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MATERNAL STRESS AND ALLERGIC DISEASE IN OFFSPRING

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Maternal stress and allergic disease in offspring

THESIS FOR DOCTORAL DEGREE (Ph.D.)

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POPULAR SCIENCE SUMMARY OF THE THESIS

Background

The development of diseases has been suggested to start already in the womb. Numerous factors in the environment can affect the unborn baby. Maternal stress has been studied in many different settings to determine how and if the mothers stress in pregnancy might affect the unborn baby. However, stress is individual and can be measured and captured in many different ways, during pregnancy, mothers-to-be might experience different stressors than non-pregnant-women. The prevalence of maternal stress can be different depending on geographical area, ethnicity and socioeconomic factors. Maternal stress during pregnancy might also be a risk factor for development of illness in the offspring by inducing changes in the fetus on growth, organs, genetics and immune responses. Since allergic diseases, abdominal pain and colic often starts in early life, theses illnesses might share a common risk factor of stress during pregnancy. Thus, the overall aim of this doctoral thesis was to evaluate maternal pre- and postpartum stress and the risk of abdominal pain and allergic disease in offspring.

All the studies in this thesis included pregnant mothers and later their infants from the Nordic population-based cohort PreventADALL. We began to study if factors in pregnancy were associated with maternal stress in mid and late pregnancy and described the prevalence of stress in the Nordic pregnant population. The findings showed that maternal stress was prevalent in 15 % of the women in mid pregnancy and decreased in late pregnancy (13%). Symptoms of allergic diseases increased the risk of stress in the mothers during pregnancy (Study 1).

We also wanted to explore if maternal stress, maternal saliva cortisol and infant saliva cortisol were correlated. The results showed that maternal stress during pregnancy and postpartum and maternal saliva cortisol at 18 weeks pregnancy were not correlated, neither were infant saliva cortisol levels in early infancy (Study 3).

The total reported prevalence of the abdominal and pain symptoms (colic, abdominal pain and other pain and infant discomforts) were reported in 26% by 3 months age. Neither infant colic (3%) or abdominal pain (22%) or other pain and discomforts (6%) affected the infant's cortisol levels. Maternal stress was not associated with any of the infant abdominal and pain symptoms (Study 2).

In Study 4, we wanted to study if maternal stress from pregnancy and early postpartum increased the odds of the presence of antibodies to common food and inhalant allergens, so called allergic sensitization, in infants at 12 months age. The results showed that stress during pregnancy and early postpartum were not associated with infant sensitization.

Conclusion

In Nordic pregnant women symptoms of allergic disease can be stressful and socioeconomic factors might contribute to increased stress. However, maternal stress in pregnancy and

postpartum does not correlate with neither maternal cortisol in pregnancy nor infant cortisol at 3 months age. Abdominal pain was the most common infant pain symptoms at 3 months age, followed by other pain and discomforts and colic. Maternal stress in pregnancy is not a risk-factor for either of these symptoms and they are not associated with infant saliva cortisol levels in early infancy. Furthermore, maternal stress in pregnancy and postpartum is not associated with allergic sensitization in their offspring at 12 months of age.

From a clinical point of view these findings could be an important information to communicate to the mothers who experience stress during pregnancy. The knowledge that maternal stress does not affect colic, abdominal pain, or allergic sensitization could relieve the mother.

POPULÄRVETENSKAPLIG SAMMANFATTNING

Utvecklingen av sjukdomar kan starta redan i livmodern och många faktorer i miljön kan påverka fostret. Mammans stress har studerats i många olika situationer och miljöer för att avgöra hur, och om mammans stress under graviditeten kan påverka fostret. Stress är individuellt och kan mätas på många olika sätt. I graviditeten kan blivande mammor känna sig stressade av andra faktorer än kvinnor som inte är gravida. Prevalensen av stress hos kvinnor i graviditet kan vara olika beroende på var man bor, etnicitet och socioekonomi. Mammans stress under graviditeten kan också vara en riskfaktor för utveckling av sjukdom hos barnet genom förändringar som påverkar fostret tillväxt, genetik och immunsvar. Eftersom allergiska sjukdomar, buksmärtor och kolik hos barn ofta börjar tidigt i livet, kanske dessa sjukdomar kan påverkas av mammans stress i graviditet.

Det övergripande syftet med denna doktorsavhandling var att utvärdera mammans stress i graviditet och första månader efter förlossning och risken för buksmärtor och allergisk sjukdom hos spädbarnet.

Alla studier i avhandling inkluderade gravida mödrar och senare deras spädbarn från den nordiska födelsekohorten PreventADALL. Vi började studera om faktorer i graviditeten var förknippade med mammans stress i mitten samt slutet av graviditeten och beskrev prevalensen av stress i den nordiska gravida befolkningen. Resultaten visade att 15% av mammorna rapporterade stress i mitten av graviditeten och 13% i slutet av graviditeten. Symtom på allergiska sjukdomar ökade risken för stress hos mammorna under graviditeten (Studie 1).

Vi ville även undersöka om mammans stress, salivkortisol och spädbarnets salivkortisol var korrelerade. Resultaten visade att mammans stress under graviditeten och stress vid tre månader efter förlossningen och mammans salivkortisolnivåer i graviditetsvecka 18 inte var korrelerade, vi fann inte heller någon korrelation mellan mammans stress och spädbarnet salivkortisolnivåer vid tre månader (Studie 3).

Den totala prevalensen av kolik, buksmärtor och annan smärta eller obehag rapporterades hos 26 % av spädbarnen vid 3 månaders ålder. Varken spädbarnskolik (3%) eller buksmärtor (22%) eller andra smärtor eller obehag (6%) påverkade spädbarnets kortisolnivåer. Mammans stress i graviditeten var inte associerad med något av spädbarnets rapporterade symptom (Studie 2).

I Studie 4 ville vi undersöka om mammans stress från graviditet och första månaderna efter förlossningen ökade oddsen för närvaron av antikroppar mot vanliga födoämnes- och inhalationsallergener så kallad allergisk sensibilisering, hos spädbarnet vid 12 månaders ålder. Resultaten visade att stress under graviditet och första månaderna efter förlossningen inte var associerad med spädbarnets sensibilisering.

Slutsats

Hos gravida kvinnor i Norden kan symtom på allergisk sjukdom vara stressande och socioekonomiska faktorer kan bidra till ökad stress. Det fanns dock ingen korrelation mellan mammans stress i graviditet eller första månaderna efter förlossningen med varken mammans kortisol nivåer i graviditetsvecka 18 eller spädbarnets kortisolnivåer vid 3 månaders ålder. Buksmärtor var de vanligaste smärtsymtomen hos spädbarn vid 3 månaders ålder, följt av annan smärta eller obehag och kolik. Mammans stress under graviditeten är inte en riskfaktor för något av dessa symtom och de är inte associerade med spädbarnets salivkortisolnivåer i tidig spädbarnsålder. Slutligen, mammans stress under graviditet och första månader efter förlossningen är inte associerad med allergisk sensibilisering hos deras spädbarn vid 12 månaders ålder.

Dessa fynd kan vara viktig information till mammor som upplever stress under graviditeten. Vetskapen om att stress inte påverkat kolik, buksmärtor eller den allergisk sensibilisering hos deras barn efter födelsen kan vara lugnande för mammor som upplevt en stressig graviditet

ABSTRACT

The theory of developmental origins of health and disease (DOHaD), hypothesize that diseases like allergy is formed in-utero and early life by interaction between adverse environmental factors and genetic predisposition.

The study population in this thesis consists of mother-child pairs from the PreventADALL cohort. The overall aim of this doctoral thesis was to evaluate maternal pre- and postpartum stress and the risk of abdominal pain and allergic disease in offspring. In Study 1, the proportion of maternal perceived stress at 18- and 34-weeks was described and the association between symptoms of allergic disease and maternal perceived stress explored in n=2164 pregnant mothers. Study 2 described the prevalence of infant colic, abdominal pain and other pain and discomforts in n=1852 infants at 3 months age and perinatal factors associated with the abdominal outcomes. The correlation between maternal perceived stress in pregnancy and postpartum, maternal saliva cortisol and infant saliva cortisol (n=1057) was explored in study 3. Finally, Study 4 investigated if maternal perceived stress during pregnancy or postpartum were associated with infant allergic sensitization at 12 months age (n=1757).

Results from Study 1 showed that maternal stress was reported by 15 % of the mothers at 18 weeks and 13% at 34 weeks. Symptoms of asthma, rhinitis and food allergy reported by the mother were associated with high perceived stress and increased stress in the third trimester. Infant colic was reported in n=59 infants, abdominal pain in n=115 and other pain and discomforts in n=119 infants at 3 months age. High maternal perceived stress was not associated with any of the abdominal and pain symptoms. Neither were the infant saliva cortisol levels at 3 months age (Study 3). Furthermore, maternal perceived stress during pregnancy and postpartum and maternal saliva cortisol at 18 weeks were not correlated, neither were infant saliva cortisol levels (n=1057). In Study 4, infants and their mothers with perceived stress were included. The maternal stress in pregnancy and postpartum were not associated with infant sensitization (n=139) at 12 months age.

Conclusion

Symptoms of allergic disease can be stressful for mothers in pregnancy. Abdominal pain is a common symptom in 3-month-old infants. Maternal stress in pregnancy is not a risk-factor for abdominal pain, other pain and discomforts or infant colic. Maternal perceived stress in pregnancy and postpartum does not correlate with neither maternal cortisol in pregnancy nor infant cortisol at 3 months age. Maternal stress in pregnancy and postpartum is not associated with allergic sensitization in their offspring at 12 months of age.

These findings could be an important information to communicate to the mothers who experience stress during pregnancy. The knowledge that maternal stress does not affect colic, abdominal pain, or allergic sensitization could relieve the mother.

LIST OF SCIENTIFIC PAPERS

I. **Olsson Mägi CA**, Bjerg Bäcklund A, Lødrup Carlsen K, Almqvist C, Carlsen KH, Granum B, Haugen G, Hilde K, Lødrup Carlsen OC, Jonassen CM, Rehbinder EM, Sjøborg KD, Skjerven H, Staff AC, Vettukattil R, Söderhäll C, Nordlund B. Allergic disease and risk of stress in pregnant women: a PreventADALL study. ERJ Open Res. 2020 Oct 13;6(4):00175-2020. doi: 10.1183/23120541.00175-2020. PMID: 33083440; PMCID: PMC7553112.

II. Despriee ÅW, **Olsson Mägi CA**, Småstuen MC, Glavin K, Nordhagen L, Jonassen CM, Rehbinder EM, Nordlund B, Söderhäll C, Carlsen KL, Skjerven HO. Prevalence and perinatal risk factors of parent-reported colic, abdominal pain and other pain or discomforts in infants until 3 months of age - A prospective cohort study in PreventADALL. J Clin Nurs. 2022 Oct;31(19-20):2784-2796. doi: 10.1111/jocn.16097. Epub 2021 Oct 26. PMID: 34704296.

