbiome composition. Yi et al., (2014) recently profiled bacterial communities in the upper respiratory tract in patients with acute viral infections including: influenza, parainfluenza, rhinovirus, coronavirus, metapneumovirus, adenovirus and respiratory syncytial virus [51]. Virus-infected individuals had an increased prevalence of *Haemophilus* and *Moraxella* but there was no specific virus-associated bacterial profile, suggesting that specific respiratory microbiomes are associated with susceptibility to viral respiratory illness, though this could be due to the low sample size of the study and large variability between viral infections and underlying diseases [51]. Interestingly, individuals with chronic respiratory bacterial infections also regularly present with two distinct microbes [68]. These microbes strongly compete with each other and are also associated with a very distinct composition of airway microbiota [68]. Germ-free mouse models that harbor no microbes in the gut or lungs, and other mouse studies have also illustrated that the presence of beneficial microbiota is critical for defense against influenza virus and certain bacterial pneumonias [69–72]. It is unclear at this point why these functional associations exist—does a lack of colonization in the gut affect the immune system, or is it due to a lack of protective microbes in the lungs? Preliminary evidence suggests that it may be a combination of both.

1.4.7 Intestinal and Respiratory Microbiomes, IgA and Asthma

The literature suggests that sIgA has a similar protective function against bacterial and viral pathogens in both the respiratory and gastrointestinal tracts. For instance, sIgA protects against respiratory tract *Mycobacteria* and *Chlamydia pneumoniae,* organisms which have been linked to a potential pathogenic mechanism leading to later-onset asthma [45,73–76]. *Mycobacteriaceae* and *Chlamydia pneumoniae* are also persistently present in the airways of the chronic asthmatic population [76–78]. Early infection with respiratory syncytial virus and human rhinovirus have also been associated with later onset and development of asthma, independent of atopy [74]. It is clear based on this large body of evidence that the development of the respiratory and gut microbiomes are linked to each other and to asthma. Because of its role in early gut microbiota and immune regulation, sIgA may be an important factor in mediating these relationships.

1.5 Summary and Gaps: IgA, the Microbiome and Asthma

Asthma is an epigenetic, multi-factorial disease with many genetic, environmental and lifestyle factors contributing to disease onset and progression. It has been established that serum and secretory IgA are separately associated with asthma and atopic disease. Studies on the gut microbiome in relation to sIgA and asthma show potential links for early life development of the immune system and microbiome that impact development of asthma later in life. However, currently there is little understanding of the differences between secretory and serum IgA and how they associate with asthma development.

Dzidic et al., (2015) found that the proportion of sIgA bound to the dominant bacteria, rather than total fecal sIgA, was associated with allergic manifestations, in particular asthma, up to 7 years of age. However, all individuals in this cohort were breastfed at the early timepoint of stool collection so there may be undiscovered differences in total sIgA between formula fed and breastfed infants [13]. As well, high total fecal IgA during infancy, which can be comprised of serum and secretory forms at a young age when the intestinal barrier is not fully developed, has been shown to be associated with reduced risk of atopic diseases at two years of age [27]. It remains to be seen whether fecal sIgA levels are predictive of asthma when breastfeeding status varies and how this relates to health care utilizations for asthma.

A recent paper by Kim et al., (2017) looked at associations between serum IgA levels and allergy/asthma in adult patients and found serum IgA was related to airway hyperresponsiveness, but only considered those with suspected asthma [26]. An older population-based study found that low serum IgA levels ($\leq$461 μg/mL) in the 18th to 23rd months of life were associated with increased cumulative incidence of asthma, atopic dermatitis and otitis media. However, this study has limitations because it was used cord blood samples which may represent both maternal and newborn contributions to total IgA [79]. In comparison, a more recent case-control study from Croatia found lower serum IgA levels in children with asthma than controls, but also that there was higher serum IgA in those with allergic asthma than those with non-allergic asthma [80]. A more rigorous prospective cohort study found that higher serum IgA levels at two months were associated with respiratory allergic symptoms and sensitization at 5 through 20 years of age [81]. Interestingly, these results with infant serum IgA levels were more significant than levels of IgA levels in milk during breastfeeding, though the breastmilk IgA concentration was inversely associated with total serum IgE and positive skin prick test at 20 years of age [81]. Many publications

also describe the association between selective IgA deficiency, which is the complete absence of serum IgA, and increased risk for asthma, but it remains to be seen if low serum IgA levels, as well as its absence, have a similar relationship to asthma [74].

There is a gap in the literature on the relationship between the maturation of IgA levels in the infant and later development of childhood asthma. To date, no large population cohort studies exist that have successfully described total levels of fecal sIgA in early life in relation to both later development of asthma and health care utilizations for asthma. As well, no studies have reported the risk of Emergency Department visits for childhood asthma with respect to serum IgA levels in a large population-based cohort. It remains to be seen how the relationships between serum IgA and fecal sIgA compare with childhood asthma outcomes. This thesis provides some much-needed insight on the associations between early life IgA levels and development of asthma.

1.6 Hypothesis and Objectives

This thesis will explore two hypothesis and objectives in separate cohort studies to further our understanding of IgA and development of childhood asthma and atopic disease.

The objective of the first study is to determine whether infants with low fecal sIgA in the first few months of life have increased odds for asthma compared to those who exhibit normal-high levels, using data gathered from the CHILD birth cohort. Our hypothesis is that infants with low fecal sIgA will have increased risk for a diagnosis of or emergency department visits for AW in childhood. Having low fecal sIgA in infancy when there is rapid development of the immune system and microbiome may be an important biological marker for aberrant development of these systems, which may

relate to later development of childhood asthma. A secondary hypothesis is that fecal sIgA is differentially associated with atopic and non-atopic AW. We test this association while controlling for the relationships between breastfeeding status, delivery mode, antibiotics exposure, gravidity, maternal asthma/allergy, maternal depression trajectories, smoke exposure, age, sex and maternal obesity on sIgA and asthma.

The second study was conducted using an Alberta Health Services (AHS) administrative health database. The objective of this retrospective cohort study is to determine whether infants with low serum IgA in the first months to years of life are at increased risk for emergency department visits asthma compared to those who exhibit normal to high levels. Our proposed hypothesis is that patients who have low serum IgA levels will be at increased risk for emergency department visits for AW in childhood. A secondary hypothesis is that serum IgA is differentially associated with atopic and non-atopic AW. We are testing this association while controlling for potential confounding pathways that include age, failure to thrive, and infant sex.

1.7 Demographic Factors Related to IgA and Asthma

The following section will outline various demographic and biological factors related to IgA (serum or secretory) and asthma that are potentially important to consider when conducting population-based studies on IgA and asthma in childhood.

1.7.1 Tobacco Smoke Exposure

Parental smoking pre- and post-natally critically impacts the development of the immune system and microbiota of the infant and increases the risk for asthma. Maternal

smoking may disturb the initial 'seeding' of the infant gut microbiota during birth. There is a significant change in adult oral and gut microbiomes after smoking cessation [82,83]. As well, notable levels of tobacco smoke metabolites are found in the meconium of infants of mothers with tobacco smoke exposure, which may have effects on the microbiome and immune system [84]. One study on mucosal immunity in infants at 12 months of age showed that maternal smoking was associated with increased total salivary IgA and an associated increase in chronic upper respiratory tract symptoms [85]. This study also showed that infants of mothers who smoked had decreased rates of successful breastfeeding initiation and stopped breastfeeding earlier [85]. Other studies have shown increased sIgA levels and a trend toward increased wheeze and lower respiratory tract symptoms in infants of mothers who smoked [86,87]. Pre- and post-natal smoke exposure has also been associated with increased risk for wheeze and asthma [88].

1.7.2 Maternal Asthma and Atopic Disease

There are high rates of correlation between maternal asthma and other atopic disease and subsequent development of asthma. Indeed, family history of allergies is one of the most important factors in the asthma predictive index, which points towards genetic heritability in immune function associated with asthma [89,90]. To date, no studies have determined associations between parental atopic diseases and immunoglobulin A levels in infants.

1.7.3 Maternal Obesity During Pregnancy and Cesarean Delivery

Maternal atopy and asthma during pregnancy are also associated with maternal distress both pre- and post-natally, and obesity [59,91–93]. Interestingly, overweight body mass index during pregnancy is linked to significant changes in the infant gut microbiome [94]. One prospective cohort study found that both high maternal pre-pregnancy weight and excessive weight gain during pregnancy were associated with a lower abundance of *Bifidobacterium* in the infant's gut at 1 month and a higher abundance of *Clostridium histolyticum* at 6 months [95]. Additionally, maternal obesity is associated with increased risk of caesarean section, which itself increases the likelihood of exposure to antibiotics during birth. Both caesarean section and antibiotic exposure are risk factors for changes in the microbiome and may also alter IgA levels and increase risk for the later development of asthma [10,29,35– 37,96,97].

1.7.4 Breastfeeding

As confirmed in many previous studies, breastfeeding has a major influence on both infant IgA levels and development of asthma, but the direction of the relationship is contentious [13,32,36,37]. Breastfeeding is associated with increased levels of fecal IgA and decreased serum IgA levels [32,36]. Although no human studies have directly determined whether breastfeeding stimulates sIgA production, increased levels of sIgA in the gut have been shown to induce a positive feedback loop for further sIgA production [98,99]. As well, breastmilk contains additional components like oligosaccharides that favour a greater abundance of *Bifidobacteria* and *Lactobacillus* [100,101]. Infants supplemented with probiotics that include species of these two genera are more likely to have higher fecal IgA than infants without supplementation

[102]. Although breastfeeding may promote proper colonization of the infant microbiome and sIgA levels in early life, evidence is still not entirely clear as to whether breastfeeding has protective abilities in development of asthma and allergy [101].

1.7.5 Maternal Depression and Anxiety

Maternal depression, anxiety or distress during and after pregnancy have been associated with increased risk for physician diagnosed childhood asthma, but these associations seem to diminish with child age [103]. As revealed by previous study from our lab, in addition to lower levels of interaction and a shorter period of breastfeeding, infants of depressed mothers also have reduced fecal sIgA levels, which may be a partial mediator in the association between maternal depression and asthma in offspring [37].

1.7.6 Antibiotic Exposure

Antibiotic usage during both delivery and within the first few months of life has large effects, decreasing species richness in the infant gut microbial composition and increasing risk for childhood asthma [104]. In particular, intrapartum antibiotics used in vaginal and C-section deliveries can significantly lower species richness and *Bacteriodetes* abundance in the 3-month period postpartum [105]. In infants, antibiotics can impact the total levels negatively and delay timing of production of sIgA. These changes in sIgA due to antibiotics were associated with decreased species diversity in the microbiome [29].

1.7.7 Multigravida

A population-based study showed that firstborn children had higher total fecal IgA than those with older siblings [36]. Additionally, multigravida is associated with maternal age, having more offspring and socioeconomic status, which are important predictors for infant outcomes including asthma. These are important factors to consider in population-based studies of asthma.

1.7.8 Immunoglobulin E

Based on the etiology of asthma, IgE is an important factor in predicting asthma and is one of the key factors in the modified asthma predictive index [90]. Elevated IgE levels are a marker of atopy or allergic sensitization [90]. IgA and IgE are intimately related in that low fecal IgA has been associated with increased risk for IgE-mediated allergic disease [27]. In comparison, both high and low serum IgA have been associated with allergic sensitization [79,81]. A significantly positive relationship exists between serum IgA and IgE levels [106].

1.7.9 Failure to Thrive

Failure to thrive is a medical diagnosis given to a child who is failing to gain appropriate height or weight, as compared to age- and gender-matched growth norms. A previous population-based study revealed that failure to thrive is significantly associated with feeding problems, however the differential diagnosis is broad and includes organic illnesses such as immunodeficiency, celiac disease, food allergies and cystic fibrosis [107]. One survey revealed that only 10% of infants with failure to thrive

are diagnosed with organic illness, with most occurrences a result of inadequate parenting, child abuse or breastfeeding difficulties [108].

1.8 Sample Size Calculation:

Estimated proportions of relative rates of asthma between IgA deficient and control individuals come from Urm et al's., 2013 study [74].

$$\text{Sample size per group needed in study based on proportions} = n = 2PI^2\left(\frac{\bar{p}(1-\bar{p})}{(p_1 - p_2)}\right)$$

PI= Power Index

To determine the sample size with a 2-sided α of 0.05 and β of 0.20 (power=80%), the Power Index (PI) will be: 1.96 + 0.84=2.80

$$\bar{p} = (p_1 - p_2)/2$$

PI= 1.96 (0.05α, two-tailed) + 0.84(0.20β, two-tailed) = 2.80

Asthma Group Size Needed:

p_1 = Asthma rates in serum IgA deficient individuals = 30.8% = 0.308

p_2 = Asthma rates in control individuals = 11.5% = 0.115

$\bar{p} = (0.308 - 0.115)/2 = 0.0965$

$n = 2(2.8)^2\left(\frac{\overline{0.0965}(1-\overline{0.0965})}{(0.308-0.115)^2}\right)$ = 36.7 individuals per group

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2.0 Study 1: Infant Fecal Secretory Immunoglobulin A in Relation to Childhood Asthma and Wheeze

2.1 Abstract

Background. Early immune maturation and gut microbial composition have a clear impact on the development of asthma and atopy in children. Low secretory Immunoglobulin A (sIgA) levels and binding patterns of sIgA to gut microbiota in infancy have been associated with the development of asthma and atopic disease in childhood. In this study, we determined if lower levels of the primary gut mucosal immunoglobulin (sIgA) during infancy was associated with the development of asthma and/or wheeze (AW) in a large prospective, normal birth cohort (CHILD).

Objective. The objective of this study was to determine whether infants with low fecal sIgA (vs normal-high) levels in the first few months of life have increased risk for development AW.

Methods. 951 infants from the CHILD study sites, Vancouver, Edmonton and Winnipeg were included based on availability of stool samples. Stool samples from infants age 2-5.5 months were chosen to limit the known effects of food introduction on mucosal immunity. Physician-diagnosed asthma and unexpected doctors visits (UVs) for AW were determined from parent report at 1, 2, 3, 4 and 5 years. Atopic sensitization status at age 1 and 3 years was determined by skin prick test. Fecal sIgA was quantified using Immundiagnostik IG sIgA ELISA kit as previously reported [1]. A 3-category variable was used: breastfed (with any fecal sIgA levels), formula fed with low sIgA levels (lowest tertile) and formula fed with normal-high fecal sIgA levels (highest 2 tertiles). Using STATA v16, logistic regression models determined the association (Odds Ratio, OR)

between low or normal-high fecal sIgA levels in non-breastfed infants and child AW in comparison to breastfed infants, adjusting for confounding factors identified based on a directed acyclic graph to determine the effect of fecal sIgA levels on childhood AW.

Results. About 7% of infants were diagnosed with asthma by age 3, and 10% at ages 4-5. In all infants, low fecal sIgA levels were found in 68% of infants who were not breastfed, 31% of infants who were partially breastfed and 14% of infants who were exclusively breastfed ($p<0.001$). When compared to breastfed infants, formula fed infants with low fecal sIgA levels had 2.13 times the odds of having an asthma diagnoses in the first 3 years of life (OR: 2.13; 95% CI: 1.03, 4.43) when controlling for confounding factors. In the absence of breastfeeding, no associations were found between unexpected healthcare utilizations for AW at age 1-3 and higher fecal sIgA or between AW at ages 4-5, and low or higher fecal sIgA. Formula fed infants with high fecal sIgA were at increased risk for atopic AW at age 1-3 years (OR: 5.45; 95% CI: 1.69, 17.31).

Conclusions. Low levels of infant produced fecal sIgA in formula-fed infants were associated with increased odds of AW, whereas normal to high levels in formula-fed infants were associated with increased odds of atopic AW in comparison to breastfed infants. Due to these associations, sIgA production in non-breastfed infants may be an important biomarker for early-onset non-atopic/atopic AW.

2.2 Introduction

Beginning prenatally through to the 1st year of life, there are key medical, lifestyle and environmental factors which contribute to the development of childhood asthma [2]. In the past 10-15 years, the human microbiome has emerged as a leading influence in the development of immune tolerance, including asthma and atopy [3]. Fecal-oral or vaginal-oral transmission ('seeding') of the microbiome during delivery and further microbiome development via breastfeeding is critical in development of 'healthy gut' microbiota. This healthy composition is linked to decreased risk of diseases ranging from depression and atopy to obesity [4].

Secretory immunoglobulin A (sIgA) is the main immunoglobulin on the mucosal surfaces and is critical to the development of early life gut microbiota composition and antigen tolerance [5]. sIgA fulfills these roles by antigen sampling, immune exclusion of pathobionts and promotion of colonization of commensal gut bacteria [6–8]. Studies in IgA deficient humans show that IgA is critical for proper gut colonization of microbes and tolerance to antigens even in spite of compensatory mechanisms by immunoglobulin M [9].

Fecal sIgA levels are linked to microbiota composition and atopy. Lower infant fecal sIgA levels at 3 months of age are associated with maternal stress, being formula-fed and having greater abundance of *Clostridioides difficile (C. difficile)* abundance in gut microbiota [1,10,11]. Infants with higher fecal IgA at 3-6 months of age had a decreased risk of developing any allergic disease by age 2 [12]. There is further evidence that different levels of binding of sIgA to bacteria in the gut is associated with allergic manifestations up to 7 years of age, in particular asthma, in a breastfed cohort [13]. It remains to be seen if total fecal sIgA levels in the first few months of life are associated with asthma when breastfeeding status varies.

Although sIgA, gut microbiota composition and atopic diseases are clearly linked, the mechanism(s) is (are) poorly understood [13,14]. The gut microbiota has been shown to have important crosstalk with the immune system and this is implicated in the pathogenesis of asthma [15]. Compared to the gut, the respiratory tract is one of the least-colonized surfaces of the body; there lies a gradient from the extensively colonized naso-oral-pharyngeal tract to the lower respiratory system, which has a low ratio of bacterial to human cells [16–19]. Factors implicated in gut microbiota composition may also have direct effects on the microbial composition in the respiratory system, evidence supports interconnected development of these microbiomes. Bacterial species which first appear in the intestine are subsequently detected in the respiratory tract, potentially due to micro-aspiration of gut microbiota as a route of colonization of airway microbiota [18]. Differences in airway microbiota are also noted in those with asthma compared to healthy individuals [20,21]. It follows that since fecal sIgA is an important marker for early gut microbial composition and mucosal immune system development, it may be an important biomarker for later development of asthma.

This study explores whether infants with low fecal sIgA in the first few months of life have increased odds for asthma/wheeze (AW) compared to those with normal-high levels, controlling for the relationships between breastfeeding status, delivery mode, antibiotic exposure, gravidity, maternal asthma/allergy, maternal depression, smoke exposure, sex, age, and maternal obesity on fecal sIgA and AW.

2.3 Methods

2.3.1 Study Population

This study was based on a sub-sample of the Canadian Healthy Infant Longitudinal Development (CHILD) study data [16,22]. The subsample includes 1,071 infants with available fecal sIgA at 3 (range 2.2 - 5.5) months from the Edmonton, Winnipeg and Vancouver sites of the CHILD birth cohort (www.childstudy.ca). Pregnant mothers were recruited, screened and enrolled with written consent from 2008 to 2012. Standardized questionnaires and lab tests were obtained previously by other CHILD investigators. This study was approved by the Ethics Committee of the University of Alberta, the University of Manitoba Human Research Ethics Board and the University of British Columbia/Children's and Women's Health Centre of British Columbia Research Ethics Board.

2.3.2 Outcomes

2.3.2.1 Physician-diagnosed Child Asthma

Childhood asthma was assessed using maternal-report of physician-diagnosed asthma during each of the questionnaires. The item was assessed as a yes/no question with no sub-questions. Since previously established asthma trajectories show transient early, prolonged early, intermediate early (atopic), late (atopic) and persistent; we created our asthma variables to account for the large shift in probability of wheezing between asthma phenotypes at different ages [23]. Our study had 2 variables: (1) physician-diagnosed asthma from the first 3 years of life and (2) physician-diagnosed asthma from years 4 and 5.

2.3.2.2 Unexpected Health-Care Visits for Asthma/Wheeze

Unexpected health-care visits (UVs) for Asthma/Wheeze (AW) were assessed by maternal-reported questionnaire in three questions: (1) unscheduled doctor visits, (2) Emergency Department (ED) visits and (3) hospital stays/admissions. Sub-questions for the reason of visit included: bad cold, fever, rash, wheezing episode, ear infection, allergy, asthma attack, chest infection, accident, coughing episode, other illness(es). UVs were considered visits for AW if the visits were for a wheezing episode and/or asthma attack and/or coughing episode. This variable was split into the two age-groups as with the physician-diagnosed asthma variable. These two variables included: (1) unexpected healthcare utilization for AW in the first 3 years of life and (2) unexpected healthcare utilization for AW in the 4^{th} and 5^{th} years of life.

2.3.2.3 Infant Atopic/Non-Atopic AW

Atopic and non-atopic AW were determined by combining atopic status (IgE skin-prick test sensitization to food or inhalant allergens; yes/no variable) from clinical assessments at 1 and 3 years with AW status (physician-diagnosed asthma and/or unexpected healthcare utilizations for AW). Six binomial variables were used to determine the differences between atopic and non-atopic AW: (1) Atopic AW vs non-atopic AW (no) from ages 1-3 using atopic status at 1 year, (2) atopic AW (yes) vs no AW from ages 1-3 using atopic status at 1 year, (3) non-atopic AW vs no AW from ages 1-3 using atopic status at 1 year, (4) atopic AW vs non-atopic AW from ages 4-5 using atopic status at 3 years of age, (5) atopic AW vs no AW from ages 4-5 using atopic status at 3 years and (6) non-atopic AW vs no AW from ages 4-5 using atopic status at 3 years. We created these groups to restrict the regression analysis to binomial outcomes which are easier to interpret compared to multinomial logistic regression models.

2.3.3 Exposures

2.3.3.1 Stool collection and storage

Infant stool was collected during the 3-month CHILD study visits as previously documented [24]. Stool samples of 5-10 g were aseptically collected from freshly soiled diapers, divided into aliquots and stored at -80°C using aseptic technique. Freezing and freeze-thaw cycles on sIgA levels show minimal impact on quantification of sIgA levels [25,26].

2.3.3.2 Extraction and analysis of sIgA

The sIgA ELISA (enzyme-linked immunosorbent assay) kit from Immundiagnostik was used to measure the amounts of fecal sIgA in mg per gram of wet feces, as per Urwin et al., (2014) [27]. Post thaw, sIgA was extracted from stool with the extraction buffer and diluted 1:125 in wash buffer. Diluted samples, controls and 100 µL standards were aliquoted into a microtiter plate, washed and incubated at 15-30°C for 60 minutes. After incubation, wells were aspirated and washed with wash buffer two times before being tapped dry. 100 µL of the conjugated anti-sIgA antibody is added and samples are again incubated at 15 to 30°C for overnight on a shaker. After the final washing and aspiration, 3,3′,5,5′-Tetramethylbenzidine (TMB) substrate is added and incubated in a dark room for 15 minutes at 15 to 30°C. Absorption is determined with an ELISA reader at 450 nm against 620 nm as the reference. Results were multiplied by the dilution factor and a standard curve was created based on kit-included controls to determine concentrations of sIgA in each sample.

The 3-month stool samples were collected at 3.7 months (2.2–8 months). Of 1,071 infants, 120 (11%) had stool collected at > 5.5 months. Fecal sIgA levels by breastfeeding status are plotted over time in Appendix B, Figure B1. The data beyond 5.5 months was excluded from the final analysis. Solid food introduction and the

ontogeny of infant sIgA production were reasons for restriction [28,29]. Breastfeeding status, known to be highly associated with fecal sIgA (and as confirmed in Appendix B, Figure B2), was treated as an exposure variable as follows to determine the differential and combined associations between maternal sIgA supplementation via breastmilk and infant sIgA production [11]. 1) exclusive and mixed breastfeed infants with any fecal sIgA levels, 2) formula fed infants with normal to high fecal sIgA levels, 3) formula fed infants with low fecal sIgA levels. The groups were mutually exclusive.

2.3.3.3 Potential covariates

Covariates obtained from the CHILD cohort questionnaires: maternal depression trajectories, infant age at stool collection, breastfeeding status at stool collection, tobacco smoke exposure, antibiotic exposure (mothers and infants), delivery mode, gravidity, number of children and pets at home, pregnancy overweight, and maternal allergy and/or asthma. These variables were created from data obtained from CHILD cohort questions.

2.3.4 Statistical Analyses

2.3.4.1 Descriptive Statistics

All infants with a fecal sample from 2-5.5 months of infant age were selected for inclusion. Tables 2.1-3 and in Appendix B (Table B2) describes the frequencies and row-percentages of demographic factors in relation to the sIgA and childhood AW. Pearson Chi χ^2 or Fisher's exact tests were run to determine the crude associations between demographic variables and fecal sIgA levels and the crude associations between demographic variables and child AW. Pearson Chi χ^2 or Fisher's exact tests were also run to see if the study population varied significantly from the initial CHILD

sample population (Appendix B; Tables B1). A p-value of < 0.05 was considered statistically significant.

2.3.4.2 Study Analysis

sIgA concentrations are not normally distributed, so non-parametric tests (Mann-Whitney U) were used to detect differences in sIgA median levels according to the AW status of the infant. The lowest tertile (0.01-3.99 mg/g feces) compared to the top two tertiles (4.01-60 mg/g feces) was used as low fecal sIgA (yes/no) combined with breastfeeding status to account for the effects of maternal sIgA provided through breastmilk. Logistic regression models used a three-way categorical variable: (1) exclusive and mixed breastfed infants with any fecal sIgA levels (0.04-60.0 mg/g feces), 2) formula fed infants with normal to high fecal sIgA levels (4.07-28.4 mg/g feces), 3) formula fed infants with low fecal sIgA levels (0.01-3.93 mg/g feces). The interaction between fecal sIgA levels and breastfeeding was significant (Appendix B, Table B3-B4).

Logistic regression was used to determine the crude and adjusted association between sIgA exposure and child AW. Our analyses followed Shrier and Platt's seminal article explaining creation of Directed Acyclic Graphs (DAGs) and use for epidemiological studies, and DAGs were created using dagitty.com [27]. DAGs are first built to select a minimum adjustment set of covariates to control for biasing pathways and avoid over-adjustment. The final model adjusted for: breastfeeding status, maternal depression trajectories, multigravida, maternal overweight or obesity during pregnancy, newborn antibiotics, prenatal smoke exposure, stratified by age of outcome (Figure 2.1). No significant interactions besides fecal sIgA and breastfeeding were found. Statistical analyses were conducted using STAT v16.0, figures were generated in GraphPad Prism 8.

