Skin Infection in Early Life, Stress Response and Asthma Development in Children

By

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A Thesis Submitted to the Faculty of Graduate Studies

In Partial Fulfilment of the Requirements

For the Degree of

MASTER OF SCIENCE

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Abstract

Historically, the primary belief has been that asthma is an atopic disease with the strongest risk factor for developing asthma being exposure to an allergen. However, researchers have begun to question that long held belief and are beginning to study other postnatal environmental factors such as stress. Research delving into maternal postnatal distress and the subsequent effects seen upon the developing neonatal immune system as it pertains to asthma has gained momentum.

With that in mind, the focus of this research was 1) to determine if skin infections are more likely to be seen in young children who have been exposed to maternal distress, 2) to determine if skin infections in children from infancy to age 2 are associated with asthma, independent of atopic dermatitis, and 3) to determine if the association between early life skin infection and asthma was independent of recent stress biomarkers such as cortisol and dehydroepiandrostrone (DHEA). To meet the objectives listed above, the 1995 SAGE (Study of Asthma, Genes and the Environment) Manitoba birth cohort of 13980 children was used. Maternal postnatal distress, skin infection and atopic dermatitis in the infant, asthma at age 11 and other risk factors for asthma were derived from Manitoba's health care databases. For objective 3, data on stress biomarkers (Cortisol/DHEA ratio) were obtained from the SAGE nested case-control study.

Multivariable logistic regression analysis confirmed the first objective that skin infections (adj. OR 1.25, 95% CI 1.13-1.39) and or atopic dermatitis (adj. OR 1.46, 95% CI 1.26-1.70) seen in children from birth to age 2 could be used as indirect markers of stress. The second objective determined that children who exhibited an early skin infection, from birth to age two, were at an increased risk for developing asthma by age

11 independent of atopic dermatitis. However, this finding was dependent upon frequency of health care use. Those children that exhibited an early skin infection and had less than 24 health care visits over 7 years were 1.33 times (95% CI 1.01-1.75) more likely to acquire asthma by age 11 than those who did not have an early skin infection. Children with fewer health care visits were 1.44 times more likely to have asthma. The third objective was not met because the association between early skin infection and asthma was not independent of the Cortisol/DHEA ratio. However, the univariate results for skin infection in the nested case-control study were not significant. The findings of this thesis may be used by family physicians or paediatricians when looking for tangible markers that may indicate infants at risk for developing asthma by school age.

Acknowledgements

This project would not have come to fruition were it not for the assistance and support of so many wonderful people. I would first like to thank my advisor, Anita Kozyrskyj, for her incredible patience, her undying certainty that I would prevail and for being such a wonderful teacher. I am exceedingly grateful to Charles Burchill at the Manitoba Centre for Health Policy for his attention to detail and his patience in teaching me SAS. For their selfless contributions I would like to thank Grace Zeng for her assistance with my understanding of SAS code and to Meghan Azad for her smile and wonderful insights.

Also, I would like to say a sincere thank you to my committee members, Brian McNeil and Lisa Lix, for their support and positive feedback.

Dedication

I dedicate this to my mom and dad for their love and encouragement and to my husband, Don, and daughters, Sophie & Maja – all this for you.

Introduction

According to the World Health Organization, asthma is the most common chronic respiratory disease seen among children and, worldwide, an estimated 300 million people currently suffer from asthma. As a result, asthma continues to be a significant health-care challenge associated with decreased productivity, an increase in health-care utilization and considerable patient morbidity and mortality.[1] In industrialized countries, epidemiological surveys indicate that the prevalence of asthma has nearly doubled since 1980. [2-4] In 1988, the Canadian prevalence of physician-diagnosed asthma in children aged 5 – 8 years ranged from 2.3% in British Columbia to 7.4% in the Maritimes.[5] These numbers auger well with data obtained from the Centers for Disease Control and Prevention which found the prevalence of asthma among children in the US to have risen from 3.6% in 1980 to 5.8% in 2003.[6] For children, in particular, the burden of asthma may affect not only the child's ability to learn, play and sleep, but it also places a burden on both direct medical costs and indirect medical costs such as those associated with missed school days.[7, 8]

A great deal of research has focused on the more tangible predispositions of asthma such as socio-demographic, genetic and environmental factors.[3,5,9] Up until recently, it was primarily believed that asthma is an atopic disease with the strongest risk factor for developing asthma being exposure to an allergen.[10] However, research is emerging that is beginning to question the importance placed on allergen exposure as the predominant risk factor in the development of asthma.[2,3,11] In an epidemiological study by Pearce, Pekkanen and Beasley[11], the authors found the connection between the prevalence of asthma and the prevalence of atopy to be, at best, tenuous and inconsistent.

The authors subsequently encouraged researchers to explore other aetiological mechanisms with respect to the development of asthma.

To that end, a gentle shift in the current paradigm of research exploring gene-environmental factors is giving way to explore immunological and inflammatory mechanisms that may underlie the origins of asthma.[9] From an immunological perspective, authors have found a link between the effects of psychosocial stress, in particular the effect of maternal depression upon the immune system of the fetus, with subsequent development of asthma and atopy.[12] The hypothalamic-pituitary-adrenal (HPA) axis lies at the core of the immune system functioning as the body's gate keeper in how it reacts to stressors by coordinating neuroendocrine and metabolic responses to stress.[13] For example, psychological stress may increase the activity of the HPA axis whereby corticotrophin-releasing hormone (CRH) and adrenocorticotropic hormone (ACTH) are released resulting in a subsequent rise in cortisol levels.[14] As for inflammatory mechanisms, there is evidence to support the hypothesis that children who have experienced a significant stressful life event, such as divorce or serious illness of a family member, are at an increased risk of developing atopic eczema.[15]

A way to further explain why a stressful life event may manifest in the skin is seen in studies outlining how psychological stress may have a negative impact on the secretion of cytokines, interleukin 1α and 8 resulting in delayed wound healing.[16] What follows is a discussion of the current research being done in an effort to uncover the immunological mechanisms responsible for asthma.

Caregiver stress and childhood asthma

For a large proportion of patients, asthma presents within the first years of life.[6] Expanding upon the research noted above that focuses on immunologic and inflammatory mechanisms, is the hypotheses that psychosocial stress such as maternal stress extending beyond the postpartum period, contributes to the development of asthma in children.[12] It has been reported that caregiver stress may predict wheeze in infants and is associated with an atopic immune profile in children.[15,17-19] The exact nature of how maternal stress acts upon a child's immune system resulting in the development of atopic dermatitis (AD) or asthma is, as yet, not clearly understood.

A prospective study designed by the W.T. Grant Foundation Asthma Risk Study employed a cohort of children at risk for developing asthma and a cohort sample of children who were at minimal risk.[20] This study monitored eleven risk factors and found that only three held statistical significance for developing asthma by age 3: frequent illness, six-month serum IgE levels and parenting difficulties. According to the authors, this was the first time that a study had shown an association between early parenting difficulties and the subsequent onset of asthma. In 2001, a paper was published that echoed the above paper's findings with respect to the three primary variables reported to predict asthma. The unique contribution of this study made upon the first was the use of an older cohort of children ranging between the ages of 6 and 8.[21]

A further prospective study by Wright *et al*., involving a high-risk birth cohort, found a positive association between caregiver stress and wheeze in infants.[17] The authors concluded that caregiver stress remained statistically significant even after

controlling for confounding factors such as parental asthma, socioeconomic status, birth weight and race/ethnicity.

It is feasible that caregiver stress could modify immune function and cytokine production in children as per a 2004 study Wright *et al*., authored in an effort to explore how caregiver stress may affect the immune system prompting a child towards subsequent development of asthma.[18] The authors discovered that caregiver stress had a significant impact on markers of the immune response in children within the first 3 years of life. In particular, an increase in both total IgE levels and TNF-α production in addition to a decreased production of INF-ɣ was demonstrated. Immunoregulatory abnormalities are a key component in the pathophysiology of atopic disease[22] resulting in the dysfunction of the HPA axis whereby a decrease in cortisol and dehydroepiandrosterone (DHEA) levels are secreted in response to HPA axis activation resulting in a decreased immune response to stressful conditions.[22]

Further studies confirmed the importance of increased IgE levels including a paper by Lin *et al*., whereby the authors found that psychosocial factors, such as maternal self-reported nervousness, may be a risk factor for increased cord IgE levels.[23] This was the first study to find a potential association between psychosocial stress and increased cord IgE levels.

The most recent addition in this line of research is a longitudinal study by Kozyrskyj *et al*., in which the authors found maternal stress, particularly when it continues beyond the postpartum period, plays a fundamental role in the development of asthma in children by age 7.[12] The noticeable strength of this paper is the use of the 1995

birth cohort of almost 14,000 children born in Manitoba making this the first study to employ a non-high risk cohort.

In animal models, maternal behaviour has demonstrated influence over the functioning of the HPA axis response to stress in the offspring.[24,25] These animal models have shown that positive early treatment of offspring (more licking and grooming of pups) results in adults that exhibit a decrease in cortisol response following response to acute stress.[24] In more recent studies, mice exposed to psychological stress were shown to develop AD-like skin lesions[26] thereby strengthening the relationship between stress and atopic development.

Stress and skin infections

As mentioned prior, research supporting the effect stress places on the immune system has branched out into more tangible outcomes such as research on psychosocial stress and wound healing. According to the authors Glaser *et al*., wound healing is significantly delayed when an individual is stressed. It would appear that the primary pathway responsible for seeing a delay in wound repair is the increase seen in cortisol levels which, in turn, suppress the proinflammatory cytokines necessary for activating the cascade of wound repair.[16]

In a paper by Douwes and Pearce, the authors explored the possibility that exposure to certain microbes early in life may protect against developing asthma, the so called "hygiene hypothesis".[10] The authors felt that while this hypothesis may explain an increase in atopy and allergic asthma, it did not explain the increase seen in asthma *not* associated with atopy. More recent studies have focused on fetal growth and asthma

whereby a relatively large birth size may be a risk factor for subsequent development of asthma later in adolescence. However, it has been reported that the population attributable risk of high fetal growth as a cause of asthma is relatively small and that prenatal events and/or early life exposures may be very important in the development of asthma.

