Association Between Early Life Grass Pollen Exposure and Cord Blood IgE and Allergic Respiratory Diseases in Childhood and Adolescents

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A thesis submitted in total fulfilment requirements for the degree of Doctor of Philosophy

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Abstract

The prevalence of allergic respiratory disease which includes asthma and hay fever in children remains high globally. Although allergic respiratory disease itself is a complex disease and influenced by environmental, familial, and behavioural factors, we still do not know what causes this disease. But we do know that understanding early life exposures is critical to reduce the burden over the life course. Mounting evidence suggests that pollen during peak pollen seasons is a major environmental trigger for allergic respiratory diseases especially in children. However, only a handful of studies have explored the association of pollen exposure in early life with the allergic respiratory disease in childhood. Moreover, its association with food allergy is gaining some interest although we still do not fully understand the biological mechanisms that pertain to such associations.

My thesis with publication will fill the gaps on current knowledge that exists on the association between pollen exposure in early life and development of allergic respiratory disease in children. I conducted a systematic review and meta-analysis to better understand season of birth as a marker of pollen exposure on cord blood at birth as this is considered a marker of subsequent allergic disease [published in Environmental Research]. The meta-analysis from 10 studies analysed the pooled effect of born in winter, spring, autumn compared to born in summer on IgE level in cord blood or at birth. The results revealed that infants born in winter had higher odds for high IgE level in cord blood or at birth, compared to infants born in summer. Winter birth could work as a proxy for outdoor exposures such as pollen, fungal, vitamin D. It could also be a proxy for indoor exposures such as house dust mites. This meta-analysis supported my hypothesis that environmental exposures during pregnancy is associated with IgE level in cord blood or at birth.

I conducted a large analysis using data from three birth cohorts: Life-style Related Factors on the Immune System and the Development of Allergies in Childhood (LISAplus) in Germany, Copenhagen Prospective Study of Asthma in Childhood (COPSAC2000) in Denmark, and Melbourne Atopic Cohort Study (MACS) in Australia and showed that birth during grass pollen season was associated with higher odds for high IgE in cord blood. In contrast, higher exposure of grass pollen during pregnancy was associated with a slightly lower odds [published in Environment

International]. These results established evidence for the association and the association was not limited to northern or southern hemisphere only. The inverse association between born in high grass pollen season and cumulative grass pollen level during pregnancy showed an effect of pollen exposure on sensitized mothers. It led to increased IgE for infants born at the start of pollen season. As the season progresses, the sensitized mothers were exposed to higher cumulative grass pollen, the immune response may lessen. Thus, infants born outside high grass pollen season, but exposed to the whole season of high grass pollen, had lower IgE.

I then wanted to explore the role of early life pollen exposure on infant food allergy. Using the population-based cohort in Melbourne called HealthNuts, I showed that pollen exposure in early life also had some associations with sensitization to foods. However, there were no clear patterns on the association with food challenge diagnosed food allergy. Still, the results were preliminary evidence for some link with pollen exposure and this needs to be replicated in other studies [this manuscript is accepted for publication in *International Journal of Environmental Health Research*].

Back to MACS cohort, I assessed several periods of pollen exposure *in utero*, at birth and at early life and the exposures were identified to have an association with allergic respiratory diseases. The association were found in different ages: in children aged 6, 12 years and adolescence aged 18 years. Maternal history of asthma or hay fever modified the association although at different time points [this was written as a thesis chapter and will be published after submission of my thesis].

Although these are preliminary results that needs supports by other studies, it is important to increase awareness of the public health implications of pollen exposure especially given that changing climatic conditions are increasing the intensity and duration of pollen seasons worldwide. With more research we will better understand the role of pollen exposure during pregnancy and early life. Public health promotion need to focus on educating the community, especially women with a history of allergic disease, on the detrimental effects of exposure during pregnancy and risk of allergic respiratory disease in childhood and later life.

Statement of Authorship

This thesis consists primarily of work by the author that has been submitted, accepted for publication or published as described in the text. Except where reference is made in the text of the thesis, this thesis contains no material published elsewhere or extracted in whole or in part from a thesis accepted for the award of any other degree or diploma. No other person's work has been used without due acknowledgment in the main text of the thesis. This thesis has not been submitted for the award of any degree or diploma in any other tertiary institution.

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Nugroho Harry Susanto

Date: 25 June 2020

Author Contribution to Publications

I am the primary author of all publications within this thesis. The inclusion of co-authors on the papers included in this thesis reflects that these papers are the result of active collaboration. As primary author, I carried out most of the work relating to developing research question, study design, data analysis and interpretation, manuscript drafting and manuscript editing, with support from my supervisors: Professor Bircan Erbas, Associate Professor Agus Salim, and Associate Professor Adrian Lowe.

Susanto, N. H., Vicendese, D., Salim, A., Lowe, A. J., Dharmage, S. C., Tham, R., . . . Erbas, B. (2017). Effect of season of birth on cord blood IgE and IgE at birth: A systematic review and meta-analysis. Environ Res, 157, 198-205. doi:10.1016/j.envres.2017.05.026

Nugroho H. Susanto led the development of this research paper, developed the methodology, undertook all aspects of the systematic review, led the writing of the manuscripts, was responsible for all edits and revisions, and submitted the manuscripts to the journals for publication.

Bircan Erbas and Agus Salim corroborated in identifying articles to be included in the systematic review.

Bircan Erbas and Don Vicendese helped assessing whether selected papers in systematic review could be included in the meta-analysis.

Don Vicendese corroborated the meta-analysis and Rachel Tham supported the quality assessment of included articles.

Bircan Erbas contributed to the development of the research question and review protocol.

Agus Salim, Adrian J. Lowe, Shyamali C. Dharmage, Caroline Lodge, Frances Garden, Katie Allen, Cecilie Svanes, Joachim Heinrich, Michael J. Abramson, and Bircan Erbas contributed in interpreting the results of systematic review and meta-analysis.

All authors contributed to the intellectual content and revising of the final draft of the manuscript.

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Nugroho H. Susanto led the development of this research paper, developed the methodology and analysis plan, led the writing of the manuscripts, was responsible for all edits and revisions, and submitted the manuscripts to the journals for publication.

Bircan Erbas, Adrian J. Lowe, and Agus Salim contributed to the development of the research question and analysis plan; and supervised the data analysis and initial manuscript drafting.

Ann-Marie M. Schoos and Hans Bisgaard contributed in analysing data from Copenhagen Prospective Study of Asthma in Childhood, a birth cohort in Copenhagen, Denmark.

Marie Standl and Joachim Heinrich contributed in analysing data from Life-style related factors on the development of the Immune System and Allergies in East and West Germany, a birth cohort in multiple cities in Germany.

All authors contributed to interpreting of the data, to the intellectual content and revising of the final draft of the manuscript.

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Presentations

- 27 June 2018. Allergy and Lung Health Unit (ALHU) seminar at the Melbourne University School of Population and Global Health. Oral presentation entitled "Association between grass pollen exposures *in utero* and early life is associated with asthma and hay fever in children and adolescence." In this presentation, I presented my early results on the last result chapter of my thesis.
- 5 April 2017. Allergy and Lung Health Unit (ALHU) seminar at the Melbourne University School of Population and Global Health. Oral presentation entitled "The Effect of Peak Pollen Season Birth and Pollen Exposures in Pregnancy on Food Allergy Outcomes at 1 Year Age". Here, I presented my early results on the third result chapter of my thesis. This manuscript is accepted for publication in International Journal of Environmental Health Research journal.
- 5 October 2016. Allergy and Lung Health Unit (ALHU) seminar at the Melbourne University School of Population and Global Health. Oral presentation entitled "Environmental grass pollen levels *in utero* and at birth and cord blood IgE: Analysis of three birth cohorts". I presented my early results on the second result chapter of my thesis. This chapter was published in *Environment International* journal.
- 20 April 2016. Allergy and Lung Health Unit (ALHU) seminar at the Melbourne University School of Population and Global Health. Oral presentation entitled "Effect of season of birth on cord blood IgE and IgE at birth: A systematic review and meta-analysis". It was a presentation on my systematic review and meta-analysis results as my first result chapter. I published this chapter in *Environmental Research* journal.

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List of Abbreviations

BMI	Body Mass Index
CI	Confidence Interval
COPSAC	Copenhagen Prospective Study of Asthma in Childhood
ERS	European Respiratory Society
GBD	Global Burden of Disease
GINA	Global Initiative for Asthma
kU/l	kilo Unit / Litre
IgA	Immunoglobin A
IgE	Immunoglobin E
IU/l	International Unit / Litre
IUIS	International Union of Immunological Societies
IQR	Inter quartile range
ISAAC	International Study of Asthma and Allergies in Childhood
LISA	Life-style related factors on the development of the Immune System and Allergies in East and West Germany
MACS	Melbourne Atopy Cohort Study
MOR	Multinomial Odds Ratio
OFC	Oral Food Challenge
OR	Odds Ratio
PALM	Pollen Associated Lipid Mediator
RCT	Randomised Controlled Trial
RR	Risk Ratio
RRR	Relative Risk Ratio
SES	Socio-Economic Status
SPT	Skin Prick Test
TOTALL	TOTal costs of ALLergic rhinitis in Sweden
TRAP	Traffic Related Air Pollution
URTI	Upper Respiratory Tract Infection
US	United States
UV	Ultraviolet

Chapter 1 Introduction

1.1. Rationale

Pollen is one of the important environmental factors that triggers allergic respiratory disease in alarming rates globally. Many types of pollen have been reported as a trigger, and the prevalent allergy are region specific (1). In the southern hemisphere, grass is the major one whereas in the northern hemisphere, birch and weed pollen are the major pollens along with grass. In North America and Europe, the estimated grass pollen allergy prevalence is approximately 40% (2).

Although there are substantial evidences from previous studies to establish pollen as risk factor for allergic respiratory diseases, almost all of them focused on childhood pollen exposure to allergic respiratory disease later in life. So far, only a few studies have explored the association between pollen exposures in early life or during pregnancy on risk of allergic respiratory disease (3-5). Early life exposures, especially environmental exposures on allergic respiratory diseases in infancy and childhood are key determinants of the pathway to progression to allergic respiratory disease in young adult (6, 7).

In my doctoral research, I aimed to determine whether intra uterine and early life pollen exposure is associated with increased risk of allergic respiratory diseases in childhood and adolescence. To assess the relative impact of different exposure windows on various markers of allergic respiratory disease at birth, infancy, childhood and adolescence, I sought to expand the evidence in multiple steps, I assessed the association of pollen exposure with multiple outcomes: first I assessed with the level of immunoglobulin E (IgE) at birth as outcome, second with food allergy at 12 months, and finally with asthma and hay fever in childhood and adolescence.

In this introductory chapter I will briefly discuss allergic respiratory diseases, focusing on asthma and hay fever, followed by IgE and food allergy, why they are important and relevant to allergic respiratory diseases. Then, a brief background on pollen and how its respirable exposure that can trigger allergic respiratory diseases. Finally, I will outline the research questions that I have addressed in this doctoral research.

1.2. Allergic Respiratory Diseases

According to the Global Initiative for Asthma (GINA), asthma is defined as "a heterogeneous disease, usually characterised by chronic airway inflammation (8). It is defined by a history of respiratory symptoms, such as wheezing, shortness of breath, chest tightness and cough that vary over time and in intensity, together with variable expiratory airflow limitation" (8). Asthma starts early in life and the age of asthma onset is reported to be earlier with a study in Canada found children under 3-year age were diagnosed with asthma (9).

Hay fever, also known as allergic rhinitis or allergic rhinoconjunctivitis, is an immune response of the upper airways induce by exposure to allergens and is characterized by sneezing, rhinorrhoea, nasal congestion, and nasal pruritus (10). Recent evidence in both children and adults suggests that hay fever is not just a disorder in nose and nasal airways, but the entire respiratory tract (11, 12). It is also known that hay fever and asthma often co-exist in individuals (11). Hence, when we talk about allergic respiratory diseases, we usually refer to asthma and hay fever.

Asthma and hay fever are complex diseases influenced by environmental, familial, and individual factors. Among the environmental factors, we know that aeroallergens are closely related with asthma and hay fever as multiple studies have reported pollen as a key environmental trigger for exacerbations of existing asthma (3, 13, 14). However, we do not know their role as early life exposures on various markers of allergies and respiratory disease.

1.3. Allergic respiratory diseases remain as a major global public health problem

The Global Burden of Disease (GBD) study estimated that worldwide there were 339.4 million people experiencing asthma (15) and another report in 2012 estimated a higher number for hay fever, around 500 million people (16). The prevalence of asthma and hay fever in children is much higher (16). In the ISAAC phase III survey, conducted between 2000 to 2002 survey, in children aged 6 to 7 years, the global prevalence of asthma and hay fever is 11.7% and 8.5%, respectively and in children aged 13 to 14 years, the prevalence of asthma and hay fever is even higher at 14.1% and 14.6% (17). Thus, it is reasonable to say that allergic respiratory diseases are a major public health problem globally.

The burden of allergic respiratory disease in Australia is substantial particularly in children. In the 2007 – 2008 National Health Survey, about one in ten or around 2 million

people had suffered from asthma in their lives. The prevalence in children aged 0 – 15 years was estimated at 10.4% and slightly lower in adults at about 9.8%. The rate of hospitalisation for asthma in children and adolescents is estimated to be 493 per 100,000 people which is much higher than adults, at 91 per 100,000 (18). In 2010 – 2011, more than half of hospital admissions due to asthma in Australia involved children aged 0 to 14 years. Children aged 15 to 18 years tended to be admitted for a longer period (19).

The estimated direct health expenditure attributed to asthma in Australia is high, around $655 million, or equal to 0.9% of total health expenditure. This health cost was only associated with direct hospital care (20%), prescription drugs (50%), and out of hospital medical care (30%) (19). There were added costs associated that was not included in that health cost; cost incurred by the families and caregivers due to loss of labour productivity and unaccounted out-of-pocket expenses (i.e. transportation cost). All of those data highlighted the importance of asthma in Australia, not only prevalent but also a substantial health and economic burden for the patients, families and communities.