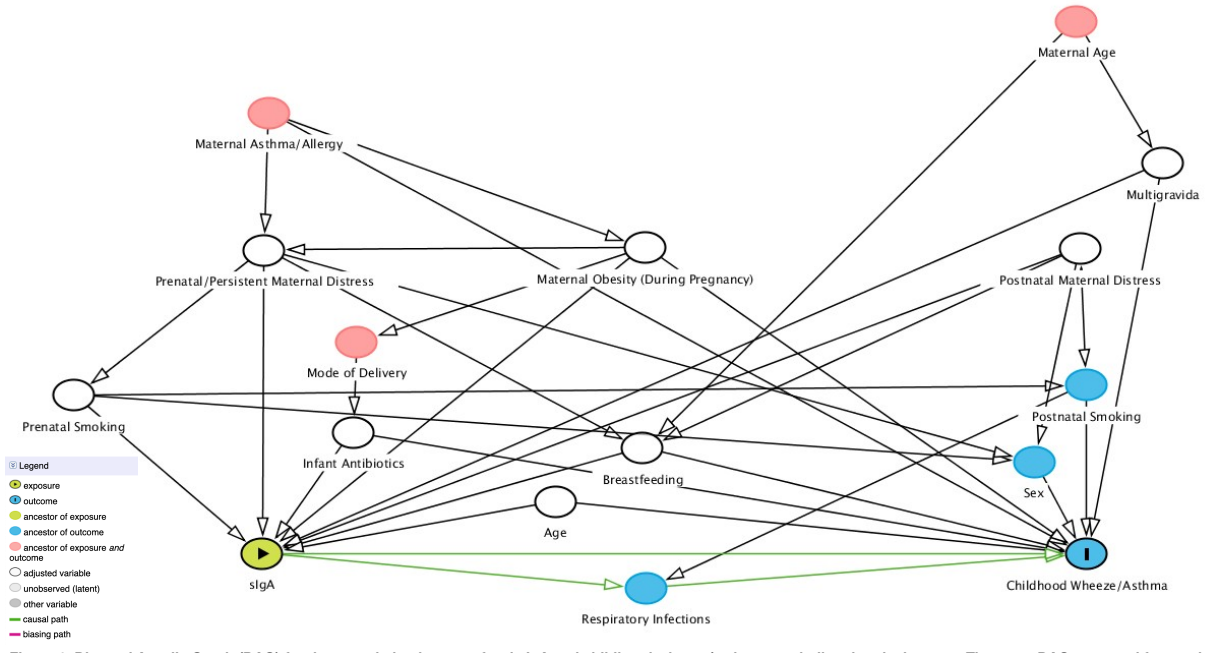

Figure 2.1. Directed Acyclic Graph (DAG) for the association between fecal sIgA and childhood wheeze/asthma was built using dagitty.com. The same DAG was used for atopic AW. Arrows between factors indicate known, consistent associations. Factors in white were adjusted for following DAG rules to determine the total effect of fecal sIgA on childhood wheeze/asthma. Green lines represent causal paths, and red lines represent biasing paths. Red circles represent ancestors of the exposure and outcome (ie, confounders), blue circles represent ancestors of the outcome, and grey circles represent unobserved variables. The minimally sufficient adjustment set represents covariates such that the adjustment for this set of variables will minimize confounding bias when estimating the association between the exposure and the outcome. The final minimally sufficient adjustment set comprised breastfeeding status, maternal depression trajectories, multigravida, maternal overweight or obesity during pregnancy, newborn antibiotics, prenatal smoke exposure, stratified by age of outcome.

2.4 Results

2.4.1 CHILD Cohort Sub-sample Characteristics

The 1,071 sub-sample of CHILD infants from Edmonton, Vancouver and Winnipeg with fecal sIgA data did not differ significantly for the majority of potential covariates in comparison to all infants in all CHILD participants (Appendix; Table B1). Breastfeeding at time of sample collection and pre- and post-natal smoke exposure were slightly higher in the entire CHILD cohort compared to the Edmonton sub-sample.

2.4.2 Study Population and Child AW

In our study, 7% had maternal-reported physician diagnosed asthma and 30% had UVs for AW from ages 1-3. In years 4-5, 9% infants had physician diagnosed asthma and 14% had an UVs for AW. 3.2% had atopic AW from ages 1-3, increasing to 4% from ages 4-5.

Tables 2.1, 2.2 and Appendix Table B2 report the population characteristics distribution according to all the outcomes. Asthma at 1-3 years was significantly more prevalent in those who were born by caesarean section ($p=0.017$) and those exposed to maternal depression ($p<0.001$) (Table 2.1). UVs for AW at ages 1-3 were more common in infants who were formula fed ($p=0.045$), were born by caesarean ($p=0.036$), had exposure pre/postnatal depression ($p=0.034$), or had post-natal smoke exposure ($p=0.005$) (Table 2.2).

Considering those with physician-diagnoses of asthma from ages 4-5, being born by caesarean ($p<0.001$), having a mother who had asthma or allergies ($p=0.035$) during pregnancy, or had pre/post-natal depression ($p<0.001$) was significantly more common (Table 2.1). Unexpected health care utilization for AW (ages 4-5) was more in those with maternal pre/postnatal depression ($p=0.030$), or prenatal smoke exposure

(p=0.005) (Table 2.2). There were similar significant differences between those with atopic AW and non-atopic AW compared to those without in either age groups (Appendix B; Table B2).

2.4.3 Study Population and Infant Fecal sIgA

In our cohort, 33% of infants were in the lowest tertile of fecal sIgA (0.01-3.99 mg/gfeces) and 67% were in the two highest tertiles of fecal sIgA (4.01-60 mg/gfeces). Table 2.1 reports the distribution of population characteristics according to the lowest tertile of fecal sIgA. Differences were observed for breastfeeding status (p<0.001), furry pets in the home (p=0.007), maternal obesity (p<0.001), prenatal smoke exposure (p=0.004) and maternal depression (p=0.001) (Table 2.1).

Table 2.1. Distribution of low fecal sIgA and physician diagnoses of asthma according to demographic and epidemiological factors (n=1071)

		Lowest tertile sIgA (% yes) n=314/951	**ChiX²****	**Physician Diagnoses of Asthma from 1-3 Years n=52/800**	**ChiX²****	**Physician Diagnoses of Asthma from 4-5 Years n=49/486**	**ChiX²****
Co-Variates		Row % (N)	p-value (X^2 exact)	Row % (N)	p-value (X^2 exact)	Row % (N)	p-value (X^2 exact)
Sex	Male	32.06 (160)	0.406	6.19 (34)	0.061	9.70 (26)	0.663
	Female	34.62 (152)		3.66 (18)		8.61 (23)	
Mode of Delivery	Vaginal	32.15 (227)	0.165	3.92 (31)	**0.017**	7.06 (29)	**<0.001**
	Elective Cesarean	31.52 (29)		8.91 (9)		22.22 (12)	

		Lowest tertile sIgA (% yes) n=314/951	ChiX²**	Physician Diagnoses of Asthma from 1-3 Years n=52/800	ChiX²**	Physician Diagnoses of Asthma from 4-5 Years n=49/486	ChiX²**
	Emergency Cesarean	40.29 (56)		8.11 (12)		11.43 (8)	
Breastfeeding Status	Exclusive	14.29 (58)	**<0.001**	7.31 (19)	0.102	12.21 (16)	0.259
	Partial	31.48 (96)		5.11 (18)		10.06 (18)	
	None	68.33 (164)		3.61 (15)		6.67 (15)	
Infant Antibiotics	No	34.09 (284)	0.701	4.94 (46)	1.00	9.39 (45)	1.00
	Yes	31.67 (19)		4.84 (3)		7.41 (2)	
Depression	None	31.4 (254)	**<0.001**	3.88 (34)	**<0.001**	7.59 (36)	**<0.001**
	Antenatal	50.88 (29)		9.68 (6)		27.27 (6)	
	Persistent	33.80 (24)		10.26 (8)		14.71 (5)	
	Postnatal	61.90 (13)		17.39 (4)		40.0 (2)	
Furry Pets in the Home	No	37.84 (165)	**0.007**	5.76 (27)	0.306	10.79 (26)	0.263
	Yes	29.5 (149)		4.36 (24)		7.96 (23)	
Smoke Exposure (Prenatal)	No	31.7 (272)	**0.004**	4.72 (44)	0.060	8.67 (43)	0.057
	Yes	47.56 (39)		9.41 (8)		18.75 (6)	
Smoke Exposure (Postnatal)	No	31.96 (249)	0.053	4.72 (40)	0.395	9.05 (41)	0.734
	Yes	39.76 (66)		6.25 (11)		10.26 (8)	
Multigravida	No	34.44 (125)	0.597	5.24 (20)	0.786	11.48 (24)	0.136
	Yes	32.77 (195)		4.86 (32)		7.67 (25)	

		Lowest tertile sIgA (% yes) n=314/951	ChiX²**	Physician Diagnoses of Asthma from 1-3 Years n=52/800	ChiX²**	Physician Diagnoses of Asthma from 4-5 Years n=49/486	ChiX²**
Maternal Overweight/Obesity (During Pregnancy)	Normal	28.24 (148)	**<0.001**	5.34 (31)	0.241	10.43 (34)	0.209
	Obese	33.17 (69)		6.19 (14)		5.04 (6)	
	Overweight	45.58 (103)		2.98 (7)		10.0 (9)	
Maternal Allergy/Asthma During Pregnancy	No	32.87 (118)	0.941	4.19 (16)	0.299	5.91 (12)	**0.035**
	Yes	33.1 (192)		5.67 (36)		11.38 (37)	
Maternal Age (Greater than Median)	No	36.48 (174)	**0.033**	7.65 (30)	0.195	10.89 (27)	0.198
	Yes	29.93 (138)		5.39 (22)		7.67 (22)	

Chi(X^2) comparison used to investigate whether distributions of categorical variables differ from one another. Fisher's exact test was used when expected frequencies were <5 in >20% of cells. **Bold indicates a significant difference between the two populations

Table 2.2. Distribution of low fecal sIgA and unexpected healthcare utilizations for AW according to demographic and epidemiological factors (n=1071)

		Lowest tertile sIgA (% yes) n=314/951	ChiX²**	UV for AW from 1-3 Years n=193/654	ChiX²**	UV for AW from 4-5 Years n=79/572	ChiX²**
Co-Variates		Row % (N)	p-value (X^2 exact)	Row % (N)	p-value (X^2 exact)	Row % (N)	p-value (X^2 exact)
Sex	Male	32.06 (160)	0.406	31.79 (110)	0.175	14.53 (42)	0.613
	Female	34.62 (152)		26.95 (83)		13.07 (37)	
Mode of Delivery	Vaginal	32.15 (227)	0.165	27.91 (139)	**0.036**	12.42 (55)	0.200
	Elective Cesarean	31.52 (29)		44.07 (26)		18.87 (10)	
	Emergency Cesarean	40.29 (56)		28.87 (28)		18.42 (14)	
Breastfeeding Status	None	68.33 (164)	**<0.001**	35.71 (55)	**0.045**	17.04 (23)	0.434
	Partial	31.48 (96)		31.01 (72)		13.47 (26)	
	Exclusive	14.29 (58)		24.63 (66)		12.30 (30)	
Infant Antibiotics	No	34.09 (284)	0.701	28.45 (167)	0.384	13.84 (71)	0.551
	Yes	31.67 (19)		35.14 (13)		17.86 (5)	
Depression	None	31.4 (254)	**<0.001**	27.64 (157)	**0.034**	12.87 (65)	**0.048**
	Antenatal	50.88 (29)		45.19 (14)		17.39 (4)	
	Persistent	33.80 (24)		37.5 (18)		18.42 (7)	
	Postnatal	61.90 (13)		57.14 (4)		50.0 (3)	

		Lowest tertile sIgA (% yes) n=314/951		UV for AW from 1-3 Years n=193/654	ChiX²**	UV for AW from 4-5 Years n=79/572	ChiX²**
Furry Pets in the Home	No	37.84 (165)	**0.007**	27.24 (82)	0.293	16.60 (43)	0.095
	Yes	29.5 (149)		31.01 (107)		11.73 (36)	
Smoke Exposure (Prenatal)	No	31.7 (272)	**0.004**	28.83 (175)	0.244	12.78 (68)	**0.005**
	Yes	47.56 (39)		37.5 (15)		30.30 (10)	
Smoke Exposure (Postnatal)	No	31.96 (249)	0.053	27.24 (152)	**0.005**	14.02 (68)	0.852
	Yes	39.76 (66)		41.76 (38)		13.25 (11)	
Multigravida	No	34.44 (125)	0.597	31.52 (81)	0.365	12.62 (27)	0.522
	Yes	32.77 (195)		28.21 (112)		14.53 (52)	
Maternal Overweight/Obesity (During Pregnancy)	Normal	28.24 (148)	**<0.001**	27.62 (108)	0.077	13.91 (48)	0.343
	Obese	33.17 (69)		27.81 (42)		10.77 (14)	
	Overweight	45.58 (103)		38.39 (43)		17.53 (343)	
Maternal Allergy/Asthma During Pregnancy	No	32.87 (118)	0.941	26.64 (65)	0.236	10.65 (23)	0.087
	Yes	33.1 (192)		31.02 (125)		15.76 (55)	
Maternal Age (Greater than Median)	No	36.48 (174)	**0.033**	31.78 (102)	0.212	13.26 (35)	0.722
	Yes	29.93 (138)		27.33 (91)		14.29 (44)	

Chi(X^2) comparison used to investigate whether distributions of categorical variables differ from one another. Fisher's exact test was used when expected frequencies were <5 in >20% of cells. **Bold indicates a significant difference between the two populations

2.4.3.1 Comparison of sIgA Levels and AW

Due to the skewed nature of the sIgA data, to compare median fecal sIgA levels between AW groups, non-parametric tests (Mann-Whitney U). Infant sIgA levels were only significantly lower in those infants who had UVs for AW in the first 3 years of life ($p<0.001$), non-atopic AW ages 1-3 ($p<0.001$), and infants with atopic AW ($p=0.04$) compared to those without AW from ages 1-3 (Figure 2.2). AW groups from ages 4-5 did not have significantly different levels of sIgA in stool (Appendix B; Figure B1).

Median sIgA levels were 0.96 mg/g feces lower for those with a physician diagnoses of asthma compared to those who did not in the first 3 years of life. Children ages 1-3 years who had an UVs for AW had median sIgA levels 2 mg/gfeces lower, those with atopic AW had 2.4 mg/gfeces lower and those with non-atopic AW had 2.3 mg/gfeces lower than those without AW. No other significant differences between median levels of sIgA were found (Appendix B, Figure B3).

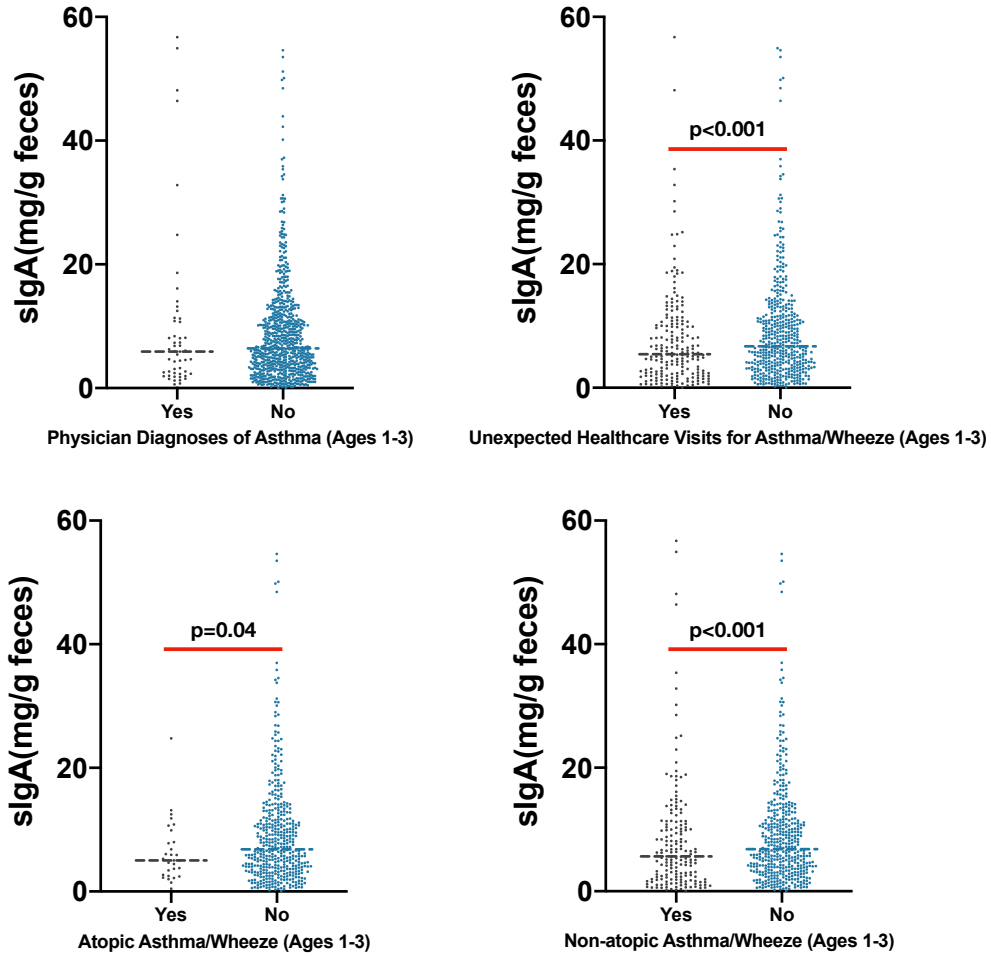

Figure 2.2. sIgA levels among AW groups from ages 1-3. UVs for AW, atopic AW and non-atopic AW had significantly lower fecal sIgA levels compared to the reference group of those without AW. P-values indicate significant differences between groups based on Mann-Whitney U tests.

2.4.4 Breastfeeding Status and sIgA Levels in Stool

Breastfeeding had a significant relationship to fecal sIgA levels. Low fecal sIgA levels were found in 68% of infants who were not breastfed, 31% of infants who were partially breastfed and 14% of infants who were exclusively breastfed ($p<0.001$) (Table 2.1). Fecal sIgA levels are highly associated with breastfeeding status (Appendix B; Figure B2). As such, we treated the exposure variable as follows to determine the

differential and combined associations between maternal sIgA supplementation via breastmilk and infant sIgA production [11]. We then created a three-way variable as follows (Figure 2.3): (1) Reference group: breastfed (mixed or exclusive) and any fecal sIgA level, (2) formula-fed, top two tertiles (normal-high) fecal sIgA, (3) formula-fed, lowest tertile (low) fecal sIgA.

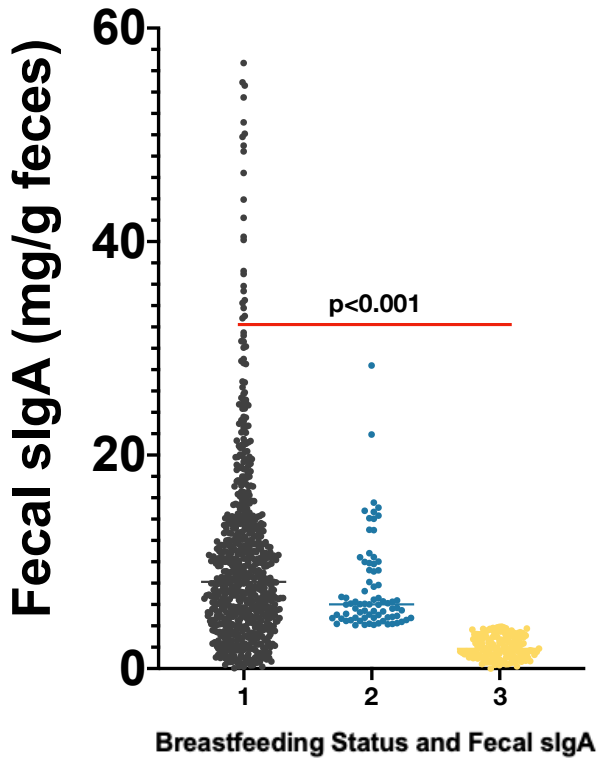

Figure 2.3. Fecal sIgA levels and breastfeeding status. (1) Breastfed, any fecal sIgA level (median: 8.00 mg/gfeces, SD: 9.89). (2) Formula-fed, top two tertiles of fecal sIgA (median: 6.01 mg/gfeces, SD: 4.25). (3) Formula-fed, lowest tertile of fecal sIgA (median: 1.86 mg/gfeces, SD: 1.03). P-values indicate significant differences between groups based on Kruskal-Wallis Test.

2.4.5 Simple and multiple logistic regression analyses

2.4.5.1 Fecal sIgA and Asthma and UVs for AW in the First Three Years of Life

Low fecal sIgA was associated with an increased odds for a physician diagnoses of asthma and UVs for AW in formula fed infants. The crude OR for asthma at 1-3 years or age when formula-fed with low fecal-sIgA was 2.40 (95% CI: 1.23, 4.66; Table 2.3), which means that with this exposure, when compared to breastfed infants with any levels of sIgA, the likelihood of physician diagnosed asthma in the first 3 years of life is 2.40 times greater than when infants are breastfed. In comparison, those who were formula-fed with normal to high fecal sIgA did not have significantly increased odds for asthma when compared to breastfed infants (OR: 1.43; 95% CI: 0.45, 4.55) (Table 2.3). When assessing odds of having an UV for AW in the first 3 years of life, formula-fed, low-fecal sIgA infants had significantly increased odds (OR: 1.64; 95% CI: 1.03, 2.61) (Table 2.4).

The final, adjusted regression model for asthma diagnoses in the first 3 years of life showed a 2.13 times (95% CI: 1.03, 4.43) increase in the odds have having an asthma diagnosis with the exposure of formula-feeding and low sIgA (Table 2.3). The final regression model for having an UVs for AW in the first 3 years of life had marginal significance (OR: 1.39; 95% CI: 0.84, 2.31), when compared to infants who were breastfed and had any level of fecal sIgA (Table 2.4). All final models adjusted for maternal depression trajectories, multigravida, maternal overweight or obesity during pregnancy, newborn antibiotics, prenatal smoke exposure.

2.4.5.2 Fecal sIgA and Asthma and UVs for AW in the Fourth and Fifth Years of Life

The same exposures were used to determine the odds of asthma diagnoses in the 4th and 5th years of life. The crude OR for odds of asthma diagnosis in ages 4-5 when formula-fed and with low fecal sIgA was 1.61 (95% CI: 0.78, 3.44) compared to those breastfed with any fecal sIgA level (Table 2.5). Formula-fed infants with normal to high fecal sIgA also did not have significantly increased odds for asthma diagnosis from ages 4-5 when compared to breastfed infants (OR: 1.15; 95% CI 0.39, 3.44) (Table 2.5). Adjusted odds ratios were also not significant.

The crude associations for formula fed infants with normal-high or low fecal sIgA on UVs for AW in the 4th and 5th years of life were also not significant. For those infants who were formula-fed and had low sIgA, there was a non-significant increase in odds for AW (OR: 1.23; 95% CI: 0.67, 2.38) compared to breastfed infants with any sIgA levels. In the same model, those who were formula-fed with normal to high levels of sIgA also had a non-significant increase in the odds of AW when compared to breastfed infants with any level of sIgA (OR: 1.25; 95% CI: 0.53, 2.95) (Table 2.6).

The finals models contained adjustment for covariates as in the models for ages 1-3 years above. Diagnosis of asthma in infants 4-5 years of age using adjusted models had no significant ORs for formula-fed, low sIgA (OR: 1.34; 95% CI: 0.58, 3.08) and formula-fed, normal to high sIgA (OR: 1.31; 95% CI: 0.41, 4.17) when compared to the reference group of breastfed infants, any level of sIgA (Table 2.5). The final regression model for having an UVs for AW in the 4th through 5th years of life had no significance in relation to formula-fed, low fecal sIgA (OR: 1.09; 95% CI: 0.53, 2.23) when compared to infants who were breastfed with any fecal sIgA level (Table 2.6). Summary forest plot of adjusted ORs for formula fed, low fecal sIgA compared to breastfed infants is in figure 2.4.

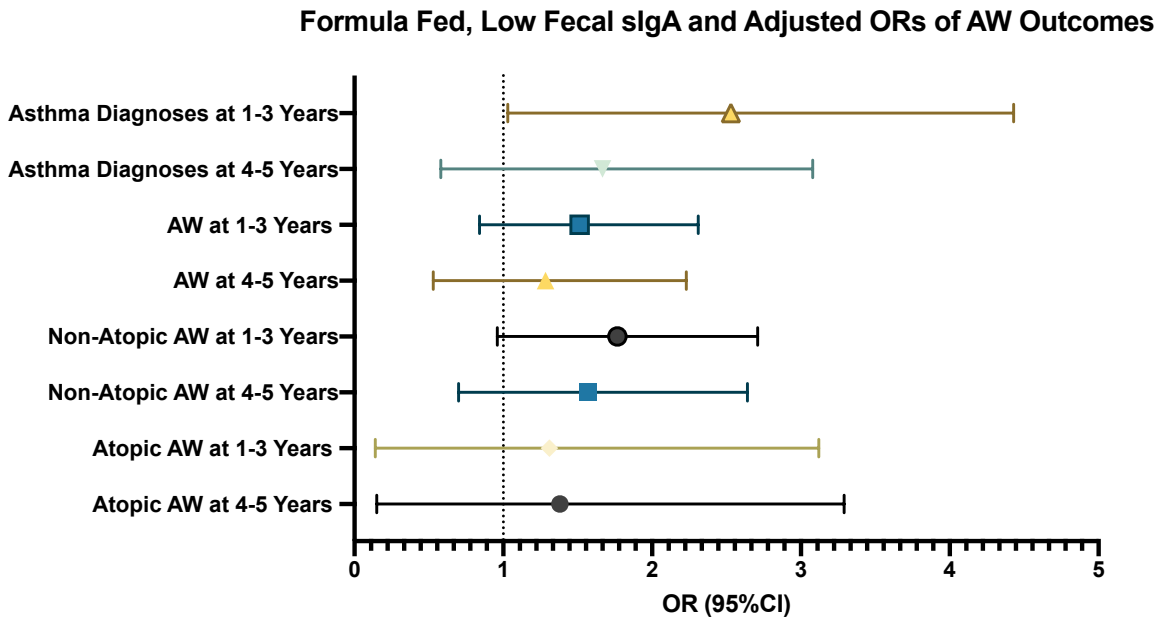

Figure 2.4. Forest plot of adjusted ORs for various AW outcomes (Tables 2.3-10) when formula fed with low fecal sIgA when compared to breastfed infants, adjusting for maternal depression (CESD Trajectories), multigravida, prenatal smoking, maternal weight during pregnancy, infant antibiotics.

2.4.5.3 Fecal sIgA and Atopic AW

In contrast to previous associations, normal-high fecal sIgA while formula fed was associated with increased odds of atopic AW compared to breastfed infants. Atopic sensitization (positive skin prick test) was used to determine the difference between atopic AW and non-atopic AW at 1-3 and 4-5 years of age.

Atopic and non-atopic AW were compared those without AW in separate models. In the following models, when infants were formula fed and had low fecal sIgA, they had marginally significantly increased odds (p=0.054) for non-atopic AW at age 1-3 years, compared to breastfed infants in crude models (OR: 1.63; 95% CI: 0.98, 2.71) (Table 2.8). This suggests that with the exposure of formula feeding and low fecal sIgA,

the likelihood of the infant having non-atopic AW in the first 3 years of life was 1.63 times greater than breastfed infants.

A different association was seen in atopic AW. Infants who were formula-fed and had normal to high fecal sIgA levels were at increased odds for atopic AW from ages 1-3 in the crude associations (OR: 2.89; 95% CI: 1.01, 8.30), when compared to breastfed infants. In the final adjusted models, the association was strengthened (OR: 5.45; 95% CI: 1.69, 17.31) (Table 2.7). In the older age group (ages 4-5), the associations were insignificant but in the same direction (Table 2.9-10; Figure 2.5).