Disruption to the immune system may also be observed through the development of skin infections. In a paper by Bockelbrink *et al*., the authors undertook a study whereby the outcome of significant stressful life events in children 2 years of age was measured by the development of atopic eczema.[15] The authors concluded that psychosocial stress, stemming from events such as separation or divorce of a child's parents, might promote the development of atopic eczema in the child. A more recent study has shown that children exposed to prenatal stress were 25% more likely to be hospitalized with a severe infectious disease.[27]

It has been shown that children with AD are especially prone to skin infections.[28, 29] One explanation for this may be the impaired skin barrier function which may predispose a child to subsequent colonization or infection by microbial organisms, such as *Staphylococcus aureus* (*S.aureus*), in early life.[30,31] The genetic mutation in a gene that codes for essential components in the epidermis results in the outer barrier of the skin being more at risk for sensitization to allergens.[32]

Biochemical measures of stress

Ilondo *et al*. explored the levels of plasma androgens typically seen in infants and children in a 1982 study. The authors found that 4 out of 5 newborns had high levels of

plasma androgens, but that after 3 months, the androgen levels were found to be uniformly low.[33] In addition, the authors found that there was a significant increase in the mean plasma DHEA levels in boys aged 8.1-10.0 years and girls aged 10.1-14.0+ years. According to the authors, by the time the children reach the age of 10, significant differences in DHEA measurement between the sexes can be seen. A study by Attanasio *et al*., also found that the DHEA response is related to pubertal developmental.[34] Cortisol measurements, on the other hand, show no significant change and levels remain fairly stable in both boys and girls before and during puberty.[33-35]

A cohort of children, aged 8 to 14 years, with AD and appropriately matched controls was employed to undergo the Trier Social Stress Test for Children (TSST-C) to determine their adrenocortical response to stress.[35] It was found that in the atopic children, their adrenocortical response to stress was attenuated suggesting that this hypo-responsive HPA axis might play a role in the stress-induced eruptions of AD symptoms. The findings in this study auger well with an earlier study by Rupprecht *et al.,* in which the authors found that adults with atopic eczema also showed a blunted response of both cortisol and ACTH when compared to the control group.[36]

Mechanism of stress upon the immune system

A proposed explanation of how maternal stress may affect a child's immune system has been outlined by von Hertzen.[37] In this review, von Hertzen hypothesises that prolonged maternal stress may result in a sustained and increased production of cortisol that may subsequently affect the developing immune system of the fetus. In a pregnant woman, the chronic increase of maternal stress hormones, primarily cortisol, and their

continual exposure upon the fetus may influence the developing immune system in such a way so as to alter the T helper cell phenotype which may lead to an increase in atopic diseases. This hypothesis is supported by animal studies in which long term changes in the HPA axis in the offspring of pregnant rats was found when the rats were subject to prenatal stress.[38] In addition, a subsequent study on pregnant rhesus monkeys found that steroidal hormones in utero could influence the fetal immune system.[39]

In a fairly recent review of prenatal depression effects on the fetus and newborn, the authors reported that children born to depressed mothers showed a biochemical/physiological profile that mimicked their mother's.[40] Of note, newborns born to mothers who had suffered both prenatal and postpartum depression had elevated cortisol levels. These mothers also demonstrated increased cortisol levels. Cortisol is able to cross the placenta and prolonged fetal exposure to elevated maternal cortisol may provide an environment in which reprogramming of the developing fetal HPA axis is allowed.

A published review explored the relationship between endogenous cortisol and stress response in infants at risk for developing allergic disease.[41] The authors found that in infants predisposed to allergic disease there was an increase in cortisol response to a stress stimulus such as a heel prick. In children and adolescents with allergic disease, the cortisol response was decreased following stressful stimuli. Two possible explanations for the two conflicting observations between infants and children were presented. The first suggested that chronic hyperactivity of the HPA axis might lead to hypocortisolism. The second, increased cortisol may be a starting point from which atopy begins. The chronic stress of having an allergic disease leads to the progression of an over-worked

HPA axis and the subsequent decrease, or blunting, of the cortisol response to stress. The authors also purported that there are three plausible mechanisms linking cortisol to the development of allergic disease such as asthma and atopy. Firstly, glucocorticoids drive the balance of Th1 to Th2 cells to more Th2 cells. Secondly, an increase in serum IgE levels has been associated with glucocorticoids. Finally, an increase in endogenous cortisol has been associated with a decrease in peripheral immune tolerance via inhibition of cytokines such as IL-10.

A study of a cohort of 8 – 16 year olds on the association between changes in saliva cortisol/DHEA (C/D) ratio and the outcome of major life events found that higher ratios predicted persistent major depression.[42] As DHEA can antagonize the actions of cortisol, the authors measured for both markers and calculated the C/D ratio. The conclusion of the study was that using the C/D ratio provided a more specific means of predicting major depression than had cortisol been measured alone as DHEA and cortisol are so intricately intertwined.

Definition of terms

Allergy is a hypersensitivity reaction initiated by immunological mechanisms.

Asthma is a chronic inflammatory lung disease that occurs most frequently in childhood and early adulthood. Physiologically, asthma is defined by variable airflow resulting in either bronchial tube spasms or the swelling of mucous membrane that gives way to the exaggerated narrowing of the airways.[2,6,11] A physician makes a pronouncement of asthma based on a clinical diagnosis that includes the patient's medical history, a physical

examination, assessment of the reversibility of airway obstruction and exclusion of alternative diagnoses that mimic asthma.[6]

Atopy is a hereditary and non-contagious tendency to become sensitized and produce IgE antibodies in response to ordinary exposure to low doses of allergens, usually proteins. As a consequence, atopic individuals can develop typical symptoms of allergic asthma, allergic rhinitis and allergic conjunctivitis, or atopic eczema/atopic dermatitis.[35] Atopy typically presents in childhood or adolescence.

The ***Hypothalamic-Pituitary-Adrenal (HPA) axis*** is made up of three components: the hypothalamus, the anterior pituitary and the adrenal cortex.[43] The three primary molecules that act upon the HPA axis include corticotrophin releasing hormone (CRH), adrenocorticotropin hormone (ACTH) and glucocorticoid hormone (cortisol in humans and corticosterone in rats) with glucocorticoids being the final end product of the HPA axis activation.[43] Activation of the HPA axis stimulates the release of the immune hormones cortisol and DHEA.[34]

Cortisol is the major adrenal glucocorticoid steroid hormone and is normally under feedback control by the pituitary ACTH and hypothalamus. Cortisol plays an important role in routine physiological functions such as metabolism and immune system functioning. Cortisol is typically released by acute stress, either physical or psychological, brought on by an event such as public speaking or visiting the dentist.[44] However, prolonged cortisol activity is associated with numerous deleterious effects such

as suppression of aspects of the immune system including decreased lymphocyte proliferation and cytokine production.[45]

DHEA, dehydroepiandrosterone, is the principal adrenal androgen and is secreted together with cortisol under the control of ACTH and prolactin. DHEA is an antiglucocorticoid and is integral in the negative feedback loop which keeps the concentration of cortisol within a very narrow range.[46]

Cortisol:DHEA ratio is an index of the net outcome of cortisol and DHEA measurements. It has been shown that patients with depression may have elevated C/D ratios.[47]

Purpose

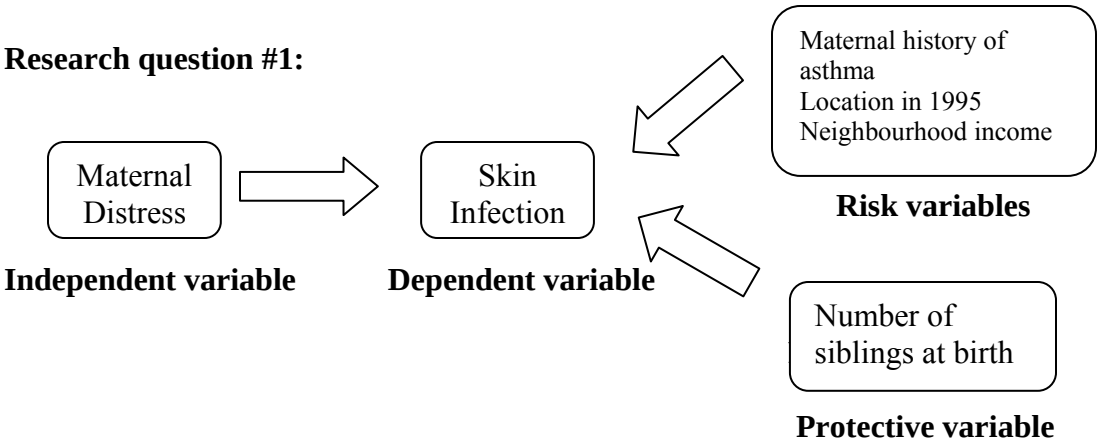
As described above, there is sufficient evidence to support the possibility that maternal distress in early life leads to the development of atopic disease in children and it is well documented that children diagnosed with AD in early life are at an increased likelihood of developing asthma. It has been suggested that continued stress upon the developing fetal immune system, particularly the HPA axis, may result in changes to the immune system leaving infants who are exposed to maternal distress at a higher risk of developing asthma via the immune pathway. More recently it has been supposed that a skin infection in early life may be seen as an early marker of an impaired skin barrier. Additionally, a child with an impaired skin barrier is more likely to be associated with asthma.

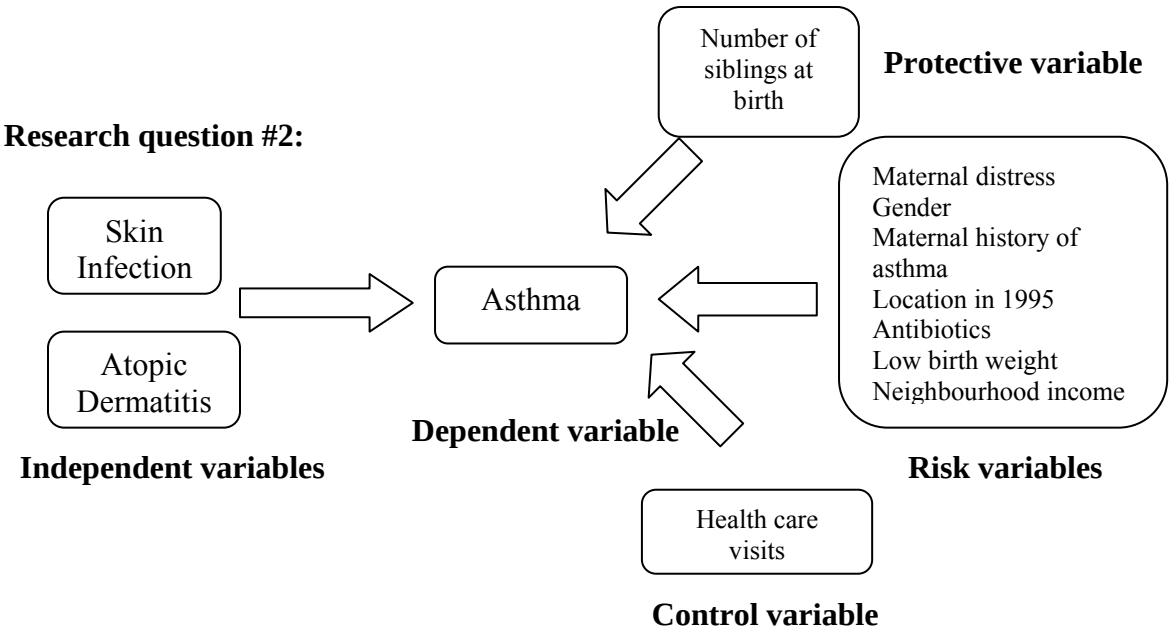
Thus, the following research questions shall be asked:

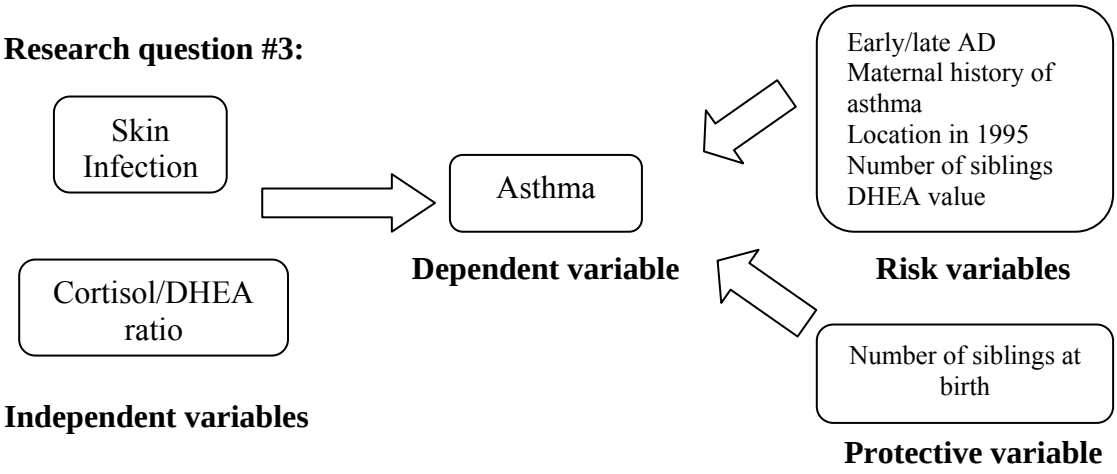
1. Are skin infections more likely to be seen in young children who have been exposed to maternal distress?
2. Are skin infections in children up to age 2 associated with asthma, independent of atopic dermatitis (AD)?
3. Is the association between early life skin infection and asthma independent of recent stress biomarkers?