III. **Olsson Mägi CA**, Wik Despriee Å, Småstuen MC, Almqvist C, Bahram F, Bakkeheim E, Bjerg A, Glavin K, Granum B, Haugen G, Hedlin G, Jonassen CM, Lødrup Carlsen KC, Rehbinder EM, Rolfsjord LB, Staff AC, Skjerven HO, Vettukattil R, Nordlund B, Söderhäll C. Maternal Stress, Early Life Factors and Infant Salivary Cortisol Levels. Children (Basel). 2022 Apr 27;9(5):623. doi: 10.3390/children9050623. PMID: 35626800; PMCID: PMC9139396.

IV. **Olsson Mägi CA,** Almqvist C, Bjerg Backlund A, Tedner G S, Lødrup Carlsen K, Rehbinder EM, Haugen G, Monceyron Jonassen C, Granum B, Staff AC, Skjervn H, Despriee ÅW, Wärberg Gerdin S, Vettukattil R, Söderhäll C, Nordlund B,. Pre- and postpartum maternal perceived stress and allergic sensitization in offspring: A observational population-based Nordic birth cohort study (PreventADALL): *Manuscript*

Scientific papers not included in the thesis:

Skjerven HO, Lie A, Vettukattil R, Rehbinder EM, LeBlanc M, Asarnoj A, Carlsen KH, Despriee ÅW, Färdig M, Gerdin SW, Granum B, Gudmundsdóttir HK, Haugen G, Hedlin G, Håland G, Jonassen CM, Landrø L, **Mägi CO**, Olsen IC, Rudi K, Saunders CM, Skram MK, Staff AC, Söderhäll C, Tedner SG, Aadalen S, Aaneland H, Nordlund B, Lødrup Carlsen KC. Early food intervention and skin emollients to prevent food allergy in young children (PreventADALL): a factorial, multicentre, cluster-randomised trial. Lancet. 2022 Jun 25;399(10344):2398-2411.

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CONTENTS

LIST OF ABBREVIATIONS

AD	Atopic Dermatitis
AS	Allergic Sensitization
CAR	The Cortisol Awakening Response
CI	Confidence Intervals
DOHaD	The Developmental Origins of Health and Disease
FGIDs	Functional GastroIntestinal Disorders
GA	Gestational Age
HSD11B2	11β-HydroxySteroid Dehydrogenase type 2 enzyme
HPA-axis	Hypothalamic-Pituitary Adrenal
ICS	Inhaled Cortico Stereoids
IgE	Immunoglobulin E
IQR	Inter quartile Range
OR	Odds ratio
PSS	The perceived stress scale
SAM	The Sympathetic AdrenoMedullary systems
SD	Standard Deviations
SES	SocioEconomic Status
SC	Saliva Cortisol
SPT	Skin Prick test

1 INTRODUCTION

Numerous factors occurring during the prenatal period may affect the children's future development of health and disease (1). In the 1980s, Barker hypothesized that the development of cardiovascular disease in adulthood was associated with geographical areas where the participants were born, where intrauterine and early life exposures such as famine and neonatal death were common (1). Later, Barker found that in-utero undernutrition caused low birth weight and lead to the suggested change later in life (2). In later years the hypothesis is now often referred to as "the developmental origins of health and disease" (DOHaD) (3), at present expanding over several other intrauterine and early life factors in epidemiological research (3, 4).

The development of allergic diseases in infancy known as the "atopic march" (5, 6) usually starts within the first months of life with atopic dermatitis and food allergy (7), later followed by allergic rhinitis and asthma. This suggests that the developmental origin of allergic diseases starts in early life, maybe even before birth and continues during the child's early life.

Maternal psychological stress in pregnancy can affect the maternal and infant HPA-axis (8), where changes in the stress hormone cortisol have been associated with an increased risk of preterm birth and low birth weight (9, 10). Interestingly, maternal prenatal stress has also been suggested to increase the vulnerability towards development of infant allergic diseases (11, 12).

The maternal subjective stress is difficult to measure. Stress-related factors, usually named stressors, are individual and can differ depending on population, socioeconomic and demographic factors. Stress is common among parents with an infant with colic, but maternal perceived stress in pregnancy and the association with early abdominal pain symptoms such as colic has not been explored in depth.

In this thesis I wish to use my clinical experience working as a pediatric nurse and explore if maternal stress in pregnancy and early postpartum influence infant health the first year of life, focusing on colic, abdominal pain and the development of allergic disease.

2 LITERATURE REVIEW

2.1 THE DEVELOPMENTAL ORIGINS OF HEALTH AND DISEASE

In the 1980s Barker found that some geographical areas with high infant mortality in the 1920s had higher rates of ischemic heart disease in the 1960s and 1970s (1). From these observations, Barker then hypothesized that malnutrition in pregnancy, low birth weight and early life exposures such as undernutrition caused changes in the body that later in life lead to coronary heart disease in adulthood (1, 13). The hypothesis has developed and is now often referred to as DOHaD theory (3). Although cardiovascular disease from early stages dominated the focus of DOHaD research, the field now includes not only epidemiological studies, but clinical and experimental research as well (3). The relationship between other intrauterine and early life factors such as stress, anxiety and depression emerged in epidemiological and clinical studies of the hypothesis (3, 4, 14). These factors are now a part of a number of suggested mechanisms that may form the epigenetic process involved in non-communicable disease development in life (15-17).

2.2 STRESS

As each person is unique, interpretation of stress in life is complex and individual. H. Selye first described stress and stress-related factors, "stressors", as the demands that causes a stress reaction (18). While stress is a normal part of life and can be beneficial, too much stress can cause physical, emotional or psychological strain (19). Previous experiences, development and situation will also determine how an individual perceives stress (20). In social and behavioral science this type of stress is referred to as *psychological stress* (20). This kind of stress occurs when the recourses, our coping factors, cannot face the demands, i.e. the stressors (21, 22). It embraces the different mental and physical conditions believed to be involved in the appraisal of stress (23). Other terms such as "stress", "perceived stress", "hassles" or "psychological stress", are also well-used in medical research.

In epidemiological research and large surveys, scales are often used to measure stress, since they are an accessible, easy and cheap methods of measuring stress (24). This thesis will focus on psychological stress, and in particular perceived stress, measured with a stress scale questionnaire.

2.2.1 Perceived stress

Maternal perceived stress during pregnancy increases the risk of maternal anxiety (25) and post-partum depression (26). Factors that can cause stress in women during pregnancy differ depending on for example, geographic location, ethnic- and socioeconomic status (27-29). The perceived stress scale (PSS) was developed by Cohen, Karmarck and Mermelsteins (30) in the 1980s and has since been well-used in both clinical settings and research to capture psychological stress. It is suitable for community samples and self-administrated samples (30).

Perceived stress measures the degree to which a person felt the past month of their life has been "unpredictable, uncontrollable and overloaded" (30). The questions include stress factors and coping factors (30).

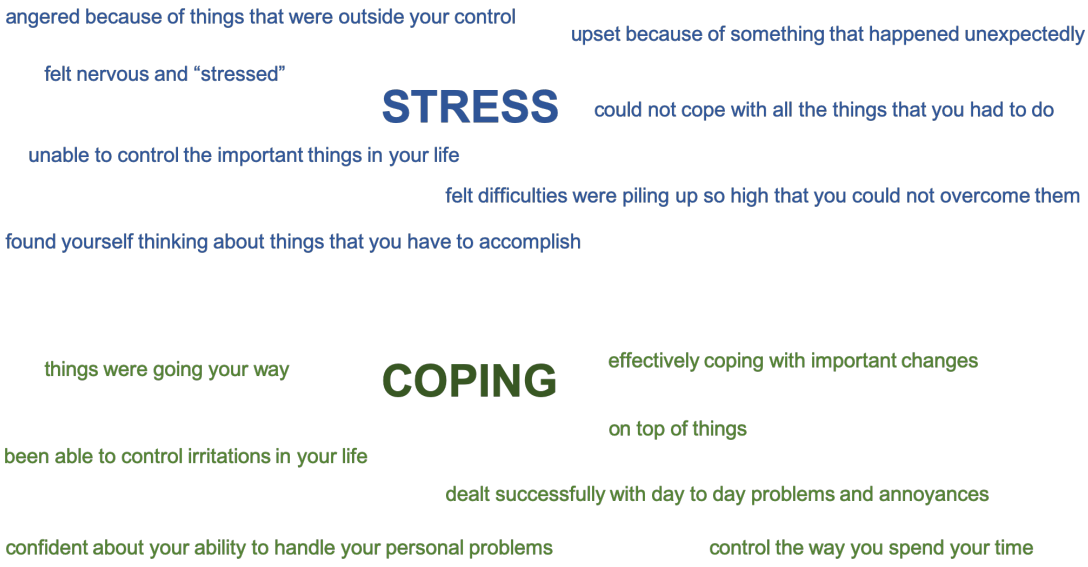

Figure 1. Stress and coping questions, in short from the perceived stress scale 14-item (30). Blue color – Stress. Green color – Coping.

In the Nordic countries there is no screening tool widely used for measuring stress in pregnancy (31, 32). Screening for early risk of postpartum depression, questions in maternity care and early pregnancy care include items such as "fear of birth", "domestic violence", "previous mental health", "lifestyle", and "work situation" (31, 32). Knowledge about stress in Nordic pregnant women and factors associated with it could benefit the women, their fetuses and individualize pregnancy healthcare.

Knowledge gap

Stress is individual and can be difficult to generalize. Measuring stress in pregnancy with a perceived stress scale could increase the knowledge of possible stressors in Nordic pregnant women and estimate the prevalence of stress in pregnancy. This knowledge could help to further understand if maternal perceived stress increases the risk of allergic disease or other illnesses in the offspring.

2.2.2 Cortisol

Cortisol, a glucocorticoid hormone secreted by the adrenal glands, is essential for several body functions including metabolism, blood pressure, stress and sleep cycle. In stress response, the physiological systems activated and involved in cortisol secretion are the nervous system and the endocrine system (33, 34). In the nervous system, the sympathetic

adrenomedullary (SAM) systems release catecholamines, noradrenaline and adrenaline (33), increasing the heart rate, blood volume, and blood pressure, signalling to the liver for an increased need for glucose in the bloodstream. In addition, the endocrine system activates the hypothalamic-pituitary adrenal (HPA) axis, that releases cortisol (34). A feedback mechanism continues to release cortisol until the (stress) situation is resolved (34). When cortisol decreases, the SAM system is downregulated with consequently normalized cortisol levels. Since cortisol is secreted in stressful situations, the hormone is a commonly used biomarker for stress.

The cortisol awakening response (CAR) displays the changes in cortisol after awakening. The cortisol levels increase on waking and rise throughout the following 30-40 minutes, then decrease throughout the day and night (35). Cortisol can be measured in blood, urine, saliva and hair. The correlation between cortisol in saliva and blood is reliable (36), making either of them suitable for sampling cortisol over a period of time period for example a day or week. The measurement of blood cortisol levels requires blood samples to be collected by venepuncture which can cause further physical pain and psychological stress to the patient. Saliva cortisol collection is on the other hand simple and painless, making it ideal for home sampling and repeated sampling of cortisol levels over the course of the day. Saliva cortisol is stable in room temperature, and can be sent from home to the testing clinic or laboratory using the normal postal service (37, 38).