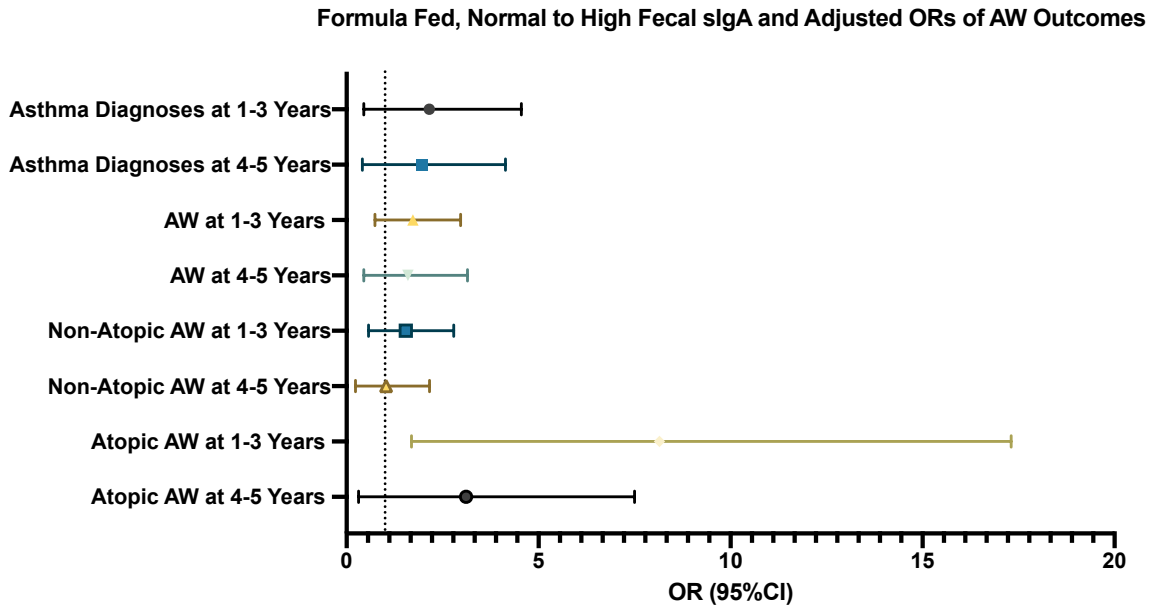

Figure 2.5. Forest plot of adjusted ORs for various AW outcomes (Tables 2.3-10) when formula fed with normal to high fecal sIgA when compared to breastfed infants, adjusting for maternal depression (CESD Trajectories), multigravida, prenatal smoking, maternal weight during pregnancy, infant antibiotics

Table 2.3. Likelihood (OR, 95% CI) of Child Asthma Diagnoses (1-3 Years Old) by Breastfeeding Status and Fecal sIgA Levels

	Asthma Diagnoses at 1-3 Years Crude OR (95% CI)	Asthma Diagnoses at 1-3 Years Adjusted OR (95% CI) (n=684)*
BF * Fecal sIgA (2-5.5 months) (Ref: Breastfed, any fecal sIgA level)		
Formula-fed, Normal-High Fecal sIgA	1.29 (0.44, 3.79)	1.43 (0.45, 4.55)
Formula-fed, Low Fecal sIgA	**2.40 (1.23, 4.66)**	**2.13 (1.03, 4.43)**
CESD Trajectories		
Antepartum (high levels during pregnancy) or Persistent (high levels throughout pregnancy and postpartum up to 2yrs of infant age)	**4.24 (1.97, 9.15)**	**5.29 (2.24, 12.05)**
Postpartum (high levels postpartum)	**2.94 (1.29, 6.68)**	**2.75 (1.13, 6.66)**
Multigravida	0.99 (0.56, 1.77)	1.17 (0.62, 2.25)
Prenatal Smoke Exposure	**3.09 (1.36, 6.98)**	**2.37 (1.13, 6.66)**
Maternal Overweight/Obesity (During Pregnancy)		
Overweight	1.28 (0.66, 2.47)	1.15 (0.70, 2.98)
Obesity	0.71 (0.31, 1.65)	0.45 (0.18, 1.17)
Infant Antibiotics	1.10 (0.33, 3.70)	0.81 (0.23, 2.90)

*Adjusted for maternal depression (CESD Trajectories), multigravida, prenatal smoking, maternal weight during pregnancy, infant antibiotics. **Bold** indicates statistical significance $p<0.05$

Table 2.4. Likelihood (OR, 95% CI) of an UV for AW (1-3 Years Old) by Breastfeeding Status and Fecal sIgA Levels

	AW at 1-3 Years Crude OR (95% CI)	AW at 1-3 Years Adjusted OR (95% CI) (n=608)*
BF * Fecal sIgA (2-5.5 months) (Ref: Breastfed, any fecal sIgA level)		
Formula-fed, Normal-High Fecal sIgA	1.47 (0.79, 2.76)	1.48 (0.74, 2.97)
Formula-fed, Low Fecal sIgA	**1.64 (1.03, 2.61)**	1.39 (0.84, 2.31)
CESD Trajectories		
Antepartum (high levels during pregnancy) or Persistent (high levels throughout pregnancy and postpartum up to 2yrs of infant age)	**2.36 (1.21, 4.57)**	2.00 (0.98, 4.08)
Postpartum (high levels postpartum)	1.57 (0.85, 2.90)	1.35 (0.69, 2.68)
Multigravida	0.85 (0.61, 1.20)	0.78 (0.53, 1.13)
Prenatal Smoke Exposure	1.48 (0.76, 2.88)	1.33 (0.64, 2.76)
Maternal Overweight/Obesity (During Pregnancy)		
Overweight	1.00 (0.66, 1.54)	1.00 (0.63, 1.60)
Obesity	**1.63 (1.05, 2.54)**	1.42 (0.87, 2.30)
Infant Antibiotics	1.36 (0.68, 2.74)	1.28 (0.62, 2.67)

*Adjusted for maternal depression (CESD Trajectories), multigravida, prenatal smoking, maternal weight during pregnancy, infant antibiotics. **Bold** indicates statistical significance $p<0.05$

Table 2.5. Likelihood (OR, 95% CI) of Child Asthma Diagnoses (4-5 Years Old) by Breastfeeding Status and Fecal sIgA Levels

	Asthma Diagnoses at 4-5 Years Crude OR (95% CI)	Asthma Diagnoses at 4-5 Years Adjusted OR (95% CI) (n=453)*
BF * Fecal sIgA (2-5.5 months) (Ref: Breastfed, any fecal sIgA level)		
Formula-fed, Normal-High Fecal sIgA	1.15 (0.39, 3.44)	1.31 (0.41, 4.17)
Formula-fed, Low Fecal sIgA	1.61 (0.78, 3.44)	1.34 (0.58, 3.08)
CESD Trajectories		
Antepartum (high levels during pregnancy) or Persistent (high levels throughout pregnancy and postpartum up to 2yrs of infant age)	**5.12 (2.10, 12.51)**	**5.78 (2.21, 15.13)**
Postpartum (high levels postpartum)	2.10 (0.77, 5.75)	2.57 (0.88, 7.54)
Multigravida	0.64 (0.36, 1.15)	0.61 (0.32, 1.17)
Prenatal Smoke Exposure	2.43 (0.95, 6.23)	1.63 (0.54, 4.94)
Maternal Overweight/Obesity (During Pregnancy)		
Overweight	0.46 (0.19, 1.12)	0.58 (0.23, 1.48)
Obesity	0.95 (0.44, 2.07)	0.82 (0.35, 1.95)
Infant Antibiotics	0.77 (0.18, 3.36)	0.62 (0.13, 2.92)

*Adjusted for maternal depression (CESD Trajectories), multigravida, prenatal smoking, maternal weight during pregnancy, infant antibiotics. **Bold** indicates statistical significance $p<0.05$

Table 2.6. Likelihood (OR, 95% CI) of an UV for AW (4-5 Years Old) by Breastfeeding Status and Fecal sIgA Levels

	AW at 4-5 Years Crude OR (95% CI)	AW at 4-5 Years Adjusted OR (95% CI) (n=486)*
BF * Fecal sIgA (2-5.5 months) (Ref: Breastfed, any fecal sIgA level)		
Formula-fed, Normal-High Fecal sIgA	1.25 (0.53, 2.95)	1.19 (0.45, 3.15)
Formula-fed, Low Fecal sIgA	1.23 (0.64, 2.38)	1.09 (0.53, 2.23)
CESD Trajectories		
Antepartum (high levels during pregnancy) or Persistent (high levels throughout pregnancy and postpartum up to 2yrs of infant age)	2.15 (0.88, 5.24)	1.92 (0.74, 4.94)
Postpartum (high levels postpartum)	1.53 (0.65, 3.61)	0.76 (0.24, 2.40)
Multigravida	1.18 (0.71, 1.94)	1.18 (0.69, 2.03)
Prenatal Smoke Exposure	**2.97 (1.35, 6.50)**	**2.84 (1.18, 6.84)**
Maternal Overweight/Obesity (During Pregnancy)		
Overweight	0.75 (0.40, 1.41)	0.48 (0.23, 1.04)
Obesity	1.31 (0.72, 2.41)	1.01 (0.52, 1.98)
Infant Antibiotics	1.35 (0.50, 3.68)	1.03 (0.34, 3.17)

*Adjusted for maternal depression (CESD Trajectories), multigravida, prenatal smoking, maternal weight during pregnancy, infant antibiotics. **Bold** indicates statistical significance $p<0.05$

Table 2.7. Likelihood (OR, 95% CI) of Atopic AW vs. No AW (1-3 Years Old) by Breastfeeding Status and Fecal sIgA Levels

	Atopic AW at 1-3 Years Crude OR (95% CI)	Atopic AW at 1-3 Years Adjusted OR (95% CI) (n=378)*
BF * Fecal sIgA (2-5.5 months) (Ref: Breastfed, any fecal sIgA level)		
Formula-fed, Normal-High Fecal sIgA	**2.89 (1.01, 8.30)**	**5.45 (1.69, 17.31)**
Formula-fed, Low Fecal sIgA	0.58 (0.13, 2.49)	0.67 (0.14, 3.12)
CESD Trajectories		
Antepartum (high levels during pregnancy) or Persistent (high levels throughout pregnancy and postpartum up to 2yrs of infant age)	2.72 (0.75, 9.89)	3.40 (0.78, 14.78)
Postpartum (high levels postpartum)	1.30 (0.29, 5.81)	1.63 (0.33, 8.00)
Multigravida	0.63 (0.29, 1.39)	0.49 (0.20, 1.20)
Prenatal Smoke Exposure	1.46 (0.33, 6.56)	1.13 (0.19, 6.61)
Maternal Overweight/Obesity (During Pregnancy)		
Overweight	1.14 (0.46, 2.86)	1.33 (0.48, 3.67)
Obesity	0.77 (0.22, 2.73)	0.79 (0.20, 3.18)
Infant Antibiotics	1.42 (0.60, 3.43)	Omitted

*Adjusted for maternal depression (CESD Trajectories), multigravida, prenatal smoking, maternal weight during pregnancy, infant antibiotics. **Bold** indicates statistical significance $p<0.05$

Table 2.8. Likelihood (OR, 95% CI) of Non-atopic AW vs. No AW (1-3 Years Old) by Breastfeeding Status and Fecal sIgA Levels

	Non-Atopic AW at 1-3 Years Crude OR (95% CI)	Non-Atopic AW at 1-3 Years Adjusted OR (95% CI) (n=526)*
BF * Fecal sIgA (2-5.5 months) (Ref: Breastfed, any fecal sIgA level)		
Formula-fed, Normal-High Fecal sIgA	1.39 (0.69, 2.81)	1.26 (0.57, 2.79)
Formula-fed, Low Fecal sIgA	**1.95 (1.23, 3.13)**	1.63 (0.98, 2.71)
CESD Trajectories		
Antepartum (high levels during pregnancy) or Persistent (high levels throughout pregnancy and postpartum up to 2yrs of infant age)	**2.03 (1.00, 4.07)**	1.59 (0.75, 3.40)
Postpartum (high levels postpartum)	1.83 (0.99, 3.39)	1.56 (0.79, 3.07)
Multigravida	0.95 (0.66, 1.36)	0.90 (0.61, 1.34)
Prenatal Smoke Exposure	1.55 (0.78, 3.09)	1.32 (0.62, 2.79)
Maternal Overweight/Obesity (During Pregnancy)		
Overweight	1.01 (0.65, 1.57)	0.97 (0.60, 1.58)
Obesity	**1.64 (1.04, 2.06)**	1.36 (0.81, 2.26)
Infant Antibiotics	1.76 (0.87, 3.54)	1.63 (0.78, 3.40)

*Adjusted for maternal depression (CESD Trajectories), multigravida, prenatal smoking, maternal weight during pregnancy, infant antibiotics. **Bold** indicates statistical significance $p<0.05$

Table 2.9. Likelihood (OR, 95% CI) of Atopic AW vs. No AW (4-5 Years Old) by Breastfeeding Status and Fecal sIgA Levels

	Atopic AW at 4-5 Years Crude OR (95% CI)	Atopic AW at 4-5 Years Adjusted OR (95% CI) (n=390)*
BF * Fecal sIgA (2-5.5 months) (Ref: Breastfed, any fecal sIgA level)		
Formula-fed, Normal-High Fecal sIgA	1.03 (0.23, 4.64)	1.53 (0.31, 7.50)
Formula-fed, Low Fecal sIgA	0.90 (0.26, 3.17)	0.70 (0.15, 3.29)
CESD Trajectories		
Antepartum (high levels during pregnancy) or Persistent (high levels throughout pregnancy and postpartum up to 2yrs of infant age)	2.19 (0.47, 10.09)	2.47 (0.47, 12.84)
Postpartum (high levels postpartum)	0.70 (0.09, 5.44)	0.69 (0.08, 5.83)
Multigravida	1.38 (0.56, 3.44)	1.11 (0.41, 2.97)
Prenatal Smoke Exposure	2.68 (0.74, 9.68)	2.07 (0.39, 10.96)
Maternal Overweight/Obesity (During Pregnancy)		
Overweight	0.29 (0.07, 1.29)	0.34 (0.08, 1.54)
Obesity	0.65 (0.19, 2.70)	0.42 (0.09, 2.00)
Infant Antibiotics	0.90 (0.12, 7.00)	0.83 (0.10, 6.79)

*Adjusted for maternal depression (CESD Trajectories), multigravida, prenatal smoking, maternal weight during pregnancy, infant antibiotics. **Bold** indicates statistical significance $p<0.05$

Table 2.10. Likelihood (OR, 95% CI) of Non-atopic AW vs. No AW (4-5 Years Old) by Breastfeeding Status and Fecal sIgA Levels

	Non-Atopic AW at 4-5 Years Crude OR (95% CI)	Non-Atopic AW at 4-5 Years Adjusted OR (95% CI) (n=441)*
BF * Fecal sIgA (2-5.5 months) (Ref: Breastfed, any fecal sIgA level)		
Formula-fed, Normal-High Fecal sIgA	0.79 (0.30, 2.12)	0.70 (0.23, 2.16)
Formula-fed, Low Fecal sIgA	1.48 (0.80, 2.76)	1.36 (0.70, 2.64)
CESD Trajectories		
Antepartum (high levels during pregnancy) or Persistent (high levels throughout pregnancy and postpartum up to 2yrs of infant age)	2.36 (0.95, 5.87)	2.41 (0.92, 6.30)
Postpartum (high levels postpartum)	1.30 (0.52, 3.26)	0.89 (0.29, 2.74)
Multigravida	0.92 (0.56, 1.50)	1.01 (0.59, 1.72)
Prenatal Smoke Exposure	2.07 (0.89, 4.81)	2.14 (0.87, 5.28)
Maternal Overweight/Obesity (During Pregnancy)		
Overweight	1.00 (0.55, 1.82)	0.71 (0.35, 1.42)
Obesity	1.39 (0.74, 2.60)	1.11 (0.56, 2.18)
Infant Antibiotics	1.01 (0.34, 3.02)	0.66 (0.19, 2.31)

*Adjusted for maternal depression (CESD Trajectories), multigravida, prenatal smoking, maternal weight during pregnancy, infant antibiotics. **Bold** indicates statistical significance $p<0.05$

2.5 Discussion

In this subsample of 951 healthy term children from a prospective birth cohort, we found significant associations between total fecal sIgA levels in the first 5.5 months of life and development of childhood AW. After exposure to lower fecal sIgA while being formula fed, infants were at significantly increased odds for having a physician diagnoses of asthma (adjusted OR: 2.13; 95% CI: 1.03, 4.43) in the first 3 years of life compared to breastfed infants. This result is consistent with previous studies citing that lower IgA in associated with increased risk for preschool age asthma [30]. In comparison, formula fed infants that had normal-high fecal sIgA had significantly increased odds of having atopic AW (ages 1-3) (adjusted OR: 5.45; 95% CI: 1.69, 17.31) compared to breastfed infants. This study is the first to report on fecal secretory IgA levels in infants in association with physician diagnoses of asthma, UVs for AW and atopic AW in a large population-based cohort. This is a key area of interest due the high cost of health-care utilization for AW and potential that early identification may promote preventive management strategies.

There was a trend for increased odds of all AW when having low fecal sIgA while formula-fed compared to breastfed infants. Formula-fed infants who had low fecal sIgA had increased odds of having a physician diagnoses of asthma, after controlling for important covariates. Previous reports link IgA in relation to later development of childhood asthma, and our findings suggest that fecal sIgA levels are related to infant AW in early life. Early wheeze is a risk factor for later development of asthma, so this is an important finding [23]. A previous study from the CHILD cohort found that in comparison to breastfed infants, those who were formula fed had increased odds of wheezing and this was in a dose-dependent manner, so our findings add an extra level to understanding why a decrease in breastfeeding is associated with increased risk for wheezing illness [31].

As highlighted in previous reports, there are difficulties in the diagnoses of preschool asthma, and the diagnosis is even less clear when it is an unexpected healthcare utilization [32]. One reason that fecal sIgA may be of value in diagnosis of asthma or an unexpected health care utilization for AW at an early age, is that IgA levels are suspected to be more related to "variable" asthma which is mediated by inflammation than "persistent" asthma caused by remodelling which is more prevalent in older age groups [31]. Furthermore, sIgA is an important factor for prevention of infection from viruses and various respiratory diseases in early life [32]. As asthma/wheezing illness in the first few years of life is largely related to respiratory infections and mucosal sIgA has been shown to play a role in prevention of respiratory infections, one explanation for our associations is that low fecal sIgA responses to microbiota could cause reduced mucosal barrier function and increased susceptibility to airway viral infections and later development of asthma [5,33]. A previous report by Dzidic et al., (2018) showed that although total sIgA levels were not related to asthma, subtle differences in microbe binding to sIgA was linked to asthma development [13]. In this study, infants were breastfed at the early time point of stool collection (1 month) and thus the maternal source of sIgA would be relevant. Feeding practices have a large effect on early life mucosal immune development and the presence of maternal milk IgA is important for the early development of mucosal immunity that is thought to protect from later development of asthma [34–37].

We found that normal-high fecal sIgA in formula fed infants was associated with atopic AW compared to breastfed infants. It has been previously reported that higher fecal IgA is associated with decreased risk for atopic disease. Kukkonen et al., (2010) showed that high fecal IgA concentration at the age of 6 months associated with decreased risk for IgE-mediated allergic disease up to 2 years of infant age, and breastfeeding had no significant confounding effects [12]. This study used a less

refined measure of total fecal IgA which may include both serum and secretory forms of IgA when the infant's mucosal barrier is not fully developed, whereas our study measured sIgA specifically, perhaps explaining our differential results. As well, there are many reports that show that high levels of mucosal sIgA could interfere with the interaction between allergens and IgE antibodies in sensitized individuals, thereby preventing allergic inflammation and clinical symptoms [32]. Despite these previous findings, it is plausible that higher fecal sIgA when formula fed may be a marker for aberrant responses of the infant immune system to microbiota, though normal to high median levels of fecal sIgA in formula fed infants were still lower than in breastfed infants with any fecal sIgA level. In 2019, Gopalakrishna et al., found that infants who were formula fed had a higher proportion of IgA that was unbound to microbes, which was associated with an increase in *Enterobacteriaceae* in the microbiota [38]. Proinflammatory Protebacteria like *Enterobacteriaceae* have also been shown to be increased in formula-fed infants compared to breastfed infants and development of a proper response of sIgA to Proteobacteria aided by passive sIgA from mothers may limit a chronic inflammatory response in infants [34,38,39,40,56]. Proteobacteria have also shown to induce IgA [41]. As well, in our CHILD cohort, previous research has shown that *Enterobacteriaceae* are over-represented in those with development of subsequent food sensitization [42]. Studies in mice models that manipulated sIgA production and breastfeeding schemes show that maternally-derived sIgA has a strong influence on infant microbiome development that was only magnified when mice reached adulthood [34]. These findings seem to indicate the importance of receiving maternally derived sIgA when the microbiome is developing. More research needs to be done to compare formula-fed infants to their breastfed counterparts to determine how their sIgA-mediated immune responses differ in relation to microbiota composition and future risk for AW.

As we hypothesized, birth characteristics and the environment appeared to affect the relationship between fecal sIgA levels and AW. We confirmed the associations between various birth characteristics on sIgA levels. Birth mode is highly associated with antibiotic exposure, and caesarean birth has additional effects on the microbiome, reducing the diversity and altering the composition in a manner which may have long-lasting effects on the immune system and IgA production [43]. A direct link between changes in the gut microbiota due to early-life antibiotic exposure and the immune response towards allergens has been confirmed by human and murine studies [44,45]. At the same time, caesarean section is associated with an increased risk for development of asthma during childhood and hospitalization due to respiratory syncytial virus infection in infancy [35,46,47]. An inverse association has been made with breastfeeding and asthma and hospitalizations for asthma [48,49].

Maternal obesity and overweight during pregnancy were also associated with decreased levels of fecal sIgA, possibly mediated through both increased risk for caesarean section which has great impacts on the microbiome and the separate effects of maternal-infant transmission of an obese microbiome to the infant [50]. Interestingly, maternal prenatal smoke exposure was more significantly associated with low fecal sIgA than postnatal smoke exposure. Two studies in particular have revealed significant changes in the gut microbiome as a result of smoking and another identified lower salivary sIgA in infants from mothers who smokers [51–53]. As well, mothers with exposure to tobacco smoke pass a significant amount of metabolites to the infant which may affect perinatal immune system programming and have long term impacts via maternal-infant fecal-oral transmission of the microbiome during birth [54,55]. Additionally, we found strong associations between maternal depression trajectories and sIgA. Previous studies from our lab, found that infants with higher exposure to maternal depressive symptoms pre and post-natally had lower fecal sIgA levels in the

first few months of life compared to those who did not [1]. Interestingly, the odds ratio for the maternal depression trajectories increased in significance in some fully-adjusted models, suggesting that sIgA may mediate the associations between maternal depression in the pre and postnatal periods and early childhood AW.

These study findings are encouraging for nursing mothers and physicians, in that the current recommendations regarding breastfeeding appear to support the development of a healthy infant mucosal immune system, which is associated with decreased odds for AW and atopic AW in early life compared to formula fed infants. Additionally, despite this, being formula fed and having low/normal-high fecal sIgA may not be a clinically significant predictor despite its statistical significance. Further research confirming this relationship is needed to fully understand the influence of fecal sIgA on development of atopic disease.

The CHILD study was designed to evaluate the developmental origins of health and disease, specifically in relation to allergy and asthma. The prospective cohort design has a number of strengths and weakness. Due to the prospective nature of this study, this study can support the temporality of the relationship between infant fecal sIgA and childhood AW and maternal report of asthma is a well validated method [23,24]. Although this cohort study was able to recruit over 3500 mothers, enough to conduct various stratification analyses with potential covariates, a limitation of this study is that stool samples were only available for 1,071 individuals which decreased our sample size significantly, despite still having adequate power to determine these associations. We also had limited information regarding environmental triggers, and our sIgA detection kit does not discriminate between sIgA free or bound to microbes which may be important based on the results of Dzidic et al., (2017) [13]. In spite of these limitations, this is a rigorous cohort study which provides important information on a potential biomarker for childhood atopic disease development. Future studies on

infant immune system development may be beneficial to confirm if fecal sIgA levels are a causative agent in this process or if it is merely a marker for aberrant gut microbiota composition and/or immune system development. Ultimately, lower fecal sIgA while formula fed is associated with reduced odds for AW in early life (Fig 2.2), whereas normal-high fecal IgA while formula fed is associated with increased odds for atopic AW compared to breastfed infants. Clearly, this is a complex topic and there are differential associations between atopic and non-atopic AW that need to be further elucidated.

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3.0 Study 2: Serum Immunoglobulin A in Relation to Emergency Department Visits for Asthma/Wheeze in Childhood

3.1 Abstract

Background. There is a large body of evidence on the association between serum immunoglobulin A (IgA), asthma, and other atopic diseases. Many previous studies have shown an association between absence of serum IgA and increased risk for asthma. Kim et al., (2017) recently showed that in a cohort of adult patients with suspected asthma, low serum IgA levels were positively related to atopic sensitisation and airway hyperresponsiveness. In contrast, Possin et al., (2011) showed that serum IgA levels were increased in those with food sensitization, but not in those with sensitizations to aeroallergens, despite the IgA levels being normal for their age. In our study, we determined associations between serum IgA in relationship to Emergency Department (ED) visits for asthma and/or wheeze (AW) and atopic AW in childhood.

Objective. The objective of this study is to determine if low serum IgA children is a useful biomarker for future ED visits for AW.

Methods. Anonymized administrative health data of 9,938 children who had serum IgA levels assessed when they were $\leq$3 years of age between April 1, 2013 and June 30, 2018 was obtained for analysis from Alberta Health Services (AHS) (Alberta, Canada). Serum IgA levels were quantified using standardized provincial lab tests. Child ED visits for AW were determined via National Ambulatory Care Reporting System using ICD-10-CA codes from ages 0-5. Using STATA v16, multiple logistic regression models determined the association (Odds Ratio, OR) between normal to

high serum IgA (top two tertiles compared to the lowest tertile) and child ED visits for AW, atopic AW, non-atopic AW, and atopic asthma and non-atopic asthma adjusting for covariates identified by directed acyclic graph.

Results. Approximately 11.4% of children had an ED visit for AW until age 3. Median levels of serum IgA from ages 1-2 years were 0.05g/L higher in those children with an ED visit for AW until age 3 (p=0.10) than in those without. When compared to infants with low serum IgA levels, infants with normal-high serum IgA levels (ages 1-2) had an adjusted OR of having an ED visit for AW of was 1.21 (adjusted 95% CI: 1.00, 1.46), controlling for confounding factors. Those with normal-high levels (from 2-3 years) also had significantly increased odds of atopic AW (adjusted OR: 1.79 (95% CI: 1.03, 3.09) when compared to those without.

Conclusions. Normal-high levels of serum IgA in the first 3 years of life appear to be positively associated with ED visits for AW and atopic AW from until age 3. Further studies are needed to define the relationships between serum IgA and ED visits for AW and atopic sensitization which may provide new insight into the development of respiratory disease and atopic illness in childhood.