Theoretical framework

In order to address the above questions, the following allows for a visual representation of the research aims of this study.







Hypothesis

The following hypotheses for this study are as follows:

1. Children who have a mother diagnosed with maternal distress in the first year of life are more likely to have a skin infection in early life.

2. Children who have a mother diagnosed with maternal distress in the first year of life are more likely to have a diagnosis of AD in early life.
3. Skin infections in early life are more likely to be associated with an outcome of asthma by age 11, independent of AD.
4. Skin infections in early life are more likely to be associated with an outcome of asthma by age 11, independent of the C/D ratio.

In order to address hypothesis 3 and 4, data analyses were divided into two distinct stages:

1. Direct markers of stress for children aged 8 –10 were determined by testing plasma for cortisol and DHEA levels to determine the C/D ratio, and
2. the direct markers of stress measures were then linked to the indirect markers of stress response during the first two years of life.

Strength of research

The strengths of this research lie in the following three improvements made upon previous literature. Firstly, this paper utilized the entire birth cohort of children born in Manitoba in 1995. Previous asthma research has employed cohorts derived from high risk groups due to the challenges of creating large cohorts. Secondly, the definition used for maternal distress employed database measures which are more robust than self-reporting questionnaires which may be subject to recall bias. Lastly, asthma diagnoses were made at age 11 in children. This is a more reliable age for ensuring a valid asthma diagnosis.

Method

Participants

This study employed data from the 1995 birth cohort from Manitoba known as the Study of Asthma, Genes and the Environment (SAGE). A total of 16,320 children were born in Manitoba in 1995; however, over the following seven years, 2,340 of the children had either died or moved out of the province and were no longer registered in the provincial healthcare registry. Thus, 13,980 children remained to comprise the birth cohort. In 2002, a nested case-control study was generated by mailing out a survey to the SAGE birth cohort. The intent of the survey was to find 1,000 children to participate in the study to see how the role of genetics and the environment could affect the development of asthma. A component of the study required a blood sample from each child for biomarker testing such as IgE levels. In all, a total of 3,598 surveys were returned with a sample of 723 being selected for the nested case-control study. The final cohort consisted of children with asthma, children with allergies but no asthma and children with neither asthma nor allergies. The children without asthma or allergies were subsequently stratified by either urban or rural location and by their neighbourhood income. A random sample from each stratum was employed allowing for a representative sampling. Upon final asthma confirmation by a pediatric allergist, the final cohort nested case-control study consisted of 246 cases and 477 controls. Informed consent was given from all participating families which included the ability of the researchers to link the child's findings to prescription and other health records from the provincial healthcare database from 1995 and onward.[48]

Ethics

There are no foreseeable ethical issues in the proposed study. The data contained in the Manitoba Centre for Health Policy (MCHP) is anonymized in such a way that any patient identifying information has been removed, but the capacity to link records has been maintained. In addition, both the University's Health Research Ethics Board and Provincial Health Information Privacy Committees must approve any research project requiring access to the administrative data contained at the MCHP.

Procedure

Stress biomarkers

Cortisol and DHEA analysis was performed on blood samples obtained from children in the SAGE cohort. This time period was termed as Wave 1 and consisted of children aged between 7 – 10 years. After venipuncture, the blood samples were spun and the plasma removed from the red cells and stored at -20^{0}C pending analysis. These plasma immune function assays were completed in Brian MacNeil's laboratory at the School of Medical Rehabilitation through routine laboratory procedures using commercially obtained enzyme linked immunosorbent assay (ELISA) kits. All plasma samples were run in duplicate using appropriate internal standards.

Administrative data

Administrative data from the Population Health Research Data Repository (Repository) residing at the MCHP were employed in this research study. The Repository contains health information primarily concerning residents of Manitoba.

Databases are gleaned from a number of sources and then grouped into the appropriate domains: Health, Education, Social, Justice, Registries and Database Support Files. For the focus of this research, data from the Health domain, containing administrative, survey and clinical data, was utilized. The data used in this study came from the following datasets:

1. Physician billing data,
2. Hospital separation abstracts and
3. Drug product information network (DPIN)

The children contained within this study were continuously registered with the Manitoba Health Services Insurance Plan (MHSIP) until 2006. These databases are reliable and valid data sources.[49,50] Anonymized personal identifiers enabled the linkage of database records and a family registration number allowed for the linkage of maternal and child records.

In order to create a working dataset containing all of the pertinent variables, record linkage between datasets was necessary. This required using the software Statistical Analysis Software (SAS) Version 9.2 to merge two or more separate sources of information together by matching the BY groups on one dataset to the corresponding BY group on the other. For example, the variable 'skin infection' from the medical claims data could be merged to asthma outcomes in 2006 using the BY variable Personal Health Information Number (PHIN). The PHIN is scrambled in order to prevent direct linkage back to the actual individual. The resulting dataset would then contain a complete list of

all observations for both skin infection data and asthma outcomes based on linkage using the scrambled PHIN.

Maternal distress

The variable maternal distress was defined based on administrative variables that included at least one physician diagnosis of a depressive or anxiety disorder *International Classification of Diseases* (9^{th} revision) (ICD-9 codes: 296, affective psychoses; 300, neurotic disorders; 308, acute reaction to stress; 309, adjustment reaction; and 311 depressive disorder not elsewhere classified) or at least one prescription for antidepressant, anxiolytic or hypnotic medication in the postpartum period and/or onwards. This database definition was validated against a subset of 454 women who had self-reported post-partum distress.[12] A specificity of 83% (95% Confidence Interval (CI), 78 – 87%) and sensitivity of 42% (95% CI, 21 – 66%) was achieved. While the higher specificity indicates that those women who self-reported no post-partum distress truly did not have post-partum distress, the moderate sensitivity may indicate that mothers who did indeed experience post-partum distress chose not to obtain health care and were therefore underrepresented. Maternal distress was a dichotomous variable defined as present in the first year of life or not present.

Asthma

An asthma diagnosis in children who had turned 11 in 2006 was defined by at least two physician visits for asthma, one hospitalization for asthma, or two prescriptions for any asthma drug (β-agonists, inhaled corticosteroids, cromones, or leukotriene

receptor antagonists). This definition was validated using a subset of 539 cohort children who had been recruited by an allergist on the basis of a high positive predictive value (94%; 95% CI, 82 to 99%) and high specificity (92%; 95% CI, 78 to 98%) and a SAS code exists for its creation.[51]

Skin infections

The skin infection diagnoses used for this study encompassed the most common skin infections encountered in children from birth to age 5. The skin infections would be severe enough that they would warrant a visit to either the family physician or, possibly, the hospital either through physician referral or a visit to the emergency department. In either case, a diagnosis would be made and the visit captured and entered into either the medical or hospital registry using the appropriate ICD-9 code. When extracting ICD-9 codes from these two registries, it was only possible to do so at the most basic level. The codes are based on a primary category (3 digits) and then sub-divided into more specific diagnosis (5 or more digits). It was not possible to pull the more specific diagnosis from the medical claims data as it was only possible to retrieve codes based on the first 3 digits of the claim. The hospital claims data allowed for up to 5 digits, but as the two datasets were merged and the majority of diagnoses were found in the medical data, diagnoses were pulled based on the 3 digit diagnosis. Thus, skin infection diagnoses were obtained using hospital and physician claims records from birth to age 5 for the relevant ICD-9 codes: 680, carbuncle and furuncle; 681, cellulitis and abscess of finger and toe; 682, other cellulitis and abscess; 684, impetigo; 686, other local infection of the skin and subcutaneous tissue; 695, erythematous conditions such as staphylococcal scaled skin

syndrome; 078, Molluscum contagiosum; 110, dermatophytosis (ringworm) and; 771, any infection specific to the perinatal period caused by bacteria or virus or, more rarely, parasites. The ICD-9 codes for the relevant skin infections are summarized in Table 1.

Table 1. List of ICD-9 codes for skin infections seen in children from birth to 5 years

Infections of skin and subcutaneous tissue
680 Carbuncle and furuncle
681 Cellulitis and abscess of finger and toe
682 Cellulitis, other
683 Acute lymphadenitis
684 Impetigo
685 Pilonidal cyst
686 Other local infections of skin and subcutaneous tissue
Other inflammatory conditions of skin and subcutaneous tissue
695 Erythematous conditions
Other diseases due to viruses and Chlamydiae
078 Molluscum contagiosum
Mycoses
110 Dermatophytosis
Certain conditions originating in the perinatal period
771 Infections specific to the perinatal period

These skin infections may be caused by bacterial, viral or even fungal agents. One of the most common skin infection seen in children is molluscum contagiosum (ICD-9 code 078), a benign viral infection which typically affects children between the ages of 2 and 5 years.[52] This infection is found most commonly in children who swim, who bathe together or who may be immune-compromised. Mollusca are dome shaped lesions found primarily on the trunk and flexural areas such as the backs of the knees and

the insides of the armpits, elbows and groin. It is not believed that this infection is more common in children with AD than those without.

Impetigo (ICD-9 code 684) is another common skin infection primarily affecting children between the ages of 2 to 6 years.[52, 53, 54] The causative agents are either *S. aureus* or *Streptococcus pyogenes* (*S. pyogenes*). These bacteria cause a superficial skin infection (impetigo contagiosa) that gives the appearance of golden-crusted plaques typically found on the face and extremities. A study done by Hayashida *et al*., reports that the odds of having a history of impetigo contagiosum were 1.8 times higher in children with AD than those without.[55] Additionally, an increased incidence of impetigo has been found in a study by Koning *et al.,* whereby the authors concluded that either parents of children with an infection were more likely to seek medical help for their child or that antibiotic resistance and virulence of *S. aureus* was to blame.

Scalp ringworm (Tinea capitis) is included within ICD-9 code for Dermatophytosis. This is a highly contagious skin infection of the scalp occurring predominately in children. While this infection had been confined to the poorest countries, it has become more prevalent in first world countries such as the United Kingdom making it a major public health concern.[52]

Under ICD-9 code 695 is Staphylococcal scaled skin syndrome (SSSS), another skin infection caused by *S. aureus.* While this condition is considered quite rare (0.09-0.13 cases per 1 million), it is found to be a disease that occurs predominately during early childhood.[56]

Atopic dermatitis working definition

AD seen in children within the first 5 years of life employed the use of ICD-9 codes; 691, atopic dermatitis; 692, contact dermatitis and other eczema and; 693, dermatitis due to substances taken internally. The inclusion of contact dermatitis is supported by a study performed by Jacob *et al*., in which the authors studied a pediatric population and found allergic contact dermatitis to be prevalent in those who also had AD.[57] As it was not possible to extract the ICD-9 code specific for AD (691.8) from the database as only 3 digit diagnosis was possible, it was necessary to create and validate a working definition of atopic dermatitis using the aforementioned diagnoses. Children are more likely to be diagnosed with AD between the ages of 2 to 5 years[58] so for the purposes of this research, both the physician diagnosed AD and working definition for AD were categorized into early AD and late AD. The category of early AD included children from birth to age 2 with late AD including children from age 3 to 5 years of age. The literature supports the importance of looking at AD at an early age as most children present with AD by age 2 and by age 3, up to 43.2% may be in complete remission.[59, 60] This same presentation holds true for eczema whereby children under the age of 23 months were showing symptoms of bronchial obstruction and allergic rhinoconjunctivitis.[61] Of the 94 children enrolled in the study, by 3 years of age, 18 of the children no longer had eczema. In the final analysis, children with an initially high eczema score were significantly associated with an increased risk of developing asthma.