2.2.2.1 Cortisol in pregnancy

Pregnancy affects the secretion of many hormones in the body. The HPA axis changes during pregnancy and post-partum period. Cortisol levels in pregnant women are three times higher than in non-pregnant women (39). Glucocorticoids in utero is one of the suggested mechanisms in the development of disease later in life (3). To protect the fetus from high levels of circulating maternal cortisol, the levels of the enzyme 11β-hydroxysteroid dehydrogenase type 2 (HSD11B2) in the placenta increases and converts most of the cortisol into cortisone (40). Although the greater part is cortisone, there is still an amount of cortisol left that reaches the fetus (40, 41). The upregulation of HSD11B2 can be reduced in mothers with anxiety and therefore increase the fetal the exposure to cortisol (42, 43). Maternal psychological well-being in pregnancy have long been suggested to affect the fetus. In pregnant women with low-risk of stress and anxiety, no significant variation in salivary cortisol levels and maternal well-being and stress has been seen (44). However, the diurnal maternal cortisol pattern in the third trimester has been associated with maternal perceived stress in all trimesters in women with low income (45). However, the correlation between cortisol levels and PSS is not well established and has previously shown inconclusive results (45).

2.2.2.2 Cortisol in infancy

In the first months of life the CAR is not yet fully visible, although lower levels towards the evening compared to the morning have been seen in infancy (46). The maturation of CAR is

difficult to detect before 12 months of age (47). In mother infant-pairs, maternal cortisol levels have been related to infant cortisol levels up to 12 months infant age (48).

Stress in infancy measured with cortisol can be challenging to measure and interpret due to the variations in the growing infant's temper, sleep and awake cycle, as well as growth and the family demographics. Different ways to study psychological stress in infancy have been used; separation, strange situations, mother-infant interaction and environments. Physical stressors include painful situations such as vaccinations and blood sampling. Infants age 3- and 6 months of age have increased cortisol levels in an observation with indoor play situations where their mothers were insensitive (49), suggesting that infant behavioral regulation was insufficient in a new environment (49). Similar to this, dysregulated mother-infant interactions in women with low income predicted an elevated infant cortisol response and reactivity at 12 weeks age (50). A meta-analysis of cortisol reactivity in infancy showed that the cortisol response was low in response to psychological stressors (51). Similar results were reported in a review (52), however physical stressors increase cortisol levels during "stressful" events in infants before six months age (52).

Knowledge gap

Perceived stress in pregnant women is individual and can increase maternal cortisol. Cortisol can pass the placenta in pregnancy and affect the fetus. In the general population, the association between perceived stress from pre- and postpartum and salivary cortisol in the mother and infant is not well elucidated.

2.3 PAIN IN INFANCY

International Association for the Study of Pain (IASP) stated that pain is subjective and an individual experience (53). It is influenced by biological, psychological and social factors, regardless of pathophysiology, and ability to communicate, and applicable on all pain conditions (53). Assessing pain with scales in the infant population is difficult (54) since the assessment is based on multifactorial parental individual experiences. In non-verbal infants, crying is one way to estimate the pain and differ between infant distress or milder discomforts (55).

2.3.1 Colic

During the first months of life, infant symptoms such as excessive crying, discomfort, fussing and abdominal pain is often described and referred to as infant colic (56, 57). Infant colic appears within the first weeks of life and persists until about 4 months age and is characterized by periods of inconsolable infant crying that appears for no obvious reason (57). The pathophysiology and cause behind infant colic is not fully understood (58), neurodevelopmental factors (59), gut microbiota (60), gastrointestinal conditions such as

allergies (61) as well as maternal anxiety disorders (62) are all proposed to be involved in the development of colic. Perinatal risk-factors of colic are to date not well-described in larger cohorts. The maternal risk factors of infant colic previously suggested are nicotine use, maternal age, socioeconomic status, maternal anxiety and parity (62-64).

The prevalence of colic ranges 2-73% (65) depending on definition and population. The ROME IV the criteria of colic, was recently revised (57), removing the stricter "Wessels rule of three" criteria of fussing, irritability or infant crying, with the duration at least three hours a day, three times a week, for three weeks of more (66).

2.3.2 Abdominal pain

Abdominal pain in infants and children is common, and usually a benign symptom or condition in infancy. Symptoms from the gastrointestinal tract without a structural or biochemical reason are referred to as functional gastrointestinal disorders (FGIDs) (57). In the pediatric emergency department, approximately 9% seek care due to acute abdominal pain (67). Most common causes and diagnoses among infants are obstipation, colic, cow's milk allergy, gastroesophageal reflux and infections (67-69). The prevalence of FGIDs depend on the disease, but ranges from 0.2-26% in infants (70), and diagnoses include diseases such as regurgitation, colic and functional vomiting, diarrhea and constipation (70).

Knowledge gap

Previous studies mainly focused on parental stress at the time of colic and abdominal pain. Maternal pregnancy stress and risk factors and stress in association of infant colic and abdominal pain in infancy are not well studied. Furthermore, infant cortisol levels and its association to colic and abdominal pain could increase knowledge on infant pain.

2.4 ALLERGIC DISEASES

Allergic diseases are among the world's most common chronic diseases. The development of allergic diseases in infancy is usually referred to as the "atopic march" (5, 6). It often starts with atopic dermatitis (AD) and food allergy, and later followed by asthma and allergic rhinitis (6, 7). Disease severity may fluctuate in life and symptoms can subside (6). Comorbidity among allergic disease is common, it has been suggested that the risk of sensitization to common foods and allergy against those foods is higher in patients with AD (71). Wheeze and sensitization in early life is associated with persistent asthma later in life (72).

2.4.1 Asthma

The world prevalence of asthma is about 4.5% in adults, but prevalence varies widely from 0.2%-21% where high income countries display the highest prevalence of asthma (73).

Asthma can debut at any time throughout life, but often begins in childhood (74). The symptoms include coughing and wheezing due to an obstruction of the airway caused by inflammation. In small children, the involuntary contractions of the airway when exposed to triggers can occur due to respiratory infections (75-77). In older children, adolescents and adults, triggers include exercise or inhaled allergens such as pollen and from animals (78-80).

Asthma is one of the most common chronic diseases in pregnant women (81). Between 2000-2016, 11% of pregnant women reported having an asthma diagnosis (82). In pregnancy the course of asthma can fluctuate, where some women experience disease improvement whereas others report worsening of symptoms (83). Still, the dispense of prescribed asthma medication seems to decrease in women during pregnancy (84) even though it is considered safe (85). Asthma in pregnancy increases the risk of pregnancy related complications in the mother and the infant (86, 87) and a well-controlled asthma could decrease the risk of pregnancy complications and infant mortality (86, 87).

2.4.2 Atopic dermatitis

Atopic dermatitis is one of the first allergic diseases that present in childhood (5-7) and is common already in infancy and later in early childhood. Atopic dermatitis usually starts within the first year of life, but is common in every age in childhood (88, 89). The lifetime prevalence is about 10-20% (90, 91). The symptoms include dry, itchy and inflamed skin. Diagnosis is determined by ocular and clinical inspection of the skin, but criteria such as Hanifin and Rajka critera (92) and United Kingdom Working Party's diagnostic criteria (93) can be used.

Atopic dermatitis is the most common skin disorder in pregnancy affecting about 30 % of women, of which many perceive first-time symptoms of the disease (94, 95), and others experience increased symptoms in mid-pregnancy (96, 97). First hand treatment of atopic dermatitis in pregnancy is use of emollients, topical stereoids, and ultraviolet B treatment to reduce itching and symptoms (98). Although few studies have evaluated the risk of components in the skin-absorbed treatment, it has been evaluated in other populations and is considered to be safe (96).

Knowledge gap

In pregnancy, symptoms of allergic diseases often fluctuate and may worsen. The association between maternal perceived stress in pregnancy and symptoms of allergic disease in pregnancy is not well understood.

2.4.3 Allergic sensitization

Foreign substances in the body such as allergens, bacteria and viruses are detected and destroyed by immunoglobins in the immune system. The development of specific

immunoglobin E (IgE) is initiated after a specific allergen is first presented to the immune system (99). The IgE production is not fully elucidated but it is suggested that production does not start in the infant in-utero (99, 100), maternal IgE detected in cord blood can be found in the infant in the first months of life (99). Sensitization in infants at 3 months of age differ from maternal sensitization during pregnancy, where maternal sensitization was more common to inhalant allergens (101). The most common sensitization found in infants are to foods, mainly to milk and egg white (101). Sensitizations to food and inhalant allergens change as the child grows older, and depending on the child's age, the prevalence in infancy and early childhood is 1-16% (101-103).

Knowledge gap

Longitudinal data on maternal stress from pregnancy and onward will increase the knowledge of immune responses of allergic sensitization in infancy.

3 RESEARCH AIMS

The overall aim of this doctoral thesis was to evaluate maternal pre- and postpartum stress and the risk of abdominal pain and allergic disease in offspring.

In particular, the doctoral thesis consisted of the following specific aims and sub-studies:

1. To analyse the prevalence of women reporting high maternal stress in pregnancy and explore whether symptoms of maternal allergic disease were associated with high perceived maternal stress in late pregnancy (Study 1) (104).
2. To evaluate the prevalence and perinatal risk factors associated with parental-reported colic, abdominal pain and pain or other discomforts in infants up to 3 months of age (Study 2) (105).
3. To explore if maternal perceived stress in or after pregnancy and saliva cortisol levels in pregnancy were associated with saliva cortisol in early infancy (Study 3) (106).
4. To investigate if maternal perceived stress in pregnancy and post-partum were associated with allergic sensitization in offspring at 12 months age and assess if the pre-specific association was more pronounced in children beloning to a household with lower socioeconomic status (Study 4).

4 MATERIALS AND METHODS

4.1 STUDY DESIGN

The design of the four substudies included in this thesis were prospective observational studies, including data collected from women at mid-pregnancy to their infant at the age of 12 months All the studies were conducted in the Preventing Atopic Dermatitis and ALLergy in children (PreventADALL) birth cohort, described elsewhere (107).

4.1.1 The PreventADALL birth cohort

PreventADALL is a Nordic multicentre, prospective, 2x2 factorial, interventional birth-cohort study with two primary objectives; to investigate the effect of primary prevention of allergic diseases by early skin care and early complementary food introduction (107). Women were recruited in Norway and Sweden between 2014-2016 (Figure 2). At birth, infants were randomly assigned to one of four intervention groups *no intervention*, *skin care*, *early complementary feeding*, or *combined skin and food intervention*. The skin care group started at two weeks infant age with oil baths and a facial cream provided by the study. The food intervention started at the three-month clinical visit. Foods were introduced weekly starting with peanut, followed by cow's milk, wheat and egg. The overall aims of PreventADALL study were to determine if allergic diseases may be prevented by skin and/or food interventions in infancy, and to identify factors early in life involved in non-communicable disease development (107). The results of the interventions showed that skin emollient and early complementary feeding did not reduce atopic dermatitis by 12 months of age (108) but a risk reduction in food allergy was seen at 3 years of age in infants fed complementary foods (109).