1.4. Immunoglobulin E

Immunoglobulin E (IgE) is a type of antibody that has an essential role in allergic diseases (20). It can induce an extremely rapid pathological responses and act as a highly sensitive immunological amplifier, capable of triggering reactions after interactions with allergens. That is why IgE is a target for pharmacological intervention (20). IgE also useful as diagnostic target for allergic diseases in later life. Many studies have used IgE in cord blood or at birth as a measure of the risk of asthma and allergies (21, 22). Maternal IgE and cord blood IgE are also highly correlated irrespective of maternal allergic status, which supports the theory that maternal environment is important for IgE in cord blood (23).

Many factors had been reported to be associated with IgE levels in cord blood, and one key environmental factor is season of birth. The findings were mixed with some studies reporting significant associations while other studies did not. Studies in the early 1990s had been carried out in many locations around the world with differences in geography, seasonality, and population characteristics from around the world (21, 24-29). Season of birth itself can act as proxy for other prenatal environmental exposures such as ultraviolet B radiation, seasonal variations in maternal nutrition, viral infections during pregnancy, or pollen exposures during pregnancy. This could point that pollen exposures during

pregnancy or at birth could influence IgE in cord blood or at birth and subsequently on later immune development and allergic respiratory diseases.

1.5. Food Allergy

The prevalence of food allergy in children varies widely among countries in many age groups (30-33). In Melbourne, Australia, the prevalence of food allergy at the age of 1 year is high at an 11% diagnosed by oral food challenge method (33).In contrast, prevalence is low, 1.4% at the age of 3 to 11 years in Portugal, 2012 – 2013 (30) and much higher at 21.6% in children aged 6 to 19 years in the USA using self-reported survey. 1.6% (32).

Food sensitisation is one of the important steps for later development of food allergy and has a significant role in the progression to other allergic diseases, such as asthma and hay fever (34). Food sensitisation was reported to be a risk factor for allergic respiratory diseases in later childhood (35), supporting the concept known as the atopic march that food allergy may progress to asthma and hay fever in later life in children and adolescents (36)

Multiple genetic and environmental risk factors are associated with food allergy. Family history of food allergy and male sex are key risk factors for increasing risk of food allergy (37-39). In contrast, the presence of older sibling and pets at home are protective (37, 40). Several studies have also reported season of birth to be important. For example, born in autumn – winter was reported as risk factor for higher odds of food allergy in children aged 1 year (41), aged 0 to 4 years (42), and aged 7 to 15 years (40). It is plausible that season of birth is a proxy for multiple environmental factors during pregnancy, such as vitamin D level and pollen exposure level. A key study in 2012 found that foetuses exposed to pollen season in the 11th week of pregnancy had higher odds of food allergy early in life (5).

1.6. Grass Pollen Exposure is a Potential Risk Factor for Child and Adolescent Food Allergy, Asthma and Hay Fever

Grass pollen (Poaceae family) is an allergen that is officially recognized by the International Union of Immunological Societies (IUIS) allergen nomenclature sub-committee. Clinically, it is the most relevant given its potent allergenic capacity when ruptured during a thunderstorm such as that was observed in the devastating 2016 Melbourne Thunderstorm asthma episode that killed 10 people and over 3,500 individuals

were admitted for respiratory distress (43). Pollination season for grass typically starts in spring, around August in Australia, and lasts until late summer, around January. Pollen peaks in Australia are usually around October to December (late spring to mid-summer), even though they vary every year in each city especially in Darwin (44). In addition, pollen grains could contain not only allergenic content, but also other protein and non-protein substances that have immunomodulatory activity, such as oxidases, proteases, pollen-associated lipid mediators (PALMs), and adenosine. These other substances may also be involved in allergic responses by altering airway epithelial function, modulating the migration of human neutrophil and eosinophil and activation of dendritic cells (DC), or stimulating CD4+ T cells priming (45).

The pollen grain as a whole may be too large to penetrate the small airways. However, wet weather during pollen season triggers pollen grains to release respirable particles that could penetrate airways to the lower parts (46). Furthermore, during thunderstorm asthma, it is hypothesized the extreme weather conditions cause pollen to rupture and release smaller respirable particles at very high volumes (46).

Several studies have found that exposure to elevated levels of pollen in early life may be associated with an increased incidence of allergic diseases. Lowe, 2012 reported that high levels of pollen exposure in the last 12 weeks of pregnancy were associated with an increased risk of asthma hospitalization in the first year of life (4). A study in Australia, found that pollen exposure around the age of 4 to 6 months in infancy increased the odds for hay fever and asthma in children aged 6 to 7 years (3). Kemp, 2009 found an interaction effect with children born in the pollen season which were exposed to high level of pollen, when having early upper respiratory tract infection had increased odds of hay fever up to 8 years of age (47). Still, there are several gaps in the existing literature to support that pollen exposure is associated with development of allergic diseases. Further research is required to better understand the key exposure periods in early life that may be a critical time when pollen exposure may lead to food allergy and allergic respiratory disease in children and adolescents.

1.7. Relevance and Importance

It is important to understand if early life exposure to grass pollen, including *in utero*, may increase the risk for food allergy and allergic respiratory diseases. Currently, management for allergic respiratory disease has advanced. Starting from clinical trials to

find out the efficacy of allergen avoidance to the clinical studies of grass pollen sublingual tablet. Knowing if pollen exposure in pregnancy and early life are associated with development of allergic respiratory diseases could help identify preventive strategies. Informing parents as to the importance of pollen exposure during pregnancy and attempt pollen avoidance activities. This may include avoiding outdoor activities during pollen season if pollen exposure is a risk factor or more outdoor activities if being expose to pollen is protective and beneficial for their children in particular time periods. For clinicians, it might help them to give evidence-based suggestion to their patients about what to do during pollen season. Lastly, for policy makers, it may lead to policies to increase public awareness of the influence of pollen season and educate vulnerable individuals about the importance of pollen count forecasts.

1.8. Aim and Objectives

The general aim of my thesis is to investigate the association of pollen exposures during pregnancy and at birth with allergic respiratory diseases in children and adolescents.

More specific objectives of my thesis are as follows:

1. To systematically synthesise the evidence as to whether season of birth is associated with IgE in cord blood at birth. (Research Question 1).
2. To investigate whether pollen count during pregnancy and at birth is associated with IgE in cord blood at birth, with comparison of results from cohorts in both the southern and northern hemispheres (Research Question 2).
3. To investigate whether pollen count during pregnancy and in early life is associated with food allergy in infancy and whether the association is modified by maternal history of allergic disease (Research Questions 3 and 4).
4. To investigate whether pollen count during pregnancy and in early life is further associated with allergic respiratory diseases in children and adolescents. In addition, to further investigate if the association is modified by maternal history of allergic diseases (Research Questions 5 and 6).

1.9. Thesis Overview

Chapter 1 is a summary of the rationale, background, aim and objectives of my thesis.

Chapter 2 is a literature review of grass pollen as a primary environmental exposure in varying exposure windows and IgE, food allergy, and allergic respiratory diseases as the outcome.

Chapter 3 gives a detailed explanation on the methods of pollen sampling of the pollen measurement data used in my thesis.

Chapter 4 is a published systematic review and meta-analysis, titled “Effect of season of birth on cord blood IgE and IgE at birth: A systematic review and meta-analysis.”, which I published in the peer-reviewed journal Environmental Research This chapter answers research question 1.

Chapter 5 is a published paper in a peer-reviewed journal Environment International, titled “Environmental grass pollen levels *in utero* and at birth and cord blood IgE: Analysis of three birth cohorts.” This chapter answers research question 2.

Chapter 6 is a paper accepted for publication, titled “Associations between grass pollen exposure *in utero* and early life with food allergy in 12-month-old infants.” This chapter answers research question 3 and 4.

Chapter 7 is a traditional thesis chapter titled “Association between grass pollen exposure *in utero* and early life is associated with asthma and hay fever in children and adolescence”. This chapter answers research question 5 and 6.

Chapter 8 is a discussion and conclusion on my thesis about the results related to the aim of my thesis.

Chapter 2. Literature Review

2.1. Introduction

Allergy is a common condition caused by immune-mediated hypersensitivity response to common environmental substances. Allergic diseases such as asthma, hay fever and food allergy are often characterized by the ability to make an IgE antibody response to environmental allergens. As such, allergic disease could be IgE mediated or non-IgE mediated. Grass pollens area is one of the important environmental aeroallergens. A number of studies have demonstrated a link between pollen exposure during peak pollen seasons and asthma-related emergency department visit in the United States (48, 49), Australia (13) and Spain (50). Approximately 20% of the population in Europe is affected by allergic diseases related to pollen (1, 51). In Australia, high concentrations of pollen were associated with the devastating thunderstorm asthma event that occurred in Melbourne on November 2016. The 2016 thunderstorm asthma event recorded 3,500 admissions to health facilities for respiratory distress and claimed 10 lives (43). Studies in Stockholm, Sweden, and Berlin, Germany reported there is an association between pollen exposure and increased risk of peanut sensitization (52, 53). With expected changes in climatic conditions that affect flowering and duration of pollen seasons, pollen exposure and burden on health is an ever growing concern globally (54).

Despite ongoing research, little is known about which critical time windows of first exposure to pollen have longer term effect to asthma or other allergy disease. Especially as most studies assessed exposure at time of the asthma or allergy related exacerbation. Although studies have examined the association between allergic diseases and pollen exposure, only a handful have assessed pollen exposure in early life and nearly nothing on *in utero exposure*. For example, a study in Sweden analysed the association between pollen exposure in the last trimester and asthma hospitalization in infants and found that being exposed to high level of pollen in the last trimester was associated with higher odds for asthma-related hospital admission in the first year of life (adjusted odds ratio (aOR) 1.35, 95% CI: 1.07, 1.71) (4). A study in Finland analysed the association between high pollen exposure *in utero* and food allergy in children and reported an association between being exposed to high level of birch and alder pollen during 11th gestational week and incidence of positive food allergy test in children aged up to 4 years (5). Another study in Melbourne, Australia analysed the association between

pollen exposure in the first 6 months of life with asthma and hay fever in children and reported increase odds of hay fever (aOR 1.14, 95% CI: 1.009, 1.29) and asthma (aOR 1.35, 95% CI 1.07, 1.72) (3).

In order to understand the association between pollen exposure in early life and allergic diseases in children, several factors need to be considered. Firstly, how the pollen exposure is measured. Secondly, how to define the outcomes. Thirdly, to take into account other factors that have been reported from many studies to be associated with allergic diseases, such as respiratory viral infections (55), air pollutants (56, 57), and tobacco smoke (58, 59). Currently there is limited evidence on the association between pollen exposure in early life with other associated factors of allergic diseases with consideration of potential confounders or effect modification.

In this review chapter, I will start by reviewing the evidence about IgE in cord blood, as it is the earliest evidence about pathways to allergic diseases in childhood (21, 60). Then I will review the definitions, prevalence and disease burden of food allergy, asthma and hay fever as the main components of allergic diseases, followed by the review of pollen as the main exposure of interest in my thesis. I will review the mechanisms by which pollen exposure might be associated with cord blood IgE and allergic diseases. Finally, I will summarize the gaps in current knowledge that will be the main topic in my thesis.

2.2. Food Allergy and Allergic respiratory disease in children: Overview

Food allergy prevalence in children varies in many age groups among different countries due to various methods of diagnosis and other etiological factors. In Melbourne, Australia, using oral food challenge, the prevalence of food allergy was 11% among 1 year old infants (33). A study in Portugal reported a low prevalence, 1.4% in children aged 3 to 11 years when diagnosed by skin prick test and/or IgE test. The prevalence was higher, 4.6%, when based on self-reported food allergy (30). A survey in 2005 in USA even reported a very high IgE diagnosed food allergy prevalence, 21.6%, in children aged 6 to 19 years (32). In general, the prevalence of food allergy is very high in many developed countries.

It was estimated that there were more than 339 million people suffering from asthma in 2017 (15), while hay fever was estimated to burden more than 500 million people in

2012 (16). Most of those cases were suspected to occur in children as in the 2000 – 2002 survey which reported that around 11% of children aged 6 to 7 years had asthma and 8% had hay fever. The prevalence was even higher in children aged 13 to 14 years (17). In Australia, asthma is also a significant problem as the asthma prevalence in children aged 0 to 15 years was estimated around 10% from the 2007 – 2008 National Health Survey (18). The high prevalence of allergic respiratory diseases contributed to a high economic burden to the patients, their families, and the government (61) and make allergic respiratory diseases major health problems globally.

In many cases, individuals of all age groups have asthma and hay fever which leads to the concept of "one airway one disease" (16). This concept is supported by evidence that allergen exposures in the upper airways could cause local inflammatory response and inflammatory response in the lower airways. The upper and lower respiratory tracts are similar in the physiological, functional, and immunological aspects (10). Finally, some people could have asthma and hay fever that leads to asthma becomes a comorbid for hay fever and vice versa. This condition shows that people in all age groups could have allergic reaction in upper and lower airways (11).

A good biomarker that is applicable in daily practice may support the many steps of allergic diseases treatment, starting from diagnosis, prognosis, and monitoring to medication (62). Many biomarkers have been studied, such as immunoglobulin E (IgE) in blood and it has been established as a good biomarker to predict the development of food allergy, asthma, and hay fever (62). Although previous studies results have been mixed, several studies have reported IgE as early as in cord blood could predict the development of allergic diseases in later childhood (21, 60).

Allergic disease itself is a multifactorial disease that is associated with multiple genetic and environmental factors. Based on the number of published systematic reviews, pollution is the major environmental determinant that has been studied for allergic disease and very few reviews on perinatal issues as risk factors (63). Aeroallergen may also be important as increasing studies are focusing on pollen exposure during peak pollen seasons with longer durations and intensity as climatic conditions also continue to change. My thesis specifically focuses on the association between outdoor pollen counts as a major environmental exposure beginning from *in utero* until early life after birth with cord blood IgE as a potential marker of allergic disease risk, then food allergy in infancy, and finally asthma and hay fever in childhood

and adolescence. Although there are plenty of evidence about pollen exposure as risk factor for allergic disease (2, 64), only a few had studied whether pollen exposure is a risk factor as early as *in utero* period.