Variables

Health care administrative data from the MCHP was used to extract the following variables: asthma diagnosed at age 11, gender, birth weight adjusted for gender and gestational age, location (rural vs urban), neighbourhood income, total number of siblings at birth, number of health care visits up to age 7 years, antibiotic use in the first year of life, history of maternal asthma and maternal distress. All of these variables had been previously created for the 1995 birth cohort.

Variables as they pertain to the research questions

Research Question #1: *Are skin infections more likely to be seen in young children who have been exposed to maternal distress?*

In order to address research question #1, the following variables were employed: history of maternal asthma, location at birth, number of siblings at birth and neighbourhood income (Table 2). With early skin infection being the dependent variable and maternal distress the independent variable, maternal asthma was included in the modelling as it is considered to be integral in the atopic pathway. The literature supports the association seen between maternal distress and atopic dermatitis and the inclusion of parental atopy as a potential confounding variable.[15] The other three demographic variables were included due to their potential association with the onset of skin infections. For example, children residing in an urban environment may be subject to overcrowding; alternatively, those living in a rural setting may be exposed to more pathogens if they live on a farm with proximity to livestock. The number of siblings a child has may influence their exposure to various skin infections and make them more

susceptible depending upon the contagious nature of the infection. The inclusion of siblings is also supported within the literature.[15] The final variable to be included in the modelling is neighbourhood income as an indirect measure of socioeconomic status.

Table 2. Dependent, independent, risk or protective variables for logistic regression analysis – research question #1

Variable Type	Variable Name	Defintion	Measure
Dependent	early skin infection	ICD-9 Codes	0 = no early skin infection 1 = early skin infection
	late skin infection	ICD-9 Codes	0 = no late skin infection 1 = late skin infection
Independent	maternal distress	physician diagnosis of depression or anxiety (specified ICD-9 codes) or a prescription for depression or anxiety related medications during three time intervals: first year of life, ages 2-4 and 507 years	0 = no post partum depression 1 = post partum depression
Risk or protective	maternal history of asthma	defined as at least one physician visit or hospitalization for asthma or one prescription for an asthma drug	0 = no maternal asthma 1 = maternal asthma
	location in 1995	urban or rural	0 = rural
	neighborhood income	based on child health quintile	0 = income ≥ $20,000 1 = income < $20,000

Research Question #2: *Are skin infections in children up to age 2 associated with asthma, independent of atopic dermatitis (AD)?*

For research question #2, early skin infection is the independent variable and the onset of asthma by age 11 years is the outcome (dependent) variable. Additional variables include: AD (early and late), maternal asthma, location at birth, number of siblings at birth, gender, maternal distress, low birth weight adjusted for gender and gestational age, neighbourhood income, courses of antibiotics in first year of life and the number of health care visits in the first 7 years of life (Table 3). AD is a known risk factor for asthma and it is important to include this variable.

All of the aforementioned variables were included on the basis of the literature.[62] As mentioned prior, these variables had been created for the 1995 birth cohort with all of the variables being dichotomous with the exception of health care visits and number of siblings being continuous variables and the number of courses of antibiotics a categorical variable. Even though the courses of antibiotics were available as a continuous variable, the categorical variable had been used in previous studies and was employed in this study.[31]

Typically, the more often a child was to visit their physician, the more likely it would be that the outcome of interest would be captured which, in this case, is development of asthma by age 11. In order to control for the potential confounding effects of detection bias, the number of health care visits was included in the analysis.

Table 3. Dependent, independent, risk or protective variables for logistic regression analysis – research question #2

Variable Type	Name	Defintion	Measure
Dependent	asthma	pediatric allergist diagnosis made at age 11 years	0 = no asthma
			1 = asthma
Independent	early skin infection	ICD-9 Codes	0 = no early skin infection
			1 = early skin infection
	late skin infection	ICD-9 Codes	0 = no late skin infection
			1 = late skin infection
	early atopic dermatitis	working definition	0 = no early atopic dermatitis
			1 = early atopic dermatitis
	late atopic dermatitis	working definition	0 = no late atopic dermatitis
			1 = late atopic dermatitis
Risk or protective	maternal history of asthma	defined as at least one physician visit or hospitalization for asthma or one prescription for an asthma drug	0 = no maternal asthma
			1 = maternal asthma
	gender	male or female	0 = female
			1 = male
	location in 1995	urban or rural	0 = rural
			1 = urban
	courses of antibiotics	courses of antibiotics received in the first year of life	0 = none
			1 = 1-2
			2 = 3-4
			3 = > 4
	number of siblings (at birth)	number of siblings at birth	continuous measure
	Low birth weight	low birth weight - adjusted for gender and gestational age	0 = normal birth weight
			1 = abnormal birth weight
	neighborhood income	based on child health quintile	0 = income ≥ $20,000
			1 = income < $20,000
Control	health care visits	number of hospitalizations or physician visits from birth to age 7 years	continuous measure

Research Question #3: *Is the association between early life skin infection and asthma independent of recent stress biomarkers?*

The variables used to investigate this research question included skin infection and the C/D ratio as independent variables and asthma as the dependent variable. Other variables included are listed in Table 4.

Table 4. Dependent, independent, risk or protective variables for logistic regression analysis – research question #3

Variable Type	Name	Defintion	Measure
Dependent	asthma	pediatric allergist diagnosis made at age 11 years	0 = no asthma 1 = asthma
Independent	C/D ratio	cortisol/DHEA (C/D) ratio - categorized using the 25, 50 and 75 percentiles	C/D low ≤ 9.4 C/D mid 14.9 ≤ 22.9 C/D high > 22.9
	early skin infection	ICD-9 Codes	0 = no early skin infection 1 = early skin infection
	late skin infection	ICD-9 Codes	0 = no late skin infection 1 = late skin infection
Risk or protective	early atopic dermatitis	working definition	0 = no early atopic dermatitis 1 = early atopic dermatitis
	late atopic dermatitis	working definition	0 = no late atopic dermatitis 1 = late atopic dermatitis
	maternal history of asthma	defined as at least one physician visit or hospitalization for asthma or one prescription for an asthma drug	0 = no maternal asthma 1 = maternal asthma
	location in 1995	urban or rural	0 = rural 1 = urban
	number of siblings (at birth)	number of siblings at birth	continuous measure
	DHEA	DHEA value in nmol/L	continuous measure

Data Analysis

Direct stress markers

The cortisol and DHEA values from the Wave 1 dataset were continuous variables and therefore tested for normality using Shapiro-Wilk with an alpha level = 0.05. Neither variable was normally distributed; therefore, the values were log transformed to achieve normal distribution. The log transform of both variables restored normal distribution, which in turn decreases the potential risk of undue influence on the outcome of the analysis due to the extreme skew of the data.

With respect to the cortisol and DHEA results, a working reference range for the Wave 1 population was established by dividing each dataset into quartiles as per previous studies.[63] Of the 555 Wave 1 cortisol samples, 278 of the values contained within the first and fourth quartiles were classified as abnormal. For wave 1 DHEA samples, 278 were contained within the 1st and 4th quartiles and deemed as abnormal. The cortisol and DHEA values were then used to create the C/D ratio (C/D ratio) which would then be used in subsequent data analysis testing. A summary of the actual mean and standard deviation for the three assays is provided in Table 5. However, the log transformed values were initially used for all major analyses.

Table 5. Descriptive statistics from wave 1 cortisol and DHEA assays

Variable	Mean	SD	n
Cortisol (nmol/L)	137.02	79.60	555
DHEA (nmol/L)	9.16	5.15	555
Cortisol/DHEA (C/D) Ratio	18.02	12.66	555

Indirect stress markers

Using record linkage, the dataset containing the children's population characteristics, such as age, was combined with the dataset containing the hospital and medical claims data for the skin infections listed in Table 1. Once the datasets were merged, skin infection data for the entire birth cohort (n = 13,980) was categorized into two age categories: early skin infection (children from birth to age 2 years) and late skin infection (children aged 3 to 5 years). Each variable was then made into a categorical variable whereby early skin infection and late skin infection were coded as present or not present with not present being the reference category.

As a pediatric allergist diagnosis of AD was available for only 281 children in the Wave 1 cohort, the creation of a working definition for AD was necessary to allow for analysis of the entire birth cohort, n=13,980. The pediatric allergist diagnosis of AD was considered the gold standard measurement and was subsequently used to validate a working definition. The database working definition for AD was based upon ICD-9 codes 691, 692 and 693 (Table 6).

Table 6. List of ICD-9 codes for atopic dermatitis seen in children from birth to 5 years

Other inflammatory conditions of skin and subcutaneous tissue (690–698)
691 atopic dermatitis and related conditions
692 contact dermatitis and other eczema
693 dermatitis due to substances taken internally

For each child that had one or more of these diagnoses, the data was grouped by age of diagnosis (Year 1 – Year 5) and coded as AD present or not present.

The one caveat in obtaining ICD-9 codes from the medical claims registry was that a code could only be extracted using three characters. This resulted in the inability to remove the diagnosis for atopic dermatitis (ICD-9 691.8) without including the diagnosis for diaper rash (ICD-9 691.0). Thus, in order to tease out diaper rash from AD, a series of combinations of variables were tested. As diaper rash typically resolves in a child by 12 months[64] and the onset of AD is typically between infancy and 5 years of age[65], the working definition for AD was achieved by combining different years and using AND/ OR statements (Table 7).

Table 7. Working definition for atopic dermatitis

Variable	Definition
Early AD	(Atop1&Atop4) OR (Atop1&Atop5) OR (Atop2&Atop4) OR (Atop2&Atop5)
Late AD	Atop3 OR Atop4 OR Atop5

The variable 'Atop' in the working definition (Table 7) above refers to the age (ie. 1 to 5) of the child. If the child had one or more diagnoses of ICD-9 codes 691 – 693 in that year, the 'Atop' variable would be coded as either 1, a diagnosis exists, or 0, no diagnosis exists.

Validation of working AD definition

As mentioned previously, only 281 children from the SAGE case-control cohort had been diagnosed with AD by a pediatric allergist. Therefore, before applying the working definition to the entire birth cohort, the working definition needed to be validated against the gold standard of pediatric allergist diagnosis. However, prior to testing the pediatric diagnosed AD children against the working AD definition, the pediatric observations had to be categorized into early and late variables. This was achieved by merging the pediatric diagnosed AD results with a 'time' variable from the SAGE case-control cohort. The 'time' variable used was age of onset of an itchy rash based upon parental recall. The itchy rash variable was then coded as being early, present from birth to age 2, or late, age 3 or more years. By merging the pediatric diagnosed AD dataset to the dataset containing the early and late onset of the itchy rash, the creation of the variable early AD was possible representing those children in the SAGE case-control cohort who had AD as per a pediatric allergist and an itchy rash having appeared between birth and age 2. Alternatively, late AD combined the AD diagnosis with the late onset of the itchy rash (3 years or more). The resulting number of observations used for sensitivity and specificity testing were: early pediatric diagnosed AD, n=488 and late pediatric diagnosed AD, n=489.

Once the gold standard was classified into either early or late AD, the working definitions for early and late AD were validated against this gold standard. The early and late AD definitions yielding the best combination of specificity and sensitivity can be seen in Table 8.