3.2 Introduction

Mucosal IgA, also known as secretory IgA (sIgA), has a role in prevention of infection and potentially in development of atopic disease [1–5]. In addition to the conventional understanding that mucosal antibodies act as neutralizing antibodies to exclude antigens and pathogens, sIgA in the gut also has antigen sampling functions and helps promote colonization of commensal bacteria in the gut microbiota [6,7].

Despite the emerging and potentially relevant roles of sIgA in prevention of asthma, respiratory infections and atopic disease, the literature on the relationships between these diseases and levels of serum (i.e. blood) IgA is less clear. One explanation that serum IgA levels may be less associated with asthma is that they only make up approximately 7% of the total IgA in the body and only loosely correlate to levels of sIgA, which is the main mediator of mucosal immunity [8,9]. One study in particular showed this using germ-free mice, revealing that with the lack of microbial stimulation of sIgA, there was an absence of gut sIgA but retention of up to half of the regular serum IgA levels [10].

Regardless of the functional differences between secretory and serum IgA, serum IgA has also previously been associated with increased risk for asthma and atopic sensitization. In 2013, Celani et al. completed a prospective cohort study of individuals with IgA deficiency, defined as an undetectable serum IgA level (<0.05 g/L), to determine their future risk of asthma and allergic diseases. Their findings suggest an increased risk of not only recurrent respiratory infections, but also atopic disease and asthma, as compared to the general population [4]. Further evidence from another study revealed that when serum IgA levels were low in infancy, asthma was more common at age 7 [11]. In contrast, other research has shown that higher serum IgA is related to an increased risk of atopic sensitization [12]. Clearly further research is

needed on the development of IgA in early life and future risk for asthma and atopic disease.

Asthma exacerbations and wheeze are frequent in childhood, can result in visits to the Emergency Department (ED), and account for a substantial amount of annual health care expenditures [13]. Pediatric asthma guidelines exist (www.cps.ca, www.worldallergy.org), but the control of asthma and wheeze in children is often poor and acute attacks of asthma or wheeze often necessitate visits to the ED. As such, it is important to study risk factors and potential biomarkers that identify odds of future asthma development risk, as well as risk for ED visits. To date, none have studied serum IgA in relation to ED visits for AW in childhood.

The objective of this study is to determine whether children with low serum IgA in the first few years of life have increased risk for ED visits for AW or atopic asthma and/or wheeze compared to those with normal to high serum IgA levels at the same time using a large, population-based administrative health database. This association is assessed while controlling for failure to thrive (FTT) and sex to attempt to determine the relationship between serum IgA and ED visits for asthma and wheeze.

3.3 Methods

3.3.1 Data Sources

This study used Alberta Health Services (AHS) anonymized administrative level health data from Alberta, Canada. Patients who had a serum IgA level assessed when they were $\leq$3 years of age during a period between April 1, 2013 and June 30, 2018 and had adequate follow up time for each of the outcomes were selected for analysis. Alberta is a province with >4 million residents and a uniform single-payer health system—the Alberta Health Care Insurance Plan (AHCIP)—that provides medically

necessary health care. The Government of Alberta is the custodian of all administrative databases used in our study. The University of Alberta Health Research Ethics Board – Health Panel approved this study (#Pro00083778) and waived participant consent since data was aggregated and anonymized to protect patient privacy. The National Ambulatory Care Reporting System (NARCS) collects information on all ED presentations and services delivered within acute care institutions in Alberta using the International Classification of Diseases diagnostic codes (Canadian Version); ICD-10-CA for April 2002-present. Each unique service contains a unique patient identifier, ED visit start/end and dates/times, diagnosis, and disposition. Additional health record level data was extracted from the Alberta provincial lab, National Ambulatory Care Reporting System (NACRS) (ED visits only) and practitioner claims (PC) (outpatient visits only). All extracted data sets contained a unifying linking identifier (ULI) which was coded as an anonymized patient identifier. Provincial lab data included serum IgA and IgE levels, the date at which the test was done, patient sex and age at the time of the test, the name of the test, the result of the test and the units of the lab test. NARCS contained data on dates of ED, ICD-10CA diagnoses codes corresponding to the ED visit, procedure codes and disposition of the ED visit. The final data set, CLM (Alberta Health Practitioner Claims) contained physician billing claim data including: date of physician billing claim, diagnoses corresponding to billing claim and health service codes which identify services performed by the health care practitioner.

3.3.2 Child AW

A pediatric AW-related visit was defined as an ED encounter that resulted in a diagnosis of asthma or wheeze (ICD-10 diagnostic codes J45.x [asthma all forms], J209 [acute bronchitis], J218 [acute bronchiolitis] and R062 [wheezing all forms] in the NARCS two first diagnosis fields). The study population was restricted to individuals

aged 0 to 5 years and who had adequate follow up time at the study end; this time varied between outcomes. Children were considered to have had asthma or wheeze if they were assigned any of these diagnostic codes within the first two principal diagnoses fields. All ED presentations were assigned a disposition code according to the manner in which they left the ED, including discharged, left against medical advice or without being seen, admitted as an inpatient, transferred to another institution, or death. Only the ED presentations with the dispositions "discharged" were used for this study to ensure the proper final diagnoses code was assigned for the ED visit.

Asthma trajectories include transient, persistent, atopic and non-atopic phenotypes, so this project included asthma variables that account for these trajectories, also taking into account how asthma and wheeze are currently diagnosed in the ED [12]. Since the diagnoses of asthma in children under the age of 5 is not straightforward, ED physicians may prefer to use terms to describe symptoms rather than assigning disease classification, so we combined the asthma/bronchiolitis/bronchitis/wheeze as an outcome of AW from ages 1 until then end of age 3 years and ages 4-5 years. Pediatric atopic AW and atopic asthma was determined by combining being in the highest tertile level of IgE ($\geq$27.2 kU/L) with AW/asthma to create an atopic AW/asthma dichotomous variable. Pediatric non-atopic AW/asthma was determined by combining not being in the highest tertile level of IgE (<=27 kU/L) with AW/ asthma to create an non-atopic AW/asthma dichotomous variable.

3.3.3 Exposures

3.3.3.1 Extraction and Analysis of IgA

Immunoglobulin measurement was performed as part of routine sample analysis by laboratories contracted to AHS. The *in vitro* serum IgA diagnostic kit

"Reagent Atellica® CH Immunochemistry / Specific Protein Test Immunoglobulin A For Atellica® CH Analyzer 1600 Tests 2 X 9.3 mL" was used following the kit protocol. Serum samples were drawn through standard phlebotomy techniques and samples delivered to the laboratory using standard clinical practice.

Serum IgA changes rapidly during the neonatal period, especially in the 1st year of life, and reaches stable adult levels around 16 years of age. Scatterplot of serum IgA levels by age is shown in Appendix C, Figure C1. As such, we stratified our analysis to serum IgA levels from ages 0 to 1, 1 to 2 and 2 to 3 years. In order determine the effect of serum IgA at each of these age ranges and ED visits for AW, we used the following variable at each age strata: 1) children with low serum IgA levels (lowest tertile) and 2) children with normal to high serum IgA levels (top two tertiles). The groups were mutually exclusive.

3.3.3.2 Definition of Potential Covariates

Covariates investigated in this study were age at serum IgA collection, age at ED visit for asthma or wheeze, sex, diagnosis of failure to thrive, IgE level and age at IgE level. Serum was extracted using standard phlebotomy techniques and the diagnostic kit used in provincial labs for determination of serum IgE levels was "Atellica® IM Total IgE (tIgE) Assay For Atellica® IM Analyzer 1400 Tests 2 X 9.3 mL". These variables were created from data obtained from the AHS provincial laboratory data, National Ambulatory Care Reporting System (NACRS) (ED visits only) and practitioner claims (PC) (outpatient visits only) and linked together by unique patient identifiers. *Sex* was designated by physician reported sex. *Immunoglobulin levels E and age at immunoglobulin level* were based on provincial lab data and age at the time of measurement. *Diagnosis of failure to thrive* (FTT) was determined using PC data by

having a diagnosis in the first two health service codes of ICD9-CA code 783.4 [Lack of expected normal physiological development in childhood].

3.3.3.3 Statistical Analyses

The latest presentation per individual was selected for those with repeated ED presentations. Table 1 and Appendix C Table C1 describes the frequencies and row-percentages of each study variable in relation to the outcomes and exposure in this study. Fisher's exact or Chi χ^2 tests were run to determine if the co-variate sampling distributions varied significantly between outcome and exposure groups. A p-value of < 0.05 was considered statistically significant. IgA concentrations were not normally distributed, so non-parametric tests (Mann-Whitney U) were used to detect differences in serum IgA medians according to the AW status of the child. Only serum IgA levels before any ED for AW were used. A logistic regression models used a binomial categorical exposure of IgA as follows: (1) normal to high serum IgA levels, 2) low serum IgA levels. The top two tertiles compared the lowest tertile was used as normal-high serum IgA (yes/no). Prototype analyses for the highest tertile compared to the lowest two tertiles (high serum IgA (yes/no)) was done (data not shown).

To determine the association between serum IgA and childhood visits to the emergency department for asthma or wheeze, our analyses followed Shrier and Platt's seminal article explaining Directed Acyclic Graphs (DAGs) creation and use for epidemiological studies. DAGs were created using dagitty.com. DAGs are first build to select a minimum adjustment set of covariates to control for biasing pathways and avoid over-adjustment. Final minimally sufficient adjustment set included: Failure to Thrive, sex, stratified by age at IgA level (0-1, 1-2, and 2-3 years) and age at diagnoses (until age 3 years, 4-5 years) (Figure 1). Statistical analyses were conducted using STAT v16.0 and figures were created using Prism v8.

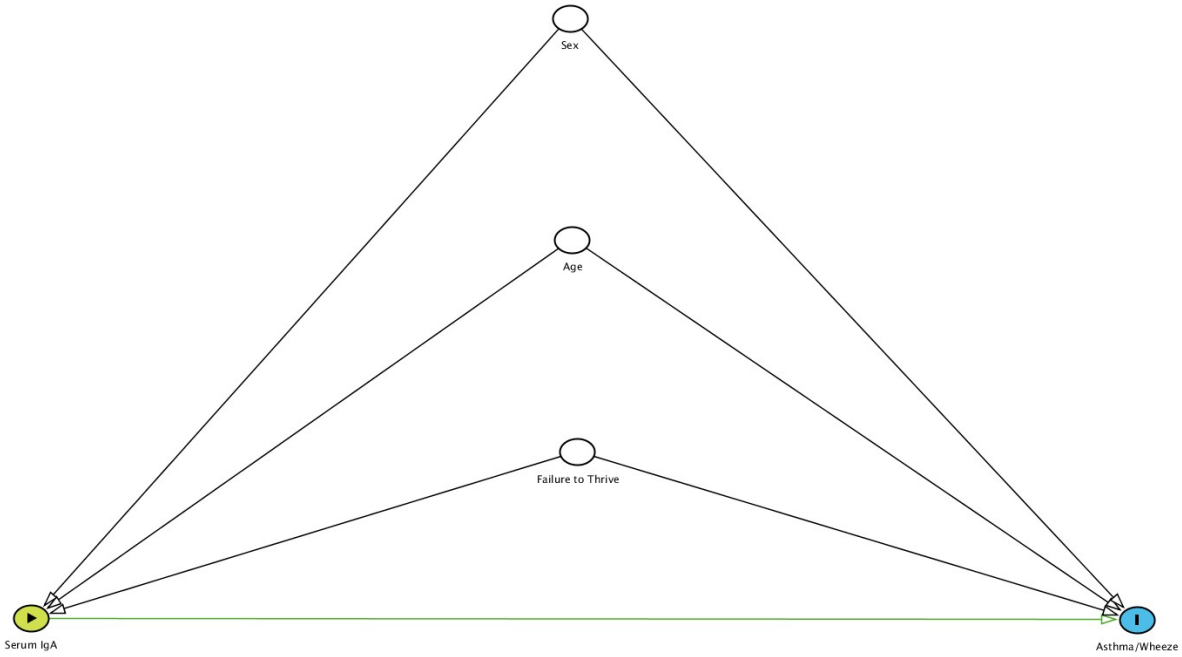

Figure 3.1. Directed Acyclic Graph (DAG) for the association between serum IgA and childhood ED visits for asthma or wheeze was built using dagitty.com. The same DAG was used for atopic AW. Green lines represent causal pathways, and red lines represent biasing paths. The minimally sufficient adjustment set represents covariates such that the adjustment for this set of variables will minimize confounding bias when estimating the association between the exposure and the outcome. The finally minimally sufficient adjustment set contained age, sex and failure to thrive. Age was taken into account by stratifying at age of ED visit and age at IgA levels.

3.4 Results

3.4.1 Study Population and ED Visits for AW

From 0 to 1 years of life, 2,040 children had a serum IgA level (median age: 0.5 years, median value: 0.23 g/L (SD: 0.38). In the 1st through 2nd years of life, there were 4,079 children with a serum IgA level with a median age of 1.5 years and median value of 0.41 g/L serum IgA (SD: 0.38). In the 2nd through 3rd years of life, there were 3,189 children with a serum IgA level with a median age of 2.5 years and median value of 0.60 g/L serum IgA (SD: 0.46). Appendix C, figure 1 shows the median level of IgA by age in years in our sample.

11.4% (until age 3 years) and 2.45% (ages 4-5 years) in our sample had ED visit for AW. 2.14% and 0.52% had atopic AW from ages 1-3 and 4-5, respectively. 3.18% and 0.37% had non-atopic AW from until age 3 and from age 4-5 years, respectively. Prevalence of atopic and non-atopic asthma were similar to atopic AW and non-atopic AW, respectively (Table 3.1). The prevalence of ED visits for AW is in line with that reported by others (www.cihi.ca).

Table 3.1 reports the distribution of normal-high children serum IgA and ED visits for AW, atopic AW and atopic asthma until age 3 years across potential covariates. Being in the top two tertiles of serum IgA from 1-2 and 2-3 years was significantly more common in males ($p<0.001$; $p=0.028$) and those without a prior diagnosis of FTT (p0.053) from ages 2-3. ED visits for AW, atopic AW and atopic asthma at until age 3 were significantly more common in male children ($p<0.001$) (Table 3.1). Most of these associations were not significant from ages 4-5 (Appendix C, Table C1). All ED presentations with a non-missing end date before June 30th, 2018 that concluded in discharge were selected for inclusion (<5% had missing end dates).

Table 3.1. Proportion of ED Visits for AW (Until age 3 years) and Potential Covariates in the 9,938 n AHS sub-Cohort with Serum IgA Samples from Ages 0-3

		Two Highest Tertiles of Serum IgA (0-1 Years) n=1,360/ 2,040	**Chi X^{2**}**	**Two Highest Tertiles of Serum IgA (1-2 Years) n=2,655/ 4,079**	**ChiX ^{2**}**	**Two Highest Tertiles of Serum IgA (2-3 Years) N = 2,506/3 ,819**	**Chi X^{2**}**	**ED Visit for AW (Until Age 3 Years) vs No AW n=1,022/ 8,937**	**ChiX ^{2**}**	**ED Visit for Atopic AW (Until Age 3 Years) vs No AW n=173/8 ,088**	**ChiX ^{2**}**	**ED Visit for Non-Atopic AW (Until Age 3 Years) vs No AW n=260/8 ,175**	**Chi X^{2**}**	**ED Visit for Atopic Asthma (Until Age 3 Years) vs No AW n=123/8 ,362**	**ChiX ^{2**}**	**ED Visit for Non-Atopic Asthma (Until Age 3 Years) vs No AW n=180/8 ,419**	**Chi X^{2**}**
Co-variates		Row % (N)	p-value (X^2 exact)	Row % (N)	p-value (X^2 exact)	Row % (N)	p-value (X^2 exact)	Row % (N)	p-value (X^2 exact)	Row % (N)	p-value (X^2 exact)	Row % (N)	p-value (X^2 exact)	Row % (N)	p-value (X^2 exact)	Row % (N)	p-value (X^2 exact)
Sex	Female	66.40 (585)	0.825	61.50 (1,131)	**<0.001**	63.69 (1,054)	**0.028**	9.28 (366)	**<0.001**	1.19 (43)	**<0.001**	2.85 (105)	0.125	0.73 (27)	**<0.001**	2.09 (79)	0.803
	Male	66.87 (775)		68.04 (1,524)		67.10 (1,452)		13.14 (656)		2.91 (130)		3.45 (155)		2.07 (96)		2.17 (101)	

		Two Highest Tertiles of Serum IgA (0-1 Years) n=1,360/2,040	Chi X²**	Two Highest Tertiles of Serum IgA (1-2 Years) n=2,655/4,079	ChiX²**	Two Highest Tertiles of Serum IgA (2-3 Years) N = 2,506/3,819	Chi X²**	ED Visit for AW (Until Age 3 Years) vs No AW n=1,022/8,937	ChiX²**	ED Visit for Atopic AW (Until Age 3 Years) vs No AW n=173/8,088	ChiX²**	ED Visit for Non-Atopic AW (Until Age 3 Years) vs No AW n=260/8,175	Chi X²**	ED Visit for Atopic Asthma (Until Age 3 Years) vs No AW n=123/8,362	ChiX²**	ED Visit for Non-Atopic Asthma (Until Age 3 Years) vs No AW n=180/8,419	Chi X²**
Diagnoses of Failure to Thrive	No	66.67 (1,214)	1.00	65.54 (2,433)	0.053	65.94 (2,379)	0.088	11.67 (961)	**0.019**	2.20 (164)	0.170	3.26 (245)	0.180	1.53 (118)	0.107	2.19 (170)	0.225
	Yes	66.76 (146)		60.49 (222)		60.19 (127)		8.73 (61)		1.39 (9)		2.30 (15)		0.75 (5)		1.49 (10)	
Age at Serum IgA Level (Years)	0-1							9.13 (164)	**0.002**	1.33 (22)	**<0.001**	2.68 (45)	**0.036**	0.88 (15)	**0.002**	1.69 (29)	0.191
	1-2							11.78 (436)		1.72 (57)		3.77 (128)		1.25 (43)		2.44 (85)	

		Two Highest Tertiles of Serum IgA (0-1 Years) n=1,360/2,040	Chi X²**	Two Highest Tertiles of Serum IgA (1-2 Years) n=2,655/4,079	ChiX²**	Two Highest Tertiles of Serum IgA (2-3 Years) N = 2,506/3,819	Chi X²**	ED Visit for AW (Until Age 3 Years) vs No AW n=1,022/8,937	ChiX²**	ED Visit for Atopic AW (Until Age 3 Years) vs No AW n=173/8,088	ChiX²**	ED Visit for Non-Atopic AW (Until Age 3 Years) vs No AW n=260/8,175	Chi X²**	ED Visit for Atopic Asthma (Until Age 3 Years) vs No AW n=123/8,362	ChiX²**	ED Visit for Non-Atopic Asthma (Until Age 3 Years) vs No AW n=180/8,419	Chi X²**
Age at Serum IgA Level (Years)	2-3							12.27 (422)		3.02 (94)		2.80 (87)		2.02 (65)		2.05 (66)	

3.4.2 Comparison of median serum IgA Levels between those with/without ED visits for AW

Considering ED visits for AW until age 3, median serum IgA levels from ages 0 to 1 were significantly higher in groups with ED visits for atopic AW and those with atopic asthma compared to those without ED visits for AW (Figure 3.2). Median levels were 0.02 g/L higher in the groups with ED visits for AW (p=0.152) compared to those with no AW. When considering median levels for children with ED visits for atopic AW, median serum IgA levels were 0.07 g/L higher (p=0.028) than those without AW. Median levels for those with an ED visit for atopic asthma were 0.09 g/L higher (p=0.035) than those without AW. Median levels of serum IgA between non-atopic AW and non-atopic asthma until age 3 were not different from those without AW. In comparison, median levels of serum IgA for those with ED visits for AW (0.02 g/L), atopic AW (0.11 g/L) or atopic asthma (0.11 g/L) from ages 4-5 were higher than those without AW, but not statistically significant (Appendix C, Figure C2).

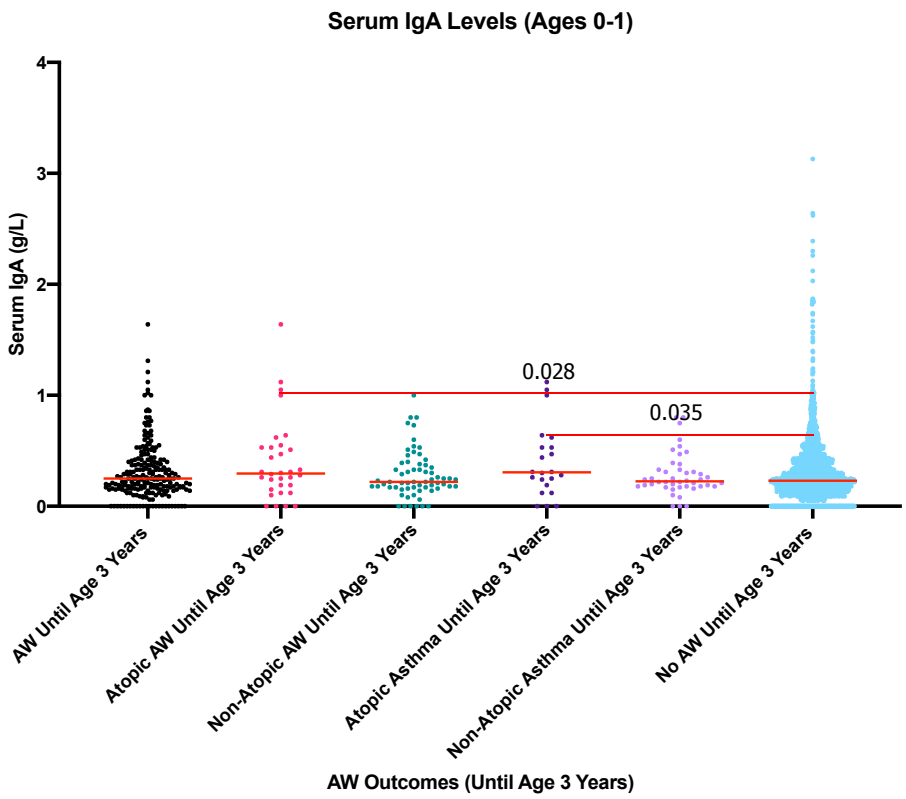

Figure 3.2. Serum IgA levels (g/L) from ages 0-1 among AW groups until age 3. P-values indicates significant differences in median values (red lines) in AW groups compared to the reference group of those without AW based on Mann-Whitney U.

Median serum IgA levels from ages 1-2 were only significantly higher in groups with ED visits for AW until age 3, compared to those without ED visits for AW (Figure 3.3). Median serum IgA levels were 0.05 g/L higher in the groups with ED visits for AW (p=0.21) compared to those with no AW. When considering median levels for children with ED visits for atopic AW, median levels were 0.12 g/L higher, but not significantly different than those without AW. Median levels for those with an ED visit for atopic asthma (not-wheeze) were only 0.07 g/L higher than those without AW. Median levels of serum IgA for those with ED visits for AW (0.02 g/L), atopic AW (0.07 g/L) or atopic

asthma (0.12 g/L) from ages 4-5 were higher but not significantly so (Appendix C, Figure C3).

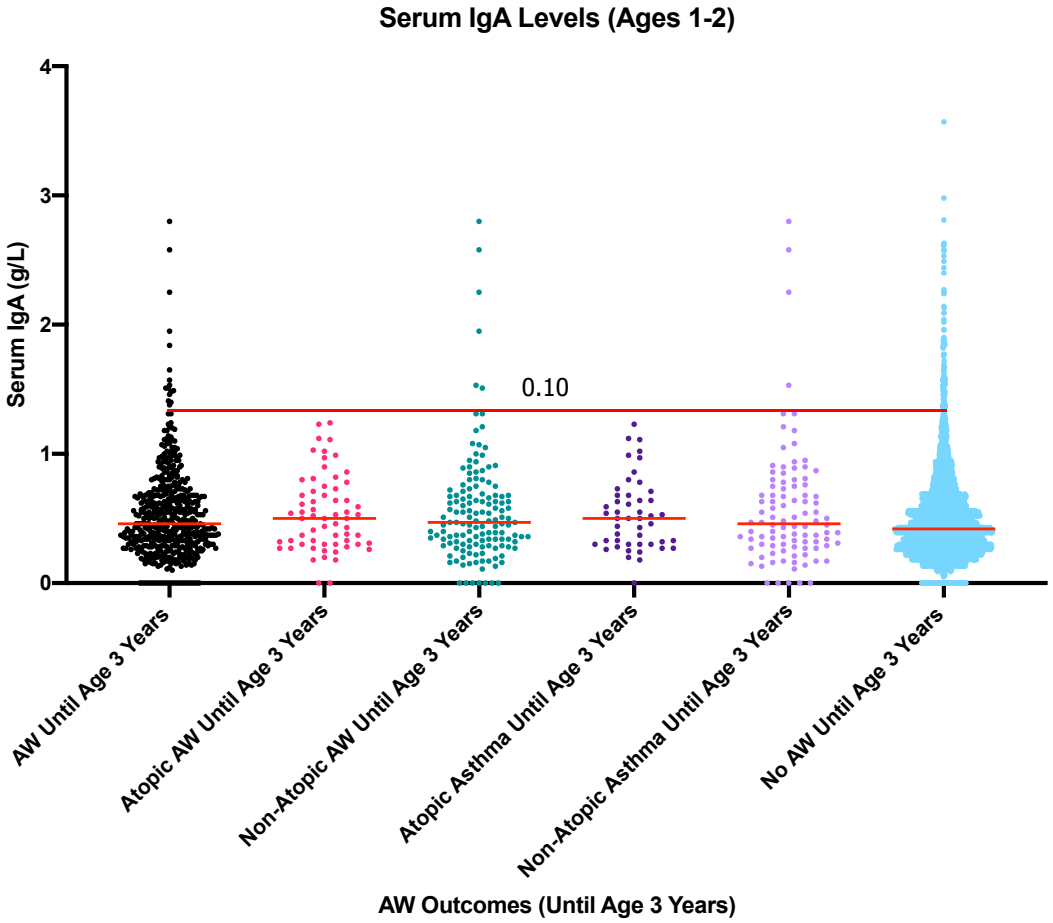

Figure 3.3. Serum IgA levels (g/L) from ages 1-2 among AW groups until age 3. P-values indicates significant differences in median values (red lines) in AW groups compared to the reference group of those without AW based on Mann-Whitney U.

Median serum IgA levels from ages 2-3 were significantly higher in groups with ED visits for atopic AW until age 3, compared to those without ED visits for AW (Figure 3.4). Median serum IgA levels were 0.03 g/L higher in the groups with ED visits for AW (p=0.2) compared to those with no AW. When considering median levels for children

with ED visits for atopic AW, median levels were 0.12 g/L higher (p=0.037) than in those without AW. Median levels for those with an ED visit for atopic asthma (not-wheeze) were only 0.07 g/L higher than those without AW. Median levels of serum IgA from ages 2-3 for those with ED visits for AW, atopic AW or atopic asthma from ages 4-5 were higher, but not significantly compared to those without AW from ages 4-5 (Appendix C, Figure C4).