To further support my hypothesis that pollen exposure in early life is associated with allergic disease in children and adolescence, I will begin my review by searching the literature on the evidence of association between pollen exposure *in utero* and cord blood IgE. By searching the current evidence of the association, I will have supporting evidence or lack of it about the association between pollen exposure and markers that predict the development of allergic disease. Then I will review the evidence of the association with food allergy in infants and children. Finally, I will review the evidence of the association with asthma and hay fever in children and adolescence. This will allow me to show what current evidence is available about this association between pollen exposure in early life and allergic disease from at birth until adolescence.

2.3. Cord blood IgE

Immunoglobulin E (IgE) is a type of antibody that has an essential role in allergic diseases. It could induce extremely rapid pathological responses and act as a highly sensitive immunological amplifier, capable of triggering reactions after interactions with allergens, which makes it a target for pharmacological intervention (20). IgE measurement itself is increasingly being used to diagnose many allergic diseases, especially chronic asthma and food allergy (65). Other than as a diagnostic tool, IgE has been established as a good biomarker to predict the prognosis of asthma severity and response to treatment by omalizumab (62). Measurement of IgE at birth can be considered as a marker of the foetal immune system. The measurement could be done using cord blood at birth or blood up till one weeks after birth because (25, 27, 28, 66, 67). Those studies measured the IgE level in cord blood or blood up till one week after birth because the IgE level in cord blood and one week blood is strongly correlated (68).

The utility of elevated cord blood IgE level as a predictor for allergic diseases later in life has been studied since 30 to 35 years ago. The studies were done in various locations globally, in different geography, seasonal characteristics, and population characteristics, but predominately in high income countries. Croner and colleague studied the association between high level of cord blood IgE and atopic dermatitis in

children in Sweden (69, 70). A study had been conducted in Netherlands on the association between cord blood IgE and allergic sensitization and wheeze (21). Sybilski and team in 2009 reported their results in Warsaw, Polland on the association between cord blood IgE and atopic symptoms (71). Another study in Europe was conducted to assess the association between cord serum IgE and allergic sensitization, and also with asthma (72). In America continent, a study on association between cord blood IgE and wheeze had been reported from United States (73). From Taiwan, a study was conducted on association between cord blood IgE and atopic dermatitis (74).

Although results from these studies were mixed because of varying methodologies, sample size and other factors, most agreed that cord blood IgE level could be a useful tool to predict allergic diseases (21, 70, 72, 74-77). Two studies by Hansen et al. concluded that although high cord blood IgE was not a good predictor for allergic diseases before 18 months of age, it was a good predictor in infants aged 18 months (78, 79). Nissen et al. found that elevated cord blood IgE was associated with food allergy and/or eczema in children aged 1.5 years and 5 years (77). Ferguson et al. found that elevated cord blood IgE was associated with wheeze and allergic sensitization in children aged 7 years (21). Other studies reported that it was not recommended to use cord blood IgE to predict allergic outcomes in childhood (71, 80-82). Edenharter et al. and Sybilski et al. even concluded that cord blood IgE capability as tool for prediction was very low (71, 80). Edenharter et al. suggested that the conflicting results due to different methods to measure allergic outcomes and allergic characteristics of the children's parents (80). With all the conflicting results, some scientists still believe that cord blood IgE have the potential to predict allergic disease, not just in infancy, but later in childhood (21, 72).

Several prenatal factors have been reported to be associated with cord blood IgE, including allergen exposure during pregnancy, parity, maternal age, sex of the infant, parent history of smoking, and season of birth (24, 26, 66, 83, 84). Most prominent reported factors are paternal and maternal history of allergic diseases and season of birth which is often a marker for respiratory viral exposure and pollen exposure. Exposure windows during pregnancy and the first 3 months of life are important for immune development and subsequent allergic diseases in childhood (85). This might reflect that high aeroallergen exposures during particular stages of pregnancy (in spring, summer or autumn) could contribute to elevated IgE levels (86). In addition to outdoor

environmental exposure, evidence suggests maternal exposure to indoor allergens such as dust mites are also associated with high IgE levels in cord blood (24).

Although season of birth was reported as one of the most frequent environmental exposure associated with cord blood IgE, only a few studies reported the direct measurement of environmental exposures during season of birth. Season of birth itself is used as a proxy for environmental exposure such as ultraviolet exposure level which itself is a surrogate measurement for vitamin D level (87). Other possible environmental exposures related to delivery season are maternal nutrition intake (88), viral infections during pregnancy (89), or pollen exposure during pregnancy. This could point that pollen exposure during pregnancy or at birth could influence IgE in cord blood or at birth and later influence immune development and allergic respiratory diseases in later life. My systematic review and meta-analysis of season of birth assessed the association between season of birth and high IgE level in cord blood or at birth. I included 20 articles for the systematic review, 10 of them for the meta-analysis and showed that born in winter had higher odds for high IgE level ($\geq$ 0.1 IU/ml), meta-analysis OR=1.24 (95%CI: 1.01 to 1.52) compared to born in summer (Chapter 4, published in Environmental Research journal in May 2017) (90).

Potential limitations with previous studies on association between season of birth and cord blood IgE or at birth was that only a few of them used the same season of birth as the reference category during analysis. There has not been any agreement about which season of birth proposed the greatest risk of high IgE in cord blood/at birth and how high the association is. For example on the variability of reference season of birth, one study compared all seasons to summer (84) but other study compared all seasons to winter (27). Sadeghnejad and team found that in United Kingdom, those born in autumn had highest odds ratio to have elevated level of IgE in cord blood compared to individuals born in winter (27). A study in Mexico reported that infants born in spring had the highest odds ratio for detectable IgE in cord blood than infants born in summer (25).

To further complicate the application of cord blood IgE to predict allergic diseases in later life, there was no consensus about the optimal cut-off to determine high level of cord blood IgE. Many of the previous studies used arbitrary level of IgE as the cut-off for high cord blood IgE. Studies in United Kingdom and in Netherlands used IgE level above 0.5 kU/l to determine elevated cord blood IgE (27, 66). Another study in

United States used the highest value of IgE as cut-off, above 1.08 kU/l (73). Other studies chose the lowest value of IgE detection in their measurement as a cut-off and the IgE measurement method in each of those studies have different limit of detection. Studies by Hernandez et al. in Mexico and Halonen et al. in United States used the lowest level of IgE detection among those studies, 0.1 kU/l (25, 91). Studies in Canada by Kaan et al. and Ferguson et al. used the highest level of IgE detection, 0.5 kU/l (21, 22). Some studies even use IgE level as continuous measurement instead (26, 28, 29).

Currently, two theories have been proposed to explain the factors that influenced cord blood IgE levels. First, as a result of direct allergen exposure *in utero*. This theory hypothesized that maternal allergens exposure could be transferred through placenta or amniotic fluid to the fetus (92-94). Second, as a direct effect of IgE transfer from mother to foetal. Bonnelykke et al. reported 46% of infants with high level of cord blood IgE were born from mothers with high level of IgE (95). Similar findings have also been reported by De Amici et al., where high maternal IgE level is highly correlated with high cord blood IgE (96). To rule out the possibility of maternofetal IgE transfer *in utero,* Bonnelykke et al. suggested to measure IgA along with IgE in the cord blood. If IgA level is very high then it can be considered the high level of IgE is because of maternal IgE transfer since foetus cannot produce IgA (95).

2.4. Food Allergy and Allergic Respiratory Diseases

2.4.1. Food allergy

2.4.1.1. Definition

Food allergy is a term that describes adverse immune reactions to food allergens, usually proteins or sometimes chemical haptens (97). The term “food” is limited to any substance—whether processed, semi-processed, or raw—that is intended for human consumption, and may include drinks, chewing gum, food additives, and dietary supplements. Non-immune-mediated adverse reactions to foods, e.g. food intolerances secondary to metabolic disorders (e.g., lactose intolerance), reactions to toxic contaminants (such as eating food contaminated by Salmonella organisms) or pharmacologically active food components (e.g. caffeine in coffee causing jitteriness, tyramine in aged cheeses triggering migraine) are not included in the definition of food allergy. Symptoms mimicking food allergy, such as auriculotemporal syndrome (a

disorder characterized by facial flushing and salivation that may follow trauma to the parotid gland), and gustatory rhinitis are also not included (97-99).

It is a well-established concept that atopic dermatitis in infancy commonly precedes and predicts the development of hay fever and asthma later in life (36, 100, 101). In the last decade, many studies have reported that food allergy can be part of the atopic march where, just like atopic dermatitis, children who have food allergy in early childhood are at increased risk of developing hay fever and asthma as they get older. There is substantial evidence to support this hypothesis. First, there are many reports on the concept of the coexistence between food allergy and allergic respiratory diseases in children and adolescents (33, 102-104). Thereafter, the research focused on food sensitization in early life as a risk factor for hay fever and asthma in older children (35, 105). And finally, children with multiple food allergies seemed to be at a higher odd of asthma-related hospitalization (106). The fact that several risk factors were similar between food allergy and allergic respiratory diseases supported the theory that children with food allergy may develop allergic respiratory diseases later in life suggesting that the association between food allergy and respiratory allergy may be due to confounding (both caused by a common set of risk factors).

Unfortunately, the mechanism remains unclear, but one possible explanation is that food allergen particles somehow enter the airways to the lungs and bind with the mast cell to trigger inflammation. This inflammation can remodel the airways and cause allergic respiratory diseases (107).

2.4.1.2. Mechanisms

The immunologic mechanisms that lead to food allergy are either IgE-mediated reactions or non-IgE mediated disorders. Sensitization can occur by contact with food allergens through the digestive tract, skin or respiratory tract (34). The manifestations of an IgE-mediated food allergy generally appear within 2 hours of exposure to the food allergens, while non-IgE mediated or combination of IgE and non-IgE mediated will usually take longer to start showing symptoms (34). From the 170 foods that have been reported as food allergens, the major ones are peanut, tree nuts, egg, milk, fish, crustacean shellfish, wheat, and soy (97). The symptoms of food allergy varied widely, it could be as mild as urticaria (hives) or severe as food induced anaphylaxis that might lead to mortality without immediate treatment.

2.4.1.3. Prevalence

The prevalence of food allergy in children was reported to be high and increasing in the last two decades (33). However, the prevalence varies by age, region and method of diagnosis. In Melbourne, Australia, the prevalence is substantial with 11% among 1-year old infants and 3.8% in children aged 4 years (33). In contrast, in some regions such as in Portugal, it is only 1.4% in children aged 3 to 11 years (30). A survey in 2005-2006 in United Stated showed a moderately high prevalence, 4.2%, in children aged 1 to 5 years (39). A more recent survey in United States, conducted in 2009 - 2010 reported a higher prevalence, 8.0% in children aged 1 to 18 years (108). A review by Nwaru and team calculated a pooled point prevalence in Europe was 6.86% in children aged 1 to 17 years (109). However, several scientists questioned the reported prevalence in children due to various study methodologies, study populations, or the methods to diagnose food allergy. For example, using self-reported as method to diagnose food allergy, most often could lead to an overestimate of food allergy prevalence (110).

Among all factors contributing to the differences in the previous reported prevalence, the main problem lies with the diagnosis of food allergy. Different methods of diagnosing food allergy lead to various reported food allergy prevalence. Without a common diagnosis method, it will be difficult to consider other factors, such as the children's age or parental history of food allergy. Nwaru et al. managed to calculate an age stratified pooled prevalence from multiple studies in Europe but they still had to separate the pooled results based on the diagnosis methods. Pooled prevalence using self-reported method is higher than symptoms plus specific IgE method (5.89% (95% CI 5.71, 6.07) and 2.66% (95% CI 1.66, 3.66), respectively) (109). An older meta-analysis by Rona and colleagues showed that the prevalence of self-reported food allergy was a lot higher (35% for allergy to any type of food) compared to those diagnosed by symptoms and sensitization tests (5%, also for allergy to any type of food) (111). Despite these differences in the reported prevalence because of food allergy due to differences in diagnosis method, there is growing community awareness and concern about food allergy in children.

Despite the high prevalence of food allergy, mortality due to an allergic reaction to food is rare. A meta-analysis from studies worldwide estimated that the mortality rate due to food allergy, defined as fatal food anaphylaxis, was 1.81 per million person-years (112). Shaker and colleagues in 2017 estimated a somewhat lower mortality rate

in the United States between 1999 and 2014, at 0.6 per million person-years (113). However, a study by Hanna et al. reported that the risk of food allergy fatality from anaphylaxis was overestimated by health practitioners. In this study, pharmacies and general practitioners' answers to the questioners revealed that they thought the risk for a child with food allergy to have a fatal anaphylaxis was higher than it supposed to be. They overestimated the risk by 13.5-fold (95% CI 5.0 to 13.6-fold) (114).

2.4.1.4. Health and economic burden

The high prevalence of food allergy leads to high financial costs. A study in 2010 estimated the direct medical cost of food allergy in children in the United States was US$ 4.3 billion annually (115). Similarly, in Europe, the cost of food allergy in children aged 7-11 years annually is much higher than children without food allergy (116). A recent study in Sweden reported similar results, with household with a food allergic children or adolescents having higher costs than families without. The study estimated a difference of €3,961 for children and €4,792 for adolescents (117). In other words, families with food allergic children or adolescents incur a higher economic burden which continues for many years.

Food allergy also impacted the quality of life, not just for the sufferers but also their families. Adolescents with food allergy experience higher emotional and behavioural problems compared to those without food allergy. A study in Australia showed that mothers of food allergic children also reported higher odds for depression (OR 4.5, 95% CI: 1.83, 11.07) and anxiety (OR 2.68, 95% CI: 1.12, 6.44) (118). Bollinger and team reported that food allergy affected daily activities in children and their families. They found that 34% of children had a problem in school attendance because of food allergy (119). Another study indicated that having multiple food allergies and receiving epinephrine worsen the quality of life (120). It is clear that food allergy causes a heavy burden and we need a better management for the sufferer and the caregiver.