Table 8. Sensitivity and specificity of AD definition as compared to physician diagnosed AD

	Estimate	95% CI
Early AD		
Sensitivity	0.56	(0.40 - 0.71)
Specificity	0.87	(0.83 - 0.89)
Late AD		
Sensitivity	0.30	(0.13 - 0.54)
Specificity	0.83	(0.78 - 0.86)

For the purposes of this study, the AD definition with the best combination of specificity and sensitivity was selected. While selecting a definition with a higher specificity over sensitivity lends itself to a more conservative dataset, it ensures that those who meet the definition for AD are truly negative and that there are few false positive results. A definition with a lower specificity would have meant more children classified as having the disease when they did not resulting in misclassification bias. This would have lead to a cohort of children falsely classified with early/late AD. Alternatively, a higher specificity also means that those children classified by the early/late AD definition are most likely those with a more severe form of the disease. Thus, the decision to err on the side of caution and use a definition with a higher specificity was selected to both minimize the potential for misclassification and ensure that any subsequent findings were would be based on a more conservative cohort. The outcomes based on this decision would be stronger owing to the fact that there would be fewer children classified as having early/late AD in the cohort, with the caveat that these children may have a more severe form of the disease. There is also the potential for the subsequent odds ratios to be underestimated when using the definition with a higher specificity and lower sensitivity.

Additionally, a definition with high sensitivity is required for prognostic value which is not the intent of this research definition.

The final working definition for early and late AD was then applied to the entire birth cohort. In observations where pediatric diagnosed AD contradicted the working AD definition, the physician diagnosed AD had precedence. The early and late AD definitions were mutually exclusive so there was no overlap within the two categories.

Regression modelling

The outcomes tested in this research were dichotomous with the dependent variable representing the desired outcome (ie. onset of asthma by age 11 or diagnosis of skin infection in early life), thus multivariable logistic regression analysis was employed utilizing the statistical software package (SAS Version 9.2; SAS Institute; Cary, NC). Confidence limits of 95% (CI 95%) were fit around the Odds Ratios (OR) generated. Logistic regression was used to investigate associations between:

1. Maternal Distress (exposure) and Early Skin Infections (outcome);
2. Maternal Distress (exposure) and Early AD (outcome);
3. Early Skin Infections (exposure) and Asthma (outcome) independent of AD,
4. Early Skin Infections (exposure) and Asthma (outcome) independent of Direct Marker of Stress (C/D ratio).

As mentioned above, in logistic regression the outcome is a dichotomous variable. This relationship may be viewed graphically as a sigmoidal curve. However, underlying all regression modelling is the use of a linear model. Therefore, the use of logistic

transformation is used to transform the sigmoidal outcome into a linear relationship. This then allowed for the use of logistic regression to explore the relationship between a binary outcome and potential explanations (independent variables). No assumptions are made pertaining to the distribution of the independent variables; however the independent variables should not be highly correlated with one another as this could affect subsequent analyses. Also, each observation should be independent with no pairing of the data as in before-after measurements.

As a large sample size was used in this study, Wald χ^2 statistics were used to test the significance of each individual variable.[66] A *P* value of 0.05 or less indicates that the variable significantly contributes to the outcome. The models were generated using stepwise regression and variables were retained if they had a *P* value of 0.05 or less. While most of the variables satisfied the level of significance stated, the variables low birth weight, maternal distress and neighbourhood income were retained based on their biological and clinical contribution as per the literature.

The possibility of interactions between all explanatory variables and independent variables was included in the modelling process. A *P* value of 0.05 was selected to evaluate statistical significance of the independent variables in the model. In order to deal with the results of interaction terms where one of the variables is a multi-category variable, mean-centering was used. This allowed for the presentation of the OR within each category. Mean centering is also a useful statistical tool used to minimize potential multicollinearity.

When presenting the analysis data in tables, the reference categories were not specifically included. The reference category for all binary variables is defined where the

variable value equals '0'. Therefore, the outcome of interest, where the variable value equals '1' is contained within the table. For example, using the variable gender, '0' represents females and '1' represents males. When reporting the OR and 95% CI in the tables in the results section for gender, only the OR and 95% CI for the outcome of interest (male) is included such that 'male gender' represents the variable value equal to '1'. The reference category for gender is female and is not included in the table as the OR for female gender is equal to 1.00. This format was used for all tables and pertains to all binary variables. A listing of all variable values is found above as per the research question being addressed.

Results

The total number of participants in the 1995 Manitoba birth cohort was 13,980. Of those, 7,114 (50.9%) were male, 908 (6.6%) were diagnosed with asthma by age 11 and 1,442 (10.4%) had a mother diagnosed with asthma. There were 2,698 (19.3%) mothers coded as exhibiting post partum distress.

Direct stress markers

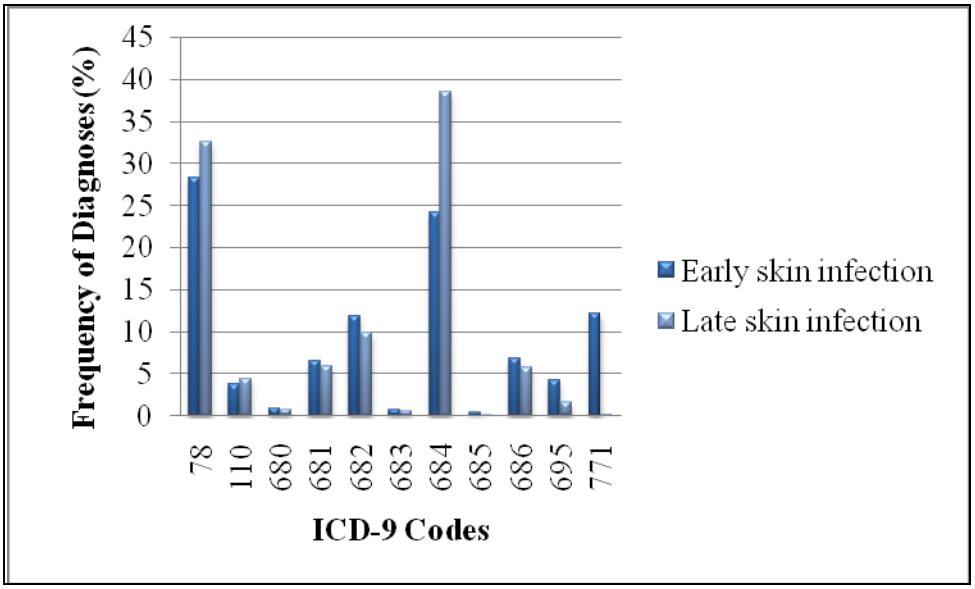
From the SAGE Wave 1 cohort, values for both cortisol and DHEA were available for 555 children. As the cortisol and DHEA results were divided into quartiles there were no outliers that were removed from the Wave 1 cohort. The children in this cohort ranged in age from 7 to 10 years (mean = 8.58, SD=0.61). For the C/D ratio, the values were categorized based on the 25, 50 and 75% percentiles. Thus, C/D high consisted of any values >22.9, C/D mid was > 14.9 and ≤ 22.9 and C/D low ≤ 14.9.

Indirect stress markers – skin infection

Using the entire birth cohort, data from the medical registry showed that there were 10,152 diagnoses for skin infections in children from birth to age 5. The hospital registry contained 295 diagnoses for skin infections for the same time period. By linking both the medical and hospital registries for skin infections, it was found that 2,678 (19.2%) children acquired at least one early skin infection (from birth to age two) and 2,156 (15.4%) a late skin infection (between the ages of 3 and 5 years). These two categories were mutually exclusive such that if a child was initially diagnosed with an early skin infection and subsequently with a late skin infection, they would only be captured in the early skin infection cohort. Of all the children in the birth cohort, 65.4% had neither and early or late skin infection.

Overall, the most frequently reported skin infection was molluscum contagiosum, a viral infection, at 22.3% and impetigo at 32.7%. The results for early and late skin infections, per ICD-9 codes, are summarized in Figure 1.

Figure 1. Number of diagnoses in children with early and late skin infections

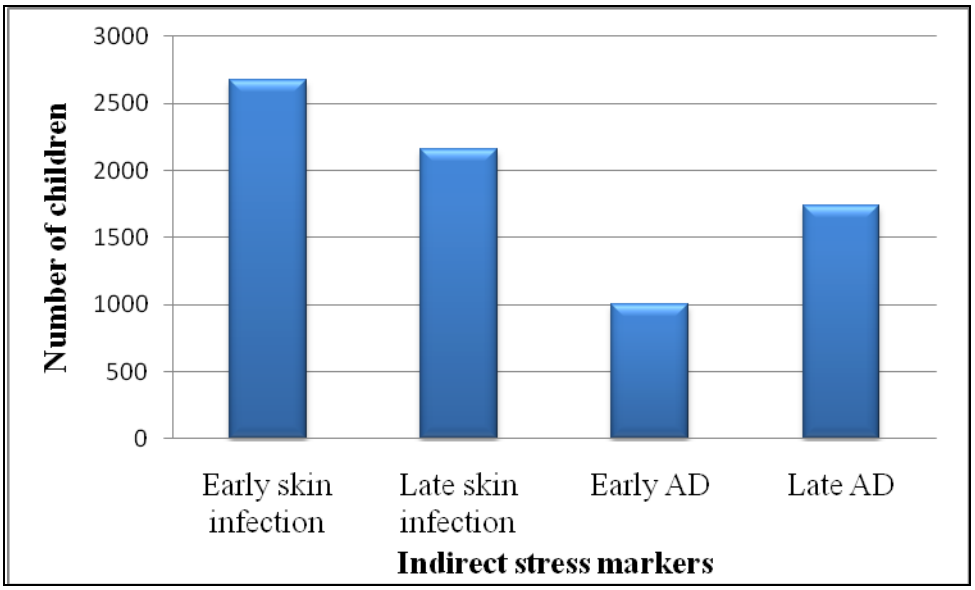


Indirect stress markers – AD

The most frequently reported dermatitis in the medical registry was contact dermatitis with 17,303 (32%) diagnoses. This was followed by 7,900 reports of ICD-9 code 691 which included atopic dermatitis, eczema and diaper rash.

After applying the working definition (Table 7) for early and late AD to the entire birth cohort, 1,006 (7.2%) of the children matched the working definition of early AD and 1,737 (12.4%) met the definition for late AD (Figure 2). The number of children who were diagnosed as having both an early skin infection and early AD was 346 (2.5%).

Figure 2. Number of children with skin infections and atopic dermatitis (AD)



Research Question #1 – Are skin infections more likely to be seen in young children who have been exposed to maternal distress?

When comparing the population characteristics of the birth cohort as they pertained to early skin infections, there was a statistically significant association (P = 0.0003) between maternal history of asthma and early skin infections (Table **9**). Children with a maternal history of asthma were significantly more likely to acquire an early skin infection (22.8%) compared with those who did not have a maternal history of asthma (18.8%). Children who lived in an urban environment were significantly more likely to have an early skin infection (19.8%) compared to children who lived in a rural area (18.3%). Also, the number of siblings at birth and low neighbourhood income were also statistically significant in relation to early skin infection.

Table 9. Distribution of population characteristics according to maternal distress, early skin infection and early atopic dermatitis.