In total, 2697 women were included; 11 of these were twin pregnancies. Women were recruited in relation to the 18-week routine ultra sound in Oslo at Oslo University Hospital, Fredrikstad at Østfold Hospital Trust (Norway) and Stockholm at Karolinska University Hospital, Ultragyn AB, Ultraljudsbarnmorskorna, Solna barnmorskemottagning, Danderyds Sjukhus, BB-Sophia MamaMia, Södersjukhuset, Södertälje sjukhus and BB-Stockholm (Stockholm) (107) from December 2014 until October 2016. Exclusion criteria in pregnancy were planning to move away from the study site within the first year of infant life, >2 fetus pregnancy and insufficient language skills (Norwegian or Swedish).

In April 2017 the last infant was born, rendering in 2397 mother/child pairs that choosing to participate in the study. Exclusion criteria at birth were severe infant disease and infant born <35 weeks of pregnancy. Three families choose to leave the study within the first year of their infants' life, leaving 2394 mother child-pairs at 12 months infant age (Figure. 3).

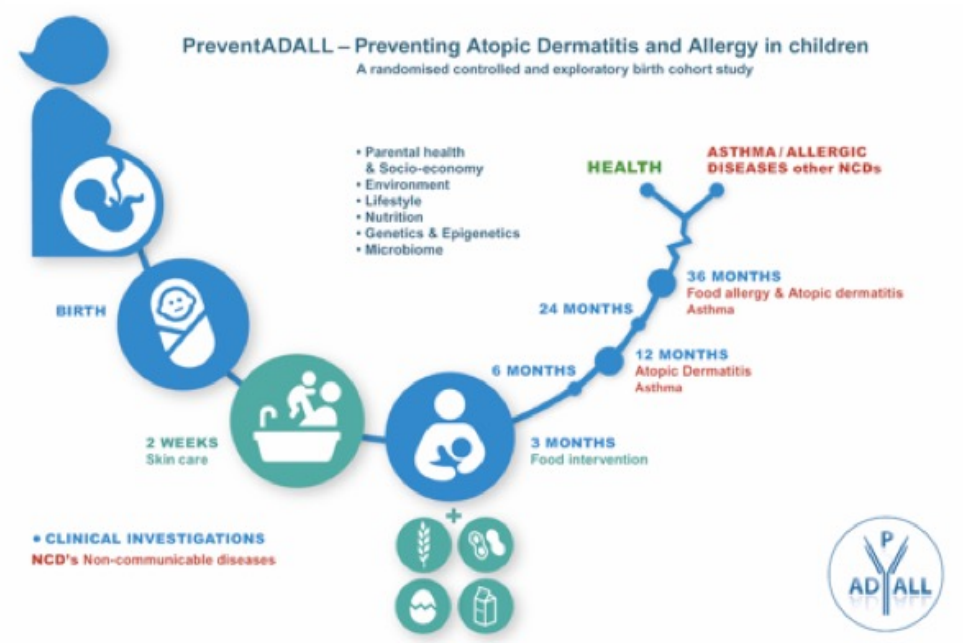

Figure 2. The PreventADALL cohort flowchart of randomization groups, clinical investigations, questionnaires and outcomes from pregnancy until 36 months of life. This thesis include data collected up to 12 months of age.

4.2 STUDY POPULATIONS

The mother-child pairs included in this thesis are presented in Figure 3. In study 1, the study population consisted of the 2164 women with PSS from both of the questionnaires in mid- and late pregnancy (104).

Study 2 included 1852 mother-child pairs from the mother child-cohort included at birth that answered the electronic questionnaire at 3 months infant age (105).

For Study 3, the infants (families) that agreed to sample infant saliva and had successful saliva cortisol (SC) measurement from the 3-month clinical visit were included (n = 1057) (106).

At the 12 months clinical visit, 1909 infants and parents attended the clinical visit. The 1757 infants that took part in the skin-prick test were eligible for inclusion in Study 4.

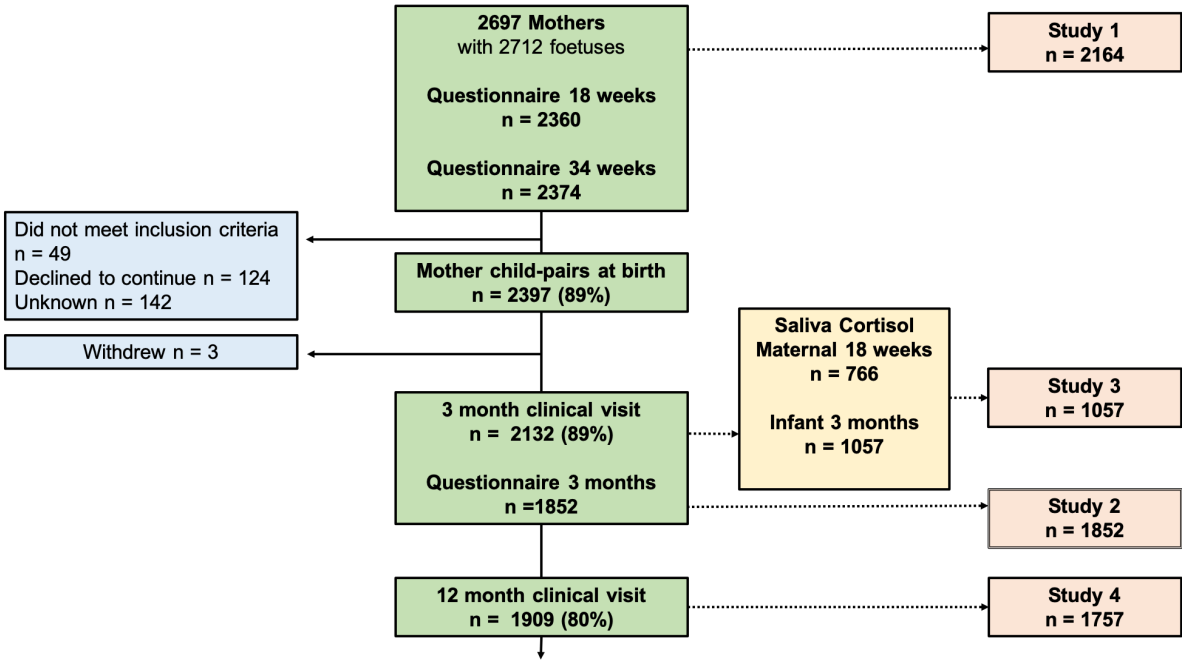

Figure 3. Flowchart of study population in the 4 studies included in this thesis.

4.3 DATA COLLECTION

4.3.1 Clinical examinations

The clinical visit in pregnancy took place between 16-22 weeks of gestation. It consisted of a short interview about the mother's health and pregnancy so far. The visit included anthropometrics, biological sampling and a home kit for biological sampling.

Within the first 24 hours after birth, study personnel visited the family at the maternity ward. The baby was included and randomized to one out of four PreventADALL intervention groups and the parents were given instructions corresponding to the intervention. Birth data and biological sampling from birth were collected from both the baby and mother. Participating midwifes collected biological sampling from the delivery room. A semi-permeable filter paper was given to the parents to collect blood in connection to the infant routine metabolic screening at 48 hours age if possible.

Three clinical visits to study center in Norway or Sweden were conducted within the first year of infant life. The first occurred at 3 months of age and included early complementary feeding (peanut) introduction. At 3, 6 and 12 months of age infants were followed according to study protocol (107) visits included infant anthropometrics, skin swabs, transepidermal water loss, physician and skin examination, lung function test (3 and 12 months), skin prick test (6 and 12 months), blood sampling (3 and 12 months), urine sampling (3, 6 and 12 months) and home kit for stool- and saliva sample. The last clinical 12-month visit was conducted in the fall of 2018.

4.3.1.1 Saliva sampling and cortisol

The inclusion of the mothers and the 3-month clinical visit for the infants included a saliva sampling using home kits. A pre-paid envelope, sampling instructions, sampling form, zip-locked plastic bag and saliva sampling set were distributed prior to testing. Mothers were instructed to sample the saliva in the morning, shortly after their own or infant's awakening, preferably after 6 AM. Thereafter the sample and sampling form were sent to the study center as soon as possible, or stored in fridge until posting. At arrival at study center, the time and date was registered and the sample was frozen until analyzed. Cortisol quantification was made at Forskningscentrum, Södersjukhuset, Stockholm with RadioImmunoAssay (RIA) (110) CORT-CT2 kits according to manufacturer's instructions. From the mothers in pregnancy 766/2697 (28.4%) had a successfully analyzed test. The infant samples resulted in 1057/2131 (49.6%) successful tests. In total 531/2394 (22.2%) mother/infant-pairs had a successfully analyzed test (Figure 3.).

4.3.1.2 Skin-prick test

Skin-prick test (SPT) was offered for all infants that attended the 12 month clinical visit. Possible interferences of SPT were infants with severe skin disease (ongoing eczema, dermatographia or urticaria), or medication including antihistamines or leukotriene receptor antagonists. The tests were applied to the volar aspect of the infant forearm, 2-3 cm from the wrist and the antecubital fossae (111, 112). The skin was marked with a pen to properly identify each test result. The distance between two allergens were at least 2 cm (111, 112). Starting with the histamine, a drop of allergen solution was placed on the skin and a metal lancet pressed through the drop (90° angle) and into the epidermis and held for approximately 1 second (111, 112). For each allergen, a new lancet was used. The excess solution was removed with a clean tissue that was held against the skin. After 15 min the test results were evaluated starting with the histamine (111, 112). The outline of the wheal was marked with a pen, blotted onto cellophane tape and transferred to the case report form. A wheal of ≥ 3 mm exceeding the negative control was considered a positive SPT in this thesis (111, 112).

4.3.2 Electronic questionnaires

The first of two electronic questionnaires was sent shortly after maternal inclusion. The questions corresponded to the mothers socioeconomic- and demographic status, previous and current health including history of allergic diseases, and a stress questionnaire. The second questionnaire was sent around pregnancy week 34. It included questions about the father, changes in the mothers socioeconomic and demographics, and changes in the mother's health including symptoms of allergic disease and maternal stress and had been used in similar population-based studies (113).

A short weekly diary was sent to the mothers by e-mail starting two weeks after birth. The questions corresponded to infant randomization group and compliance to the intervention. An electronic questionnaire similar to the ones in pregnancy was sent quarterly at 3, 6, 9, and 12 months postpartum. The questions corresponded to the last 3 months and included; infant

health, symptoms of allergic disease, infant diet, family socioeconomic and demographic questions, and maternal stress.

4.3.2.1 Maternal perceived stress

Perceived stress scale (PSS)(30) with 14 items were a part of the electronic questionnaires. Answers are rated on a 5-point Likert scale (0=never, 1=almost never, 2=once in a while, 3=often, 4=very often). Questions 4-7, 9, 10 and 13 were coded in reverse. Perceived stress scale is not a diagnostic instrument, therefore no predefined cut off score for stress exist, but higher scores increases the likelihood of stress (30). Validity and reliability were first assessed by the authors (30, 114). PSS has been used worldwide and translated into many different languages, including Swedish and Norwegian (115-117).