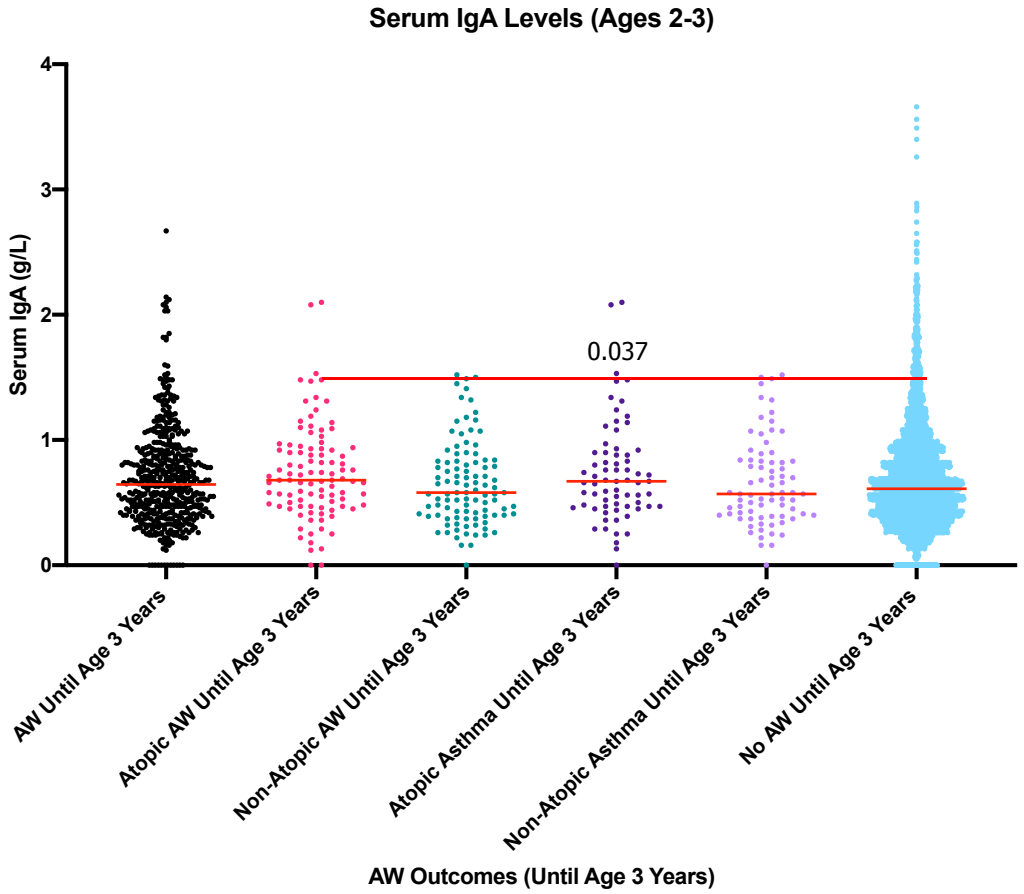

Figure 3.4. Serum IgA levels (g/L) from ages 2-3 among AW groups until age 3. P-values indicates significant differences in median values (red lines) in AW groups compared to the reference group of those without AW based on Mann-Whitney U.

3.4.3 Simple and multiple logistic regression analyses

As stated in the methods, the top two tertiles (normal-high) of serum IgA from ages 0-1, 1-2 and 2-3 years were compared to the lowest tertile. With this exposure, we looked at the odds of ED visits for AW, atopic AW and atopic asthma until age three. The logistic regression analysis for outcomes from ages 4-5 is shown in Appendix C. Range and mean of those in the lowest tertile for ages 0-1 years was 0 to 0.17 g/L and 0.08 g/L, from 2-3 years it was 0 to 0.39 g/L and 0.35 g/L and from 2-3 years it was 0 to 0.39 g/L and 0.25g/L. Range and mean of those in the top two tertiles for ages 0-1 years was 0.18 to 3.13 g/L and 0.41 g/L, from 2-3 years it was 0.4 to 9.92 g/L and 0.70 g/L and from 2-3 years it was 0.4 to 9.64 g/L and 0.82 g/L.

3.4.4 Serum IgA and ED Visits for AW

Normal to higher serum IgA levels from ages 1-2 was associated with increased odds of ED visits until age 3 years for AW, compared to those in the lowest tertile of serum IgA levels. The crude OR for an ED visit for AW (until age 3) when having normal/high serum IgA in the 1st through 2nd year of life was 1.24 (95% CI: 1.03, 1.49; Table 3.3), and this association was significant. The variables identified via DAG directed in the methods as minimally sufficient to adjust for, included: a diagnosis of FTT and sex. A final regression model for ED visits for AW (until age 3) showed a 1.21 (95% CI: 1.00, 1.46) increase in the odds of having an ED visit for AW with the exposure normal-high serum IgA (Ages 1-2) compared to those in the lowest tertile after adjustment for FTT and sex (Table 3.3). In comparison, having normal to high serum IgA from ages 0-1 and 2-3 was not associated with a significantly increased odds of an ED visit until age 3 after adjusting for sex and FFT, although the later showed a trend (p=0.12) (Table 3.2, 3.4). Similar but not significant associations were seen in the final regression model for ED visit for AW in ages 4-5 (Appendix C, Tables C2-C16).

Table 3.2. Odds of Emergency Department Visits for Asthma and/or Wheeze (Until Age 3 Years) After Exposure to the Two Highest Tertiles of Serum IgA (Ages 0-1)

Ref: Lowest Tertile Serum IgA (0-1 Years)	ED Visit for AW vs No AW (Until Age 3 Years) Crude OR (95% CI)	ED Visit for AW vs No AW (Until Age 3 Years) Adjusted OR (95% CI) (n=2,040)*
Normal-High Serum IgA (0-1 Years)	1.08 (0.80, 1.46)	1.08 (0.80, 1.46)
Failure to Thrive	0.72 (0.55, 0.95)	0.66 (0.40, 1.11)
Sex (Ref: Female)	**1.48 (1.29, 1.69)**	**1.49 (1.11, 2.00)**

Bold indicates p<0.05. *Adjusted for sex and failure to thrive

Table 3.3 Odds of Emergency Department Visits for Asthma and/or Wheeze (Until Age 3 Years) After Exposure to the Two Highest Tertiles of Serum IgA (Ages 1-2)

Ref: Lowest Tertile Serum IgA (1-2 Years)	ED Visit for AW vs No AW (Until Age 3 Years) Crude OR (95% CI)	ED Visit for AW vs No AW (Until Age 3 Years) Adjusted OR (95% CI) (n=4,079)*
Normal-High Serum IgA (1-2 Years)	**1.24 (1.03, 1.49)**	**1.21 (1.00, 1.46)**
Failure to Thrive	0.72 (0.55, 0.95)	0.98 (0.71, 1.35)
Sex (Ref: Female)	**1.48 (1.29, 1.69)**	**1.47 (1.22, 1.79)**

Bold indicates p<0.05. *Adjusted for sex and failure to thrive

Table 3.4. Odds of Emergency Department Visits for Asthma and/or Wheeze (Until Age 3 Years) After Exposure to the Two Highest Tertiles of Serum IgA (Ages 2-3)

Ref: Lowest Tertile Serum IgA (2-3 Years)	ED Visit for AW vs No AW (Until Age 3 Years) Crude OR (95% CI)	ED Visit for AW vs No AW (Until Age 3 Years) Adjusted OR (95% CI) (n=3,819)*
Normal-High Serum IgA (2-3 Years)	1.16 (0.93, 1.44)	1.14 (0.92, 1.42)
Failure to Thrive	0.72 (0.55, 0.95)	0.75 (0.49, 1.14)
Sex (Ref: Female)	**1.48 (1.29, 1.69)**	**1.35 (1.12, 1.62)**

Bold indicates p<0.05. *Adjusted for sex and failure to thrive

3.4.5 Serum IgA and ED Visits for Atopic/Non-Atopic AW

Atopic and non-atopic AW were compared to those without AW in separate models. The same exposures were used to determine the odds of ED visits for atopic AW until age 3 and normal-high serum IgA was associated with increased odds of atopic AW. The crude OR for risk of an ED visit for atopic AW for those with normal-high serum IgA when compared to low serum IgA (Ages 2-3) was 1.85 (95% CI: 1.07, 3.21; Table 3.9), and this association was significant. After adjusting for covariates, the final regression model for atopic AW showed a 1.79 (95% CI: 1.03, 3.09) times increase in the odds of having an ED visit for atopic AW with exposure to normal-high serum IgA (ages 2-3) when compared to those with low serum IgA (Table 3.9). The directions of the associations were similar but the associations were non-significant when considering serum IgA from 0-1 and 1-2 years of age, and atopic AW (Tables 3.5 and 3.7). In comparison, adjusted ORs for ED visits for non-atopic AW with normal-high serum IgA compared to those with low serum IgA, were decreased compared to atopic AW outcomes (Tables 3.6, 3.8, and 3.10). Associations for atopic and non-atopic AW from ages 4-5 were also in a similar direction with exposure to normal-high serum IgA from 0-1, 1-2 and 2-3 years of age, compared to those with low serum IgA, but were not statistically significant (Appendix C; Tables C2, C16).

Table 3.5. Odds of Emergency Department Visits for Atopic AW (Until Age 3 Years) After Exposure to the Two Highest Tertiles of Serum IgA (Ages 0-1)

Ref: Lowest Tertile Serum IgA (0-1 Years)	ED Visit for Atopic AW vs No AW (Until Age 3 Years) Crude OR (95% CI)	ED Visit for Atopic AW vs No AW (Until Age 3 Years) Adjusted OR (95% CI) (n=1,872)*
Normal-High Serum IgA (0-1 Years)	1.53 (0.68, 3.42)	1.53 (0.68, 3.42)
Failure to Thrive	0.63 (0.32, 1.23)	1.45 (0.55, 3.80)
Sex (Ref: Female)	**2.49 (1.76, 3.53)**	**2.42 (1.08, 5.42)**

Bold indicates p<0.05. *Adjusted for sex and failure to thrive

Table 3.6. Odds of Emergency Department Visits for Non-Atopic AW (Until Age 3 Years) After Exposure to the Two Highest Tertiles of Serum IgA (Ages 0-1)

Ref: Lowest Tertile Serum IgA (0-1 Years)	ED Visit for Non-Atopic AW vs No AW (Until Age 3 Years) Crude OR (95% CI)	ED Visit for Non-Atopic AW vs No AW (Until Age 3 Years) Adjusted OR (95% CI) (n=1,900)*
Normal-High Serum IgA (0-1 Years)	1.21 (0.70, 2.08)	1.20 (0.70, 2.08)
Failure to Thrive	0.70 (0.41, 1.18)	0.81 (0.35, 1.90)
Sex (Ref: Female)	1.22 (0.95, 1.57)	1.18 (0.71, 1.96)

Bold indicates p<0.05. *Adjusted for sex and failure to thrive

Table 3.7. Odds of Emergency Department Visits for Atopic AW (Until Age 3 Years) After Exposure to the Two Highest Tertiles of Serum IgA (Ages 1-2)

Ref: Lowest Tertile Serum IgA (1-2 Years)	ED Visit for Atopic AW vs No AW (Until Age 3 Years) Crude OR (95% CI)	ED Visit for Atopic AW vs No AW (Until Age 3 Years) Adjusted OR (95% CI) (n=3,671)*
Normal-High Serum IgA (1-2 Years)	1.30 (0.78, 2.16)	1.22 (0.73, 2.03)
Failure to Thrive	0.63 (0.32, 1.23)	1.25 (0.56, 2.77)
Sex (Ref: Female)	**2.49 (1.76, 3.53)**	**2.46 (1.39, 4.37)**

Bold indicates p<0.05. *Adjusted for sex and failure to thrive

Table 3.8. Odds of Emergency Department Visits for Non-Atopic AW (Until Age 3 Years) After Exposure to the Two Highest Tertiles of Serum IgA (Ages 1-2)

Ref: Lowest Tertile Serum IgA (1-2 Years)	ED Visit for Non-Atopic AW vs No AW (Until Age 3 Years) Crude OR (95% CI)	ED Visit for Non-Atopic AW vs No AW (Until Age 3 Years) Adjusted OR (95% CI) (n=3,746)*
Normal-High Serum IgA (1-2 Years)	1.22 (0.87, 1.71)	1.21 (0.86, 1.70)
Failure to Thrive	0.70 (0.41, 1.18)	1.15 (0.67, 1.99)
Sex (Ref: Female)	1.22 (0.95, 1.57)	1.10 (0.78, 1.54)

Bold indicates p<0.05. *Adjusted for sex and failure to thrive

Table 3.9. Odds of Emergency Department Visits for Atopic AW (Until Age 3 Years) After Exposure to the Two Highest Tertiles of Serum IgA (Ages 2-3)

Ref: Lowest Tertile Serum IgA (2-3 Years)	ED Visit for Atopic AW vs No AW (Until Age 3 Years) Crude OR (95% CI)	ED Visit for Atopic AW vs No AW (Until Age 3 Years) Adjusted OR (95% CI) (n=3,453)*
Normal-High Serum IgA (2-3 Years)	**1.85 (1.07, 3.21)**	**1.79 (1.03, 3.09)**
Failure to Thrive	0.63 (0.32, 1.23)	0.62 (0.23, 1.70)
Sex (Ref: Female)	**2.49 (1.76, 3.53)**	**2.60 (1.66, 4.09)**

Bold indicates p<0.05. *Adjusted for sex and failure to thrive

Table 3.10. Odds of Emergency Department Visits for Non-Atopic AW (Until Age 3 Years) After Exposure to the Two Highest Tertiles of Serum IgA (Ages 2-3)

Ref: Lowest Tertile Serum IgA (2-3 Years)	ED Visit for Non-Atopic AW vs No AW (Until Age 3 Years) Crude OR (95% CI)	ED Visit for Non-Atopic AW vs No AW (Until Age 3 Years) Adjusted OR (95% CI) (n=3,453)*
Normal-High Serum IgA (2-3 Years)	0.99 (0.64, 1.54)	0.98 (0.64, 1.52)
Failure to Thrive	0.70 (0.41, 1.18)	0.56 (0.21, 1.54)
Sex (Ref: Female)	1.22 (0.95, 1.57)	1.18 (0.81, 1.72)

Bold indicates p<0.05. *Adjusted for sex and failure to thrive

3.4.5 Serum IgA and ED Visits for Atopic/Non-Atopic Asthma

Serum IgA and ED visits were studied in atopic and non-atopic asthma (not wheeze), and compared to those without AW in separate models. After adjusting for FTT and sex, the final regression models showed only a marginally significant increase in the odds of having an ED visit for atopic asthma with exposure to normal-high serum IgA, compared to those in the lowest tertile of serum IgA from ages 0-1, 1-2 or 2-3 (Tables 3.11, 3.13, 3.15). When considering those with exposure to normal-high serum IgA compared to those with low levels of serum IgA, the odds ratios were reduced in most models for an outcome of an ED visit for non-atopic asthma compared to ED visits for atopic asthma (Tables 3.11-16). In the crude associations, being male was significantly associated with increased odds of atopic asthma (OR: 2.89; 95% CI: 1.88, 4.44) but not non-atopic asthma (OR: 1.04; 95% CI: 0.77, 1.19) compared to females (Tables 3.11-16). Associations for atopic and non-atopic asthma from 4-5 years of age were also in a similar direction with exposure to normal-high serum IgA from 0-1, 1-2 and 2-3 years of age, compared to those with low serum IgA, but were not statistically significant (Appendix C; Tables C2-C16). Summary forest plots of the adjusted ORs of each of the AW outcomes (until age 3) are shown below (Figure 3.5-7) and in Appendix C for ages 4-5.

Table 3.11. Odds of Emergency Department Visits for Atopic Asthma (Until Age 3 Years) After Exposure to the Highest Tertile of Serum IgA (Ages 0-1)

Ref: Lowest Tertile Serum IgA (0-1 Years)	ED Visit for Atopic Asthma vs No AW (Until Age 3 Years) Crude OR (95% CI)	ED Visit for Atopic Asthma vs No AW (Until Age 3 Years) Adjusted OR (95% CI) (n=1,929)*
Normal-High Serum IgA (0-1 Years)	1.73 (0.63, 4.70)	1.71 (0.63, 4.67)
Failure to Thrive	0.48 (0.20, 1.19)	0.79 (0.18, 3.40)
Sex (Ref: Female)	**2.89 (1.88, 4.44)**	**3.57 (1.20, 10.58)**

Bold indicates p<0.05. *Adjusted for sex and failure to thrive

Table 3.12. Odds of Emergency Department Visits for Non-Atopic Asthma (Until Age 3 Years) After Exposure to the Highest Tertile of Serum IgA (Ages 0-1)

Ref: Lowest Tertile Serum IgA (0-1 Years)	ED Visit for Non-Atopic Asthma vs No AW (Until Age 3 Years) Crude OR (95% CI)	ED Visit for Non-Atopic Asthma vs No AW (Until Age 3 Years) Crude OR (95% CI) (n=1,947)*
Normal-High Serum IgA (0-1 Years)	1.97 (0.94, 4.13)	1.97 (0.94, 4.13)
Failure to Thrive	0.67 (0.77, 1.40)	1.02 (0.40, 2.61)
Sex (Ref: Female)	1.04 (0.77, 1.40)	1.04 (0.57, 1.90)

Bold indicates p<0.05. *Adjusted for sex and failure to thrive

Table 3.13. Odds of Emergency Department Visits for Atopic Asthma (Until Age 3 Years) After Exposure to the Highest Tertile of Serum IgA (Ages 1-2)

Ref: Lowest Tertile Serum IgA (1-2 Years)	ED Visit for Atopic Asthma vs No AW (Until Age 3 Years) Crude OR (95% CI)	ED Visit for Atopic Asthma vs No AW (Until Age 3 Years) Adjusted OR (95% CI) (n=3,800)*
Normal-High Serum IgA (1-2 Years)	1.29 (0.72, 2.32)	1.21 (0.67, 2.16)
Failure to Thrive	0.48 (0.20, 1.19)	0.89 (0.32, 2.49)
Sex (Ref: Female)	**2.89 (1.88, 4.44)**	**2.79 (1.42, 5.49)**

Bold indicates p<0.05. *Adjusted for sex and failure to thrive

Table 3.14. Odds of Emergency Department Visits for Non-Atopic Asthma (Until Age 3 Years) After Exposure to the Highest Tertile of Serum IgA (Ages 1-2)

Ref: Lowest Tertile Serum IgA (1-2 Years)	ED Visit for Non-Atopic Asthma vs No AW (Until Age 3 Years) Crude OR (95% CI)	ED Visit for Non-Atopic Asthma vs No AW (Until Age 3 Years) Crude OR (95% CI) (n=3,846)*
Normal-High Serum IgA (1-2 Years)	1.13 (0.75, 1.69)	1.14 (0.76, 1.71)
Failure to Thrive	0.67 (0.77, 1.40)	1.09 (0.56, 2.12)
Sex (Ref: Female)	1.04 (0.77, 1.40)	0.94 (0.63, 1.41)

Bold indicates p<0.05. *Adjusted for sex and failure to thrive

Table 3.15. Odds of Emergency Department Visits for Atopic Asthma (Until Age 3 Years) After Exposure to the Two Highest Tertiles of Serum IgA (Ages 2-3)

Ref: Lowest Tertile Serum IgA (2-3 Years)	ED Visit for Atopic Asthma vs No AW (Until Age 3 Years) Crude OR (95% CI)	ED Visit for Atopic Asthma vs No AW (Until Age 3 Years) Adjusted OR (95% CI) (n=3,569)*
Normal-High Serum IgA (2-3 Years)	1.58 (0.85, 2.95)	1.53 (0.82, 2.84)
Failure to Thrive	0.48 (0.20, 1.19)	0.66 (0.21, 2.12)
Sex (Ref: Female)	**2.89 (1.88, 4.44)**	**2.74 (1.59, 4.72)**

Bold indicates p<0.05. *Adjusted for sex and failure to thrive

Table 3.16. Odds of Emergency Department Visits for Non-Atopic Asthma (Until Age 3 Years) After Exposure to the Two Highest Tertiles of Serum IgA (Ages 2-3)

Ref: Lowest Tertile Serum IgA (2-3 Years)	ED Visit for Non-Atopic Asthma vs No AW (Until Age 3 Years) Crude OR (95% CI)	ED Visit for Non-Atopic Asthma vs No AW (Until Age 3 Years) Crude OR (95% CI) (n=3,570)*
Normal-High Serum IgA (2-3 Years)	0.88 (0.53, 1.44)	0.87 (0.52, 1.43)
Failure to Thrive	0.67 (0.77, 1.40)	0.60 (0.19, 1.90)
Sex (Ref: Female)	1.04 (0.77, 1.40)	1.21 (0.78, 1.89)

Bold indicates p<0.05. *Adjusted for sex and failure to thrive

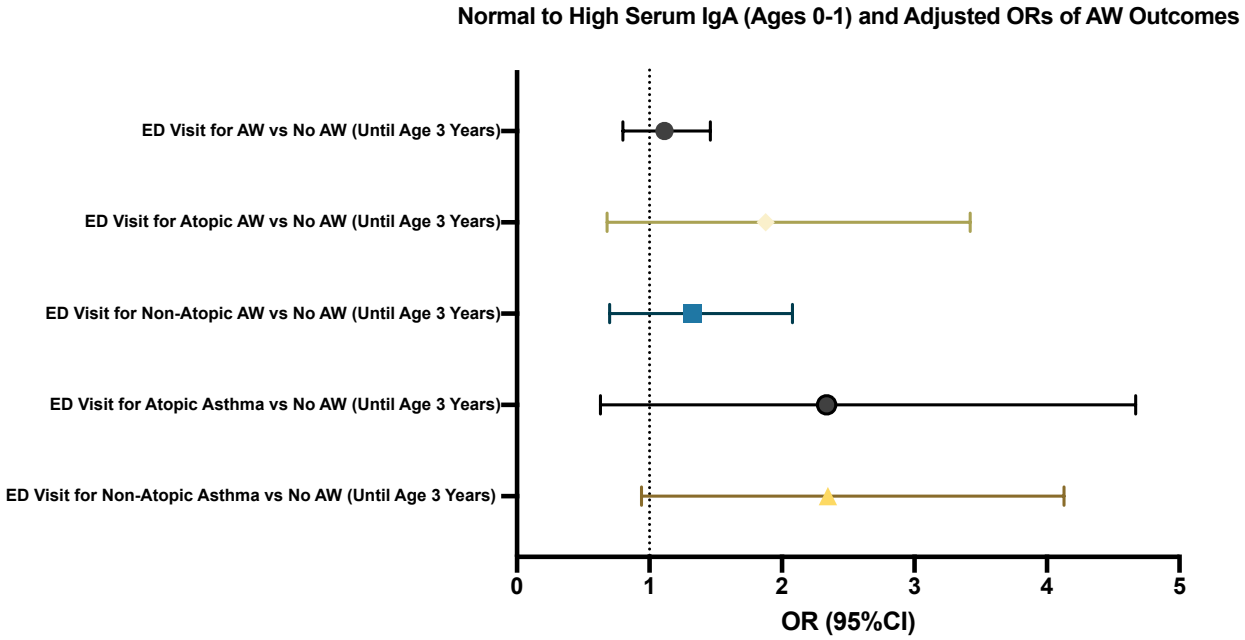

Figure 3.5. Forest plot of adjusted ORs for AW outcomes until age 3 in children with normal to high serum IgA levels (g/L) compared to those with low serum IgA levels from ages 0-1, adjusted for failure to thrive and sex.

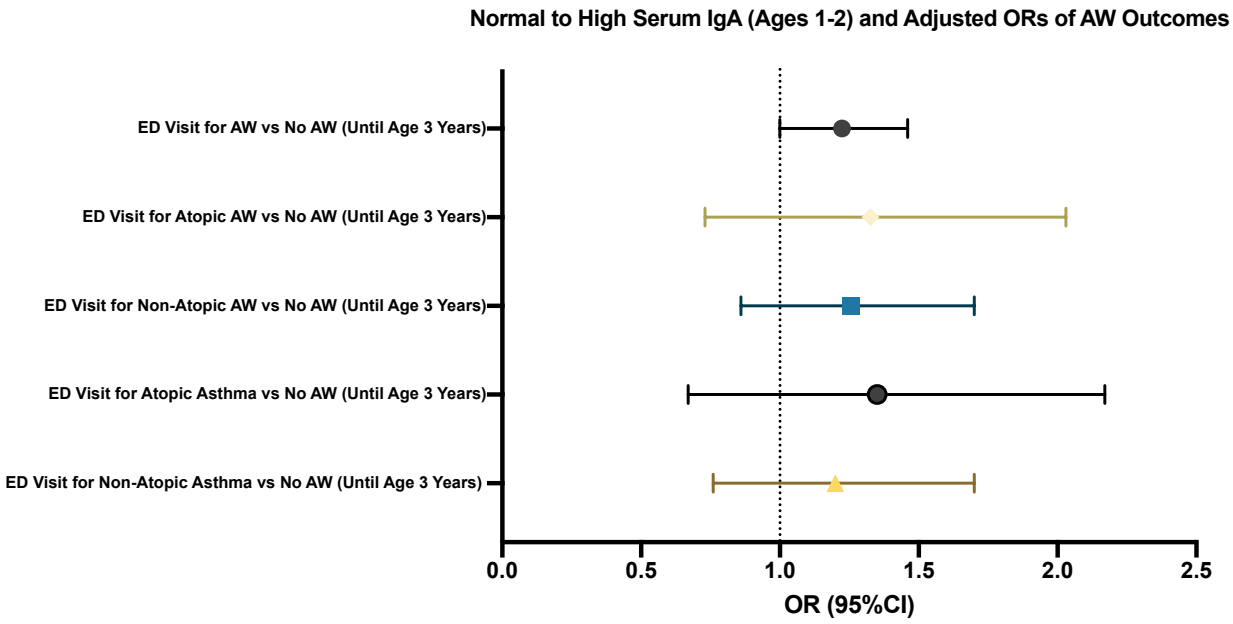

Figure 3.6. Forest plot of adjusted ORs for AW outcomes until age 3 in children with normal to high serum IgA levels (g/L) compared to those with low serum IgA levels from ages 1-2, adjusted for failure to thrive and sex.