			Maternal		Early skin		Early atopic	
		Missing	distress	*P* value	infection	*P* value	dermatitis	*P* value
Total participants = 13980			2698 (19.3)		2678 (19.2)		1006 (7.2)	
Maternal history of asthma	No		2231 (17.9)		2338 (18.8)		879 (7.1)	
	Yes	73	394 (27.3)	<0.0001	328 (22.8)	0.0003	120 (8.3)	0.077
Location in 1995	Rural		1073 (17.8)		1100 (18.3)		358 (5.9)	
	Urban		1625 (20.4)	0.0001	1578 (19.8)	0.0188	648 (8.2)	<0.0001
Number of siblings (at birth)	Mean ± SD		1.1 (1.44)	0.0147	1.3 (1.59)	0.0016	1.07 (1.51)	0.0006
Neighbourhood income <$20,000	No		1951 (18.7)		1673 (16.1)		695 (6.7)	
	Yes	41	738 (21.2)	0.0013	993 (28.5)	<0.0001	308 (8.8)	<0.0001

In order to address the first research question as to whether or not early skin infections, and then early AD, were more likely to be seen in young children who had been exposed to maternal distress, multivariable models were tested. Based on the associations seen in Table 9, the variables maternal history of asthma, living in an urban environment, having more than one sibling and low neighbourhood income were found to be significantly associated with an increased likelihood of developing an early skin infection. In multivariable analysis (Table 10), children with a history of maternal distress were 25% more likely to develop an early skin infection (adjusted OR 1.25, 95% CI 1.13–1.39) as compared to those who did not.

Table 10. Likelihood of early skin infection according to maternal distress and other variables.

Variable	Early Skin Infection	
	OR	95% CI
Maternal distress	1.25	(1.13 - 1.39)
Maternal history of asthma	1.23	(1.08 - 1.41)
Urban location in 1995	1.11	(1.01 - 1.21)
Number of siblings (at birth)	1.05	(1.02 - 1.08)
Neighbourhood income <$20,000	2.02	(1.85 - 2.22)
Total participants = 13,868		

These same variables were then used to investigate for any associations with early AD (Table 11). As was seen with early skin infection, a significant relationship was observed between maternal distress and the odds of developing AD within the first two years of life (adjusted OR 1.46, 95% CI 1.26-1.70). In contrast to the above analysis for early skin infection, maternal history of asthma had no influence on the likelihood of developing early AD.

Table 11. Likelihood of early atopic dermatitis according to maternal distress and other variables.

Variable	Early AD	
	OR	95% CI
Maternal distress	1.46	(1.26 - 1.70)
Maternal history of asthma	1.12	(0.92 - 1.37)
Urban location in 1995	1.35	(1.18 - 1.55)
Number of siblings (at birth)	0.98	(0.93 - 1.02)
Neighbourhood income <$20,000	1.34	(1.16 - 1.54)
Total participants = 13,868		

Research Question #2 – Are skin infections in children up to age 2 associated with asthma, independent of AD?

When testing for an association between either early skin infections or early AD and the outcome of asthma by age 11, the number of children within the birth cohort from 1995 to 2006 decreased from 13,980 to 13,720 children. This represented the number of children remaining in the province of Manitoba with provincial health insurance coverage.

The characteristics of the birth cohort as they pertain to the onset of asthma by age 11 are summarized in Table 12. Children with an early skin infection were significantly more likely to have asthma (8.0%) when compared to those without an early skin infection (6.3%) ($P = 0.0011$). This finding held true for late skin infection as those children who had a late skin infection were significantly more likely to have asthma than not (7.7% vs 6.4%, $P = 0.0266$). It was not surprising to find that children with asthma were significantly more likely to have early or late AD ($P < 0.0001$) or a maternal history of asthma ($P < 0.0001$). Gender was significantly associated with asthma as boys were more likely to have asthma (8.0%) than girls (5.2%). One category of antibiotics was significantly associated with asthma: those children who had received more than four courses of antibiotics within the first year of life were more likely to have asthma (8.9%) than those who received 3 or fewer (6.3%). Children with asthma had significantly more health care visits (72.8 vs 52.8 visits, $P < 0.0001$) and had fewer siblings than those without asthma (0.9 vs 1.2 siblings, $P < 0.0001$). The variables not significantly associated with asthma were low birth weight and low neighbourhood income.

Table 12. Distribution of population characteristics according to the onset of asthma by age 11

		No Asthma	Asthma	*P* value
Total Participants = 13,720		12,812	908	
Early skin infection	No	10,398 (93.7)	697 (6.3)	
	Yes	2414 (92.0)	211 (8.0)	0.0011
Late skin infection	No	10,851 (93.6)	744 (6.4)	
	Yes	1,961 (92.3)	164 (7.7)	0.0266
Early atopic dermatitis	No	11,974 (94.0)	763 (6.0)	
	Yes	838 (85.2)	145 (14.8)	<0.0001
Late atopic dermatitis	No	11,269 (93.8)	751 (6.3)	
	Yes	1543 (90.8)	157 (9.2)	<0.0001
Maternal history of asthma	No	11508 (94.0)	733 (6.0)	
	Yes	1238 (87.7)	174 (12.3)	<0.0001
	missing	66	1	
Location in 1995	Rural	5653 (95.2)	286 (4.8)	
	Urban	7159 (92.0)	622 (8.0)	<0.0001
Number of siblings (at birth)	Mean ± SD	1.2 (1.48)	0.9 (1.20)	<0.0001
Gender	Female	6391 (94.8)	351 (5.2)	
	Male	6421 (92.0)	557 (8.0)	<0.0001
Maternal distress	No	10372 (93.6)	704 (6.4)	
	Yes	2440 (92.3)	204 (7.7)	0.0115
Number of health care visits	Mean ± SD	52.8 (28.32)	72.8 (35.55)	<0.0001
Low birth weight	No	10998 (93.5)	765 (6.5)	
	Yes	1814 (92.7)	143 (7.3)	0.1854
Neighborhood income <$20,000	No	9564 (93.3)	688 (6.7)	
	Yes	3212 (93.7)	217 (6.3)	0.4353
	missing	36	3	
Number of courses of antibiotics received in first year of life	0	4347 (94.8)	239 (5.2)	
	1-2	4755 (93.1)	351 (6.9)	0.3527
	3-4	2089 (92.9)	160 (7.1)	0.3006
	>4	1621 (91.1)	158 (8.9)	<0.0001

The variables that showed strong associations with the onset of asthma by age 11, as seen above, also proved significant using univariate logistic regression (Table 13). By analyzing these variables through univariate logistic regression the strength of the

associations could be gauged through the generation of the unadjusted (unadj.) OR and 95% CI. Children with an early skin infection were 30% (unadj. OR 1.30, 95% CI 1.11-1.53) more likely to develop asthma than those with did not have an early skin infection. Living in an urban environment increased the odds of developing asthma by 72% (unadj. OR 1.72, 95% CI 1.49-1.98). Asthma was inversely associated with siblings, such that, the more siblings a child had, the less likely they were to asthma by age 11.

Table 13. Likelihood of asthma at 11 years of age - Univariate analysis

Variable	Asthma	
	OR	95% CI
Early skin infection	1.30	(1.11 - 1.53)
Late skin infection	1.22	(1.02 - 1.45)
Early AD	2.72	(2.24 - 3.29)
Late AD	1.53	(1.28 - 1.83)
Maternal history of asthma	2.21	(1.85 - 2.63)
Urban location	1.72	(1.49 - 1.98)
Number of siblings (at birth)	0.86	(0.81 - 0.91)
Male gender	1.58	(1.38 - 1.81)
Maternal distress	1.23	(1.05 - 1.45)
Number of health care visits	1.02	(1.02 - 1.02)
Low birth weight	1.13	(0.94 - 1.36)
Low neighborhood income	0.94	(0.80 - 1.10)
Courses of antibiotics		
1-2	1.07	(0.93 - 1.23)
3-4	1.10	(0.92 - 1.31)
> 4	1.45	(1.22 - 1.74)
Total participants = 13,720		

After establishing the significance of each of the variables, and the strength of the association through univariate logistic regression, it was possible to determine if some of the variables could be deemed confounders as they were significant with both the

outcome variable (asthma at age 11) and the exposure variable (early skin infection). The variables that met the criteria for confounding included: maternal history of asthma, living in an urban location in 1995, having more than one sibling, having a mother with a history of maternal distress, the number of health care visits within the first 7 years of life and having received more than 4 courses of antibiotics within the first year of life.

Based upon the above findings, it was then possible to test for interactions between the aforementioned confounding variables and the exposure variable (early skin infection). All interactions were non-significant except for the interaction between the number of health care visits and early skin infection ($P = 0.0149$). Thus, the number of health care visits was subsequently categorized using the percentiles and added into the final models based upon those criteria.

With all variables in place, multivariable logistic regression analysis was performed to test the second research question of the thesis which was to determine if skin infections (exposure) in children from infancy to age 2 were associated with asthma (outcome), independent of AD.

The initial construct involved the addition of the variables in a forward stepwise regression. As seen in the univariate analysis, early skin infection was significantly associated with the onset of asthma with those children who exhibited an early skin infection being more likely to be diagnosed with asthma by age 11 (unadj. OR 1.30, 95% CI 1.11–1.53). After the addition of early AD, the likelihood of children with an early skin infection fell to a 19% likelihood of acquiring asthma; however, it was still a significant variable. The subsequent addition of the variables urban location and male gender dropped the odds ratio of early skin infection to just under the significant level

(adj. OR 1.17, 95% CI 0.99-1.38). The variables maternal asthma, low birth weight, maternal distress or antibiotic usage in the first year had minimal impact on the contribution of early skin infection and the outcome of asthma. However, the addition of the continuous variable, the number of siblings a child in the study had, raised the significance of early skin infection to a significant status (adj. OR 1.19, 95% CI 1.01-1.41). The number of siblings had proven to be a protective factor in the likelihood of developing asthma with the addition of each consecutive sibling decreasing the probability of developing asthma by 11%. The addition of the number of health care visits to the model decreased the contribution early skin infection made the most with the odds ratio dropping from 1.14 (95% CI 0.96-1.34) to 0.99 (95% CI 0.84-1.17).

With the information garnered from above, a further set of analysis was performed. Even though low birth weight and maternal distress were not statistically significant in the model, it was felt that their addition was important from a biological perspective. Low neighbourhood income did not contribute greatly to the model and was found not to be statistically significant. A history of maternal asthma did contribute significantly to the model (adj. OR 2.10, 95% CI 1.76-2.52) as did the antibiotic usage variable with more than 4 courses of antibiotics having the strongest association with the outcome (adj. OR 1.63, 95% CI 1.31-2.02). As with the models run prior, the addition of the number of health care visits decreased the OR of early skin infection by the largest degree. The OR for number of health care visits was significant (adj. OR 1.02, 95% CI 1.01-1.02) and this value stayed fairly consistent throughout subsequent modelling.

Due to the interaction between early skin infection and the number of health care visits, the data was categorized based on the percentiles for the number of health care visits (9, 17, 24, 35, 49 and 67 respectively).

Each model was run in full using the aforementioned criteria for centering. When testing the interaction terms, the odds ratio for (health care visits*early skin) was approximately one (adj. OR 0.99, 95% CI 0.99-1.00) as both the interaction variable and the health care visit variable, at each cut-off value, are '0'. Thus, the effect of early skin infection at that reference value for visits was the only main effect.