4.3.2.2 Infant pain, colic and abdominal pain

The 3-months questionnaire contained the question "*in the last 3 months, did the infant have any of the following*"? with the exclusive reply options "*Colic*", "*abdominal pain (not colic)*", "*pain or other discomforts you have consulted the health care services for*".

4.3.3 Exposures

Study 1 explored maternal factors in pregnancy, allergic diseases and symptoms of allergic disease as exposures. The use of analgesic medication, diagnosis of asthma, eczema, rhinitis, food allergy and anaphylaxis were self-reported by the mother in the electronic questionnaires. Symptoms of allergic diseases were restricted to women who had a self-reported doctor-diagnosed allergic disease.

Study 2 included self-reported perinatal covariates and potential exposures from pregnancy body mass index, living area, sick-leave, allergic disease, high PSS, and nicotine use of the mother. Information about the partner/father included age, allergic disease, education and nicotine use. In Study 2 the definition of allergic disease corresponded to any self-reported doctor-diagnosed asthma, atopic dermatitis, rhinitis, food allergy or anaphylaxis reported by the mother and defined by the PreventADALL study group (107).

The PSS outcome in this study was low or high PSS. One SD above mean from the 18 weeks total score was defined as high PSS (equal to >29).

For **Study 3** PSS at pregnancy week 18- and 34-week GA age as well as 3 months postpartum, continuous and categorical, was used as exposure. Factors explored with the outcome from pregnancy and onward were: medically induced labor, birth mode, birth weight, breast feeding status, colic, abdominal pain, other pain and discomforts, nicotine exposure and PreventADALL skin intervention.

In **Study 4** maternal stress was defined by PSS scores at 18- and 34 weeks of pregnancy and at 3 months postpartum. PSS scores were used as continuous variable and in percentiles (<25th, 25th-49th, 50th-75th and >75th) with the $<25^{th}$ percentile was used as reference. In

stratification for socioeconomic status (SES), low SES was defined as yearly family income less than 600.000 NOK/SEK at 18 weeks pregnancy. Sensitivity analysis: Positive allergic sensitization (AS) defined by at least 2 mm wheal size to any of the tested allergens.

4.3.4 Outcomes

The outcome for **Study 1** was maternal perceived stress by pregnancy week 18 and 34. PSS from both time-points used as a continuous variable, the highest possible score was 56. The categorical variable; low or high PSS, corresponded to 1 SD above mean PSS at 18 weeks (equal to or >29).

Outcome for **Study 2** were colic, abdominal pain or other pain or discomforts. It was based on the mothers self-reported answer from the 3-month electronic questionnaire corresponding to either, infant "*Colic*", infant "*Abdominal pain (not colic)*", or "*Pain or other discomforts you have consulted the health care service for*". The last option was rephrased into "pain and other discomforts".

The main outcome for **Study 3** was infant salivary cortisol levels at three months of age given as nmol/L. Quartiles of infant saliva cortisol (nmol/L) were used as the main outcome when exploring early life factors. The groups were divided as follows <25th percentile (reference); *≤5.50 nmol/L (n = 266)*, 25th-50th percentile; *5.51–15.35 nmol/L (n = 263)*, 50th-75th percentile:*15.36–24.93 nmol/L (n = 265)* and >75th percentile: *≥24.94 nmol/L (n = 263).*

In **Study 4** positive allergic sensitization with a SPT wheal size equal to or larger than 3 mm, was used as outcome.

4.4 STATISTICAL ANALYSIS

The statistical analysis in Study 1-4 were all preformed in Statistical Package for the Social Sciences (SPSS) version 25 or later (IBM, Chicago IL, USA). In Study 1-4 continuous variables were presented as mean and SD and categorical variables as number and percentages (%). Independent t-tests and Chi-square tests were used to describe differences in continuous and categorical variables between populations, respectively, all presented with p-values. In addition, study 2-4 used both the latter descriptives as well as median and inter quartile range to present continuous variables. Non-parametric tests and Mann-Whitney U-tests were used on continuous variables not normally distributed. Significance level were set at the <0.05 for all studies in this thesis.

4.4.1 Study 1

Univariate regression analysis was presented with odds ratio with 95% confidence intervals (CI). Statistically significant self-reported factors from pregnancy from univariate analysis were included in Models I and II in the multivariate regression. Model I was adjusted for maternal self-reported factors at inclusion (lower income and previous pregnancies), and Model II for self-reported factors in the late pregnancy (moving to new residence, changes in employment and use of analgesics). The multivariate binary regressions were used to

compare the Odds Ratio (OR) of high stress in mothers with symptoms of allergic disease and the reference. Linear regression analyses were used to explore if associations identified in binary regression analyses were similar in univariate and multivariate linear regression. The linear regressions were presented with unstandardized β coefficient with 95% confidence intervals (95 % CI) and p-values.

4.4.2 Study 2

To describe the prevalence of colic, abdominal pain and pain and other discomforts descriptive statistics; frequency, number and percent were used for all three outcomes separately and together. Logistic regression was conducted to explore the relationship between the exposure and outcome variables. Univariate and multivariate logistic regression was presented with OR, 95% confidence intervals (CI) and p-values. The variables with p-values <0.1 from univariate regression were included in an adjusted regression model. Covariate were numbers of pregnancies, maternal marital status and maternal PSS at 34 weeks, obtained from the univariate analysis.

4.4.3 Study 3

Saliva cortisol levels were presented with median, and interquartile range (IQR). Spearman's non-parametric correlations describe the correlations between infant SC levels and maternal PSS in pregnancy and postpartum and the maternal SC levels in Study 3. Data was presented with Spearman's coefficient rho (r) and p-value. Multinomial logistic regression was used to explore the association between early life factors and iSC levels. The reference category was <5.50 nmol/L (n = 266). Data was presented with OR, 95% CI and p-values.

4.4.4 Study 4

Maternal PSS were presented with median and IQR. The differences in maternal PSS in pregnancy and post-partum of sensitized and non-sensitized infants at 12 months, stratified for SES, was conducted with Mann-Whitney U-test. Univariate and multiple logistic regression were used to explore the association of maternal PSS and allergic sensitization. Data is presented as OR with 95% CI and p-value.

Study	I	II	III	IV
Design	Observational study	Observational study	Observational study	Observational study
Population	Pregnant mothers n = 2164	Mother/child-pairs n = 1852	Mother/child-pairs n = 1057	Mother/child-pairs n = 1757
Data source	Questionnaire 18- and 34 weeks. Inclusion visit mother 18 weeks.	Questionnaire 18- and 34 weeks, and 3 months. Inclusion visit mother 18 weeks and infant at birth.	Questionnaire 18- and 34 weeks, and 3 months. Inclusion visit mother 18 weeks and infant at birth. Clinical visit 3 months. Home sampling.	Questionnaire 18- and 34 weeks, and 3 months Inclusion visit mother 18 weeks and infant at birth. Clinical visit 12 months.
Data analysis	Descriptive Logistic and linear regression	Descriptive Logistic regression	Descriptive Multinomial logistic regression	Descriptive Logistic and linear regression

Figure 4. Summary of included studies.

4.5 ETHICAL CONSIDERATIONS

Research in pregnancy and infants should be done with careful ethical consideration. The infant cannot defend itself and parental consent on infant participation in a cohort such as PreventADALL is mandatory. Prior to the clinical visits included in this thesis, families were informed about the expenditure of time calculated for the visit, and the included procedures. According to the Declaration of Helsinki (118) participants involved in medical research have the right to self-determination. This can be challenging when young children and infants are involved in research. Every infant was assessed as an individual at each visit. If the infant showed signs of unease, agitation or was scared, tired or hungry the procedures were upheld or stopped. The parents were encouraged to call the study centers with questions or concerns about the study and the procedures included. The phoneline was operated daily until all infants had turned 12 months. All families were welcome to book a visit with the study personnel if they had a suspicion the infant might be atopic or reacting to any of the interventions included in the study.

The ethics application for PreventADALL and informed consent was written in line with the Declaration of Helsinki – ethical principles for medical research including human subjects (118). Ethical approval was granted by the Regional Committee for Medical and Health

Research Ethics in Norway, 8 December 2014 (2014/518) and in Sweden by the Swedish Ethical Review Authority, 25 March 2015 (former Regional Ethics committee in Stockholm) (2014/2242-31/4). Overview of ethical permissions and amendments are presented in Table 5.

Study	Ethical approvals	Amendment
I	**Norway:** *2014/518* **Sweden:** *2014/2242-31/4*	Requirements of the General Data Protection Regulations law, 2015; *2015/738-32.* Collaborating ultrasound-, midwifery-, and maternity wards, 2015-2016; Ultragyn AB, Ultraljudsbarnmorskorna och Solna barnmorskemottagning, *2015/1244-32.* Danderyds Sjukhus; *2015/1387-32.* BB-Sophia (including changes in recruitment strategy); *2016/412-32.* MamaMia; *2016/617-32.* Södersjukhuset; 2016/802-32. Södertälje sjukhus and BB-Stockholm; *2016/1703-31.* The Swedish Pregnancy Register, 2018; 2018/2492-32.
II	**Norway:** *2014/518* **Sweden:** *2014/2242-31/4*	Recruitment of collaborators; oral provocations and food allergy diagnostics, 3 years, *2019-01386.*
III	**Norway:** *2014/518* **Sweden:** *2014/2242-31/4*	Follow-up questionnaires; 4-, 5-, 6 years; *2019-01386.*
IV	**Norway:** *2014/518* **Sweden:** *2014/2242-31/4*	Changes in principal investigators, 2020; *2020-02305.*

Table 5. Overview of ethical permissions and amendments.

The informed consent was written in both Norwegian and Swedish. Almost all mothers obtained the written informed consent prior to the visit and were encouraged to read it and talk to their partners about the study before enrollment. The consent was signed twice; first at 18 weeks pregnancy by the mother prior to data sampling at the inclusion visit. Secondly, at inclusion of the baby at birth, both parents had to read and sign the informed consent. In line

with PreventADALL steering group's recommendations the PhD student attended a 2-day course in Good Clinical Practice (GCP) at Karolinska Trial Alliance in April 2016.

All electronic data submitted by study personnel and electronic questionnaires and diaries submitted by the mothers were directly obtained and stored at University of Oslo, Services for Sensitive data (TSD). Its security met the Norwegian and Swedish requirements of the General Data Protection Regulations law (GDPR) that was adopted in April 2016.

5 RESULTS

5.1 MAIN FINDINGS

5.1.1 Maternal stress and allergic diseases

5.1.1.1 Prevalence of high stress

The mean PSS score in pregnancy was 21.1 (SD 7.2) at 18 weeks gestational age (GA) and 20.3 (SD 7.2) at 34 weeks GA. The prevalence of high stress in pregnancy in PreventADALL was 15% at 18 weeks GA and 13% by 34 weeks GA. High stress at both time points in pregnancy was reported by 7% (145/2164) of the women.

5.1.1.2 Associated factors

In univariate analysis, low family income was associated with high stress at both 18- and 34-weeks pregnancy from univariate analysis (Figure 6.). Doctor's diagnosis of allergic rhinitis ever, use of analgesics, the use of inhaled corticosteroids (ICS), moving to a new residence, changes in employment, low education and unemployment were associated with high stress at 34 weeks only.