2.4.1.5. Risk factors

Food allergy is also associated with genetic and environmental factors. Sex, hereditary and race were some of the reported genetic factors thought to be related with food allergy. Boys have higher risk of food allergy (121, 122). History of allergic diseases, not just food allergy, in parents or siblings has also been reported to be associated with higher risk of food allergy (37, 122, 123). Other studies reported

ethnicity as a risk factor for food allergy, where non-white children had higher rates or risk of food allergy compared to white children (124-127).

Environmental factors are also important, and these include variables such as hygiene level, ultraviolet ray level, and pollen. Children who were exposed to better hygiene lifestyle or more sterile environment, might actually have higher risk for food allergy (16). This hypothesis is supported by results from two studies that showed children with food allergy have lower number of intestinal microbiome (the Clostridia class), considered to be the results of better hygiene or more sterile lifestyle, compared to children without food allergy (128, 129). Ultraviolet ray exposure which related to vitamin D level, may be important as higher exposure to ultraviolet ray will increase vitamin D level, which has been associated with a lower the risk of food allergy (130-132). Pollen exposure may be a risk factor for peanut food allergy because the protein structure in pollen is similar with the protein structure in peanut (52, 53). Pollen and food allergy associations will be described further in section 2.6.2. As pollen is the primary exposure variable in my thesis.

2.4.2. Asthma

2.4.2.1. Definition

Based on a consensus by Global Initiative for Asthma in 2018, the definition of asthma is a "*heterogeneous disease, usually characterized by chronic airway inflammation. It is defined by the history of respiratory symptoms such as wheeze, shortness of breath, chest tightness and cough that vary over time and in intensity, together with variable expiratory airflow limitation*" (8). Symptoms and expiratory airflow limitation may go away quickly with medication and sometimes will not recur for months. Asthma symptoms may lead to poor outcomes such as emergency department visits, hospitalizations or deaths.

Respiratory symptoms such as wheezing, shortness of breath, chest tightness, and cough are all thought to be related to asthma diagnosis when we consider the nature, timing and triggers of these symptoms. How individuals respond to treatment is also critical (133). Although physical examinations are often normal, they could reveal comorbidities to support the diagnosis of asthma, e.g. atopic dermatitis, allergic rhinitis, etc. The diagnosis of asthma is especially challenging in children aged 5 years and younger, since the recurrent respiratory symptoms such as wheezing and cough are also

common in children without asthma, especially in the 0 to 2-year-old age-group, as wheeze caused by respiratory tract infection. In addition, measuring expiratory airflow limitation or bronchodilator responsiveness is known to be difficult in children aged 5 years or younger (8).

Children with asthma could experience worsening of asthma symptoms after being exposed to situations which may trigger their asthma such as allergen exposure. This event is called asthma exacerbation, defined by Global Initiative for Asthma (GINA) guidelines as "episodes of progressive shortness of breath, cough, wheezing or chest tightness, or a combination of these symptoms" (8). Sometimes asthma exacerbation becomes so severe that the treatment of asthma reliever is no longer enough, and the sufferer then needs to be treated at hospital to prevent worsening condition or even fatality. This event is called asthma-related hospitalization. A number of studies have examined the impact of short-term variations in environmental triggers, including pollen, on short term risk of asthma exacerbation and asthma-related hospitalization a. In my thesis, I only focus on early life pollen exposure as a risk factor for asthma diagnosis.

2.4.2.2. Mechanisms of asthma

The immunopathology of asthma is complex and multifactorial; therefore, the underlying mechanism is poorly understood. Current understanding of the underlying mechanisms of asthma is that it could be allergic dependent, non-allergic dependent, or both. The allergic dependent mechanism, also called eosinophilic asthma, is initiated after contact to an allergen which triggers stimulation of the dendritic cells (133). The non-allergic dependent mechanism or non-eosinophilic asthma is reported in some cases of patients having neutrophil-predominant disease, or because of bacterial colonization in bronchiectasis, or the effect of corticosteroids. Mixed mechanism do exist when allergic dependent and non-allergic dependent occur simultaneously and triggered mixed granulocytic inflammation or changes in the inflammatory profile (133). Asthma in children is predominantly allergic dependent and the resulting eosinophilic airway inflammation and structural airway wall changes are collectively called airway remodelling (134).

2.4.2.3. Prevalence of asthma

The Global Burden of Disease Study estimated that more than 339 million people ever had asthma in 2017 (8, 135). A global report from the International Study of

Asthma and Allergies in Childhood (ISAAC) phase III, conducted between 5 to 10 years after phase I, gave the distribution of asthma prevalence in each region of the world (17). Children aged 6-7 years were chosen because this age group tends to have higher prevalence of asthma and higher hospital admission rates (136). The lowest prevalent was in the Indian sub-continent (6.1% in female and 7.4% in male) and the highest prevalence was in the Oceania (19.2% in female and 24.3% in male). The data showed that asthma prevalence ranged widely between regions, with the highest prevalent region having more than three-fold prevalence compared to the lowest prevalent region (17). In older children aged 13-14 years, the lowest prevalence was still in Indian sub-continent (8.6% in female and 5.4% in male) the highest prevalence was no longer in Oceania but in North America (19.8% in female and 23.3% in male) (17).

In Australia, a national health survey between 2007 and 2008 reported that around 2 million people have asthma and the prevalence in children aged 0 – 15 years was higher than in adults (10.4% vs 9.8%, respectively) (18). A comparison of global asthma prevalence between 1990 and 2015 showed that asthma prevalence increased by 12.6%. Mortality caused by asthma is rare for all age groups, the mortality rates of asthma in the 2010 report was lower compared to pneumonia and chronic obstructive pulmonary disease (137). The mortality rate has declined by 26.7% in the span of 25 years (15). Although there were no specific mortality rates for asthma in children, reports indicated that death of asthma in children is rare at around 0.7 per 100,000 children (138).

2.4.2.4. Health and economic burden

Asthma is a substantial burden on society as a whole from the individual and carers. Major direct costs in children with asthma including expenses related to the treatment of asthma (inpatient care, emergency visits, physician visits, nursing services, etc.) (139). There are indirect costs too, and these include productivity loss of the carer (school days lost, travelling, waiting time, caretaker need to take time off to look after the children) (139). The estimated cost of asthma per year in high income countries in 2008 in US dollars was estimated to be $654 million in Canada, $1,413 million in Switzerland, and 8,256 million in United States (139). As a result, in Australia the asthma-related hospitalization rate is estimated to be much higher in children and adolescents than in adults, 493 vs 91 per 100,000, respectively, the burden cost is also

very high (18). The 2013 report from Australian Institute of Health and Welfare revealed that more than half of hospital admissions due to asthma in Australia involved children aged 0 to 14 years. The report also revealed that adolescents aged 15 to 18 years tended to be admitted to hospital for a longer period. The total direct cost of asthma in Australia was estimated to be around AU$655 million or equal to 0.9% of total health expenditure (19).

Asthma also affects the quality of life of the sufferer and their families. Asthma substantially impairs the activities of daily life (140, 141), the quality of sleep (142, 143), and of course school attendance (144). Although the estimated disability-adjusted life-years (DALY) for all age caused by asthma in 2016 was slightly lower compared to the estimated DALY in 1990, the global disease burden report in 2017 still puts asthma as ranked as 28th of the leading cause of DALY in the world (15, 135).

2.4.2.5. Risk factors associated with asthma in children and adolescents

Asthma is multifactorial and associated with complex interactions between genetic predisposition and environmental exposures. Both protective and predisposing factors involved in the development of asthma have been identified by many studies. A substantial number of genes and many polymorphisms have been reported to be associated with the onset of asthma, although none of them, either alone or in combination, is able to predict the occurrence of disease (145, 146). In this section I focus on the major environmental factors that cause or trigger asthma exacerbations and or symptoms in asthma in children because my thesis focuses on outdoor pollen exposure and allergic respiratory disease outcomes. Environmental factors such as allergens, tobacco smoke, respiratory infections and air pollution have all been shown to be associated with the onset of asthma in children. Both prenatal and postnatal exposures to cigarette smoke increase the risk of the child to become asthmatic and to experience severe asthma exacerbations (59, 147, 148).

Indoor allergens (dust mites, molds and animal dander) and outdoor allergens (pollens and molds) have also induced sensitization and triggered asthma exacerbations (149). According to the hygiene hypothesis, microbial diversity early in life may either induce or protect from asthma or lead to the development of allergic diseases later in life (150). Repeated episodes of infection with rhinovirus and respiratory syncytial virus (RSV) in early life are associated with the risk of recurrent wheezing and asthma in children, and also with early allergic sensitization (151). In the concept of atopic

march, allergic sensitization is a major risk factor for the development of asthma. Subjects with polysensitization, combined with food allergy, could present with more severe asthma, which highlighted the additive feature of the atopic march (152).

Aeroallergens such as pollen have been associated with asthma exacerbations requiring emergency department visit and subsequent admissions (49, 153, 154). Moreover, consecutive days of high pollen coupled with changing climatic conditions have been associated with the TA event in Melbourne, Australia.

While higher level of pollen exposure was associated with an increase in asthma-related hospital admission, pollen exposure was the main risk factor for thunderstorm asthma in Australia in November 2016 (4, 13, 43, 64). An in-depth review concerning pollen exposure as the risk factor for asthma is discussed in section 2.5.3.

2.4.3. Hay fever

2.4.3.1. Definition

Hay fever, also known as allergic rhinitis or allergic rhinoconjunctivitis, is an immune response after exposure to allergens and is characterized by sneezing, rhinorrhea, nasal congestion, and nasal pruritus (10). Recently, there is growing focus on the "one-airway" conceptualization of respiratory allergy, with the connectedness of upper-airways (with symptoms expressed as hay fever) being connected and impacting on lower-airway conditions (symptoms expressed as wheeze and asthma) (11, 12). Hay fever is the most common type of chronic rhinitis, while rhinitis itself in general is an inflammation of the nasal mucosa.

The underlying mechanism of hay fever involves multiple reactions of inflammatory cell types in immune system after exposure to allergens. In general, after being exposed to allergens, the immune system in human body will be activated and cause symptoms such as itch, rhinorrhea, or mucous secretion (155). Following these symptoms, the immune system will trigger a late chain reactions which cause clinical symptoms such as nasal congestion that persist in longer time than the earlier symptoms (155).

The diagnosis of allergic rhinitis requires a detailed history and physical examination of the patient. History taking is needed to identify the classical symptoms of hay fever such as sneezing, rhinorrhea, nasal congestion or nasal pruritus, which will

be further confirmed by physical examination of outward signs, nose, ears, sinuses, posterior oropharynx, chest, and skin. Currently, the primary diagnostic test for hay fever is a skin prick test. This test is done by pricking the skin on the forearm or back to expose the patient into the extract of suspected allergens (10).

2.4.3.2. Prevalence

Like asthma, the high prevalence of hay fever in children is reported worldwide. ISAAC phase three survey in 2002-2004, using self-completed written questionnaires, estimated that the global prevalence of hay fever in children aged 6 to 7 years was 8.5% and the prevalence was higher in children aged 13 to 14 years, 14.6%. (17). In contrast, a different finding was reported by study in 2016 in Wuhan, China, where the prevalence of doctor diagnosed hay fever decreased with increasing age. That study reported a much lower prevalence of new cases of hay fever in children aged 13 to 14 years (2.86%), but much higher prevalence in children aged 2 to 6 years (25.92%) (156).

In Australia, the prevalence of hay fever based on self-reports in 2014-2015 was 12.6% in male and 9.1% in female children aged 0 to 14 years. The same survey estimated about 4.5 million people of all age in Australia had hay fever, or equal to 20% prevalence nationally, with a higher prevalence in adult people aged 30 years or older than in children aged 0 to 14 years (157). Earlier, Kim and colleagues, showed that the prevalence of doctor diagnosed hay fever was higher in children younger than 19 years compared to adults in South Korea (158). Based on this prevalence data, just like food allergy and asthma, hay fever remains a global public health problem.

2.4.3.3. Health and economic burden

Roger et al. conducted a study in Spain in 2011-2012 and found that children with more severe hay fever have higher loss of academic productivity and daily activities (159). Another study in Changsa, China, in 2011-2012 provided evidence that hay fever influenced school aged children's sleep, emotion and memory (160). A survey in Australia between 2009 and 2010 revealed that 42% people in all age groups had work or school interference caused by hay fever and 33% had moderate to extreme interference of sleep (161). Several studies had similar conclusion that hay fever causes worsening of quality of life and that successful treatment will improve the quality of life (159, 162, 163). In brief, having hay fever especially during peak seasons causes a significant depreciation in the quality of the daily activities and sleep but successful management may improve the situation.

Although the economic burden of hay fever is not as high as asthma, it is still a substantial burden. Estimates of the direct medical cost of hay fever in United States in 2010 was around US$ 3.4 billion annually. Almost half of the direct medical cost went to medication prescriptions, which placed hay fever in the fifth ranks for overall economic burden of illness among chronic conditions in United States (164). The TOTALL study (TOTal costs of ALLergic rhinitis in Sweden) gave a more detailed economic cost of hay fever, the direct cost was €210.3 and indirect cost was €750.8 per person. As a result, the indirect cost was 3.6 fold of the direct cost and presenteeism represented the largest portion (70%) of the total cost (165). A study in 2011 in United Kingdom, reported an estimated total cost of hay fever was £1.25 billion per year (166). A national report in 2011 in Australia reported an increase in the medication cost of hay fever by 2.1 fold in 2010 compared to 2001 (167). A substantial increase, even after considering the increased price of medications.

2.4.3.4. Risk factors associated with hay fever

Multiple environmental risk factors for the diagnosis or self-reported hay fever have been reported such as pollen allergens, air pollution, and tobacco smoke. The most recent study being a meta-analysis conducted by Zou and colleagues showed that air pollution was a significant risk factor for reported hay fever in Europe (168). Another meta-analysis by Chong and team estimated a significant increasing odds for the manifestation of hay fever (OR 1.34, 95% CI: 1.18, 1.54) for smokers (169).