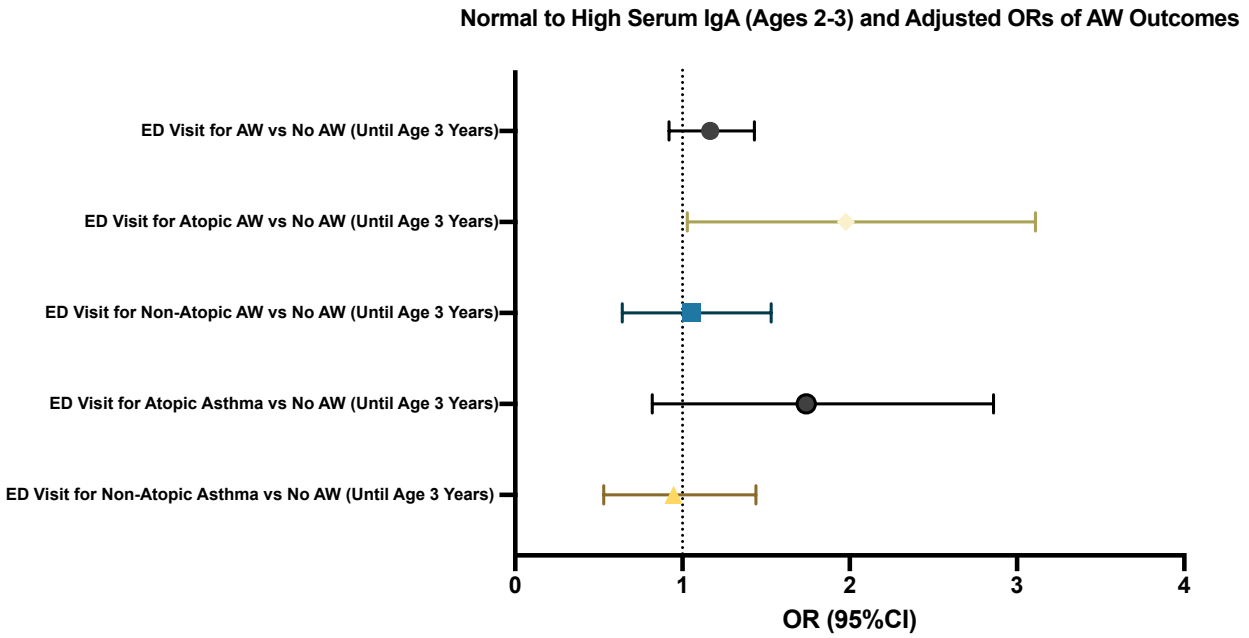

Figure 3.7. Forest plot of adjusted ORs for AW outcomes until age 3 in children with normal to high serum IgA levels (g/L) compared to those with low serum IgA levels from ages 2-3, adjusted for failure to thrive and sex.

3.5 Discussion

In this large, population-based cohort of 9,938 Albertan children, we found some significant associations between being in the top two tertiles of serum IgA and ED visits for AW. Being in the top two tertiles of serum IgA from ages 1-2 was associated with increased odds of having an ED visit for asthma or wheeze until age 3, compared to those in the lowest IgA tertile (adjusted OR: 1.21; 95% CI: 1.00, 1.46). A similar association was seen with normal to high serum IgA (ages 2-3) and atopic AW until age 3 (adjusted OR: 1.79; 95% CI: 1.03, 3.09). There was a corresponding trend of associations observed between normal-high serum IgA from ages 0-1, 1-2, and 2-3 and ED visits for AW, atopic AW and non-atopic AW until age 3, but the majority of these associations did not reach statistical significance. Based on our models, the trend suggested that being in the top two tertiles of serum IgA in the

first 3 years of life was associated with increased odds of ED visits for AW, atopic AW or atopic asthma until age 3, and from 4-5, compared to those in the lowest tertile of serum IgA. This is the first-time serum immunoglobulin A levels in early childhood have been studied in relation to ED visits for AW in early life in a large population-based cohort. This is a key area of interest because of the high cost of healthcare utilization for AW and need to characterize risk factors for childhood presentations of AW to the ED.

In comparison to our findings, some previous studies have found that lower serum IgA levels are associated with an increased risk for wheeze, asthma or allergic disease. These studies include the findings of Ludviksson et al., (1992), which found that low serum IgA in infancy was associated with increased risk for asthma at age 7, though the diagnoses of asthma was heavily weighted towards atopic dermatitis or sensitization, and was not based on ED visits [11]. As well, IgA and IgE levels were determined from cord blood and may partially represent the mother [10]. A study by Janzi et al., (2009) also reported that individuals with a complete absence of serum IgA at 4 years of age had increased risk of atopic disease and infection, though they did not report on asthma as an outcome [15]. Given this literature, we expected that serum IgA would be inversely associated with AW. By contrast, we found that normal-high concentrations of serum IgA were marginally associated with increased odds of AW within the 1st through 3rd years of life. These findings raise the question of why higher levels of serum IgA would be associated with increased risk for ED visits for AW and with ED visits for atopic asthma/asthma. One explanation is that undetectable serum IgA (IgA deficiency) is inherently different from having low serum IgA. Completely undetectable levels may be a marker of immune system dysfunction and genetic abnormality, whereas low levels of serum IgA may indicate a properly functioning immune system with an absence of infection. Previous reports

have shown that partial IgA deficiency (low but not absent levels) in childhood was not associated with increased risk for atopic disease in comparison to complete IgA deficiency [15].

More recently our findings of normal-high serum IgA being associated with asthma have been supported. In particular, Pesonen et al., (2011) found that higher serum IgA at 2 months of age was associated with development of atopic sensitization at 5, 11 and 20 years of age [16]. These findings were independent of breastfeeding, parental atopic disease, sex and tobacco smoke exposure, of which some may have been confounders in our study, but these results support the associations we found. Thus, having serum IgA levels that are within normal-high range may be an important biomarker not only for the development of respiratory illness, but atopic respiratory illness in early childhood. We addressed the temporality of relationships in our study by limiting our analysis to individuals with serum IgA testing before any ED visits for asthma or wheeze, but these individuals may have had various undetected respiratory illnesses before the IgA testing happened, which could have been involved in this association. For example, respiratory infections like respiratory syncytial virus (RSV), which are common in infancy and can contribute to asthma exacerbations, are known to increase serum IgA levels after infection, and this increase may last up to a few weeks [17–19]. This relationship between RSV and production of IgA following infection might also explain the stronger associations between normal-high serum IgA and atopic AW, compared to the association between normal-high serum IgA and AW without designation of atopic status. A previous study by Schauer et al., (2002) showed that RSV infection in the 1st year of life was one of the most important risk factors for allergic sensitization (determined by allergen specific IgE values) in children [20]. As well, mouse models of RSV infection reveal that infection can promote airway hyperresponsiveness and resultant increase in Th2-mediated cytokine production with

exposure to various allergens [21]. This relationship between normal-high serum IgA and ED visits for atopic asthma/wheezing is an important finding. Previously published trajectories reveal that presence of early atopic sensitization in addition to AW is one of the best predictors of persistent asthma, though our associations were strongest with AW until age 3, in comparison to ages 4-5 which is more highly associated with persistence [22].

In comparison to serum IgA, sIgA is the main mediator of mucosal homeostasis and defence and serum IgA levels may not reflect the function of sIgA. Indeed, different induction mechanisms have been elucidated and serum IgA arises from B-cells in the bone marrow, whereas sIgA arises from B-cell production in the mucosal lamina propria, although there is clonal relatedness between the two forms [23]. Although sIgA is not generally considered to be implicated in inflammatory reactions, but serum IgA is capable of initiating inflammatory reactions, serum IgA may act as a second line of defence when there is breach of the mucosal barrier by infectious agents [8,24,25]. This functional distinction may explain why total fecal IgA and salivary sIgA were lower in children who developed subsequent atopic disease [5,26]. Previous findings by Ladjemi et al., (2018) showed that there is down regulation of the polymeric immunoglobulin receptor protein (pIgR), the protein responsible for transport of sIgA into the mucosal surfaces, in the bronchial epithelium of patients with asthma compared to healthy controls [27]. However, asthma severity was not considered, and corticosteroid treatment may have confounded these results. This reduction of pIgR could result in reduced sIgA-mediated protection on the mucosal surfaces, increasing risk for infection and inflammation in the airways, and increasing risk for development of asthma. Knockout of pIgR in murine models has revealed a significant increase in serum IgA

in knockout mice compared to controls, which provides a potential explanation for our results [28].

This AHS cohort study was designed to determine the preliminary associations between immune system development in early life and the development of asthma in childhood. The experimental design has both strengths and a number of weaknesses. The strengths of this study include both its large sample size and standardized testing. As well, we limited measurement of serum IgA to before AW presentations to the ED, which increases our ability to draw temporal relationships, and the range and mean of serum IgA values were comparable to a previous Iranian cohort [29]. However, despite the standardization of tests, there is variability in the measurements of serum IgA, as testing was done for varying indications, in various labs across the province, by different technicians, and using different machines. Additionally, there are many inherent limitations with studies based on administrative health care data, since the records primarily represent an account of the illness experienced, and not a set of standardized questionnaires validated for research. The records were also limited to the individuals under study and no information was collected about parental history of atopic disease, breastfeeding status, home environment, or birth mode, which are a few of many factors related to IgA levels and asthma. Moreover, since serum IgA levels are not routinely taken and are indicated for in the case of chronic infections or suspected immunodeficiency, those who have serum IgA levels tested may already be at increased risk for asthma or other atopic diseases. Although prevalence was higher for ED visits during the ages of serum IgA used, likely indicating some confounding of this nature, our results are similar to reported national averages (www.cihi.ca). Overall, our data must be interpreted in light of these limitations. Nevertheless, this is an important study that provides insight on early development of the immune system and future risk for wheeze, atopy and asthma.

In conclusion, normal-high levels of serum IgA in early life are associated with a increased odds for ED visit for AW and atopic AW in childhood. Further studies are needed to link these associations to see if they hold when controlling for various other pre/postnatal influences on the development of the immune system and AW. Future studies comparing the development of serum and secretory IgA, and risk of atopic illness in a prospective, healthy birth cohort would provide key insights into the developmental origins of these complex diseases.

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Chapter 4: Conclusions

4.1 Key Findings and General Conclusions

This thesis was conducted to help understand the relationship between IgA--both serum and secretory—and asthma and wheeze in childhood. We completed two separate cohort studies. Chapter 2 describes the associations between infant fecal secretory IgA in the 1st year of life and childhood asthma and wheeze in the first 5 years of life in a prospective, normal birth cohort. Chapter 3 evaluates the associations between serum IgA and the first 3 years of life and ED visits for asthma and wheeze in the first 5 years of life in a retrospective administrative health database cohort. The major findings will be summarized first in this chapter and then we will outline strengths and limitations in relation to our CHILD study then our AHS study. The final sub-section of this chapter will discuss the implications of this research and potential areas for future research.

Key finding #1 Fecal sIgA in formula fed infants in infancy is differentially related to atopic and non-atopic AW in comparison to breastfed infants.

Asthma is one of the most common chronic childhood diseases and is a huge burden on society. Research focusing on the developmental origins of asthma is being pursued to elucidate areas for future strategies for prevention or to mitigate severity. In the past 10 years, the development of the gut microbiome has been revealed as a key influence on the etiology of asthma and atopic disease [1]. One of main mediators of the effects of the development of the gut microbiota on asthma is sIgA [2–4]. This study expands on our knowledge of the relationship between early life sIgA and future odds of developing childhood asthma.

We showed that lower fecal sIgA in formula fed infants was associated with increased risk for non-atopic AW, whereas normal-high fecal sIgA was associated with

increased risk for atopic AW in comparison to breastfed infants with any level of fecal sIgA. Previous research identified that low sIgA is associated with increased risk for asthma and atopic disease [5,6], whereas others have shown that high mucosal IgA is associated with increased risk of allergic sensitization and asthma [7]. sIgA may impact the development of asthma in a number of ways. Variations in intestinal colonization patterns implicated in allergic disease and asthma may be mediated through sIgA binding to microbes [4]. Although these studies add to the literature on the relationship between fecal sIgA in infancy and childhood atopic disease while controlling for breastfeeding status to varying degrees, none report on how formula fed and breastfed infants differ. Since the main source of sIgA in infancy is through maternal breastmilk, we hypothesized that in the case of formula feeding when an infant relies on endogenous production of sIgA, which matures throughout the 1st year of life, there may be further differences in risk of atopic disease compared to breastfed infants who receive supplementation of maternally derived, protective sIgA. Since, formula feeding is already strong predictor of childhood wheeze, differences in total sIgA in formula fed infants, may be an important biomarker for aberrant development of the infant immune system.

We speculate that low fecal sIgA may be a marker for low mucosal immune response to microbes, resulting in reduced mucosal barrier function and increased susceptibility to viral airway infections [8,9]. By contrast, higher fecal sIgA in formula fed infants may be a marker for inflammatory Proteobacteria and an over-representation of *Enterobacteriaceae,* a gut microbe found in infants who develop food sensitization in childhood [10,11].

This work leads to interesting questions on how infant feeding, mucosal immune development and the microbiome coordinately impact the development of asthma and atopic disease. There is potential, as with all epidemiological studies, that an

unmeasured confounding variable could lead to these associations. In particular, we were unable to measure what microbes were bound to fecal sIgA in our population, a potential key consideration [4]. This more basic association may be important in a clinical context. Even after controlling for the effects of multiple co-variates, total fecal sIgA in formula fed infants was associated with early asthma and atopic AW. Interventions such as promotion of breastfeeding or even partial breastfeeding may serve as a useful tool for asthma prevention.

Key finding #2 Normal-high serum IgA is associated with increased risk for ED visits for AW and atopic AW in childhood.

Varying levels of serum IgA have previously been associated with asthma, atopic sensitization and infection [12,13]. Although previous results are conflicting, our results support that normal-high levels are associated with ED visits for respiratory illness and atopic disease in childhood. The most prominent studies linking low serum IgA to atopic diseases are those on IgA deficiency, which increases the risk of atopic diseases [14]. As well, when serum IgA levels are present, other studies have associated lower serum IgA with increased risk of allergic disease at age 7 [15]. In comparison, higher serum IgA has also been associated with later development of atopic diseases in several studies [12,16,17]. As addressed in Chapter 3, this may be due to early infection with RSV, which has been shown to raise serum IgA and IgE levels and increase risk for later development of asthma, although research on this relationship is contentious [18–21].

Although our study aids in establishing the relationship serum IgA and atopic disease, it should be kept in mind that serum IgA levels likely do not accurately reflect the function of sIgA at the mucosal surfaces. sIgA has a larger role in immunological tolerance to food antigens and commensal microbes, and as such is more functionally

relevant than levels of serum IgA in development of various atopic diseases. The role of serum IgA is less well understood, but it has been suggested to act as a second line of defence against invasive bacterial infections with a mucosal surface origin [22]. It follows that the relationship of higher serum IgA with atopy may reflect decreased sIgA at mucosal surfaces. A clearer understanding of the relationship between IgA, sIgA and atopy is needed to understand the relevance of IgA levels in development of immune tolerance and their ability to predict clinical outcomes. Regardless, this research highlights important findings for clinicians; although undetectable levels of serum IgA is clearly linked with asthma risk, when serum IgA is present but low, the clinical significance is unclear.

These findings reveal the intricate relationship between IgA in early life and the etiology of childhood atopic disease. Importantly, we showed that fecal sIgA levels may be more predictive of development of childhood asthma than serum IgA levels. We examined these two cohorts using similar outcomes and exposures, and we were interested in particular in the comparison between serum IgA in the first year of life and our fecal sIgA sample. In this time frame, fecal sIgA was more highly associated with childhood AW than serum IgA levels at one year. This interpretation should be taken lightly though as serum IgA may have a similar or greater predictive value but was confounded by the limitations in our dataset. Despite this, we showed similar trends between serum and sIgA, in particular on the association with atopic AW, though the relationships between the two different isoforms and atopic disease may have divergent functional explanations. Overall, both serum IgA and secretory IgA may be important biomarkers for clinicians to aid in early identification and treatment of those prone to develop atopic diseases like asthma.

4.2 Strengths and Limitations

The strengths and limitations of this thesis will be mentioned briefly in this section then outlined more extensively in the next sub-sections on bias and confounding. Epidemiological research has intrinsic and inevitable sources of bias. This thesis used two different cohort designs; each has different drawbacks. The CHILD study is a prospective, normative birth cohort. The main objective was to determine the relationship between early fecal sIgA levels and AW in childhood. The AHS study is a retrospective cohort study developed to determine the association between serum IgA level and emergency department visits for asthma and wheeze in childhood.

4.2.1 General Strengths and Limitations of the CHILD Cohort

The CHILD cohort has multiple strengths. The cohort is representative of the general Canadian population based on the variety of recruitment methods and high rate of retention [23,24]. Because of the large sample size and extensive, accurately documented data on covariates, the sample size was sufficient to adjust for many important covariates when using multivariate regression analyses. The prospective nature of this study allows us to comment on the temporality of the relationships and verify that various pre and post-natal exposures came prior to the emergency department visits or diagnoses of asthma and wheeze. Despite this, we cannot comment fully on the causal relationships, as there are potentially unobserved sources of bias in our study.

In the CHILD study (Chapter 2), many co-variates and outcomes of interest (ED visits/physician diagnoses of AW) relied on data collected from standardized questionnaires. While standardization improves accuracy, questionnaires relied on completeness and correct reporting by mothers, which is an inherent limitation. More objective measures of co-variates and AW would have helped to increase the reliability

and validity of our findings. Regardless of these limitations, this is a rigorously completed cohort study which contributes novelty to the literature on the relationship between secretory sIgA and AW in childhood.

4.2.1.1 Selection Bias in the CHILD Cohort

Prior to the initial recruitment period for CHILD, specific inclusion and exclusion criteria were used to recruit participants from the general Canadian population using multiple methods to control selection bias [23,24]. However, there are some discrete differences in the recruited population that may limit generalizability, including more individuals from the CHILD study are white, urban and from a higher socioeconomic status than the rest of Canada [25]. The loss to follow up is low in the CHILD study, with 92% of mother-child pairs retained when infants reached 1 year of age [24,25]. Although this one year evaluation lacked information from the Toronto study site (777/3,296 total participants), study bias was minimal because the same recruitment and selection methods were used at all sites. One potential source of selection bias is due to issues with stool collection from breastfeeding infants (harder to extract from diapers), but this was controlled by adjustment for breastfeeding status.

4.2.1.2 Measurement Bias in the CHILD Cohort

Measurement bias is the systematic inconsistency in measurements between groups of interest. The prospective, normal subject nature of the CHILD cohort minimizes this type of bias. Standardized operating procedures for biological samples and self-report questionnaires for study participants also serve to reduce systematic inconsistencies between groups, though our study could have benefited further from a formal physician diagnosis of AW instead of relying on a maternal report of physician diagnoses. Despite this maternal-report of childhood asthma is a relatively well-

validated method [24]. As well, although infant fecal samples were not all collected at the same time, the SyMBIOTA research team had all fecal sIgA steps performed by the same research technician which helps to minimize systematic differences between samples. Further strengths of our study include the use of an ELISA kit to detect specifically sIgA in our 3-month stool collection. Despite this, the kit used for detection of fecal sIgA is unable to discriminate between sIgA bound and unbound to bacteria which may be more important than total levels of fecal sIgA in relation to development of asthma as reported by Dzidic et al., (2017) [4].

4.2.1.3 Confounding Bias in the CHILD Cohort

As with all epidemiological studies, we aimed to reduce the effects of confounding between our exposure and an outcome. In chapter 2, using our Directed Acyclic Graph (DAG), we mapped the relationship between various covariates in our study and their effects on the relationship between the total effect of fecal sIgA on asthma development. This visual representation of the relationships between variables helped us understand relationships between covariates and control for potential sources of bias in our exposure-outcome relationships [26]. Proponents of DAGs argue that they are more robust in helping determine causal relationships than other model building methods such as purposeful model building, because over- or under-adjustment bias can be avoided by proper identification of a minimally sufficient adjustment set. Despite our robust approach to model adjustment, there was limited access to data on potentially important issues affecting our relationship of interest. These include: timing of food introduction, probiotic supplementation, fiber intake and types of formula used. Efforts to control for some of these measures included: tests of confounding for duration of breastfeeding, and adjustment for age at fecal sIgA samples.

Inherent to all representations of complex biological phenomena are over-simplifications or misspecifications regarding the relationships of interest. As such, there are some limitations of this approach in comparison to more conventional approaches, but, since both sIgA and respiratory illness have been well described, we are confident our approach addresses a significant amount of the confounding present in this cohort study.

4.2.2 General Strengths and Limitations of the AHS Cohort

The AHS cohort has both a number of strengths and limitations. Retrospective cohort design with database linkage is a cost-effective and valid method to evaluate the use of ED visits for asthma [27]. As well, our sample size was large and there was continuity of data over a relatively long time period (5 years). Other strengths of the study include validated methods used to determine serum immunoglobulin levels and characterization of ED visits for both wheeze and asthma. Unfortunately, missing or conflicting data within the records could result in bias and loss to follow up can result in information bias.

4.2.2.1 Selection Bias in the AHS Cohort

Since the AHS cohort was based on patients with available serum immunoglobulin A levels in the first 18 years of life between April 1st, 2013 to June 30th, 2018, there is a large risk for selection bias. The opportunistic sampling method was based on the availability of testing which precludes randomization. This healthcare record data also only reflects children who sought healthcare for symptoms, increasing the likelihood that asymptomatic children were excluded. As well, demographics other than age and sex were not available, limiting our ability to account for many potential confounders. Since immunoglobulin testing is not done routinely in the general

population, it is likely that individuals in our cohort have a higher burden of diseases associated with possible abnormal immunoglobulin levels. In particular, our group of study reflects individuals that have increased risk of acute and chronic infections, immunologic and autoimmune disorders, liver and renal dysfunction, metabolic derangement, including malnutrition and diabetes, and certain cancers [13]. The plausible higher disease burden in this study population over the general population could have introduced significant bias.

4.2.2.2 Measurement Bias in the AHS Cohort

There are a number of factors that introduce potential measurement bias to the AHS cohort. Due to the association of low immunoglobulin A levels with respiratory infection, previous testing of these levels may influence health care providers to more readily describe symptoms of asthma as bronchitis or bronchiolitis or vice versa. As well, health care providers in the emergency department do not provide a formal evaluation for asthma, resulting in the potential for a primary diagnosis that captures the patient presentation (e.g. wheeze) but does not accurately reflect the long term clinical picture. Resultantly, there is a large potential for heterogeneity in the diagnosis of asthma/wheeze/bronchitis/bronchiolitis. We controlled for this by combining all these common terms into a single AW variable. We also are aware that in spite of there being a fairly standardized protocol for analysis of IgA levels, analyses were done at different sites and times, potentially influencing results.

4.2.2.3 Confounding Bias in the AHS Cohort

Confounding is a significant risk in this epidemiological study. As with our CHILD study, to account for confounding bias in our AHS study we used DAGs to identify

minimally sufficient adjustment sets while determining the effect of serum sIgA on ED visits for AW and this has the same strengths and limitations as above.

As mentioned previously, in contrast to the CHILD study, there were substantially less demographic characteristics observed in this study that could bias the relationship between IgA levels and asthma. These limitations stem from inherent healthcare database issues that reflect opportunistic data collection; not an ideal study design. This study design and lack of information likely contributed to bias in our estimates, and this limitation is especially clear in the 1st year of life, when feeding modes and smoke exposure significantly impact serum IgA levels, immune system development and respiratory illness. Interpretation of study results should reflect an understanding of these limitations.

4.3 Significance and Clinical Relevance

Although IgA, gut microbiome and immunity have been studied in animal models and various cohort studies, this is the first study to combine two cohort studies to report associations between serum IgA and fecal sIgA and asthma and wheeze in the first 5 years of life in humans. Since animal models are beneficial to determine mechanisms but sometimes not applicable to humans, our study greatly adds to the literature on the link between IgA and asthma in humans. Additionally, since there is a difference between serum IgA and fecal sIgA, being able to compare these two exposures with similar outcomes of asthma and wheeze in children in two cohort studies increases the novelty of this work.

Since a randomized control trial testing this association would be impossible, the combined findings of the CHILD and AHS study provide some of the strongest possible evidence for these associations. This study provides a significant addition to the literature on the interrelation between IgA and asthma. In addition, the asthma

model delineated in this thesis connected differences in infant gut IgA and serum IgA to child atopy, in turn providing insight on potential infant biomarkers to predict development of other common atopic chronic conditions in children. These findings should also encourage interventions aimed at breastfeeding promotion as a potential means to reduce the risk of developing asthma.

4.4 Implications for future research

Despite the large body of data on IgA and asthma, these findings reveal that more studies on the relationships between IgA in early life and development of asthma, wheeze and other atopic conditions are needed. In particular the differential influence and mechanisms of IgA and sIgA in relation to the development of asthma and atopic disease need further exploration. It would be beneficial to know the optimal levels and/or binding of sIgA that promote a healthy infant gut microbiome composition, and how serum IgA is related.

4.5 Concluding Remarks

This thesis investigated the association between serum and secretory IgA and childhood respiratory disease in the first few years of life. We found that when a formula-fed infant, low fecal sIgA is significantly associated with childhood asthma/wheeze, when controlling for various covariates. As well, we found that serum IgA in the first three years of life is marginally associated with AW in the first 5 years of life. With these population-level findings, this study highlights the importance of early infant immune system development as it may contribute to the life-long health of children.

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Appendix A:

Approval Form

Date:	October 24, 2018
Study ID:	Pro00083778
Principal Investigator:	Elizabeth Hicks
Study Title:	Characterizing IgA deficiency: associated diseases
Approval Expiry Date:	Wednesday, October 23, 2019

Thank you for submitting the above study to the Health Research Ethics Board - Health Panel. Your application, including the following, has been reviewed and approved on behalf of the committee;

- Protocol (10/9/2018)
- Health Data Variables Requested for IgA Characterization (10/14/2018)

The Health Research Ethics Board assessed all matters required by section 50(1)(a) of the Health Information Act. It has been determined that the research described in the ethics application is a secondary analysis of anonymized administrative health data for which subject consent for access to personally identifiable health information would not be reasonable, feasible or practical. Subject consent therefore is not required for access to personally identifiable health information described in the ethics application.

In order to comply with the Health Information Act, a copy of the approval form is being sent to the Office of the Information and Privacy Commissioner.

A renewal report must be submitted next year prior to the expiry of this approval if your study still requires ethics approval. If you do not renew on or before the renewal expiry date (Monday, October 14, 2019), you will have to re-submit an ethics application.

Approval by the Health Research Ethics Board does not encompass authorization to access the patients, staff or resources of Alberta Health Services or other local health care institutions for the purposes of the research. Enquiries regarding Alberta Health approvals should be directed to (780) 407-6041. Enquiries regarding Covenant Health approvals should be directed to (780) 735-2274.

Sincerely,

Anthony S. Joyce, PhD.
Chair, Health Research Ethics Board - Health Panel

Note: This correspondence includes an electronic signature (validation and approval via an online system).

Notification of Approval (Renewal)

Date:	October 11, 2019
Amendment ID:	Pro00083778_REN1
Principal Investigator:	Elizabeth Hicks
Study ID:	MS2_Pro00083778
Study Title:	Characterizing IgA deficiency: associated diseases
Approval Expiry Date:	Friday, October 9, 2020

Thank you for submitting this renewal application. Your application has been reviewed and approved.

This re-approval is valid for another year. If your study continues past the expiration date as noted above, you will be required to complete another renewal request. Beginning at 30 days prior to the expiration date, you will receive notices that the study is about to expire. If you do not renew on or before the renewal expiry date, you will have to re-submit an ethics application.

All study-related documents should be retained so as to be available to the Health REB upon request. They should be kept for the duration of the project and for at least 5 years following study completion.

Sincerely,

Anthony S. Joyce, PhD.
Chair, Health Research Ethics Board - Health Panel

Note: This correspondence includes an electronic signature (validation and approval via an online system).