Table 14. Likelihood of asthma, excluding maternal asthma in children at age 11 by number of hospitalizations or physician visits from birth to age 7

Health care visit percentiles (n)	1% (9)		5% (17)		10% (24)		25% (35)		50% (49)		75% (67)	
Variables	Asthma		Asthma		Asthma		Asthma		Asthma		Asthma	
	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI
Early skin infection	1.46	(1.05-2.03)	1.40	(1.03-1.88)	1.34	(1.02-1.77)	1.26	(0.99-1.59)	1.16	(0.94-1.42)	1.04	(0.87-1.25)
Late skin infection	1.16	(0.81-1.69)	1.14	(0.82-1.60)	1.13	(0.83-1.53)	1.10	(0.84-1.43)	1.06	(0.85-1.33)	1.02	(0.84-1.23)
Early AD	2.22	(1.80-2.73)	2.22	(1.80-2.73)	2.22	(1.80-2.73)	2.22	(1.80-2.73)	2.22	(1.80-2.73)	2.22	(1.80-2.73)
Late AD	1.52	(1.25-1.83)	1.52	(1.25-1.83)	1.52	(1.25-1.83)	1.52	(1.25-1.83)	1.52	(1.25-1.83)	1.52	(1.25-1.83)
Urban location	1.35	(1.16-1.58)	1.35	(1.16-1.58)	1.35	(1.16-1.58)	1.35	(1.16-1.58)	1.35	(1.16-1.58)	1.35	(1.16-1.58)
Male gender	1.52	(1.32-1.75)	1.52	(1.32-1.75)	1.52	(1.32-1.75)	1.52	(1.32-1.75)	1.52	(1.32-1.75)	1.52	(1.32-1.75)
Low birth weight	0.98	(0.81-1.19)	0.98	(0.81-1.19)	0.98	(0.81-1.19)	0.98	(0.81-1.19)	0.98	(0.81-1.19)	0.98	(0.81-1.19)
Maternal distress	0.96	(0.81-1.14)	0.96	(0.81-1.14)	0.96	(0.81-1.14)	0.96	(0.81-1.14)	0.96	(0.81-1.14)	0.96	(0.81-1.14)
Number of siblings (at birth)	0.92	(0.86-0.97)	0.92	(0.86-0.97)	0.92	(0.86-0.97)	0.92	(0.86-0.97)	0.92	(0.86-0.97)	0.92	(0.86-0.97)
Low neighborhood income	0.86	(0.73-1.01)	0.86	(0.73-1.01)	0.86	(0.73-1.01)	0.86	(0.73-1.01)	0.86	(0.73-1.01)	0.86	(0.73-1.01)
Courses of antibiotics												
1-2	1.11	(0.94-1.33)	1.11	(0.94-1.33)	1.11	(0.94-1.33)	1.11	(0.94-1.33)	1.11	(0.94-1.33)	1.11	(0.94-1.33)
3-4	0.90	(0.73-1.13)	0.90	(0.73-1.13)	0.90	(0.73-1.13)	0.90	(0.73-1.13)	0.90	(0.73-1.13)	0.90	(0.73-1.13)
>4	0.88	(0.69-1.12)	0.88	(0.69-1.12)	0.88	(0.69-1.12)	0.88	(0.69-1.12)	0.88	(0.69-1.12)	0.88	(0.69-1.12)
Total participants = 13,615												

For these two final models, maternal asthma was added to one model (Table 15), but not the other (Table 14) to see what effect maternal asthma had on early skin infection. As Table 14 shows, without the maternal asthma variable, the significance of early skin infection, when tested at the 1% percentile for number of health care visits,

was significant (adj. OR 1.46, 95% CI 1.05-2.03). As Table 15 shows, after the addition of the maternal asthma variable, early skin infection retained its significance within the model (adj. OR 1.44, 95% CI 1.04-2.01). In both of these tables, early skin infection is no longer significant when the number of health care visits reaches 35, independent of maternal asthma.

For the final two models, the other significant variables included atopic dermatitis, living in an urban location, male gender, having more than one sibling, maternal asthma and having a neighbourhood income of less than $20,000. None of the antibiotic variables were significant nor was low birth weight or maternal distress.

When the above model was run using the centering technique at the designated health care visit percentiles, the OR for early skin infection was 1.46, 1.40, 1.34, 1.26, 1.16, and 1.04 respectively. Upon review of the ORs, a definite trending for early skin infection was evident such that as the number of health care visits increased the OR for early skin infection decreased with early skin infection just losing significance (adj. OR 1.26, 95% CI 0.99-1.59) at the 25% (n = 35) reference level. This same negative trending for the early skin infection OR was also observed in the final model (Table 15).

Table 15. Likelihood of asthma in children at age 11 by number of hospitalizations or physician visits from birth to age 7

Health care visit percentiles (n)	1% (9)		5% (17)		10% (24)		25% (35)		50% (49)		75% (67)	
Variable	Asthma		Asthma		Asthma		Asthma		Asthma		Asthma	
	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI
Early skin infection	1.44	(1.04-2.01)	1.38	(1.02-1.87)	1.33	(1.01-1.75)	1.25	(0.98-1.59)	1.15	(0.94-1.41)	1.04	(0.87-1.24)
Late skin infection	1.18	(0.81-1.71)	1.15	(0.83-1.62)	1.13	(0.83-1.54)	1.10	(0.84-1.44)	1.06	(0.85-1.33)	1.01	(0.83-1.23)
Early AD	2.26	(1.83-2.78)	2.26	(1.83-2.78)	2.26	(1.83-2.78)	2.26	(1.83-2.78)	2.26	(1.83-2.78)	2.26	(1.83-2.78)
Late AD	1.50	(1.24-1.81)	1.50	(1.24-1.81)	1.50	(1.24-1.81)	1.50	(1.24-1.81)	1.50	(1.24-1.81)	1.50	(1.24-1.81)
Urban location	1.34	(1.15-1.56)	1.34	(1.15-1.56)	1.34	(1.15-1.56)	1.34	(1.15-1.56)	1.34	(1.15-1.56)	1.34	(1.15-1.56)
Male gender	1.53	(1.33-1.77)	1.53	(1.33-1.77)	1.53	(1.33-1.77)	1.53	(1.33-1.77)	1.53	(1.33-1.77)	1.53	(1.33-1.77)
Low birth weight	0.97	(0.80-1.18)	0.97	(0.80-1.18)	0.97	(0.80-1.18)	0.97	(0.80-1.18)	0.97	(0.80-1.18)	0.97	(0.80-1.18)
Maternal distress	0.95	(0.80-1.18)	0.95	(0.80-1.18)	0.95	(0.80-1.18)	0.95	(0.80-1.18)	0.95	(0.80-1.18)	0.95	(0.80-1.18)
Number of siblings (at birth)	0.92	(0.87-0.97)	0.92	(0.87-0.97)	0.92	(0.87-0.97)	0.92	(0.87-0.97)	0.92	(0.87-0.97)	0.92	(0.87-0.97)
Low neighborhood income	0.85	(0.72-1.00)	0.85	(0.72-1.00)	0.85	(0.72-1.00)	0.85	(0.72-1.00)	0.85	(0.72-1.00)	0.85	(0.72-1.00)
Courses of antibiotics												
1-2	1.11	(0.93-1.32)	1.11	(0.93-1.32)	1.11	(0.93-1.32)	1.11	(0.93-1.32)	1.11	(0.93-1.32)	1.11	(0.93-1.32)
3-4	0.89	(0.71-1.10)	0.89	(0.71-1.10)	0.89	(0.71-1.10)	0.89	(0.71-1.10)	0.89	(0.71-1.10)	0.89	(0.71-1.10)
>4	0.85	(0.67-1.08)	0.85	(0.67-1.08)	0.85	(0.67-1.08)	0.85	(0.67-1.08)	0.85	(0.67-1.08)	0.85	(0.67-1.08)
Maternal asthma	1.86	(1.55-2.23)	1.86	(1.55-2.23)	1.86	(1.55-2.23)	1.86	(1.55-2.23)	1.86	(1.55-2.23)	1.86	(1.55-2.23)
Total participants = 13,615												

In the two final models, with and without maternal asthma, the association between a child having an early skin infection and the likelihood of their being diagnosed with asthma by age 11 was highest when the number of hospitalizations or physician visits was lowest. By the time a child had, on average, accrued up to 35 hospitalizations or visits, the association between early skin infection and the outcome of asthma became non-significant. At a reference point of <10 visits, 92% of 217 children lived in a rural area whereas only 8% (17) children lived in an urban area. Children living in a rural area would include First Nations children and/or children visiting a nursing station.

Using the median value (49) for the number of health care visits, children who had a mother with a history of asthma were 64% more likely to have either been hospitalized or seen a physician greater than 49 times.

Research question #3 – Is the association between early life skin infection and asthma independent of recent stress biomarkers?

A final analysis was conducted to test for an association between the indirect or direct stress variables and an outcome of asthma by age 11 using the SAGE case-control cohort (Table 16). Neither the indirect stress markers, early skin infection and/or late skin infection, nor the direct stress marker, C/D ratio, showed any significant association with an outcome of asthma by age 11.

Table 16. Likelihood of asthma at age 11 in relation to indirect and direct stress variables

Variables	Asthma	
	OR	95% CI
Early skin infection	1.71	(0.98-2.97)
Late skin infection	0.98	(0.54-1.76)
C/D high	1.22	(0.64-2.33)
C/D med	1.31	(0.70-2.45)
C/D low	1.15	(0.61-2.19)
Total participants = 488		

Initially, the abnormal C/D ratio was based on the 1st and 4th quartile being coded as abnormal. As that variable proved insignificant, the raw non-transformed C/D ratio was used and the variable further categorized according to the 25, 50 and 75 percentiles (9.4, 14.9, 22.9 respectively) (Table 16). As the number of participants in the SAGE cohort was significantly lower, 488 versus 13,980, the variables gender, low birth weight, maternal distress, low neighbourhood income and number of health care visits were omitted from the final models. None of the aforementioned variables, except for the

number of heath care visits, were significantly associated with an outcome of asthma in the nested case-control. From a biological perspective, the biomarker DHEA was included in the model to account for increasing DHEA levels with advancing age as per the literature. Even though none of the C/D variables were statistically significant, the highest C/D ratio produced the largest odds ratio across all of the models run after early skin infection was added to the model (adj. OR 1.41, 95% CI 0.71-2.80).

Early skin infection was close to being significant (adj. OR 1.71, 95% CI 0.98-2.97) in the above model (Table 16) when only the C/D variables were included in the model. Upon the addition of the other variables (Table 17), early skin infection dropped in significance by 23% (adj. OR 1.48, 95% CI 0.83-2.65).

Table 17. Likelihood of asthma at age 11 in relation to indirect and direct stress variables and C/D ratio

Variables	Asthma		Asthma		Asthma	
	OR	95% CI	OR	95% CI	OR	95% CI
Early skin infection					1.48	(0.83-2.65)
Late skin infection					0.94	(0.51-1.74)
Early AD			2.59	(1.32-5.11)	2.55	(1.29-5.04)
Late AD			0.45	(0.13-1.56)	0.45	(0.13-1.56)
Urban	2.53	(1.52-4.20)	2.54	(1.52-4.24)	2.52	(1.50-4.21)
Number of siblings (at birth)	0.85	(0.67-1.07)	0.85	(0.67-1.07)	0.85	(0.67-1.08)
Maternal asthma	1.53	(0.86-2.73)	1.6	(0.89-2.90)	1.52	(0.84-2.76)
DHEA level	1.02	(0.97-1.06)	1.02	(0.97-1.07)	1.02	(0.97-1.07)
C/D high	1.33	(0.68-2.62)	1.35	(0.69-2.67)	1.41	(0.71-2.80)
C/D med	1.31	(0.69-2.48)	1.26	(0.66-2.42)	1.3	(0.68-2.51)
C/D low	1.08	(0.55-2.12)	1.03	(0.52-2.04)	1.07	(0.54-2.13)
Total participants = 487						

Discussion

This study examined whether indirect (skin infection, atopic dermatitis) and direct (C/D ratio) markers of stress measured at critical developmental stages in childhood, infancy and pre-puberty, were able to predict the onset of asthma at 11 years of age. The objectives were to be met in three phases: 1) to determine if indirect markers of stress during the first two years of life were elevated in response to maternal postnatal distress, 2) to see if children who exhibited an increase in early skin infections will be at an increased likelihood for developing asthma by age 11, independent of AD, a well known risk factor for asthma and 3) to explore the relationship between early skin infections, direct measures of recent stress and the outcome of asthma by age 11.