Obviously, aeroallergen exposure is also associated with hay fever and seasonal allergic rhinitis because of the intensity and duration of peak pollen season. However, only two studies have assessed the impact of early life pollen exposure on hay fever and reported different findings. Kihlström et al. found no association between pollen exposure and hay fever (170). In contrast, Erbas et al. found that pollen exposure in early life was a risk factor for hay fever at aged 6-7 years (adjusted OR 1.14, 95% CI: 1.009, 1.29) (3). A further review of association between pollen exposure and hay fever can be found in section 2.5.4.

2.5. Pollen Exposure

2.4.4. A brief introduction about pollen

Pollen is one of the important environmental factors related to human health. Evidence to date suggests that pollen is a risk factor for allergic respiratory disease (13, 154, 171-173). Pollen itself consists of many types with each prominent type specific to a region (1).Of all species, grass pollen is considered as the leading aeroallergen worldwide with estimated pollen sensitization or allergy prevalence around 35% in North America and Europe (2). Grass pollen (from the Poaceae family) is already registered by the International Union of Immunological Societies (IUIS) allergen nomenclature sub-committee as an allergen (2).

The pollination season in each region commences at differing times and its duration varies in line with the flowering seasons of the plant sources in that region (174). Even in one country, the pollen season could vary between cities such as Melbourne and Sydney in Australia. The pollen season begins with dispersing a small amount of pollen and then during the peak pollen season will gradually continue to disperse and peak for several days with the highest number of pollen grains occurring during this period. The pollen season will end when the source plant stops producing pollen. Pollen exhibit a diurnal cycle which the pollen concentration rising during the day and then decreasing during the afternoon (175). For example, in Australia, the pollination season for grass usually starts in early spring which is around August. This pollination season lasts until late summer or around January in the next year. And the peak of pollen season in Australia are usually around October to December (late spring to mid-summer), this is particularly the case for the grass pollen season. This timeline of pollen varies every year in each city in Australia (44). It may vary a couple of days at the start and a couple of days at the end but not much more than that. Hence, I used different timepoint of pollen season when I analysed the association between pollen exposure in three birth cohorts in chapter 5, a Result chapter on the association between pollen exposure during pregnancy and level of IgE in cord blood.

The ability of pollen to disperse large amounts of particles into the atmosphere determines the pollen concentration in the air thus this ability relates to the pollen exposure in humans (176). This ability of pollen to disperse depends on physical characteristics of the pollen grain and meteorological factors. The size of pollen is one of the physical characteristics. Pollen size determines how long pollen stays in atmospheres that pollen with smaller size will stay longer in atmospheres (176, 177). A

pollen grain has the largest physical size among all aeroallergens. Pollen size varies between 10 and 100 μm but when the pollen ruptures, the fragments can be between 30 nm to 5 μm (178-181). Hence, when we talk about pollen as an aeroallergen, we talk about pollen as a whole or as fragments. As an entity pollen remains in the air for a short time period but if ruptured it will remain in the air longer.

2.4.5. Pollen measurement

Temperature, relative humidity, wind direction and speed and rainfall preceding the pollen season and during the pollen season are the most important meteorological factors that relate to the way pollen will disperse in the air (176, 182). Many studies show evidence that the increase of temperature is associated with increase in pollen counts (183). Kuparinen and colleagues suggested that increase in air temperature could increase pollen dispersal (183). Global change in climatic conditions can impact pollen dispersal, for example increasing temperature and changes in precipitation (184). Therefore, climate change has the potential to cause changes on the production, distribution and dispersion of pollen which will result in longer duration and intense pollen seasons thereby exposing susceptible populations longer.

Pollen as one of the vast number of aeroallergens can be measured using air sampling. There are multiple techniques of air samplings, but Levetin (2004) classified these techniques into two methods, either active or passive. Passive sampling is a method to measure pollen using gravity while active sampling can measure pollen using impaction, impingement, or filtration (185-187).

Passive sampling is the simplest method to measure pollen from air. This method relies solely on the gravity effect to capture pollen from the air into a prepared solution by exposing the prepared solution into outdoor or indoor atmosphere. The prepared solution could be a microscope slide or petri dish containing agar. This is a relatively simple measure of sampling, but the outcome is qualitative because this method does not measure the pollen per volume of air. However, this method tends to capture pollen that is larger in size due to gravitational force used to capture the pollen. Few studies use this method.

In contrast, active sampling does not use gravity to capture the pollen. Instead, it uses inertia to capture pollen from the air. Active sampling is a quantitative measure of sampling because this technique also measures the volume of the air sampled into the

measurement device. The main difference between each active sampling methods is how each method capture pollen into the prepared solution such as microscopic slide or liquid media (176, 185). Impaction sampling method captures pollen into solid media, such as microscopic slide. Impingement sampling method draws air through water or dilute buffer and captures pollen into liquid media. Filtration sampling method captures the pollen by trapping them in a fibrous or porous substrate. Thus, multiple factors affect the performance of sampling method and we need to choose the sampling method that is most appropriate for the type of aeroallergen that we want to measure.

Size and shape of the sampler inlet, velocity and direction of the wind are some of the factors that some researchers suggest being considered when choosing the sampling method for pollen measurement (185). Other researchers consider particle size as the most important factor to consider when choosing the appropriate sampling method (176). Still, all those factors lead to Burkard volumetric spore trap being the widely used sampler for pollen measurement. My thesis also used this active method of pollen sampling and the measured by the Burkard volumetric data. Further details of Burkard volumetric spore trap will be discussed in Methods chapter of my thesis.

Once the pollen is captured using any of these methods, samples are then analysed using several methods. The researcher will choose the analysis methods based on the sampling method and the information they would like to extract from the samples. Direct microscopy is the most often used method to analyse the captured pollen from a Burkard volumetric spore trap. Direct microscopy method is used to identify the pollen from the morphological and measure pollen manually. To get the most accurate results of pollen measurement is by counting the pollen on all field of the microscopy slide (185). However, this is rarely done as it is time consuming, instead the pollen measurement usually done by counting the pollen on some parts of the microscopy slide (188, 189). I elaborate further on direct microscopy analysis in the Methods chapter of my thesis.

2.4.6. Mechanism underlying pollen and how it may lead to allergic respiratory disease

Protein of pollen has been recognized as the allergen of allergic disease and already registered by the International Union of Immunological Societies (IUIS). There are additional factors other than protein that are involved in the allergic mechanism caused

by pollen. Some of those factors are the size of the pollen particles, protein of pollen washes out rapidly, and the pollen particles contain foreign protein, glycoprotein and lipids (190). The pollen grain as a whole with its size from 10 μm up to 100 μm, may be too large to penetrate the small airways. However, wet weather during pollen season triggers pollen grains to release respirable particles that could penetrate deep into the airways. These respirable particles size is considerably smaller than the whole pollen grain, between 30 nm to 5 μm (180, 181). Furthermore, during thunderstorm asthma, it is hypothesized the extreme weather conditions cause pollen grains to rupture and release those smaller respirable particles at very high volumes (46, 191).

One theory of how the protein of pollen triggers immune response is due to concomitant exposure to toll like receptor ligands (190). However, this exposure must happen in close physical distance(192, 193). When the protein of pollen is inhaled to the nose, the protein contains high concentration of ligands that will be recognized by immune system in nose. Along with the protein of pollen, pollen particle also contains allergens or other foreign parts that have the ability to induce immune response. The presence of ligands and protein in the same particle means they are close enough to trigger the response of T helper 2 cells (194).

It is well accepted that pollen as an aeroallergen triggers allergic symptom by inhalation. Some researchers argue that inhalation is not the only route of exposure for pollen to cause an allergic respiratory response (190). Initially, the sensitization phase occurs over long period, so it is hard to say that during this phase people being expose to pollen only through by inhalation. Additionally, there are cases of mite allergy after eating mite-infested flour (195-197). Thus, it is possible there are other routes of exposure for pollen allergy to contribute to an allergic reaction. Among all possible alternate routes of exposure, the most obvious ones are transdermal and sublingual. Hence, exposure to outdoor pollen might be higher due to multiple routes of exposure.

2.4.7. Season of birth as a proxy for pollen exposure

Many researchers have examined the association between season of birth and allergic respiratory disease. A study in Kazakhstan reported the highest number of children aged 1 to 17 years with seasonal hay fever was born in the summer season (198). Another study, conducted in Taiwan, found that children aged 7 to 15 years born in autumn had a higher odds of asthma compared to children born in spring (OR 1.11, 95% CI: 1.07, 1.28) (199). Researchers also examined the association between season

of birth and food allergy. Bird and team found that in children aged 5 to 8 years, born in winter had the highest prevalence of peanut and egg allergy compared to born in other seasons (200).

Keet and colleagues reported that in United States, children born in the autumn season with history of eczema had higher odds for food allergy compared to children born in autumn season without history of eczema (OR 1.39, 95% CI: 1.23, 1.57 vs 1.01, 95% CI: 0.82, 1.24, respectively) (201). A study in Japan, among infants aged 0 – 1 year, infants born in autumn to the winter season had a higher odds of food allergy compared to those born in spring to summer season although not significant (OR 1.32, 95% CI: 0.97, 1.80) (41). Mullins and team conducted a study in Australia, found similar results that there were more children born in autumn/winter season than born in spring/summer season among children aged 0 – 4 years who were diagnosed with food allergy (42). Mullins and team's study method is a simplistic way of examining exposure as data from four seasons is essentially categorised as two seasons .

In addition, researchers also examined the association between season of birth and IgE (at birth) as biomarker of allergic disease in later life. A study in Mexico found that children born in spring had higher odds to have detectable level of cord blood IgE compared to those born in summer, although this association was no longer significant in the adjusted analysis for various confounding factors (OR 1.64, 95% CI: 1.03, 2.62 vs 1.60, 95% CI: 0.99, 2.58) (25). Another study in Iran reported that children born in winter season had lower cord blood IgE level than children born outside of the winter season (26). In contrast, two separate studies in United States and Netherland found no association between season of birth and cord blood IgE level (66, 202). Few studies used the same season of birth as the reference category when examining risk. For example, one study compared all seasons to summer (84) and another compared all seasons to winter (27). Furthermore, the studies that reported significant associations did not agree as to which season of birth had the greatest risk of high IgE, or how large was the actual risk. Despite the evidence from the previous studies are still not strong enough about the association between season of birth and allergic diseases, many researchers still want to know the reason behind these associations. Furthermore, multiple studies in their analysis had adjusted their results for season of birth (203).

Multiple theories had been suggested to explain the association between season of birth and allergic disease. Some researchers theorized that season of birth is a proxy for

ultraviolet exposure which responsible for the development of vitamin D in human body (42, 200, 201, 203, 204). Thysen and colleagues suggested that this association is actually mediated by changes in immune system (205). Finally, some researchers hypothesized that the association is because the season of birth overlaps with pollen season (198, 206, 207). All those theories essentially based on a premise that season of birth is just a proxy for main exposure which mean we should measure the main exposure itself.

In my first result chapter, chapter 4, I systematically reviewed the previous studies that analysed the association between season of birth and IgE level in cord blood or at birth. Then I did a meta-analysis in the same result chapter to get the pooled effect size of the association (90). My meta-analysis using data from Denmark, Germany, and Australia, to capture whether the association is consistent with variations in these three different regions. The meta-analysis results provide stronger evidence to support the theory that season of birth is actually a proxy for pollen exposure.

2.5. Association between pollen exposure and allergic disease

2.5.1. Pollen exposure and cord blood IgE

No studies to date have investigated the association between pollen exposure at birth and level of cord blood IgE. The closest attempt performed in previous studies were on the association between born in pollen season and cord blood IgE. To my knowledge, only two previous studies examined the distribution of high cord blood IgE level at each month of birth but both lack data on pollen concentrations (69, 208). A study of 1,652 children in Sweden reported that children born in the grass pollen season, around June to August, had a slightly higher cord blood IgE levels, but this association was not significant (OR 1.03, 95% CI: 0.74, 1.45) (69). Another study of 5,353 children in Belgium showed that children born in grass pollen season, mid-May to mid-July, had higher odds of higher cord blood IgE levels compared to children born in the rest of the year, although not significant (OR 1.21, 95%CI: 0.88, 1.66) (208).

A similar situation is seen on the association between pollen exposure during pregnancy and IgE level at birth. Little is known about cord blood IgE when exposed to high levels of pollen during pregnancy. To my knowledge, only two studies have reported the effect of exposure to pollen during pregnancy and risk of allergic disease

in childhood. One study reported that high levels of exposure to pollen in the last 12 weeks of pregnancy were associated with an increased risk of asthma hospitalization during the first year of life (4). Another reported a trend that high exposure to birch pollen during pregnancy increased the risk of sensitization to birch pollen at 5 years of age (209). Although Heinrich and team examined the impact of prenatal exposure to indoor allergens derived from mites and cats, not pollen, their results supported theory that exposure during pregnancy affects infants immune system (210).

Based on those three previous studies, I hypothesized that pollen exposure may also be important during the pregnancy, especially during the 2nd and 3rd trimesters where the immune system develops and among women with a history of allergic disease (211). I wanted to investigate what role pollen concentrations in the outdoor environment have during pregnancy, at birth during months where outdoor levels of pollen peak (pollen seasons) on allergic diseases. To test my hypothesis, I analysed data from three birth cohorts from three countries, Germany, Denmark, and Australia in Chapter 5.

2.5.2. Pollen exposure and food allergy

Oral allergic syndrome (pollen-food-related syndrome) is common where pollen-food cross-reactivity occurs and sensitization occurs through the respiratory track (98). Multiple studies in children have reported increased risk of asymptomatic peanut sensitization and pollen sensitization. Asarnoj and colleagues reported this association with birch pollen in a birth cohort in Stockholm, Sweden and Niggeman and colleagues reported the same association with grass pollen in Berlin, Germany (52, 53). Although these studies did not assess pollen exposure nor sensitization *in utero* or first year of life it is possible that exposure to high pollen in earlier life further exacerbates this risk.