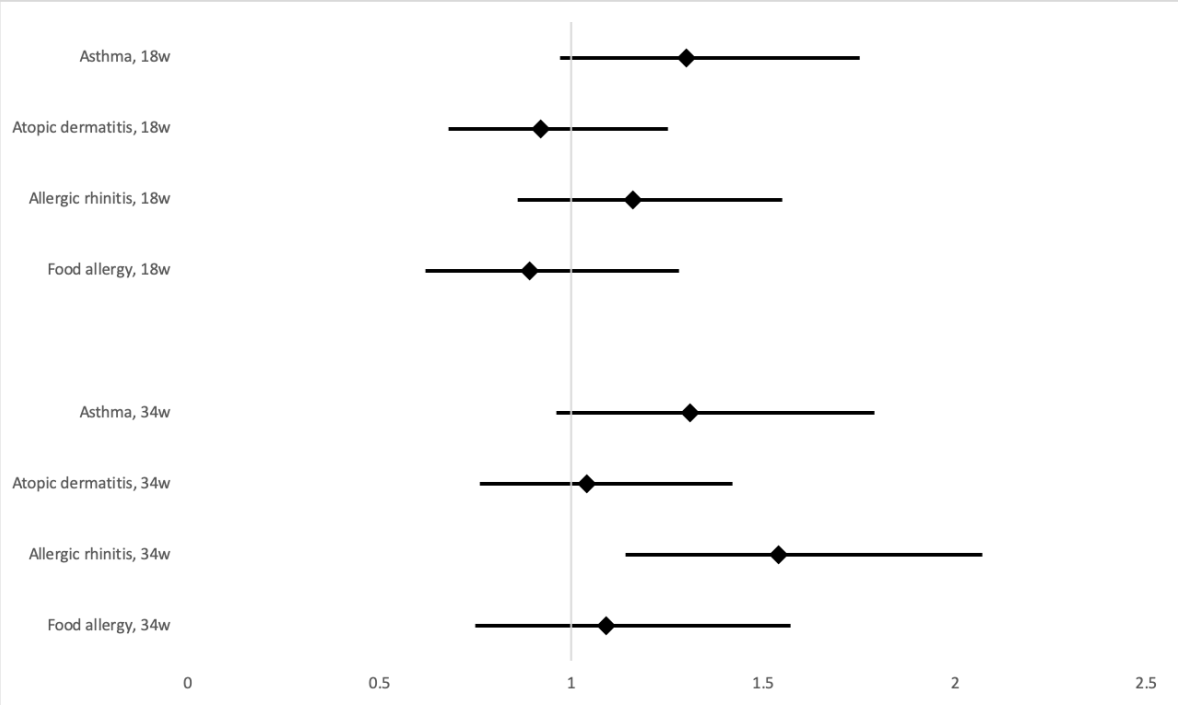

Figure 6. Univariate logistic regression of self-reported maternal allergic diseases, ever, at 18- and 34 weeks and high perceived stress (reference, low stress).

5.1.1.3 Allergic diseases

At the time of inclusion doctors-diagnosed; asthma was reported by 17% (375/2164), atopic dermatitis by 20% (426/2164), rhinitis by 23 % (440/2164) and food allergy by 14 % (281/2164) of the women. Rhinitis was the only self-reported doctors-diagnosed allergic

disease statistically significant in the high stress group in univariate logistic regression at 34 weeks of pregnancy (OR. 1.54 95 % CI 1.14-2.07).

High stress was significantly associated with self-reported symptom of allergic diseases asthma and food allergy in adjusted models: I and II, and allergic rhinitis in adjusted models: I (Figure. 7).

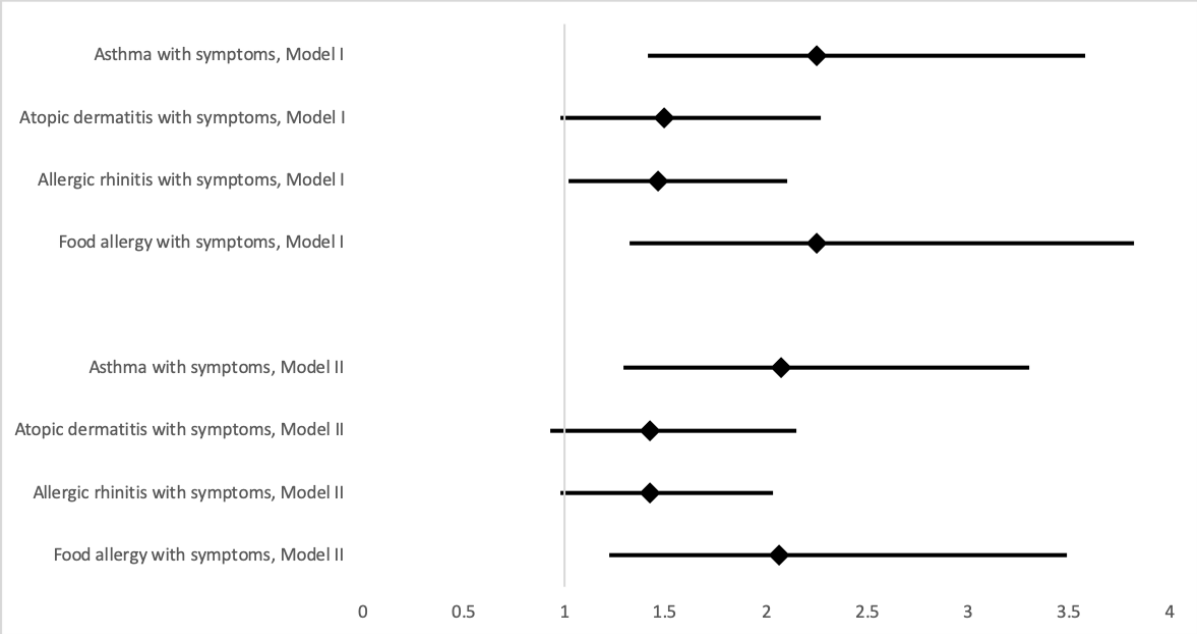

Figure 7. Adjusted logistic regression of allergic disease with symptom and maternal high perceived stress in late pregnancy (reference: low stress).

The adjusted linear regression showed similar results as the logistic regression. Increased PSS scores at 34 weeks of pregnancy were associated with all allergic symptoms accept rhinitis (Figure 8).

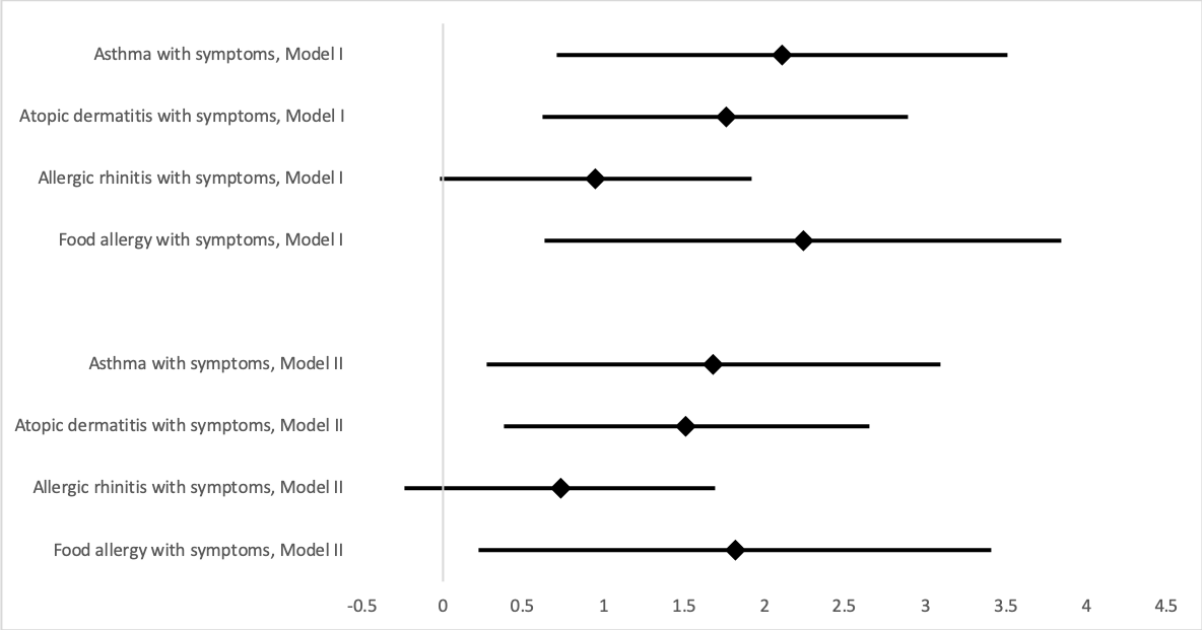

Figure 8. Adjusted linear regression of allergic disease with symptom and maternal perceived stress in late pregnancy.

5.1.2 Maternal stress and infant saliva cortisol

5.1.2.1 Maternal PSS and maternal saliva cortisol

Perceived stress was reported by 95% (1002/1057) of the mothers at 18 weeks pregnancy and 96% (1016/1057) at 34 weeks pregnancy and 88% (933/1057) 3 months post-partum. The median maternal perceived stress score was 21.00 (IQR 9.00) at 18 weeks; 20.00 (IQR 10.00) by 34 weeks, and 18.00 (IQR 10.00) at 3 months postpartum.

Of the 1057 infants included in Study 3, 531 (50.8 %) of their mothers had SC-level available from 18 weeks pregnancy. The maternal median SC levels were 29.60 nmol/L. There was no correlation between maternal perceived stress at any time point and maternal SC levels at 18 weeks pregnancy (maternal morning samples: 18w, r = -0.08, 34w, r = -0.04, 3m r = -0.01).

5.1.2.2 Infant saliva cortisol and maternal stress

The median SC level among the 1057 infants was 15.30 nmol/L (IQR 15.35) at 3 months of age. Infants with morning samples (05:00-10:59) had median SC levels of 14.00 nmol/L (IQR 13.40) (n = 551 52.1 %), while infants with other sampling time (11:00-04:59) had a median SC levels of 13.20 nmol/L (IQR 16.1) (n = 79, 7.47%). The remaining infants had missing sampling time, with median SC levels of 17.70 nmol/L (IQR 18.30) (n=427, 40.4%)

The infant's saliva cortisol levels were not significantly associated with maternal perceived stress at any time point in pregnancy or by three months postpartum (all infant samples: 18w r = 0.00, 34w r = -0.01, 3m r = -0.04).

5.1.3 Perinatal factors and infant abdominal- and pain outcomes

5.1.3.1 Prevalence of colic, abdominal pain and other pain and discomforts

Abdominal pain was the most common symptom among the three (22.4 %), followed by other pain or discomfort reported in 6.4% and infant colic in 3.2%. In total, the symptoms were reported in 26% of the 1852 included infants at 3 months age.