Appendix B:

Table B1

Comparison between the general CHILD cohort and the 1071 infant CHILD sub-Cohort with available Fecal sIgA Samples

		CHILD Cohort (Three Sites*) % N = 2,502	sIgA Sample Available % (95% CI) N = 1071	ChiX²**
Co-Variates		Row % (N)	Row % (N)	p-value(X^2)
Asthma Diagnoses from Ages 1-3	No	56.64 (1,322)	43.36 (1,012)	0.253
	Yes	50 (52)	50 (52)	
Asthma Diagnoses from Ages 4-5	No	55.23 (612)	44.77 (496)	0.094
	Yes	62.88 (83)	37.12 (49)	
UV for AW from Ages 1-3	No	53.18 (535)	46.82 (471)	0.165
	Yes	57.08 (262)	42.92 (197)	
UV for AW from Ages 4-5	No	54.65 (605)	45.35 (502)	0.157

		CHILD Cohort (Three Sites*) % N = 2,502	sIgA Sample Available % (95% CI) N = 1071	ChiX²**
	Yes	60 (123)	40 (82)	
Sex	Male	56.63 (722)	43.37 (553)	0.542
	Female	57.85 (678)	42.15 (494)	
Mode of Delivery	Vaginal	57.61 (1,079)	42.39 (794)	0.701
	Elective Cesarean	56.65 (132)	43.35 (101)	
	Emergency Cesarean	55.19 (186)	44.81 (151)	
Breastfeeding Status	Exclusive	25.28 (91)	74.72 (269)	**<0.001**
	Partial	45.06 (292)	54.94 (356)	
	None	62.42 (431)	37.58 (431)	
Infant Antibiotics	No	56.97 (1,238)	43.03 (935)	0.319
	Yes	52.59 (71)	47.41 (64)	
Depression	None	57.12 (1,200)	42.88 (901)	0.752
	Antenatal	54.23 (77)	45.77 (65)	
	Persistent	54.02 (94)	45.98 (80)	
	Postnatal	59.65 (34)	40.35 (23)	
Smoke Exposure (Prenatal)	No	57.19 (1,281)	42.81 (959)	**0.023**
	Yes	48.26 (89)	51.74 (89)	
Smoke Exposure (Postnatal)	No	55.12 (1,072)	44.88 (873)	0.053
	Yes	49.58 (178)	50.42 (181)	
Multigravida	No	56.01 (489)	43.99 (384)	0.382
	Yes	57.83 (942)	42.17 (687)	

		CHILD Cohort (Three Sites*) % N = 2,502	sIgA Sample Available % (95% CI) N = 1071	ChiX^{2**}
Maternal Overweight/Obesity (During Pregnancy)	Normal	57.55 (804)	42.45 (593)	0.909
	Overweight	56.5 (300)	43.5 (231)	
	Obese	56.97 (327)	43.03 (247)	
Maternal Allergy/Asthma During Pregnancy	No	56.63 (517)	43.37 (396)	0.945
	Yes	56.48 (845)	43.52 (651)	
Maternal Age (Greater or less than median)	No	56.55 (814)	43.45 (603)	0.955
	Yes	55.5 (290)	44.5 (221)	

Table B2. Distribution lowest fecal sIgA, atopic and non-atopic asthma/wheeze according to demographic and epidemiological factors (n=1071)

		Lowest tertile sIgA (% yes) n=314/951	**ChiX²****	**Atopic AW vs no AW (Ages 1-3) n=26/455**	**ChiX²****	**Atopic AW vs no AW (Ages 4-5) n=23/463**	**ChiX²****	**Non-Atopic AW vs no AW (Ages 1-3) n=175/604**	**ChiX²****	**Non-Atopic AW vs no AW (Ages 4-5) n=78/518**	**ChiX²****
Co-Variates		Row % (N)	p-value (X^2 exact)	Row % (N)	p-value (X^2 exact)	Row % (N)	p-value (X^2 exact)	Row % (N)	p-value (X^2 exact)	Row % (N)	p-value (X^2 exact)
Sex	Male	32.06 (160)	0.406	7.47 (18)	0.087	6.33 (15)	0.167	30.31 (97)	0.441	15.59 (41)	0.731
	Female	34.62 (152)		3.74 (8)		3.54 (8)		27.46 (78)		14.51 (37)	
Mode of Delivery	Vaginal	32.15 (227)	0.165	4.82 (17)	0.204	3.87 (14)	**0.046**	27.27 (126)	**0.018**	12.56 (50)	**0.015**
	Elective Cesarean	31.52 (29)		11.76 (4)		11.90 (5)		45.45 (25)		24.49 (12)	
	Emergency Cesarean	40.29 (56)		7.35 (5)		6.78 (4)		27.59 (24)		22.54 (16)	
Breastfeeding Status	Non	68.33 (163	**<0.001**	7.22 (7)	0.434	5.41 (6)	0.953	36.62 (52)	**0.042**	18.60 (24)	0.358
	Partial	31.48 (96)		6.75 (11)		4.58 (7)		28.97 (62)		15.12 (26)	
	Exclusive	14.29 (58)		4.10 (8)		5.03 (10)		24.60 (61)		12.90 (28)	
Infant Antibiotics	No	34.09 (284)	0.701	5.73 (24)	0.619	4.82 (20)	1.00	27.52 (150)	0.112	15.24 (71)	0.984
	Yes	31.67 (19)		0.00 (0)		4.35 (1)		40.00 (14)		15.35 (4)	
Depression	None	31.4 (254)	**<0.001**	5.22 (21)	**0.053**	4.83 (20)	0.243	27.01 (141)	0.059	14.16 (65)	0.287
	Antenatal	50.88 (29)		5.56 (1)		6.25 (1)		41.38 (12)		28.57 (6)	
	Persistent	33.80 (24)		6.67 (2)		3.45 (1)		40.43 (19)		17.65 (6)	

			Lowest tertile sIgA (% yes) n=314/951	ChiX²**	Atopic AW vs no AW (Ages 1-3) n=26/455	ChiX²**	Atopic AW vs no AW (Ages 4-5) n=23/463	ChiX²**	Non-Atopic AW vs no AW (Ages 1-3) n=175/604	ChiX²**	Non-Atopic AW vs no AW (Ages 4-5) n=78/518
	Postnatal	61.90 (13)		40.0 (2)		25.00 (1)		50.00 (3)		25.00 (1)	
Furry Pets in the Home	No	37.84 (165)	**0.007**	5.53 (12)	0.858	5.50 (11)	0.674	26.79 (75)	0.264	18.88 (44)	**0.034**
	Yes	29.5 (149)		5.15 (12)		4.65 (12)		30.94 (99)		12.14 (34)	
Smoke Exposure (Prenatal)	No	31.7 (272)	**0.004**	5.62 (24)	0.647	4.64 (20)	0.135	28.16 (158)	0.208	14.37 (69)	0.085
	Yes	47.56 (39)		8.00 (2)		11.54 (3)		37.84 (14)		25.81 (8)	
Smoke Exposure (Postnatal)	No	31.96 (249)	0.053	5.74 (23)	0.755	5.37 (21)	0.554	26.32 (135)	**<0.001**	15.14 (66)	0.955
	Yes	39.76 (66)		3.85 (2)		2.94 (2)		43.82 (39)		15.38 (12)	
Multigravida	No	34.44 (125)	0.597	7.26 (13)	0.252	4.05 (7)	0.481	29.66 (70)	0.765	15.74 (31)	0.735
	Yes	32.77 (195)		4.71 (13)		5.52 (16)		28.53 (105)		14.64 (47)	
Maternal Overweight	Normal	28.24 (148)	**<0.001**	5.71 (16)	0.857	6.27 (18)	0.203	27.07 (98)	0.092	14.33 (45)	0.569
	Obese	33.17 (69)		6.48 (7)		1.92 (2)		27.34 (38)		14.29 (17)	
	Overweight	45.58 (103)		4.48 (3)		4.17 (3)		37.86 (39)		18.82 (16)	
Maternal Allergy/Asthma During Pregnancy	No	32.87 (118)	0.941	5.65 (10)	0.940	3.89 (7)	0.367	25.78 (58)	0.210	11.73 (23)	0.097
	Yes	33.1 (192)		5.82 (16)		5.78 (16)		30.56 (114)		17.41 (54)	
Maternal Age (Greater than Median)	No	36.48 (174)	**0.033**	5.50 (12)	0.853	4.72 (10)	0.820	31.79 (96)	0.127	16.18 (39)	0.504
	Yes	29.93 (138)		5.91 (26)		5.18 (13)		26.16 (79)		14.08 (39)	

Table B3. Test of Interaction between Fecal sIgA (2-5.5 months) and breastfeeding status at stool collection

	UV for AW at 1-3 Years (95% CI)
Model 1: Crude OR (Low Fecal sIgA (2-5.5 Months))	**1.54 (1.07, 2.22)**
Model 2: Adjusted for Breastfeeding	1.36 (0.92, 2.01)
Model 3: Breastfeeding * Low Fecal sIgA (2-5.5 Months) (Reference: Breastfed, Normal-High Fecal sIgA)	
Breastfed*Low Fecal sIgA (2-5.5 Months)	1.69 (0.77, 3.71)
Formula Fed*Normal-High Fecal sIgA (2-5.5 Months)	1.48 (0.96, 2.29)
Formula Fed*Low Fecal sIgA (2-5.5 Months)	**1.89 (1.21, 2.94)**

Table B4. Test of Interaction between Fecal sIgA (2-5.5 months) and breastfeeding status at stool collection

Variable	Interactions with fecal sIgA
Breastfeeding status at stool collection	**0.036**

Note: Interactions between a covariate and fecal sIgA variable were tested. When interaction terms with $p<0.05$ were found, the interaction term for each was reported.

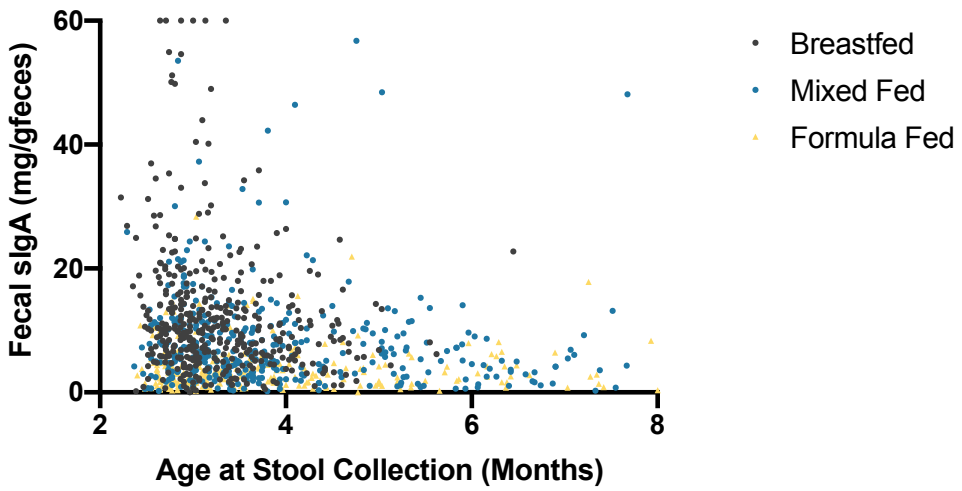

Figure B1. Fecal sIgA levels (mg/gfeces) by feeding mode and infant age at time of stool collection.

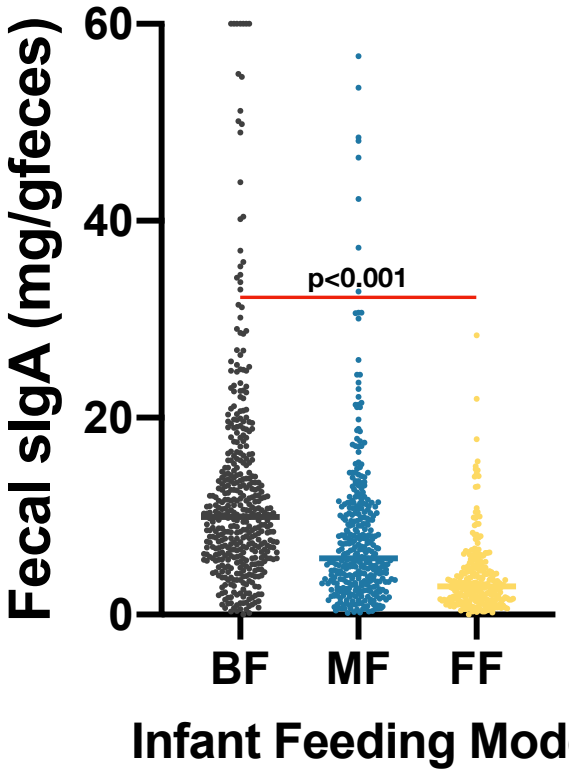

Figure B2. Fecal sIgA levels (mg/gfeces) by feeding mode at time of stool collection. BF = Breastfed; MF = Mixed Fed; FF = Formula Fed. P-values indicate significant differences between groups based on Kruskal-Wallis Test.

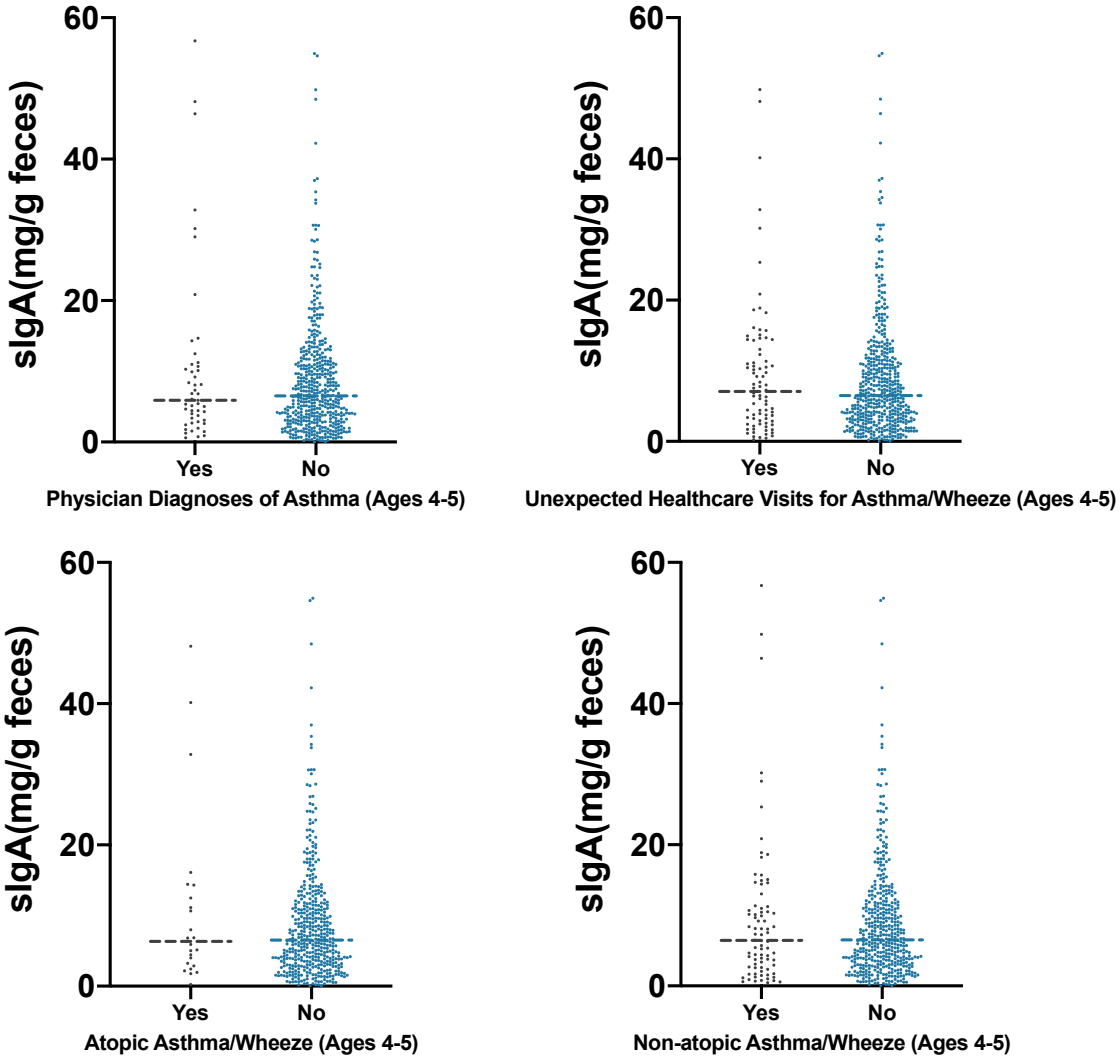

Figure B3. sIgA levels among AW groups from ages 4-5. UVs for AW, atopic AW and non-atopic AW had significantly lower fecal sIgA levels compared to the reference group of those without AW.

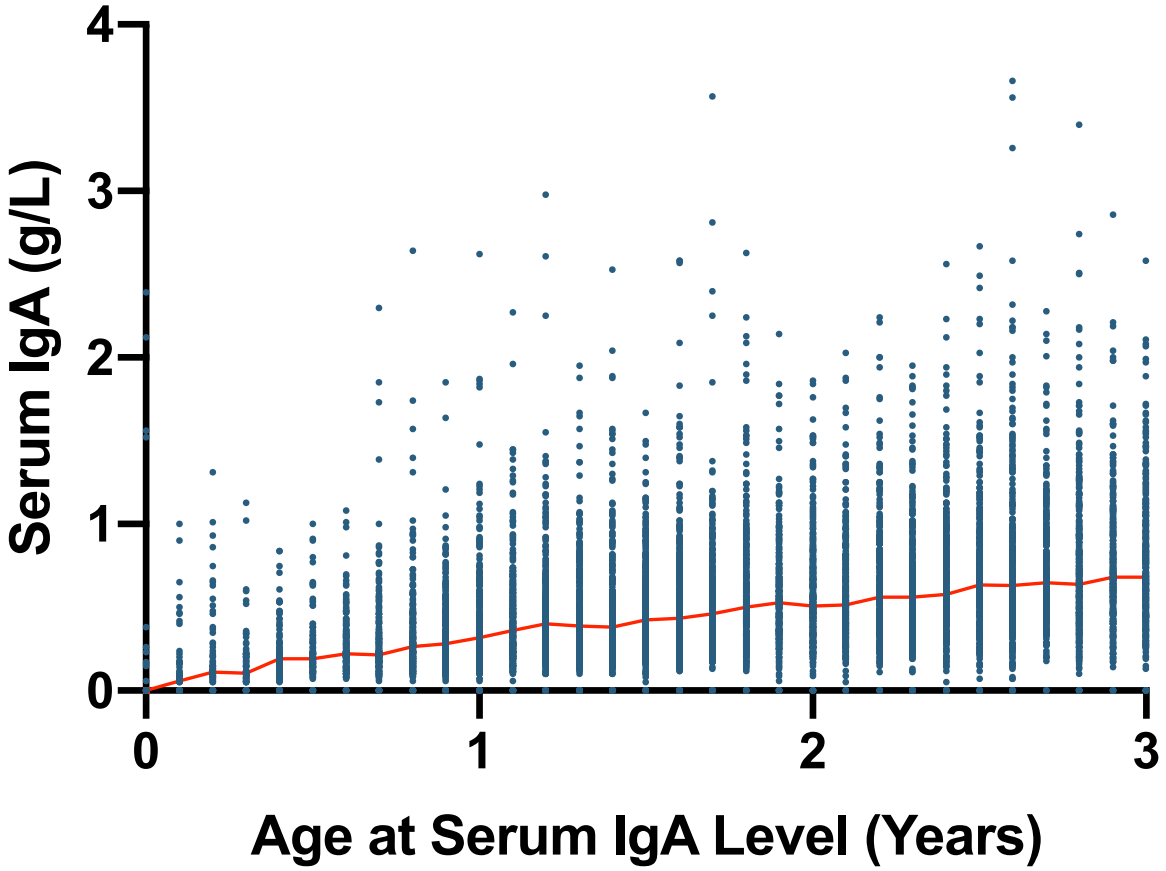

Figure C1. Serum IgA levels (g/L) by age in years from ages 0-3. Red line indicates median serum IgA level (g/L) by time of serum IgA collection (years). 5 data points were outside the scale of the graph.

Table C1. Proportion of ED Visits for AW (Ages 4-5) and Potential Covariates in the 9,938 n AHS sub-Cohort with Serum IgA Samples from Ages 0-3

		Two Highest Tertiles of Serum IgA (0-1 Years) n=1,360/2,040	ChiX^{2**}	Two Highest Tertiles of Serum IgA (1-2 Years) n=2,655/4,079	ChiX^{2**}	Two Highest Tertiles of Serum IgA (2-3 Years) N = 2,506/3,819	ChiX^{2**}	ED Visit for AW (Age 4-5 Years) vs No AW n=219/8,937	ChiX^{2**}	ED Visit for Atopic AW (Age 4-5 Years) vs No AW n=46/8,764	ChiX^{2**}	ED Visit for Non-Atopic AW (Age 4-5 Years) vs No AW n=32/8,750	ChiX^{2**}	ED Visit for Atopic Asthma (Age 4-5 Years) vs No AW n=41/8,791	ChiX^{2**}	ED Visit for Non-Atopic Asthma (Age 4-5 Years) vs No AW n=25/8,775	ChiX^{2**}
Co-variates		Row % (N)	p-value (X^2 exact)	Row % (N)	p-value (X^2 exact)	Row % (N)	p-value (X^2 exact)	Row % (N)	p-value (X^2 exact)	Row % (N)	p-value (X^2 exact)	Row % (N)	p-value (X^2 exact)	Row % (N)	p-value (X^2 exact)	Row % (N)	p-value (X^2 exact)
Sex	Female	66.40 (585)	0.825	61.50 (1,131)	**<0.001**	63.69 (1,054)	**0.028**	2.08 (82)	**0.044**	0.41 (16)	0.195	0.36 (14)	0.951	0.33 (13)	0.105	0.26 (10)	0.665
	Male	66.87 (775)		68.04 (1,524)		67.10 (1,452)		2.74 (137)		0.61 (30)		0.37 (18)		0.57 (28)		0.31 (15)	

		Two Highest Tertiles of Serum IgA (0-1 Years) n=1,360/2,040	ChiX2**	Two Highest Tertiles of Serum IgA (1-2 Years) n=2,655/4,079	ChiX2**	Two Highest Tertiles of Serum IgA (2-3 Years) N = 2,506/3,819	ChiX2**	ED Visit for AW (Age 4-5 Years) vs No AW n=219/8,937	ChiX2**	ED Visit for Atopic AW (Age 4-5 Years) vs No AW n=46/8,764	ChiX2**	ED Visit for Non-Atopic AW (Age 4-5 Years) vs No AW n=32/8,750	ChiX2**	ED Visit for Atopic Asthma (Age 4-5 Years) vs No AW n=41/8,791	ChiX2**	ED Visit for Non-Atopic Asthma (Age 4-5 Years) vs No AW n=25/8,775	ChiX2**
Diagnoses of Failure to Thrive	No	66.67 (1,214)	1.00	65.54 (2,433)	0.053	65.94 (2,379)	0.088	2.52 (208)	0.118	0.48 (39)	0.769	0.40 (32)	0.175	0.48 (39)	0.769	0.31 (25)	0.257
	Yes	66.76 (146)		60.49 (222)		60.19 (127)		1.57 (11)		0.29 (2)		0.00 (0)		0.29 (2)		0.00 (0)	
Age at Serum IgA Level (Years)	0-1							1.67 (30)	**<0.001**	0.28 (5)	**0.002**	0.34 (6)	0.963	0.28 (5)	**0.011**	0.23 (4)	0.871
	1-2							2.03 (75)		0.33 (12)		0.38 (14)		0.30 (11)		0.30 (11)	
	2-3							3.31 (114)		0.86 (29)		0.36 (12)		0.74 (25)		0.30 (10)	

Chi(X2) comparison used to investigate whether distributions of categorical variables differ from one another. Fishers exact test was used when expected frequencies were <5 in >20% of cells. **Bold indicates a significant difference between the two populations.

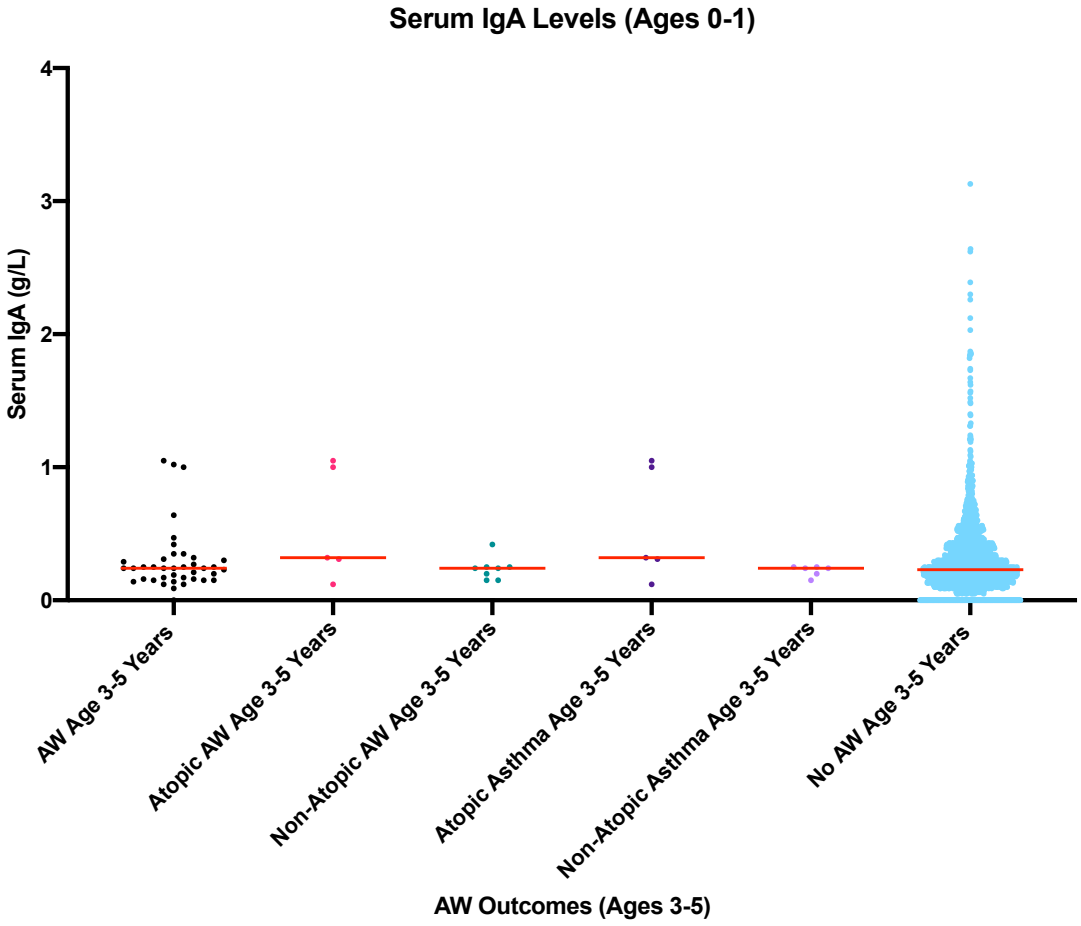

Figure C2. Serum IgA levels (g/L) from ages 0-1 among AW groups from ages 4-5 years. AW groups compared to the reference group of those without AW based on Mann-Whitney U.