Still, only a few studies have investigated potential association between pollen exposure during pregnancy or in early life with food allergy. A study in Finland found that children who had their 11^{th} gestational week in April to May (high birch and alder pollen seasons in Finland) had a higher odds of food sensitization up to 4 years of age (5). It is understood that the liver produces IgE around this period and the lung and spleen contribute thereafter (211). The same study in Finland also reported that the pollen season and food sensitization associations were modified by parent's history of allergic manifestation such as hay fever or pollen allergy (5). Although the study in Finland showed that exposure during the pollen season was important, they did not have actual pollen measurements to assess actual pollen exposure effects, and they did not

assess clinical food allergy outcomes. The only other study that analyzed the association between grass pollen exposure in early life and food sensitization in children was a cohort study in Melbourne, Australia that reported no association between grass pollen exposure in the first 3 months and food sensitization assessed by skin prick test in children aged 2 years (3). Thus, there is not enough evidence to conclude that pollen exposure in early life is associated with food allergy and more studies are needed to test this hypothesis further with large sample sizes and sufficient data on exposure and outcome.

2.5.3. Pollen exposure and asthma

Compared to studies on the association between pollen exposure and cord blood IgE or food allergy, more studies were conducted on the association between pollen exposure and asthma. Increasingly, the changes in climatic conditions are resulting in higher pollen exposure. A meta-analysis of 14 studies showed an increase of 1.88% (95% CI: 0.94, 2.82%) asthma related emergency department visit per an increase of 10 grass pollen grain/m^3 (64). Thien and colleagues reported a thunderstorm asthma event in Melbourne in November 2016 due to extremely high level of pollen (43) and an increase in respiratory related emergency departments presentations and asthma related hospital admission but this was not done in children. Based on all the available evidence, there is already a consensus that asthma patients should have inhaled corticosteroid treatment during the pollen season. The consensus even recommends the treatment should be continued until 4 weeks after the end of the pollen season (133).

To the best of my knowledge only a couple studies examined the association between pollen exposure in earlier life and asthma, especially in the southern hemisphere. Erbas and team found that pollen exposure during the first 4 to 6 months of life in children aged 6-7 years was associated with asthma (OR 1.35, 95% CI: 1.07, 1.72) (3). The other study in Sweden, examined the association between exposure to high level of pollen during pregnancy and risk of asthma related hospitalization. This study found that high level pollen exposure during last 12 weeks of pregnancy was associated with increased odds of asthma hospitalization (OR 1.35, 95% CI: 1.07, 1.71). On the other hand, high level pollen exposure during first 3 months of life was associated with a decreased odds of asthma hospitalization (OR 0.76, 95% CI: 0.59, 0.98) among children with smoking mothers (4). These studies suggest pollen exposure is important for allergy disease. I want to go further by assessing pollen exposure during

pregnancy association with allergy disease. If this doctoral study can show the evidence for the association then it could support the idea to modify behaviour regarding pollen exposure during pregnancy.

2.5.4. Pollen exposure and hay fever

Similar to asthma, many studies have analysed the association between pollen exposure and hay fever in children and adolescents. Bauchau and colleagues reported pollen as the main exposure for hay fever in Europe (212). In the Southern Hemisphere, especially in Australia, pollen exposure is high and have major implications for health and quality of life. Many studies have found a significant association between pollen exposure and hay fever (213). Another study in United States reported increase in total pollen count was associated with increase prevalence of hay fever in children (214). De Weger and colleagues suggested that pollen count could predict the severity of hay fever symptoms up to 5 days in advance in teenagers, young adults, and adults (215). In conclusion, there is a really strong evidence to support the association between pollen exposure and hay fever, even to say that expose to pollen is one of the main etiological factors for hay fever.

To the best of my knowledge, only one study analysed the association between pollen exposure in early life and hay fever. It is the same study that analysed the association between pollen exposure in early life and asthma. Erbas and team found that pollen exposure in the first 3 months of life is associated with higher odds for hay fever in children aged 6 to 7 years (OR 1.14, 95% CI: 1.009, 1.29) (3).

2.5.5. Potential confounders and effect modifiers

Technically, there are few factors that can be considered as a true confounder in the pollen exposure pathway. The definition of confounder is “The extraneous factors that are responsible for difference in disease frequency between the exposed and unexposed”. To act as a confounding variable, the factor must be both associated with the outcome (cord blood IgE) and the exposure (pollen during pregnancy) (216). Thus, no factor listed below could become a “true” confounder between pollen exposure and IgE, food allergy, or allergic respiratory disease. Because they are neither a) a cause of the exposure (pollen exposure during pregnancy) or b) likely to vary with season of conception. For example, it is unlikely that maternal history of allergic disease will impact the month of conception of the child, which will then impact the pollen exposure during pregnancy. If by chance a third factor that influences the outcome happens to be

associated with exposure, it will bias the associations and adjustment for this will provide a better estimate of the causal effect.

Sternthal et al. (2011) observed that traffic related air pollution was related to cord IgE levels. As pollution also follows a seasonal pattern in many cities, it is possible that pollution exposure in pregnancy is associated with birth during the pollen season. As such, pollution exposure, and other environmental factors that vary with the seasons, including heat and humidity, may act as confounding factors. Due to the limited availability of data, I did not consider this related data in my thesis. It is why I could not exclude the possibility of residual confounding, particularly by environmental exposures that vary across seasons, such as pollution. However, I have included the following factors in the following sub chapters as potential confounder to be consistent with others in the literature.

2.5.5.1. Maternal history of smoking during pregnancy

A study in Boston, United States, found that mothers' history of smoking during pregnancy was associated with higher odds of their infants to have detectable level of cord blood IgE (84). A study on twin children aged 3 to 9 years in Denmark found that children with mothers who smoked during pregnancy had higher odds for asthma (OR 1.7, 95% CI: 1.42, 2.04) (217). Another study among school-aged children in California, United States reported an interaction effect between smoking exposure *in utero* and active smoking in children to increase the risk for developing asthma (218). In addition, many studies considered maternal history of smoking during pregnancy as a confounder. Thus, they adjusted their analysis for maternal history of smoking during pregnancy (203, 219-221). Based on all these previous studies, I adjusted my analysis in chapter 5, 6 and 7.

2.5.5.2. Maternal age

Maternal age could be a marker of parity and economic status. Results from previous studies are mixed with some showing an association with lower cord blood IgE and some other reported no association with food allergy. A study in Iran reported that being an older mother was associated with lower level of cord blood IgE (26). A study in Melbourne, Australia showed that maternal age was not an associated factor for developing egg allergy in infants aged up to 1 year (38). Karr and colleagues found no association between maternal age and asthma or hay fever among twin children aged 3 – 9 years (217). Despite the contradicting results from all those studies, other studies

considered maternal age as confounder in their analysis (203, 220, 222). Thus, I adjusted my analysis in result chapter 5, 6, and 7 for maternal age in order for my analysis results to be comparable with previous studies results.

2.5.5.3. Maternal history of allergic diseases

I considered maternal history of hay fever and food allergy as effect modifiers for the association in my result Chapter 5, 6, and 7, based on results from other studies and our own when we considered early life pollen exposure (37, 122). Maternal history of asthma and hay fever were also considered as potential effect modifiers for the association because previous studies had reported that history of asthma or even allergic diseases in mother were associated with asthma in children (3, 37, 85, 223). I only adjusted and analyzed the interaction with maternal history of asthma and hay fever, instead of father's, based on a meta-analysis results that showed maternal history of asthma is a bigger risk factor for asthma in children (224).

2.5.6. Gaps in knowledge

My literature review shows that many studies have examined the association between pollen exposure and allergic respiratory disease and therefore, I am confident in that outdoor pollen, especially in regions where it is substantially high during peak pollen season is an important risk factor for allergic respiratory disease. Nevertheless, much work still needs to be done on early life exposure and on the role it has on cord blood IgE and food allergy outcomes. None of the previous studies directly measured the pollen exposure, they used pollen season or season of birth as a proxy for pollen exposure.

Windows of exposure during pregnancy and the first 3 months of life are important for immune system development and subsequent allergic diseases in childhood (85). High aeroallergen exposures to pollens and fungi during certain stages of pregnancy (in spring, summer or autumn) could contribute to elevated levels of IgE (225). Combined with shared genetic predisposition, this would further mediate the allergen response. These are important factors that enable a better understanding of maternal environment and at which timepoint that may subsequently alter the development of the child's immune system and potentially be modified to reduce risk of allergic respiratory diseases in children.

Despite many previous studies on this topic, a few studies aimed at pollen exposure in early life. To add to the dilemma, all these studies used different periods of early life to measure the extent of pollen exposure (3-5). One study, that measured the main exposure per each month of birth and reported that exposure in the 11th week of gestational age as an important and critical timepoint (5). Another study measured the main exposure during the last 12 weeks of pregnancy and first 3 months of life (4). One study used the first 3 months of life and first month 4 to 6 of life as critical timepoint for the main exposure of analysis (3). I wanted to extend on these studies and assess these exposure windows concurrently from intra-uterine exposure, at birth, first 7 days, 3 months and 6 months exposure to actual levels of grass pollen in the air. This has never been done before.

2.6. Summary and contribution to knowledge

In summary, in this literature review this work confirms the evidence on the association between pollen exposure and allergic respiratory disease at multiple timepoints in life. This chapter starts by reviewing the association between pollen exposure and IgE at birth because IgE at birth is an indicator of early phase of immune system development that relates to allergic disease in later life (67). There are no previous studies that analysed the association between pollen exposure *in utero* or at birth with IgE at birth. All the previous studies used season of birth or birth in pollen season as a proxy for pollen exposure.

Next, this chapter reviews the published studies on the association between pollen exposure and food allergy focusing on food allergy in infants as this is where behaviour modification could be beneficial with respect to exposure. Food allergy is an important outcome in its own right and may be a predictor for development of allergic respiratory disease. Studies did suggest an association between high levels of pollen exposure with food allergy in childhood. The problem is, there is lack of evidence on whether pollen exposure *in utero* or early life is associated with food allergy in childhood. Only one study addressed the question of the association between pollen exposure *in utero* or early and food allergy, but this was not done in infants and children were much older. This doctoral study intends to extend on this study by assessing the association between pollen exposure during pregnancy and food allergy.

Finally, this chapter shows an abundance of evidence of pollen exposure as a risk factor for developing asthma and hay fever later in childhood but little on pollen exposure in early life. This chapter confirms two published studies that analysed the association between pollen exposure *in utero* or early life and asthma or hay fever in childhood. This doctoral study intends to extend on these two studies to try and clarify where the critical exposure window may begin during pregnancy and early life.

Through this doctoral study, this study aims to fill in the gaps in knowledge that this literature review chapter shows. Throughout all this thesis result chapters, it explores if the impact of pollen exposure starts very early, as early as *in utero*. By analysing the association between pollen exposure and multiple outcomes, this doctoral study aims to show that pollen exposure may have a bigger impact than what previous studies showed. In the first results chapter (chapter 4 of this thesis) the meta-analysis from 10 studies showed that birth in the winter season is associated with higher odds for having higher level of cord blood IgE in northern and southern hemispheres. These findings were published in *Environmental Research* journal. Later, this study extended on the meta-analysis results and addressed the association between pollen exposure (as opposed to season of birth) and cord blood IgE in chapter 5 (This chapter was published in *Environmental International*). For this analysis, this doctoral study used data from three birth cohorts: Melbourne Atopic Cohort Study (MACS) in Melbourne, Australia; Copenhagen Prospective Study of Asthma in Childhood (COPSAC2000) cohort in Denmark, and Life-style Related Factors on the Immune System and the Development of Allergies in Childhood (LISA) cohort in Germany. This doctoral study answers the question on the association with food allergy in chapter 6 (This chapter is accepted for publication in *International Journal of Environmental Health Research*). In that chapter, this study assesses the association in a population-based cohort study of children in Melbourne called HealthNuts. In the last result chapter, chapter 7, this study assesses the association with asthma and hay fever based on the follow up visit at 6, 12 and 18 years of age from children in MACS cohort.

Chapter 3. Methods

In this thesis, the primary exposure is outdoor pollen count to evaluates its association with multiple outcomes which are IgE level in cord blood, food allergy, asthma and hay fever. In chapter 4, it described the methods for the systematic review and meta-analysis between season of birth and IgE level in cord blood. In chapter 5, it detailed the methods to analyse the association between pollen count and IgE level in cord blood using three different birth cohorts. Next, chapter 6 explained the methods to analyse the association between pollen count and food allergy in one-year infants using HealthNuts cohort. Finally, chapter 7 described the methods to analyse the association between pollen count and asthma and hay fever. As the methods to analyse the association are already explained in each related result chapter, this methods chapter will describe the methods that was being used to measure the pollen count.

3.1. Sampling outdoor pollen grains

Daily pollen grains levels were sampled using Burkard 7-day volumetric spore trap. Burkard spore trap is one of the major types of sampling devices for monitoring outdoor aeroallergen particles (226). Aeroallergen data collection using a Burkard spore trap already certified by the American Academy of Allergy, Asthma, and Immunology (226). This Burkard spore trap is a suction slit impact factor based (Figure 1) which it practically draws 10 litres of air per minute into a 14 mm by 2 mm size orifice. The airborne particles in the air are impacted on a microscope slide under the orifice. The slide itself moves past the orifice at 2 mm/hour over 24-hour period (185).

Figure 1. Burkard 7 day recording volumetric spore trap (accessed from http://burkard.co.uk/product/7-day-recording-volumetric-spore-trap/ on 18 December 2019).

The pollen count for my analysis in chapter 5, 6, and 7 was measured from the Burkard spore trap located on the rooftop of the Earth Sciences building at the Parkville campus of the University of Melbourne. This Burkard spore trap site is 15 metres above ground in order to get a location that is clear of building and tree but not higher to avoid getting a high proportion of tree pollen as per guidelines of World Allergy Organisation (226). For chapter 5 and 7, I used pollen count measured daily between October 1991 and January 1994. For chapter 6, I used pollen count measured daily during the peak pollen season each year, 1st of October to 31st of December, in 2007 to 2010.