5.1.3.2 Maternal stress and perinatal factors

High maternal stress was reported by 14.4 % (n = 184) of the mothers to infants with neither of the 3 pain conditions at pregnancy week 18, and 11.9 % (n=157) at 34 weeks of pregnancy. In all pain groups, high maternal PSS by 34 weeks was more frequent than in the non-pain group. Univariate analysis showed that mothers with self-reported maternal allergic diseases and maternal sick leave in pregnancy had higher odds of reporting all three pain outcomes in their infant independently. Birth method with vacuum extractor and forceps increased the odds of reported abdominal pain and other pain and discomforts in the infant. Mothers that were cohabitant with their partners were more likely to report infant colic. The odds of mothers reporting infant abdominal pain was lower in the Swedish population than the Norwegian population.

Maternal stress and cohabitant mothers were not associated with the any pain outcomes in multiple regression. Maternal allergic diseases and sick leave were still statistically significant in all three pain groups in adjusted analysis.

5.1.4 Maternal stress and infant allergic sensitization

5.1.4.1 Study population and maternal PSS

The median maternal PSS scores by pregnancy week 18 and 34 and at 3-month post-partum were similar between infants with positive and negative allergic sensitization. The mothers of the included infants had median PSS score of 21.00 (IQR, 10.00) at 18 weeks, 20.00 (IQR, 10.00) at 34 weeks, and 18.00 (IQR, 9.00) 3 months post-partum.

5.1.4.2 Allergic sensitization

A positive SPT was found in 139 of 1757 (7.9%) infants. Allergic sensitization to egg white (n = 75), peanut (n = 52) and cow's milk (n = 20) were most common. A total number of 36 infants were polysensitized. Any sensitization was associated with being the family's first-born child and randomized to non-food intervention group in PreventADALL.

In the univariate and adjusted logistic regression there was no association between maternal pregnancy nor post-partum PSS scores and allergic sensitization at 12 months of age.

5.1.4.3 Allergic sensitization and socioeconomic status

Mann-Whitney U-tests showed that maternal median PSS scores at 18 weeks and 34 weeks were significantly higher in infants belonging to a family with low socioeconomic status

compared to reference (infants with high socioeconomic status and negative AS), p-values <0.04. (Figure 9.)

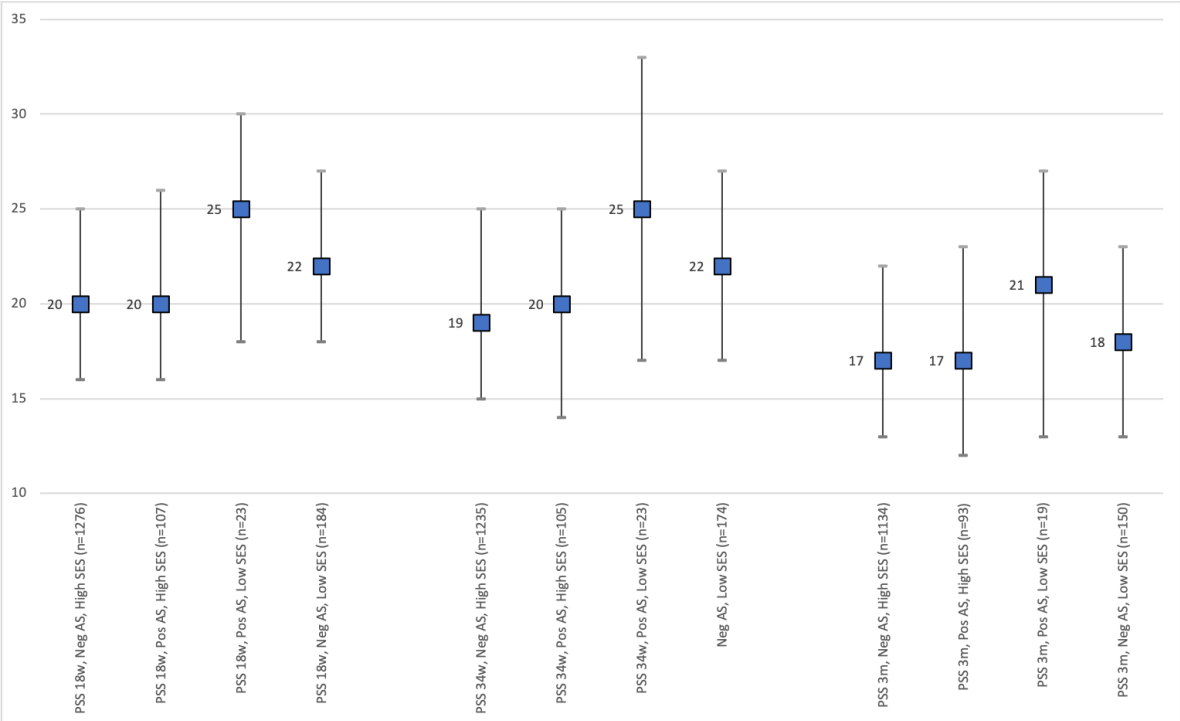

Figure 9. Median maternal perceived stress (PSS) (blue square) at 18- and 34-weeks of pregnancy and 3 months postpartum and interquartile range (error lines) in groups of infant allergic sensitization (AS) stratified for socioeconomic status; high or low.

However, when maternal perceived stress scores were stratified for socioeconomic status this did not increase the risk of AS in the infants in pregnancy or postpartum (Figure 10.).

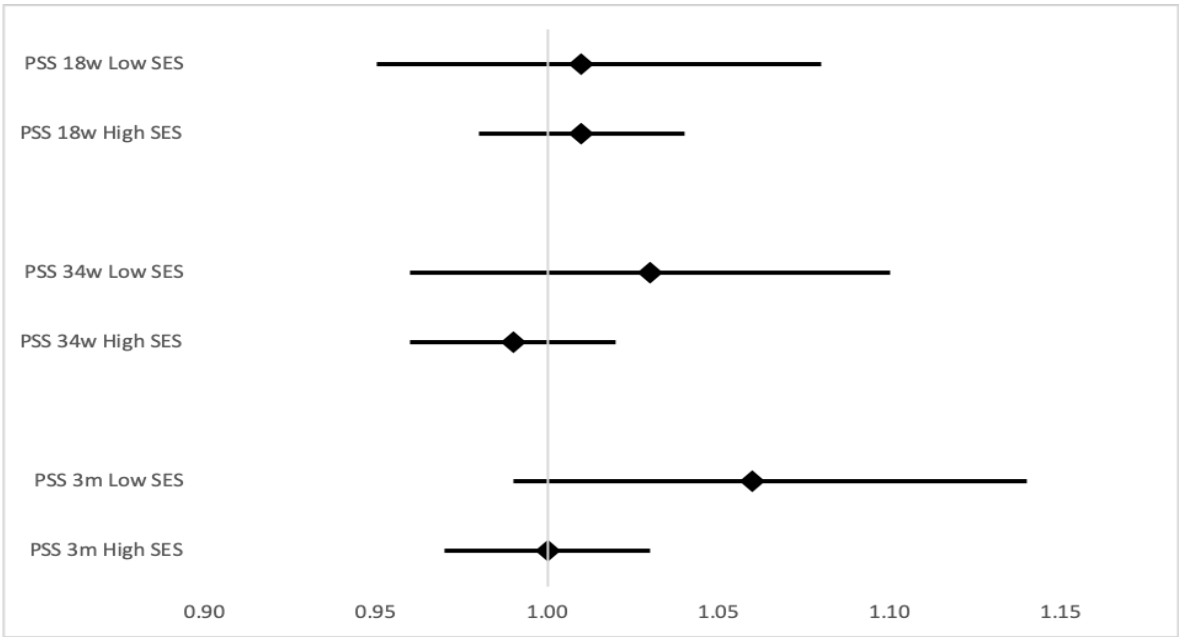

Figure 10. Forest plots showing logistic regression analysis of maternal perceived stress, stratified by socioeconomic status, and risk of allergic sensitization in offspring at 12 months age.

6 DISCUSSION

6.1 MAIN FINDINGS

The overall aim of this thesis was to evaluate maternal pre- and postpartum stress and the risk of abdominal pain and allergic disease in offspring. The main findings of this thesis showed that high perceived stress in pregnancy were prevalent in 15% (n=328) at 18 weeks and 13 % (n=287) in late pregnancy and associated with maternal symptoms of allergic disease. However, maternal saliva cortisol levels were not associated with maternal perceived stress in pregnancy neither at three months post-partum, nor with their infant saliva cortisol levels at three months of age. No associations were found between maternal perceived stress in pregnancy or infant salivary cortisol levels at 3 months of age, and infant colic/abdominal pain at three months of age. Maternal perceived stress was not associated with allergic sensitization in their infant at 12-months of age. Although maternal perceived stress was associated with lower socioeconomic status, no association was found between maternal perceived stress and allergic sensitization in stratified analysis for socioeconomic status.

6.1.1 Maternal perceived stress and allergic diseases in pregnancy

In Study 1, high stress was reported by 15% by 18 weeks and 13% by 34 week. In a Canadian population where low, moderate and high stress was measured with PSS 4 items, 17% reported high PSS (119). The women with high stress were younger, had lower education level and family income and were not living with a partner (119). Biresaw et al. (120) measured stress in pregnant women in Ethiopia during the Covid-19 pandemic, where the overall prevalence was 13.7%. The women with increased odds of high PSS were students, in the second or third trimester, or had a prenatal depression (120). In a Saudi Arabian study with pregnant women, factors that increased stress in pregnancy were not brushing teeth, chronic disease, diabetes in pregnancy and sleep deprivation, among 33 % of the women reporting high stress in their study (28). Both the Saudi Arabian study and the Ethiopian study used PSS with 10 items and a similar cutoff score (28, 120). In this thesis, associated factors with high stress in late pregnancy (34 w) in Study 1 were socioeconomic factors, low education, low income, unemployment, previously pregnant, changes in employment, moving to new residence, and medical factors, inhaled corticosteroids, allergic rhinitis, and the use of analgesics. Socioeconomic factors seem to be a common factor among women with increased stress in pregnancy. Comparing and interpreting perceived stress between groups of pregnant women should be done with caution. Stress is individual and no in-dept knowledge about the cause of stress is known. To collect individual factors of stress in pregnancy, future studies could include qualitative data to further explore maternal stressors and coping factors in pregnancy.

In this thesis, women with self-reported symptoms of asthma, atopic dermatitis and food allergy experienced an increased perceived stress. Previous studies reporting increased stress and symptoms of allergic diseases in pregnancy are lacking. These findings are, to the best of my knowledge, novel. Allergic diseases can improve or worsen in pregnancy. In Kircher et

al. 34% of women with asthma in pregnancy reported an improvement, while 36% reported a worsened asthma (121). Atopic dermatitis in pregnancy showed a worsening of symptoms in 50% of women usually occurring before 20 weeks of GA (97). Changes in medication and poor adherence to medication during pregnancy occur (84) even though adherence and compliance is considered important and medications are considered safe (122, 123). In asthma, the worsening of symptoms can be caused by various reasons (121, 124, 125). In the United States, smoking and obesity was associated with poor asthma control in pregnancy (125). Pregnant women with food allergy have reported reduced quality of life (126) and individualized diets can be hard to provide (127, 128). The possible reason for worsening of symptoms was not evaluated in the questionnaires for this thesis. Other chronic diseases and increased stress has previously been studied in the adult population. The findings that symptoms of allergic diseases increase stress in pregnancy are novel findings (Study 1).