This thesis was successful in showing that skin infections and or AD seen in children from birth to age 2 could be used as an indirect marker of stress, thus satisfying the first objective of the hypothesis. The analysis showed that a child whose mother had postpartum distress was 1.25 times more likely to have a skin infection in the first 2 years of life than a child whose mother did not exhibit postpartum distress. A child whose mother had postpartum distress was also more likely to have AD (adj. OR 1.46, 95% CI 1.26-1.70) as opposed to a child whose mother did not report having postpartum distress in the first year of life. These findings are consistent with those found by other researchers who have demonstrated that the likelihood of developing AD is increased following the effect of family stressors.[15,18, 67] The ICD-9 codes selected for skin infections represented the most common skin infections seen in early childhood, thus meeting the criteria for face validity.[68,29,55,69-71] New mothers with postpartum depression are less likely to interact with their infants resulting in increased stress levels in the

infant.[37,72,73,40,74] This research now provides additional support that skin infections seen in early life can be used as a marker for a stress response in an infant.

Using the final model based on the number of health care visits and including all variables, the second objective was met. Children who exhibited an early skin infection were at a 33% increased likelihood for developing asthma by age 11 independent of the addition of early AD (adj. OR 1.33, 95% CI 1.01-1.75). The association remained significant until the number of health care visits reached the reference value of 25%. Despite the caveat regarding the interaction, the analysis shows that the association with skin infection within the first 2 years of life is independent of early AD. The OR for early AD consistently remained statistically significant, across all reference levels, with an OR of 2.26 (95% CI 1.83-2.78). This is an important finding as skin infections could be confused with AD,[75,76] a well known risk factor asthma.[77,61]

By including the health care visits into the analysis, it was possible to account for detection bias whereby the more often a child was hospitalized or seen by a physician, the greater the likelihood that the outcome of interest would be captured. In these analyses, the opposite was observed with the strength of association decreasing between the dependent and outcome variables as the number of health care visits increased. There could be a study design explanation for these findings such as children who had lower health care usage were more likely to live in a rural area: of the 217 children who had less than 10 visits to a health care provider, 200 (92%) were from a rural location. This lower utilization may represent a pattern typical of children living on reserve who more often see a salaried physician in remote nursing stations. However, analyses were adjusted for urban-rural residence making this an unlikely explanation.

In order to understand the association between skin infection and asthma, the following biological pathways are presented. Hypotheses on the psychoneuro-immunologic origins of asthma involving the skin are still evolving. Animal studies have described the impact of prenatal stress on the developing immune system of the fetus[78] and these biochemical changes have been documented in humans, as well.[79] According to one author, the skin may be viewed as a 'neuro-endocrine organ'.[80] The skin expresses the equivalent of the HPA axis that can communicate with the HPA axis of the brain. Similar to the workings of stress on the HPA axis of the brain and its subsequent influence on inflammatory disorders of the skin such as AD, [22,81,82] environmental stressors may lead to the development of skin infections. It is plausible that stress-induced changes in the immune system, brought on by maternal stress, may be manifesting in such as way as to make infants more susceptible to skin infections.[83] In this regard, skin infection in the infant is behaving as a marker of early life stress in the skin infection and asthma association.

A further biological pathway for the skin infection and asthma association may directly involve the permeability of the newborn skin. Stress alteration of the HPA axis may act upon regulatory molecules pertaining to the integrity of the epidermal barrier.[84,85] Findings on the immunogenetics of asthma and AD have identified the epithelium as a common pathway for the development of both of these diseases.[86] It is plausible that skin infections early in life are manifestations of an impaired barrier function of the epithelium, including the gastrointestinal epithelium, which leads to allergen penetration and subsequent inflammation. Of note, the early infection findings were independent of the AD and asthma association. Further, early and not late skin infection was associated

with asthma, consistent with the one year time period after birth during which the barrier function of the skin matures to adult levels.[84]

In some of the preliminary models, early skin infection is significant until antibiotic usage is added to the model. The use of antibiotics may disrupt the normal flora in the gut of the infant which may then interfere with the integrity of the skin barrier. A paper by Warner et al. describes how up to 80 – 100% of subjects with AD may have cutaneous colonization with *S. aureus* compared with 5 – 30% of healthy controls.[71] This data supports the use of early skin infection and early AD as early predictors of asthma. The addition of antibiotics in the first year of life may result in a decrease in a skin infection caused by *S. aureus* thus rendering skin infection as non-significant.

Neither of the following variables changed the association between skin infection and an outcome of asthma: maternal asthma and low birth weight. Prenatal stress has been associated with an increased risk for preterm birth and subsequently low birth weight.[87] It is possible that children within this birth cohort were either premature or small for their gestational age. As such, they could be more susceptible to a skin infection if their immune system were not fully functional. However, all analysis was done utilizing a robust variable that accounted for birth weight, gestational age and gender.[88,89] At no time did this variable significantly contribute to any of the models run.

It was important to access whether the association between skin infections and asthma was independent of recent stress markers. There is much support within the current literature base to encourage the exploration of using direct stress markers, such as cortisol and DHEA, in determining an outcome of asthma. As evidenced by other

researchers, maternal distress can have an impact of the developing immune system of the fetus, which in turn, may result in the subsequent development of asthma in later years.[90,17, 91] As an example of the effect of stress upon direct stress markers, changes in cortisol and DHEA levels have been seen in patients who have undergone a traumatic event,[92, 93] Further, a recent paper has shown that children with asthma are less likely to exhibit a strong cortisol response to an acute stressor in contrast to children without asthma who are able to elicit an elevated cortisol response.[94] A final example showing the effects of stress upon a child's immune system describes how cortisol's ability to function effectively is minimized due to the harsh family climate the child is surrounded by.[95] However, in our study, although the association was in the right direction it was not significant.

Unlike the first and second objectives which utilized the entire birth cohort of 13,780 children, the third objective was only able to use data from the SAGE nested case-control study where n=488. In both univariate and multivariable modelling, neither early nor late skin infection nor C/D ratio showed any statistical significance whatsoever with an outcome of asthma by age 11. The lack of significance for early skin infection is contrary to the significance seen when applied to the full cohort.

A key strength in using a birth cohort is that it is the best study design to assess for a relationship between exposure and outcome; use of provincial databases and data linkage; and access to national funding. Additionally, the validity of both the skin infection and AD variables is free from recall bias as the data for both was culled from physician diagnoses. One of the primary difficulties in research that incorporates AD is the inherent difficulty in standardizing the diagnostic criteria for AD. This research was

fortunate in that there was a nested case-control study within the birth cohort containing children who had been diagnosed with AD by a pediatric allergist. These children could then be used to validate a working definition that could subsequently be applied to the entire birth cohort. This validation for the AD definition encompassed both face and concurrent validity. Face validity was achieved by extracting the relevant ICD-9 codes from both the medical and hospital registries. The selection of the time period for health care visits for early AD also met face validity, as children between the ages of 2 to 5 years are more likely to be seen for a diagnosis of AD.[58] The inclusion of physician diagnosed AD served as the 'gold standard' by which to compare the database AD definition allowing for the ability to generate sensitivity and specificity as a measure of that validity. Similar to other research, no association between early AD and the number of siblings a child had in 1995, their birth weight and the use of antibiotics in the first year of life was found.[96]

In conclusion, it has been documented that skin infections seen in children are on the rise, particularly for infants.[54] Even though the overall incidence rates for skin infection has decreased, incidence rates for specific skin infections, such as impetigo, are on the rise. According to Mohammedamin *et al*., 29% of children between 1-4 years of age were diagnosed with impetigo. These finding are similar to those found regarding the incidence of asthma. According to Gershon *et al.*, there has been an increase in the incidence of asthma seen in children between 1996 and 2005.[97] As mentioned in the introduction, some researchers propose that infections in early life may actually protect against asthma.[10,69] The conclusions of this thesis actually shows the opposite: children

up to the age of two who exhibit an early skin infection are actually at an increased likelihood for developing asthma by age 11.

Study Limitations

The primary advantage of using the 1995 birth cohort is that it allows for the study of a non-high risk group of pre-pubescent children in addition to allowing for greater generalization to the pediatric population at large. A non-high risk group is defined as children not born solely to mothers who have asthma or a history of asthma.

However, as with all studies, certain limitations are inherent. With respect to cortisol levels, some results may be elevated simply through the act of obtaining the blood sample. For a child, having a veni-puncture to acquire the blood sample may be an extremely stressful event. The result would be activation of the child's immune system to elicit an increase in their total cortisol measurement.[34,98] Salivary cortisol would have been the preferred specimen as it allows for a "stress-free" sample. However, salivary and blood concentrations of cortisol and DHEA are highly correlated.[42] The timing of the sample draw for Wave 1 was fairly consistent, but not timed.

The interpretation of data found within this study when compared with other epidemiological studies may be hindered owing to the varying definitions of asthma used.[3] Even though the asthma definition employed for this study was validated on children at age 7 the measure remains valid as asthma is a chronic disease.

While much progress has been made in validating the diagnostic criteria used for AD it is still a diagnosis that is based upon clinical presentation and, as a result, is subject to interpretation. While diagnostic criteria may not be as crucial in the physician's office,

the use of a standard definition for research is paramount to allow for consistency.[60] Thus the validation of the AD definition employed against the physician diagnosed AD may be subject to misclassification. When testing the database definition of early AD against the physician diagnosed gold standard, a specificity of 87% was obtained. This ensured that when the database definition was employed, a positive result meant a high probability of the presence of early AD. While this may ensure a low Type I error rate (false positives), it may also mean that the children with the most severe AD were coded as positive for early AD within the criteria of this study. Thus, the results of this study may reflect only those children with the most severe form of early AD. In addition, it may also be possible that some of the children diagnosed with a skin infection may have actually been misdiagnosed AD which could, again, positively skew the results.

The dataset utilizing the physician-diagnosed AD for sensitivity and specificity testing may be subject to physician misclassification particularly in the late AD definition thereby resulting in a falsely lowered sensitivity.

The data for skin infection is dependent upon the parent bringing their child to their physician for a consultation. Some of the skin infections, such as molluscum contagiosum, may have resolved spontaneously; subsequently, treatment would not have required. It is likely then that there may be an underreporting of the number of skin infections that a child in this study has experienced in early life. Additionally, ICD-9 classification codes were used which, while highly specific, are not as sensitive[99] resulting in the potential failure of capturing more atypical skin infections.

Skin infection diagnosis pulled from the hospital registry was dependent upon the skin infection coding as either the primary or secondary diagnosis. Therefore, if the

physician visited a child in the hospital for a diagnosis other than skin infection and subsequently noticed the child had a skin rash, the coding of the rash would perhaps be diagnosis 3 or greater. As a result, this incident would not be captured in this data.

Future Directions

At this point in time, the underlying mechanism(s) of asthma are not fully known or clearly understood. What is known, however, is that asthma is reaching almost epidemic proportions in industrialized nations such as Canada resulting in enormous expenditures of health-care dollars. In the US, asthma is the third leading cause of hospitalization among those under 18 years of age.[6]

The results of this study will go towards supporting the growing body of literature that favours the priming of the fetal immune system resulting in increased asthma susceptibility. This study will build upon the study recently published by Kozyrskyj *et al*., [12] by further exploring the relationship between prolonged maternal distress and the developing immune system of the fetus and the subsequent development of asthma in children by age 11.

By having a clearer understanding of the risk factors and pathways that influence the development of childhood asthma, perhaps more intuitive primary-prevention strategies may be developed. Ultimately, the direct goal of being able to decrease the incidence of asthma worldwide would be achieved by targeting these at-risk events. [100]

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