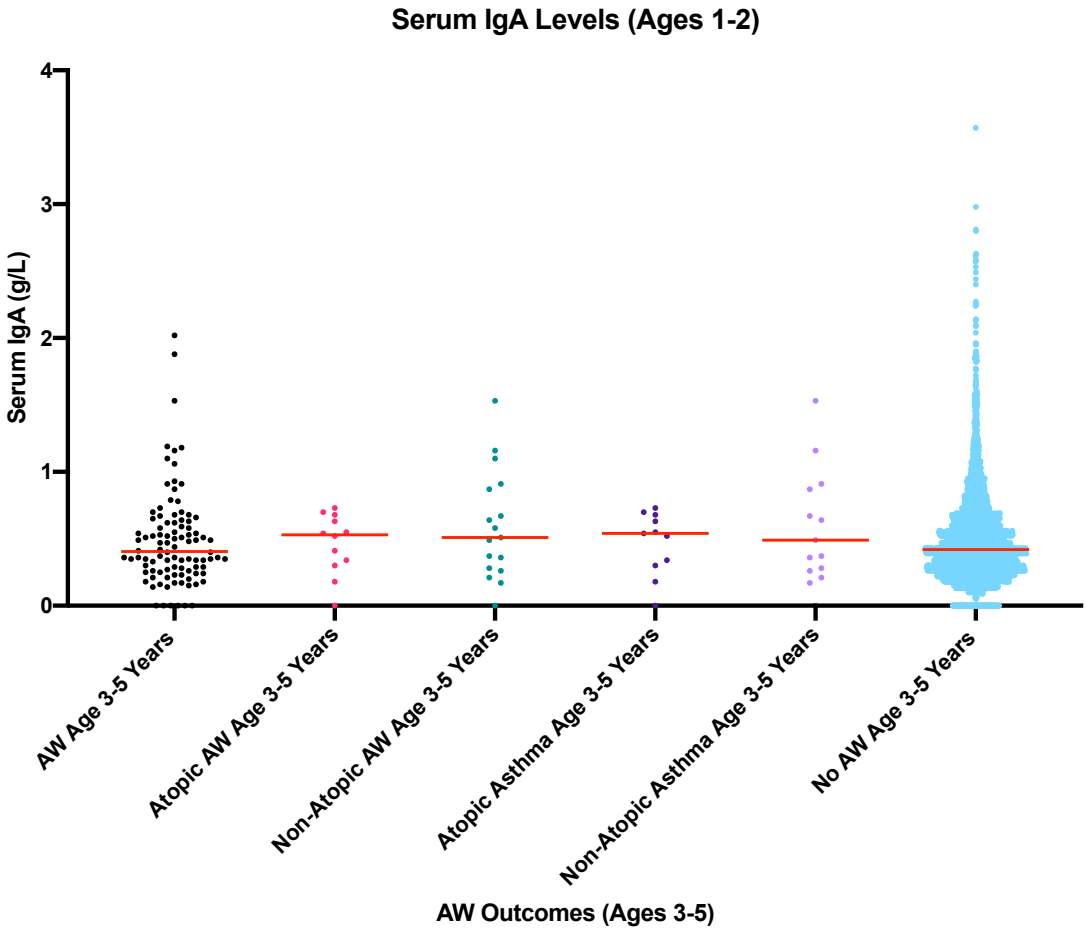

Figure C3. Serum IgA levels (g/L) from ages 1-2 among AW groups from ages 4-5 years. AW groups compared to the reference group of those without AW based on Mann-Whitney U.

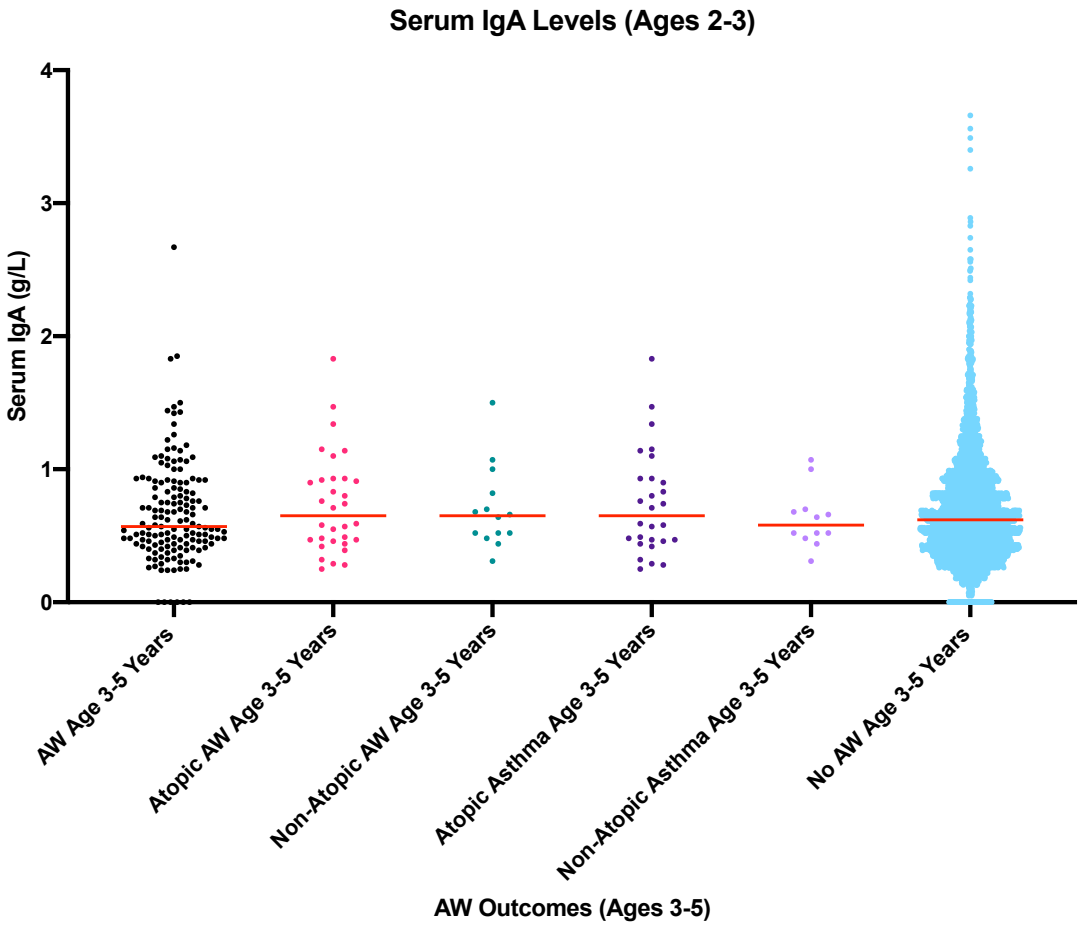

Figure C4. Serum IgA levels (g/L) from ages 2-3 among AW groups from ages 4-5 years. AW groups compared to the reference group of those without AW based on Mann-Whitney U.

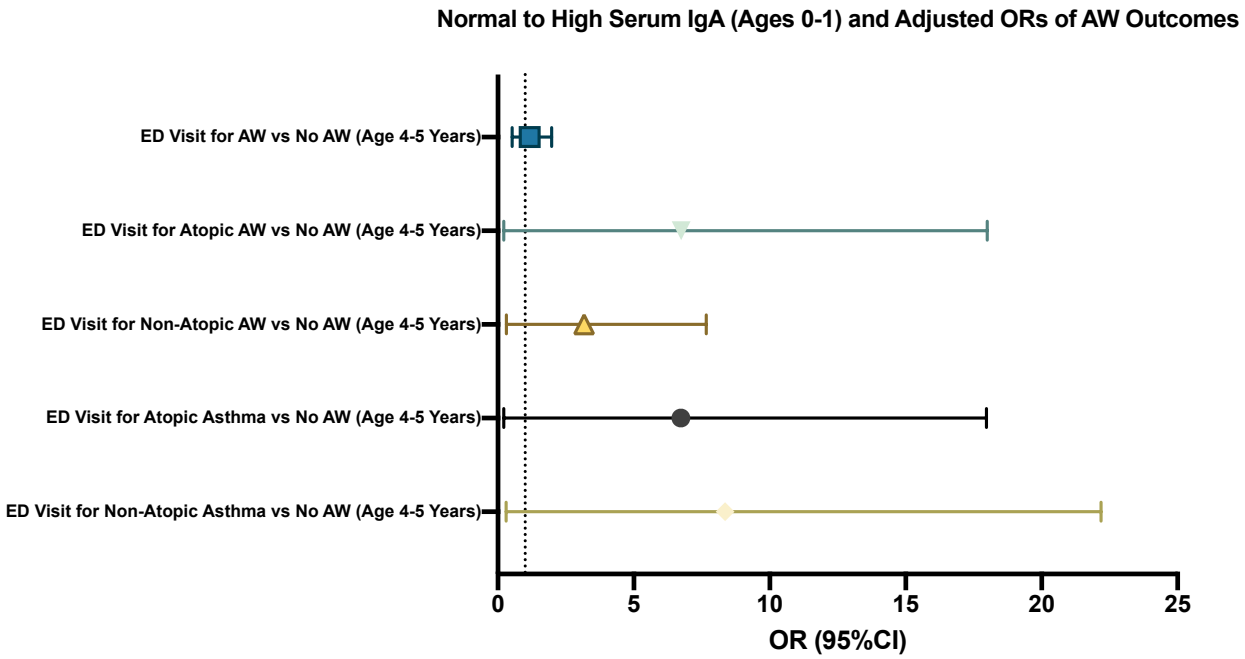

Figure C5. Forest plot of adjusted ORs for AW outcomes ages 4-5 years in children with normal to high serum IgA levels (g/L) compared to those with low serum IgA levels from ages 0-1, adjusted for failure to thrive and sex.

Table C2. Odds of Emergency Department Visits for Asthma and/or Wheeze (Age 4-5 Years) After Exposure to the Two Highest Tertiles of Serum IgA (Ages 0-1)

Ref: Lowest Tertile Serum IgA (0-1 Years)	ED Visit for AW vs No AW (Age 4-5 Years) Crude OR (95% CI)	ED Visit for AW vs No AW (Age 4-5 Years) Adjusted OR (95% CI) (n=2,040)*
Normal-High Serum IgA (0-1 Years)	1.00 (0.52, 1.98)	1.00 (0.52, 1.98)
Failure to Thrive	0.62 (0.33, 1.14)	0.67 (0.20, 2.19)
Sex (Ref: Female)	**1.33 (1.00, 1.75)**	1.12 (0.59, 2.13)

Bold indicates p<0.05. *Adjusted for sex and failure to thrive

Table C3. Odds of Emergency Department Visits for Atopic Asthma and/or Wheeze (Age 4-5 Years) After Exposure to the Two Highest Tertiles of Serum IgA (Ages 0-1)

Ref: Lowest Tertile Serum IgA (0-1 Years)	ED Visit for Atopic AW vs No AW (Age 4-5 Years) Crude OR (95% CI)	ED Visit for Atopic AW vs No AW (Age 4-5 Years) Adjusted OR (95% CI) (n=2,010)*
Normal-High Serum IgA (0-1 Years)	2.02 (0.23, 18.1)	1.97 (0.22, 17.70)
Failure to Thrive	0.53 (0.13, 2.20)	1 (Omitted)
Sex (Ref: Female)	1.49 (0.81, 2.74)	3.10 (0.35, 27.8)

Bold indicates p<0.05. *Adjusted for sex and failure to thrive

Table C4. Odds of Emergency Department Visits for Atopic Asthma (Age 4-5 Years) After Exposure to the Two Highest Tertiles of Serum IgA (Ages 0-1)

Ref: Lowest Tertile Serum IgA (0-1 Years)	ED Visit for Non-Atopic AW vs No AW (Age 4-5 Years) Crude OR (95% CI)	ED Visit for Non-Atopic AW vs No AW (Age 4-5 Years) Crude OR (95% CI) (n=2,013)*
Normal-High Serum IgA (0-1 Years)	1.51 (0.30, 7.52)	1.57 (0.31, 7.80)
Failure to Thrive	1 (Omitted)	1 (Omitted)
Sex (Ref: Female)	1.02 (0.51, 2.06)	0.11 (0.01, 0.90)

Bold indicates p<0.05. *Adjusted for sex and failure to thrive

Table C5. Odds of Emergency Department Visits for Atopic Asthma (Age 4-5 Years) After Exposure to the Highest Tertile of Serum IgA (Ages 0-1)

Ref: Lowest Tertile Serum IgA (0-1 Years)	ED Visit for Atopic Asthma vs No AW (Age 4-5 Years) Crude OR (95% CI)	ED Visit for Atopic Asthma vs No AW (Age 4-5 Years) Adjusted OR (95% CI) (n=2,012)*
Normal-High Serum IgA (0-1 Years)	2.02 (0.22, 18.1)	1.97 (0.22, 17.70)
Failure to Thrive	0.60 (0.14, 2.49)	1 (Omitted)
Sex (Ref: Female)	1.71 (0.89, 3.31)	3.10 (0.35, 27.8)

Bold indicates p<0.05. *Adjusted for sex and failure to thrive

Table C6. Odds of Emergency Department Visits for Non-Atopic Asthma (Age 4-5 Years) After Exposure to the Highest Tertile of Serum IgA (Ages 0-1)

Ref: Lowest Tertile Serum IgA (0-1 Years)	ED Visit for Non-Atopic Asthma vs No AW (Age 4-5 Years) Crude OR (95% CI)	ED Visit for Non-Atopic Asthma vs No AW (Age 4-5 Years) Crude OR (95% CI) (n=2,013)*
Normal-High Serum IgA (0-1 Years)	2.53 (0.29, 21.7)	2.64 (0.31, 22.72)
Failure to Thrive	1 (Omitted)	1 (Omitted)
Sex (Ref: Female)	1.19 (0.54, 2.66)	1 (Omitted)

Bold indicates p<0.05. *Adjusted for sex and failure to thrive

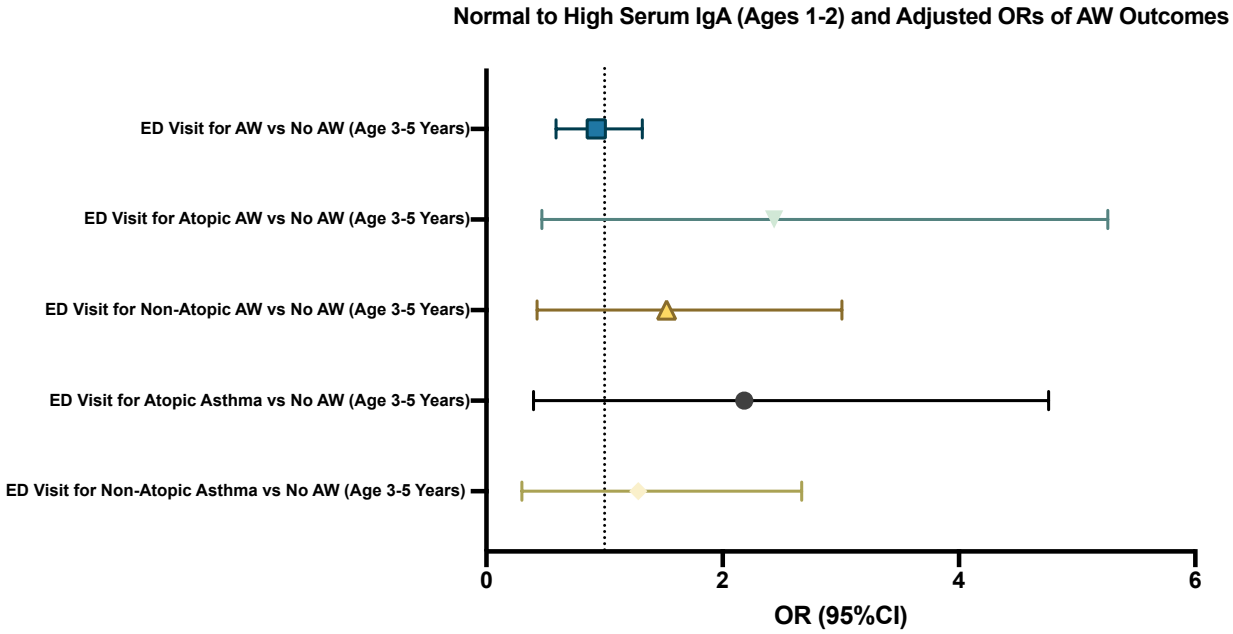

Figure C6. Forest plot of adjusted ORs for AW outcomes ages 4-5 years in children with normal to high serum IgA levels (g/L) compared to those with low serum IgA levels from ages 1-2, adjusted for failure to thrive and sex.

Table C7. Odds of Emergency Department Visits for Asthma and/or Wheeze (Age 4-5 Years) After Exposure to the Two Highest Tertiles of Serum IgA (Ages 1-2)

Ref: Lowest Tertile Serum IgA (1-2 Years)	ED Visit for AW vs No AW (Age 4-5 Years) Crude OR (95% CI)	ED Visit for AW vs No AW (Age 4-5 Years) Adjusted OR (95% CI) (n=4,079)*
Normal-High Serum IgA (1-2 Years)	0.90 (0.61, 1.35)	0.88 (0.59, 1.32)
Failure to Thrive	0.62 (0.33, 1.14)	0.87 (0.42, 1.80)
Sex (Ref: Female)	**1.33 (1.00, 1.75)**	1.42 (0.94, 2.16)

Bold indicates p<0.05. *Adjusted for sex and failure to thrive

Table C8. Odds of Emergency Department Visits for Atopic Asthma and/or Wheeze (Age 4-5 Years) After Exposure to the Two Highest Tertiles of Serum IgA (Ages 1-2)

Ref: Lowest Tertile Serum IgA (1-2 Years)	ED Visit for Atopic AW vs No AW (Age 4-5 Years) Crude OR (95% CI)	ED Visit for Atopic AW vs No AW (Age 4-5 Years) Adjusted OR (95% CI) (n=4,011)*
Normal-High Serum IgA (1-2 Years)	1.67 (0.50, 5.54)	1.56 (0.47, 5.20)
Failure to Thrive	0.53 (0.13, 2.20)	1 (Omitted)
Sex (Ref: Female)	1.49 (0.81, 2.74)	2.38 (0.64, 8.84)

Bold indicates p<0.05. *Adjusted for sex and failure to thrive

Table C9. Odds of Emergency Department Visits for Atopic Asthma (Age 4-5 Years) After Exposure to the Two Highest Tertiles of Serum IgA (Ages 1-2)

Ref: Lowest Tertile Serum IgA (1-2 Years)	ED Visit for Non-Atopic AW vs No AW (Age 4-5 Years) Crude OR (95% CI)	ED Visit for Non-Atopic AW vs No AW (Age 4-5 Years) Crude OR (95% CI) (n=4,013)*
Normal-High Serum IgA (1-2 Years)	1.19 (0.45, 3.13)	1.13 (0.43, 2.97)
Failure to Thrive	1 (Omitted)	1 (Omitted)
Sex (Ref: Female)	1.02 (0.51, 2.06)	1.94 (0.68, 5.54)

Bold indicates p<0.05. *Adjusted for sex and failure to thrive

Table C10. Odds of Emergency Department Visits for Atopic Asthma (Age 4-5 Years) After Exposure to the Highest Tertile of Serum IgA (Ages 1-2)

Ref: Lowest Tertile Serum IgA (1-2 Years)	ED Visit for Atopic Asthma vs No AW (Age 4-5 Years) Crude OR (95% CI)	ED Visit for Atopic Asthma vs No AW (Age 4-5 Years) Adjusted OR (95% CI) (n=4,020)*
Normal-High Serum IgA (1-2 Years)	1.46 (0.43, 4.98)	1.37 (0.40, 4.70)
Failure to Thrive	0.60 (0.14, 2.49)	1 (Omitted)
Sex (Ref: Female)	1.71 (0.89, 3.31)	2.15 (0.57, 8.11)

Bold indicates p<0.05. *Adjusted for sex and failure to thrive

Table C11. Odds of Emergency Department Visits for Non-Atopic Asthma (Age 4-5 Years) After Exposure to the Highest Tertile of Serum IgA (Ages 1-2)

Ref: Lowest Tertile Serum IgA (1-2 Years)	ED Visit for Non-Atopic Asthma vs No AW (Age 4-5 Years) Crude OR (95% CI)	ED Visit for Non-Atopic Asthma vs No AW (Age 4-5 Years) Crude OR (95% CI) (n=4,020)*
Normal-High Serum IgA (1-2 Years)	0.97 (0.33, 2.89)	0.89 (0.30, 2.67)
Failure to Thrive	1 (Omitted)	1 (Omitted)
Sex (Ref: Female)	1.19 (0.54, 2.66)	**4.56 (1.00, 20.62)**

Bold indicates p<0.05. *Adjusted for sex and failure to thrive

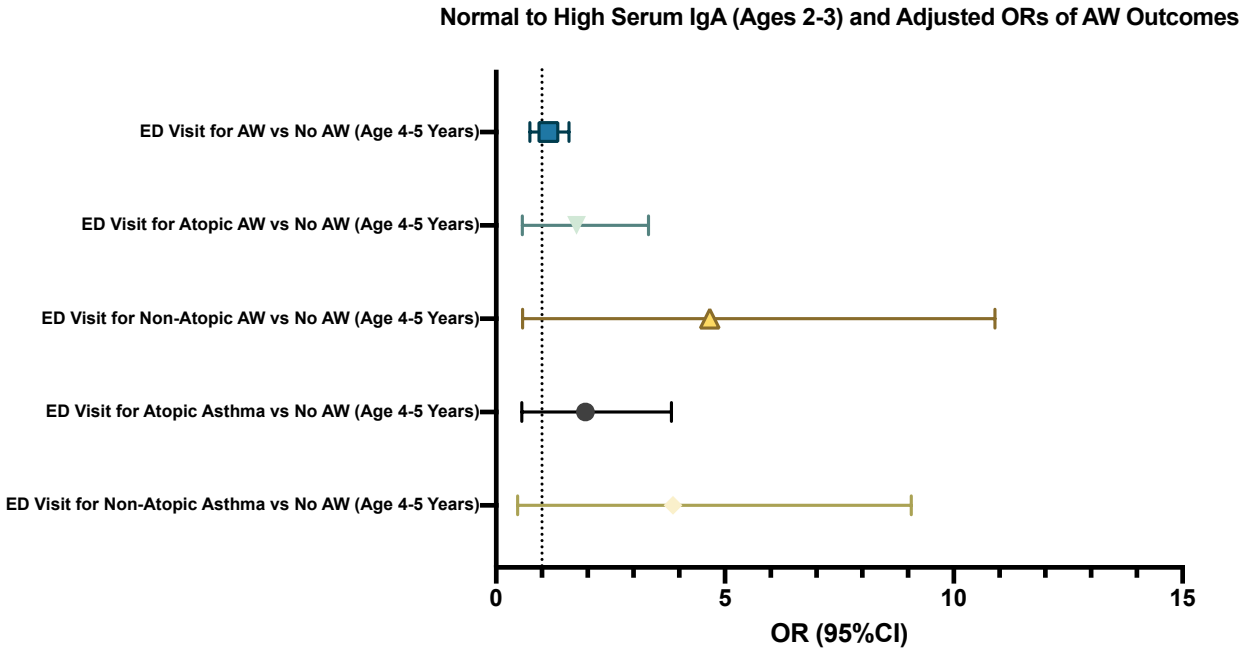

Figure C7. Forest plot of adjusted ORs for AW outcomes ages 4-5 years in children with normal to high serum IgA levels (g/L) compared to those with low serum IgA levels from ages 2-3, adjusted for failure to thrive and sex.

Table C12. Odds of Emergency Department Visits for Asthma and/or Wheeze (Age 4-5 Years) After Exposure to the Two Highest Tertiles of Serum IgA (Ages 2-3)

Ref: Lowest Tertile Serum IgA (2-3 Years)	ED Visit for AW vs No AW (Age 4-5 Years) Crude OR (95% CI)	ED Visit for AW vs No AW (Age 4-5 Years) Adjusted OR (95% CI) (n=3,819)*
Normal-High Serum IgA (2-3 Years)	1.10 (0.75, 1.60)	1.09 (0.74, 1.59)
Failure to Thrive	0.62 (0.33, 1.14)	0.95 (0.48, 1.87)
Sex (Ref: Female)	**1.33 (1.00, 1.75)**	1.25 (0.91, 1.72)

Bold indicates p<0.05. *Adjusted for sex and failure to thrive

Table C13. Odds of Emergency Department Visits for Atopic Asthma and/or Wheeze (Age 4-5 Years) After Exposure to the Two Highest Tertiles of Serum IgA (Ages 2-3)

Ref: Lowest Tertile Serum IgA (2-3 Years)	ED Visit for Atopic AW vs No AW (Age 4-5 Years) Crude OR (95% CI)	ED Visit for Atopic AW vs No AW (Age 4-5 Years) Adjusted OR (95% CI) (n=3,723)*
Normal-High Serum IgA (2-3 Years)	2.53 (0.58, 10.95)	2.52 (0.58, 10.9)
Failure to Thrive	0.53 (0.13, 2.20)	1.59 (0.48, 5.23)
Sex (Ref: Female)	1.49 (0.81, 2.74)	1.25 (0.63, 2.51)

Bold indicates p<0.05. *Adjusted for sex and failure to thrive

Table C14. Odds of Emergency Department Visits for Atopic Asthma (Age 4-5 Years) After Exposure to the Two Highest Tertiles of Serum IgA (Ages 2-3)

Ref: Lowest Tertile Serum IgA (2-3 Years)	ED Visit for Non-Atopic AW vs No AW (Age 4-5 Years) Crude OR (95% CI)	ED Visit for Non-Atopic AW vs No AW (Age 4-5 Years) Crude OR (95% CI) (n=3,709)*
Normal-High Serum IgA (2-3 Years)	1.48 (0.57, 3.88)	1.38 (0.57, 3.33)
Failure to Thrive	1 (Omitted)	1 (Omitted)
Sex (Ref: Female)	1.02 (0.51, 2.06)	1.05 (0.42, 2.63)

Bold indicates p<0.05. *Adjusted for sex and failure to thrive

Table C15. Odds of Emergency Department Visits for Atopic Asthma (Age 4-5 Years) After Exposure to the Two Highest Tertiles of Serum IgA (Ages 2-3)

Ref: Lowest Tertile Serum IgA (2-3 Years)	ED Visit for Atopic Asthma vs No AW (Age 4-5 Years) Crude OR (95% CI)	ED Visit for Atopic Asthma vs No AW (Age 4-5 Years) Adjusted OR (95% CI) (n=3,741)*
Normal-High Serum IgA (2-3 Years)	1.48 (0.57, 3.88)	1.48 (0.56, 3.87)
Failure to Thrive	0.60 (0.14, 2.49)	1.82 (0.55, 6.06)
Sex (Ref: Female)	1.71 (0.89, 3.31)	1.55 (0.73, 3.33)

Bold indicates p<0.05. *Adjusted for sex and failure to thrive

Table C16. Odds of Emergency Department Visits for Non-Atopic Asthma (Age 4-5 Years) After Exposure to the Two Highest Tertiles of Serum IgA (Ages 2-3)

Ref: Lowest Tertile Serum IgA (2-3 Years)	ED Visit for Non-Atopic Asthma vs No AW (Age 4-5 Years) Crude OR (95% CI)	ED Visit for Non-Atopic Asthma vs No AW (Age 4-5 Years) Crude OR (95% CI) (n=3,728)*
Normal-High Serum IgA (2-3 Years)	2.08 (0.47, 9.15)	2.06 (0.47, 9.07)
Failure to Thrive	1 (Omitted)	1 (Omitted)
Sex (Ref: Female)	1.19 (0.54, 2.66)	1.29 (0.47, 3.55)

Bold indicates p<0.05. *Adjusted for sex and failure to thrive