6.1.2 Maternal stress and infant salivary cortisol

The results from Study 3 showed that perceived maternal stress by 18- and 34 weeks pregnancy and 3 months postpartum was not associated with maternal salivary cortisol levels in mid-pregnancy (week 18). In another Swedish study, perceived stress score and morning cortisol had an negative association (n=91) (129). In their study, women had similar socioeconomic characteristics as the women included in PreventADALL (129), but higher median PSS score. Similar results to the ones in Study 3 were seen in Voegtline et al (44) where maternal saliva cortisol levels were sampled at mid-day in low-risk pregnant women but other scales measuring several psychological factors were used. No association was found between cortisol levels and mood except for minor associations in pregnancy week 30-32 and anxiety and depression (44). Women in households with low income could still possibly still be at risk for increased cortisol. In the study by Scheyer et al. (45), mothers in low-income households with increased perceived stress and post-partum depressive symptoms showed increased saliva cortisol levels in pregnancy. In low-income Mexican women and their infants, the mother-infant interaction elevated the total infant cortisol response and reactivity in early infancy (50). In Study 3, the infants cortisol levels at 3 months of age showed no correlation to maternal perceived stress or maternal salivary cortisol levels during pregnancy. This may suggest that the level of perceived maternal stress during pregnancy in the general population is not sufficient enough to disrupt the HPA axis in neither the mother, nor the infant.

6.1.3 Perinatal factors and infant abdominal and pain outcomes

In the 3 month old infants, none of the abdominal and pain outcomes (colic, abdominal pain and other pain and discomforts) were associated with high maternal stress in pregnancy.

Phycological maternal stress in the third trimester has previously been associated with maternal reported of infant gastrointestinal illness in another large study with 3000 participants with American origin (130). In contrast to the mothers in the PreventADALL cohort the mothers in the previous study were younger, had lower education, and smoked to a

larger extent (130). In addition, a different scale for stress was used, capturing hassles (130). Colic and cow's milk intolerance in the first month of an infant's life was found to be associated with higher maternal stress in their study (130).

In Study 2, the only pregnancy risk factors for the abdominal outcomes (colic, abdominal pain and other pain and discomforts) were maternal sick leave in pregnancy and maternal allergic diseases in pregnancy. These factors could be interpreted as incipient stressors. In Study 1 symptoms of allergic disease were associated with high stress. We did not include symptoms in Study 2. Known socioeconomic stressors (income and education) did not associate with the outcomes. Sick leave in pregnancy could be a financial strain. A trend towards high maternal stress was seen for mothers in the groups reporting either colic or abdominal in their infants. However, in the adjusted analyses high maternal stress was not associated to colic or abdominal pain in the infants in Study 2.

Both psychosocial and psychological factors in pregnancy have previously been associated with infant colic (131). A smaller European study (n=272) by Takács et al. (132) on infant temperamental unpredictability showed that prenatal maternal stress was associated with the perceived social support in pregnancy. Even though no association was found between maternal stress and the abdominal pain outcomes in the infants in Study 2, the fussy, crying behavior in the infant associated with colic and abdominal pains could be perceived as a stressor. This suggests that a challenging pregnancy could perhaps promote maternal postpartum stress.

Perhaps the future in colic and abdominal pain is in the infant gut microbiome. In a Finnish study, 212 newborn infants were included and provided a sample of the first meconium (133). One year later, 19 infants had developed colic in early infancy and their first meconium differed from the controls, suggesting colic development could be related to gut microbiome and present already at birth (133).

6.1.4 Maternal stress and infant allergic sensitization

Perceived maternal stress was not associated with allergic sensitization in the infant or with allergic sensitization in infants with low socioeconomic status in Study 4. These findings are in line with several other studies. In a European study by Smejda et al. (134) with 370 mother-infant pairs, food allergy based on medical history in the one-year old children was not associated with maternal stress. In this study, additional stress scales were used but no association was detected (134). The urban environment and childhood asthma (URECA) cohort, included pregnant women and later their infants from families with at least one parent with allergic disease and residing in low-income urban neighborhoods in the Baltimore, Boston, New York City and St Louis area (135). In their infant population, there was no association with maternal stress and infant allergic sensitization by 3 years infant age (136). Interestingly, maternal stress at infant age 2 and 3 years was associated with recurrent wheeze in the infants by 3 years (136). The 10-year follow-up visits in the same cohort showed that maternal cumulative stress from pregnancy until 3 years post-partum were again associated

with wheeze among the children in low-income families (137). In addition, allergic sensitization against inhalant allergens (s-IgE) were inversely associated with maternal stress (137). This may indicate a cumulative effect of factors and exposures associated with low socioeconomic status. Low income families are at higher risk for air pollution, poor housing, smoking and reduced access to healthcare which are risk-factors of asthma (138). However, healthy habits, treatments and services cannot alone be explained by education, income and occupation. Long-term exposures to life-style choices influenced by either high or low socioeconomic status and stress may affect the risk of allergic diseases and disease development during infancy, and allergic symptoms later in child's life. The development of health and disease cannot be confirmed nor denied solely on the first 12 months of infant life.

6.2 STRENGTH AND LIMITATIONS

The strength of the present thesis is the prospective design and the large general study population recruited in pregnancy. The electronic questionnaires included detailed information about maternal and infant health from pregnancy and onward, including perceived stress. Power was not calculated for each study included in the thesis; however, all studies include a large sample size from the general population. Furthermore, the data collection in PreventADALL was standardized and study personnel had regular meetings and workshops.

It is likely that perceived stress in pregnancy would be associated with symptomatic allergic diseases in populations similar to PreventADALL. However, it should be noted that the perceived stress scale used in these studies is a validated measurement for stress but not a diagnostic instrument (30). The generalizability of these results, including high stress in the PreventADALL population should be interpreted with caution. Saliva was sampled in the participating mother's and infant's home environment, and the sampling procedure was unlikely to cause stress to either of them. The report of colic, abdominal pain, and other pain and discomforts in the questionnaire is a strength, misclassification of colic is common (139). Furthermore, abdominal pain before introduction to solid foods has never been reported before. The skin-prick test was done by trained study personnel in Sweden and Norway and is an objective measurement of allergic sensitization rather than self-reported symptoms by parents.

The following limitations in this thesis should be noted. The questionnaires did not include detailed information about the severity of allergic disease. Self-reported information about having symptoms of allergic diseases, without self-perceived options for disease severity, makes it difficult to assess allergic disease symptoms in relation to perceived stress. The proportion of allergic diseases and high education level among included women in PreventADALL, mainly living in urban areas, indicates selection towards higher socioeconomic status, which can affect the generalizability of the findings of this thesis. There was no detailed information about the cause of the abdominal or pain and discomfort outcome which makes the prevalence difficult to generalize to other infant populations with pain, discomfort or abdominal symptoms. Saliva sampling time and date was missing for several infants and their mothers. The PreventADALL interventions could have affected the outcome of allergic sensitization at 12 months. However, there were no interaction between PSS and either of the randomization groups at any time point. One may speculate that the accumulative exposure of stress and other environmental and genetic factors is relatively short in relation to development of allergic sensitization in the infant.

7 CONCLUSIONS

In Nordic pregnant women, symptoms of allergic disease can be perceived as stressful and socioeconomic factors might contribute to increased stress. Women experiencing stress in pregnancy could benefit from strategies for stress reduction (Study 1).

Maternal perceived stress in pregnancy and postpartum in Nordic women was not associated with their infant's salivary cortisol levels at three months of age. Neither was maternal perceived stress in mid and late pregnancy and postpartum and maternal morning or other sampling time associated with salivary cortisol levels by 18 weeks GA (Study 3).

Before solid food introduction, abdominal pain was the most common infant pain symptoms reported by mothers in their three-month-old infants (22%). Other pain and discomforts (6%) and colic (3%) were reported by the mothers at the same time. Maternal perceived stress in pregnancy is not associated with infant pain outcomes (colic, abdominal pain or other pain and discomforts) in this Nordic population. Allergic heredity and maternal sick leave in pregnancy were associated risk factors with colic, abdominal pain and other pain and discomforts (Study 2). Infant salivary cortisol levels by three-months-age was not associated with colic, abdominal pain or other pain and discomforts (Study 3).

Maternal PSS scores in pregnancy and postpartum in this Nordic cohort is not associated with allergic sensitization in their offspring at 12 months of age. Association between low socioeconomic status and perceived stress score was found independently of allergic sensitization. However, perceived stress stratified for socioeconomic status showed no association to infant allergic sensitization at 12 months. These findings are supported by previous studies (136, 140).

8 POINTS OF PERSPECTIVE

8.1 IMPLICATION FOR CLINICAL PRACTICE

From a clinical point of view this thesis demonstrates that; experiencing symptoms of allergic disease can be perceived as stressful in Nordic pregnant women and that socioeconomic factors might contribute to increased stress. Pregnant women might benefit from stress reducing activities and extra healthcare visits in pregnancy to reduce symptoms of allergic diseases.

Maternal stress in pregnancy or early postpartum does not appear to increase the odds of infant colic or abdominal pain by three months age, nor allergic sensitization at 12 months age in this population.

8.2 FUTURE RESEARCH

Socioeconomic status is a commonly associated factor for perceived stress in mothers and could promote adverted health in their children. Longitudinal prospective research could be helpful in order to determine if exposure to low socioeconomic status in the Nordic countries contribute to adverse health later in childhood or early adulthood in their offspring.

Qualitative studies could increase the knowledge about pregnancy-related stressors in women, with focus on chronic diseases such as allergic diseases. This could be beneficial for women in child bearing age, and in the planning of their health care before, during and after pregnancy. A larger cohort study with women from either early pregnancy or before conception and onward trough pregnancy, birth and the first year after delivery could perhaps increase the understanding of the course of allergic diseases before, during and after pregnancy.

Infant gut microbiota should be considered in future research on infant colic and abdominal pain (133). Infant stool samples from birth and onward, prospective data on infant colic, abdominal pain and allergies are all available in the PreventADALL cohort and could confirm recent findings on gut microbiome diversity in infants with and without colic (133).

Wheeze and asthma in children are associated with maternal stress in a high risk, low income population (136, 140). The mechanism behind this does not seem to be associated with allergic sensitization in early childhood. Longitudinal data from birth, childhood and onward could perhaps be used to investigate infant wheeze and the asthmatic phenotypes affected by maternal stress (136, 140) in the general population included in PreventADALL.

On the other hand, The PreventADALL study contains detailed clinical and self-reported information about the participants from pregnancy, early infancy and onward. It has been suggested that objective measurements such as IgE in sera or SPT rather than parental reported infant allergic disease could be useful to determine the association between allergic diseases and maternal stress (12). PreventADALL included food allergen molecules in sera (101) and a rigorous definition of food allergy by 3 years of age (109). Future research could

investigate the self-reported avoidance of common foods in infancy such as cow's milk, peanut, egg and wheat and the overlap with infant sensitization (confirmed by IgE in sera or SPT) and its association to maternal stress in pregnancy. Participants with both self-reported and confirmed allergy and pregnancy stress from the same cohort could add important knowledge.

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