3.2. Identification and measurement of outdoor pollen grains

The aeroallergen on the slides were identified using microscopy method. Pollen grains were identified and counted under 400x magnification of microscopy. Only a subset of the sample in the slide were identified and counted due to the length of time of time to identify and count the entire slide (185). There are multiple methods to analyse a subset of the slide but analysing the slide at 4-mm intervals is considered to give a better result and give data on pollen concentration every 2 hours per day (185). Pollen grains were identified using accredited identification manuals and reference slide.

Chapter 4 Effect of season of birth on cord blood IgE and IgE at birth: A systematic review and meta-analysis

This result chapter consists of a peer reviewed published article on systematic review and meta-analysis at the beginning of this doctoral study. This article aimed to answer the first research question.

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Effect of season of birth on cord blood IgE and IgE at birth: A systematic review and meta-analysis

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ABSTRACT

Background: Elevated cord blood IgE is important on the pathway to allergic disease. The association between season of birth and infant cord blood IgE is not well-established. Study findings differ on which birth season is associated with higher cord blood IgE risk and its magnitude. We conducted a systematic review and meta-analysis of studies on season of birth and cord blood IgE.

Methods: We searched Medline, Web of Science, Scopus and ProQuest Health databases, and reviewed reference lists of articles that met the inclusion criteria. All included studies measured IgE as a binary variable using various cut-off values. We performed multivariate-random-effects meta-analysis to handle an exposure with multiple categories of Season of Birth.

Results: Our search identified 275 records and 10 had sufficient data to be included in a meta-analysis. Relative to summer, winter birth had the greatest odds of high IgE (≥ 0.1 IU/ml), meta-analysis OR = 1.24 (95%CI: 1.01–1.52). A similar OR, was found for IgE ≥ 0.5 IU/ml, OR = 1.30 (95%CI: 0.99–1.71).

Conclusions: A winter season of birth was associated with statistically significant higher odds of elevated cord blood IgE at cut-off ≥ 0.1 IU/ml but borderline at cut-off ≥ 0.5 IU/ml. This winter effect is likely to be a marker for a range of other environmental exposures during specific stages of pregnancy, such as aeroallergen exposures, maternal infections and vitamin D levels. Further research is required to support our finding and to identify the exact mechanisms that lead to the winter season of birth effect on circulating IgE levels, as this may have implications for allergic disease prevention.

1. Introduction

Globally, allergic respiratory diseases continue to be a major public health burden in children. To date, we still do not have a full understanding of the causes of allergic respiratory diseases. However, we know that environmental factors, such as pollens, moulds, air pollutants and respiratory viruses, play a major part, along with genetic predisposition (Erbas et al., 2013). The adverse impact of outdoor exposures may begin at any time, with high risk windows for exposure including pregnancy, shortly after birth or within the first two years of

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life (Algert et al., 2012; Erbas et al., 2013).

In many studies of high risk children (at least one parent with history of allergic disease) and allergies in early life levels of Immunoglobulin E (IgE) measured in cord blood/at birth have shown to be a useful measure of subsequent risk of asthma and allergies (Ferguson et al., 2009; Kaan et al., 2000). A recent study by Bundhoo et al. showed that maternal IgE and cord blood IgE were highly correlated irrespective of the allergic status of the mothers suggesting that the maternal environment is important (Bundhoo et al., 2015).

Many factors influence IgE levels in cord blood with one of them being season of birth. Many studies have examined the association between season of birth and high cord blood IgE with mixed results. Studies have been conducted since the early 1990s in a range of locations with differences in geography, seasonal characteristics, and population characteristics from regions all over the world, but predominately in high income countries. Few used the same season of birth as the reference category when examining risk. For example, one study compared all seasons to summer (Scirica et al., 2007) and another compared all seasons to winter (Sadeghnejad et al., 2007). Furthermore, the studies that reported significant associations did not agree as to which season of birth had the greatest risk of high IgE, or how large was the actual risk.

The association between season of birth and allergic respiratory diseases has been studied extensively. It is hypothesized that season of birth is an important proxy for exposures at time of conception during pregnancy. For example, season of birth may act as a proxy for maternal exposures to UV-B as higher exposures during pregnancy may lower risk of subsequent allergic disease (Camargo et al., 2011). It may also be a proxy for the seasonal variation of maternal nutrient intake (Watson and McDonald, 2007). Mother's response to viral or bacterial infections in autumn and winter might lead to increased immune response in infants born in spring and summer (Sullivan Dillie et al., 2008).

It is important to synthesise these findings to clarify which months pose the greatest risk, whilst acknowledging differences in latitude, climatic conditions and populations. In doing so, we may be better placed to understand the environmental conditions that contribute to cord blood IgE levels. For example, if cord blood IgE is particularly high in infants born in spring compared to other seasons, then this may point to specific environmental allergens at birth or a particular stage during pregnancy which have adverse effects on subsequent immune development. In this review, we attempt to qualitatively synthesise all of the studies that have examined the association between the season of birth and high IgE in cord blood or at birth, and where possible, quantify the magnitude of these associations by using meta-analytic techniques.

2. Methods

In this systematic review and meta-analysis, we followed the MOOSE guidelines for identifying observational studies for systematic review and meta-analyses (Stroup et al., 2000).

2.1. Inclusion criteria

In order for a study to be included in this review, it had to focus on humans, be published in English, and measure the association between the outcome of IgE in cord blood, or in the first week of life, and the season of birth as the exposure. We considered the first week because evidence suggests a significant correlation between IgE in both cord blood and blood in the first 5 days of life (Hansen et al., 1992). Only studies that provided effect sizes, or sufficient data to allow effect sizes to be calculated, were included in the meta-analysis.

2.2. Search strategy

A literature search was conducted using bibliographic databases: Medline (PubMed), Web of Science, Scopus, Google Scholar and ProQuest Health. The search terms are listed in the online Sup. Table 1. An additional manual search was also conducted based on citations of all articles that met the inclusion criteria. The abstracts of all identified articles were reviewed by authors BE, NHS and AS for initial inclusion; then full papers were reviewed by NHS and BE based on the inclusion criteria.

2.3. Assessment of quality and risk of bias

Two authors (NHS and RT) independently assessed the quality of each study using a validated quality assessment framework (Zaza et al., 2000) in combination with the Cochrane Collaboration risk of bias assessment (Sterne and Reeves, 2014). Quality was assessed using a checklist: description of the study population and how they were selected; how exposure (season of birth) was measured and whether this was valid and/or reliable; whether the outcome (cord blood IgE or a week after birth IgE) measures were valid and/or reliable; the appropriateness of the statistical testing; the appropriateness of controlling potential confounders (maternal age, gestational age, birth order, maternal ethnicity, maternal allergic disease during pregnancy, parental atopy); appropriateness of controlling for potential bias (i.e. selection bias, recall bias, measurement bias, analytic biases related to sample sizes and statistical methods); and whether problems with data analysis limited the interpretation of the results. Any differences between reviewers were resolved by discussion and consensus that led to agreement on final scores. Any paper that reported results as descriptive statistics and correlations which matched their aims were also assessed.

2.4. Data extraction

We extracted the following data from each study: study design; country and whether the city was urban or rural; sample size, IgE measurement method, the grouping of IgE levels and the cut-off that was used for the categorisation if any were reported in the paper, month and/or season of birth; effect estimates including 95% confidence intervals (CI); potential variables considered as confounders and/or effect modifiers.

2.5. Statistical analysis

Three authors (BE, NHS and DV) assessed whether papers could be included in the meta-analysis. Since the exposure of interest (seasons of birth) has four categories, which means that from each study we need to extract three odds-ratios that compare three seasons against the reference season (e.g. Summer). Generally speaking, odds-ratios from the same study are correlated and this within-study correlation cannot be handled by standard meta-analysis. We therefore decided to conduct multivariate random-effects meta-analysis that were developed to handle meta-analysis with multiple outcomes and/or exposure with multiple categories (White, 2011).

One key requirement of multivariate random-effects meta-analysis was determining the amount of correlation among odds-ratios from the same study. To compute the correlations, we needed the raw counts of subjects for each season of births and outcome category (low/high IgE). Hence, only studies that reported these raw counts could be included in our meta-analysis. Studies without this information were excluded and these include studies that reported adjusted or unadjusted odds ratios without providing subject counts by season of birth and outcome. For studies that reported the counts by month of birth, we aggregated the count by season of birth according to the study location. Studies used different cut-offs to define high IgE levels and to investigate the impact of using different cut-offs on our meta-analysis estimate, we performed two separate meta-analyses involving: (a) studies that used IgE $\geq$ 0.1 IU/ml and (b) studies that used IgE $\geq$ 0.5 IU/ml. We chose these two cut-offs as these were the cut-offs used by the majority of studies

included in the review. We used summer as the reference category for our analysis, because none of the studies reported a summer birth associated with the highest cord blood IgE, while some reported significant evidence of summer having the lowest cord blood IgE (Hernandez et al., 2013; Kuiper et al., 2006; Sadeghnejad et al., 2007; Scirica et al., 2007). The multivariate random-effect model allowed us to model between-studies heterogeneity. Due to the multiple group comparisons, the DerSimonian and Laird method (DerSimonian and Laird, 1986) could not be used for the random-effects model and instead a restricted maximum likelihood (REML) approach was used (White, 2011). The I^2 statistics for the multi group comparisons were calculated and a restricted likelihood ratio test for heterogeneity between studies was performed (Jackson et al., 2013).

Using multivariate meta-regression (White, 2011), we also investigated the potential role of latitude as effect modifier. The reasoning behind this analysis was that birth in winter, in a lower latitude like Mexico (Hernandez et al., 2013) may have a different influence than birth in winter in a much higher latitude such as Sweden (Scirica et al., 2007), as the contrast between winter and summer in these two places can be quite different. For studies that recruited participant from a single city, the latitude of that city was used in the meta-regression, while for studies that had participants from more than one city, a mean latitude was used. All of the meta-analysis and meta-regression was performed using the mvmeta command in Stata™ version 14.0 (StataCorp, Texas, TX, USA) statistical package.

3. Results

3.1. Search results

Our search found 388 records. After removing duplicate entries, there were 272 unique records. We assessed 31 records as full articles and found 3 additional records from the reference lists. Once two authors (BE and NHS) had reviewed the full texts, 14 were excluded because they did not have any relevant results about the association between season of birth and IgE at birth. Twenty articles were included in the systematic review and ten in the meta-analysis (Fig. 1).

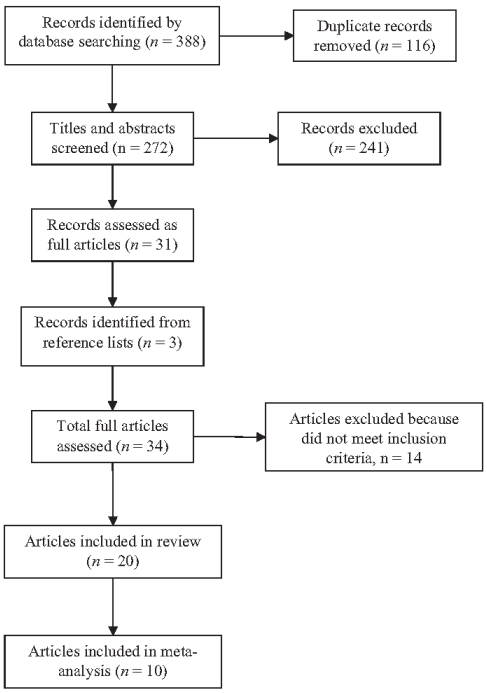

Fig. 1. Flow diagram of record selection.

3.2. Quality assessment

Using the quality assessment framework most studies scored quite highly (above 70%) (Bergmann et al., 1995; Bjerke et al., 1994; Croner and Kjellman, 1992; Ferguson et al., 2009; Halonen et al., 1991; Hansen et al., 1992; Hernandez et al., 2013; Kaan et al., 2000; Kerkhof et al., 2005; Kimpen et al., 1987; Kuiper et al., 2006; Lendor et al., 2008; Sadeghnejad et al., 2007; Schönberger et al., 2005; Scirica et al., 2007; Sternthal et al., 2009; Van Gool et al., 2004; Zittermann et al., 2004) as they identified potential biases, discussed limitations and assessed the role of other variables as confounders and/or effect modifiers. The two studies that scored low (Croner and Kjellman, 1986; Nabavi et al., 2013) were characterized by limited reporting of the sample, use of only correlational statistical methods, and did not identify and/or adjust for variables that might lead to potential over-estimation of the levels of association, namely gestational age, maternal atopy or maternal allergic disease. Most studies that were chosen for meta-analysis scored highly (Ferguson et al., 2009; Hernandez et al., 2013; Kaan et al., 2000; Kerkhof et al., 2005; Kimpen et al., 1987; Kuiper et al., 2006; Sadeghnejad et al., 2007; Scirica et al., 2007; Sternthal et al., 2009) except for one (Croner and Kjellman, 1986) which reported results as correlational statistics. This study was included as the associations between season of birth and IgE were calculated from the data reported.

3.3. Characteristics of the studies

The 20 articles included in the review were derived from 18 studies (two pairs of articles used data from the same studies). Detailed description of all studies included in the review are provided in Table 1. All the analyses were cross-sectional in design, but the majority were nested within birth-cohorts (17 of 20). Most of the articles (13 of 20) recruited both high risk subjects, defined as at least one first-degree atopic family member, and low risk subjects, defined as absence of atopic family member (Bergmann et al., 1995; Bjerke et al., 1994; Croner and Kjellman, 1986; Hernandez et al., 2013; Kerkhof et al., 2005; Kuiper et al., 2006; Lendor et al., 2008; Nabavi et al., 2013; Sadeghnejad et al., 2007; Schönberger et al., 2005; Scirica et al., 2007; Sternthal et al., 2009). Three of them recruited high risk subjects only (Ferguson et al., 2009; Kaan et al., 2000; Van Gool et al., 2004) and four of the articles did not mention the characteristics of the subjects (Halonen et al., 1991; Hansen et al., 1992; Kimpen et al., 1987; Zittermann et al., 2004). The sample sizes considered varied between 96 (Zittermann et al., 2004) and 6401 subjects (Bergmann et al., 1995). The majority of the studies were conducted in the United States of America and the Netherlands, followed by Germany, Canada, Sweden, and Denmark (4, 4, 2, 2, and 2 out of 20 studies, respectively). Most of the studies were conducted in an urban area (16 of 20) (Bergmann et al., 1995; Bjerke et al., 1994; Croner and Kjellman, 1986, 1992; Ferguson et al., 2009; Halonen et al., 1991; Hansen et al., 1992; Hernandez et al., 2013; Kaan et al., 2000; Kimpen et al., 1987; Lendor et al., 2008; Nabavi et al., 2013; Scirica et al., 2007; Sternthal et al., 2009; Van Gool et al., 2004; Zittermann et al., 2004) and the rest of them were conducted in both urban and rural areas (Kerkhof et al., 2005; Kuiper et al., 2006; Sadeghnejad et al., 2007; Schönberger et al., 2005).

Most of these 20 studies reported IgE as a dichotomous variable, but cut-off levels varied (Bergmann et al., 1995; Bjerke et al., 1994; Croner

Table 1
Summary of the characteristics including populations and IgE measurements of studies included in the review.

Author and year	Location & study year	Sample size	Exposure definition	Outcome definition	Effect size	Included in meta-analysis
Continuous measurements						
Nabavi et al. (2013)	Semnan, Iran (2006–2007)	181	Season of birth: Winter, Spring, Summer, Autumn	Cord blood IgE (continuous level)	Effect size estimates for cord blood IgE level; Ref: born outside winter season: -0.227 (SE: 0.056) IU/ml Adjusted for: parity, maternal age, type of delivery, maternal IgE, allergy during pregnancy	No
Van Gool et al. (2004)	Limburg and Noord-Brabant, Netherland (Oct 1997–Apr 2000)	178	Season of birth: • First (Jan–Mar) • Second (Apr–Jun) • Third (Jul–Sep) • Fourth (Oct–Dec)	Total blood IgE at 1 week of age (continuous level)	Regression coefficient for ln IgE ku/l Ref: Oct-Dec: Jan–Mar -1.68 (SE 0.46); Apr–Jun -0.67 (SE 0.46); Jul–Sep -0.42 (SE 0.42) Adjusted for: parity, mother age, mother IgE, smoking during pregnancy, father allergy, child's gender, type of feeding	No
Zittermann et al. (2004)	Bonn, Germany (No data)	96	Summer half year, Winter half year	Cord blood IgE (continuous level)	No effect size reported	No
Categorical measurements						
Hernandez et al. (2013)	Mexico (No data)	613	Season of birth: Summer, Autumn, Winter, Spring	Detectable total cord blood IgE at birth (cut-off 0.1 IU/ml)	Effect size OR; Ref Summer; Spring 1.60 (0.99–2.58), Autumn 1.44 (0.89–2.31), Winter 1.53 (0.98–2.40) Adjusted for: treatment, mother allergy, child's gender, birth order, pesticide in home, socioeconomic level.	Yes
Halonen et al. (1991)	Arizona, USA (May 1980–Oct 1984)	1074	Month of birth: Jan–Dec	Detectable cord blood IgE (cut-off 0.1 IU/ml)	No effect size; only showed proportion in each birth month but not with IgE levels.	No
Scirica et al. (2007)	Boston, USA (Sept 1994–Jun 1996)	874	Season of birth: Winter, Spring, Summer, Fall	Detectable cord blood IgE (cut-off 0.2 IU/ml)	Effect size unadjusted OR; Ref Summer; Autumn 1.54 (1.00–2.36), Winter 1.12 (0.74–1.70), Spring 1.45 (0.97–2.17)	Yes
Bergmann et al. (1995)	5 cities in Germany (Jan–Dec 1990)	6401	Month of birth: Jan–Dec	Total cord blood IgE (cut-off 0.35 kU/l)	No effect size and no reported data; only mentioned that highest undetectable CB IgE is in Jan and the lowest in Aug	No
Ferguson et al. (2009)	Vancouver & Winnipeg, Canada (Oct 1994–Aug 1996)	289	Season of birth: Winter, Spring, Summer, Fall	Detectable cord blood IgE (cut-off 0.5 kU/l)	No effect size reported for season of birth on cord blood IgE.	Yes
Sadeghnejad et al. (2007)	Isle of Wight, UK (Jan 1989–Feb 1990)	798	Season of birth: Winter, Spring, Summer, Fall	Cord serum IgE (cut-off 0.5 kU/l)	Effect size unadjusted OR; Ref Winter: Summer 0.99 (0.63–1.57), Autumn 1.56 (1.02–2.37), Spring 1.05 (0.67–1.64)	Yes
Kuiper et al. (2006)	Netherlands (1997–2002)	529	Season of birth: Winter, Spring, Summer and Autumn	Total IgE from blood stratified into different cut-offs, 0.5–0.9 IU/ml	Effect size OR; Ref. Spring; Cut-off 0.9 IU/ml: Summer 1.35 (0.58–3.16), Autumn 1.42 (0.57–3.58), Winter 1.47 (0.62–3.52). Adjusted for: maternal asthma, paternal and/or sibling asthma, number of siblings, maternal intake supplement during pregnancy	Yes
Schönberger et al. (2005)	Maastricht, Netherlands (No data)	174	Season of birth as: Spring/summer and Autumn/winter	Total IgE at birth (cut-off 0.5 IU/ml)	No effect size; reported about multiple logistic regression analysis, but no results mentioned	No
Kerkhof et al. (2005)	Netherlands (May 1996–Dec 1997)	1027	Season of birth Winter, Spring, Summer and Autumn	Blood IgE at 1 week (cut-off 0.5 IU/ml)	Effect size OR; Ref Spring; Results also stratified by mother's allergic history. Results for all: Summer 1.3 (0.7–2.5), Autumn 1.2 (0.6–2.2), Winter 1.0 (0.6–1.9) Adjusted for: presence of sibling, cat during pregnancy, dog during pregnancy, gestational age, birth weight, mother age, mother smoking during pregnancy, caesarean section, born in hospital,	Yes
Kaan et al. (2000)	Vancouver & Winnipeg, Canada (Oct 1994–Aug 1996)	384	Season of birth: Winter, Spring, Summer, Fall	Total cord blood IgE (cut-off 0.5 kU/l)	No effect size but raw data reported	Yes
Bjerke et al. (1994)	Aarhus, Denmark (Feb 1990–May 1991)	2631	Season of birth: Winter, Spring, Summer, Autumn	Cord serum IgE (cut-off 0.5 kU/l)	No effect size reported	No
Croner and Kjellman (1992)	Sweden (Dec 1974–Dec 1975)	1654	Month of birth: Jan–Dec	Elevated cord blood IgE (cut-off 0.9 kU/l)	No effect size; only mentioned that the proportion of high CB IgE is similar for births in Mar–May and Aug–Oct	No
Croner and Kjellman (1986)	Sweden (Dec 1974–Dec 1975)	1652	Month of birth: Jan–Dec	Cord blood IgE (cut-off 0.9 kU/l)	No effect size reported	Yes

(continued on next page)

Table 1 (*continued*)

Author and year	Location & study year	Sample size	Exposure definition	Outcome definition	Effect size	Included in meta-analysis
Kimpen et al. (1987)	Belgium (1985)	5353	Month of birth: Jan–Dec	Abnormal cord blood IgE level (cut-off 1.0 IU/ml)	No effect size; reported number of cases per month	Yes
Sternthal et al. (2009)	Boston, USA (Aug 2002–Jan 2007)	478	Season of birth: Winter, Spring, Summer, Fall	Cord blood IgE (low and high, cut-off 1.08 IU/ml)	No effect size reported	Yes
Hansen et al. (1992)	Denmark (Nov 1983–Oct 1984)	2814	Month of birth: Jan–Dec	Elevated cord blood IgE (cut-off > mean + 2 SD)	No effect size but data provided in graph	No
Lendor et al. (2008)	New York, USA (Jan 1998–Jul 2006)	275	Season of birth: winter and non-winter	Cord blood IgE (no further data)	No effect size; only mentioned that no association between winter birth and CB IgE (data not shown)	No

and Kjellman, 1986, 1992; Ferguson et al., 2009; Halonen et al., 1991; Hansen et al., 1992; Hernandez et al., 2013; Kaan et al., 2000; Kerkhof et al., 2005; Kimpen et al., 1987; Kuiper et al., 2006; Lendor et al., 2008; Sadeghnejad et al., 2007; Schönberger et al., 2005; Scirica et al., 2007; Sternthal et al., 2009), while three of the remaining studies treated IgE as a continuous variable (Nabavi et al., 2013; Van Gool et al., 2004; Zittermann et al., 2004). IgE was measured from cord blood (80%) (Bergmann et al., 1995; Bjerke et al., 1994; Croner and Kjellman, 1986, 1992; Ferguson et al., 2009; Halonen et al., 1991; Hansen et al., 1992; Hernandez et al., 2013; Kaan et al., 2000; Kimpen et al., 1987; Lendor et al., 2008; Nabavi et al., 2013; Sadeghnejad et al., 2007; Scirica et al., 2007; Sternthal et al., 2009; Zittermann et al., 2004) or 1-week-old infant blood (20%) (Kerkhof et al., 2005; Kuiper et al., 2006; Schönberger et al., 2005; Van Gool et al., 2004). Most studies used assays manufactured by Pharmacia to measure IgE (Bergmann et al., 1995; Croner and Kjellman, 1986, 1992; Ferguson et al., 2009; Halonen et al., 1991; Hansen et al., 1992; Hernandez et al., 2013; Kaan et al., 2000; Sadeghnejad et al., 2007; Scirica et al., 2007; Van Gool et al., 2004). One study used RIA assay manufactured by DPC (Zittermann et al., 2004), another used ELISA manufactured by Pishtazteb (Nabavi et al., 2013), and one study used two assays which are Pharmacia CAP and Diagnostic Product IRMA (Lendor et al., 2008). Four studies used in house assays (Bjerke et al., 1994; Kimpen et al., 1987; Kuiper et al., 2006; Schönberger et al., 2005) and one study did not describe the assay they used (Kerkhof et al., 2005). Although those studies used assays by different manufacturers or in house, except for one study which did not mention the assay, they all used similar and comparable assays (Bousquet et al., 1990).

3.4. Overall results

Six of twenty articles reported the effect size of season of birth on IgE as categorical but different reference categories were used (Table 1): three articles used summer as the reference season of birth (Hernandez et al., 2013; Scirica et al., 2007; Zittermann et al., 2004), two used spring (Kerkhof et al., 2005; Kuiper et al., 2006), and one used winter (Sadeghnejad et al., 2007). One article reported the effect size from a linear regression by dividing time of birth into January – March, April – June, July – September, and October – December (Van Gool et al., 2004). Two articles reported no numbers in text, tables or figures and just reported that they found no associations between season of birth and cord blood IgE levels (Bergmann et al., 1995; Lendor et al., 2008). One article included the number of births for each month in a table, but did not undertake any further analysis and only reported that similar levels of cord blood IgE were observed among children born in March–May compared to August–October (Croner and Kjellman, 1992) (Table 2).

Five articles only showed the data per month of birth (Bjerke et al., 1994; Croner and Kjellman, 1986; Halonen et al., 1991; Hansen et al., 1992; Kimpen et al., 1987). A study of 1652 births only reported the distribution of children born in each month and proportions of IgE ≥0.9 kU/l (Croner and Kjellman, 1986). Kimpen et al. (1987) studied 5353 births, reporting the distribution of children born in each month and proportion of IgE > 1.0 IU/ml and concluded that more children with elevated IgE were born in spring (March, April, and May). Halonen et al. (1991) studied 697 children, but only reported the proportion of

Table 2
Pooled ORs and 95% CIs of the meta-analysis.

Season of birth	Cut-off ≥ 0.1 IU/ml 10 studies		Cut-off ≥ 0.5 IU/ml 8 studies	
	OR (95% CI)	*p*-value	OR (95% CI)	*p*-value
Spring	1.05 (0.81–1.35)	0.71	0.91 (0.67–1.24)	0.57
Autumn	1.17 (0.92–1.48)	0.20	1.13 (0.82–1.55)	0.45
Winter	1.24 (1.01–1.52)	0.04	1.30 (0.99–1.71)	0.06

children born with detectable IgE ≥0.1 IU/ml in each month. Hansen et al. (1992) also only reported the proportion of 2814 children who were born with elevated IgE ≥ 0.5 IU/ml in each month and stratified the data by sex. Bjerke et al. (1994) only reported the proportion of 2631 children who were born with elevated IgE > 0.5 kU/l in each month.

Five articles reported the association between the birth season and proportions with total IgE greater than a predefined level (Ferguson et al., 2009; Kaan et al., 2000; Nabavi et al., 2013; Schönberger et al., 2005; Sternthal et al., 2009). Kaan et al. (2000) reported 27.2% of children who were born in winter had IgE ≥0.5 kU/l, 10.8% of children who were born in spring had IgE ≥0.5 kU/l and there was a significant variation in proportions of children with detectable IgE by season of birth without reporting effect sizes in text or tables. Schönberger et al. (2005) reported 20% born in spring/summer and 27% born in autumn/winter had IgE ≥0.5 IU/ml and reported no association between season of birth and high IgE (only in text with no differences or estimated effect sizes reported in text or tables). Sternthal et al. (2009) reported the highest proportion of all children who had high IgE were born in winter (33%), lowest were born in summer (17%), but the differences were not significant without any estimated effect sizes reported in text or tables. Ferguson et al. (2009) reported the highest proportion of all children who had detectable IgE were born in winter (40%), lowest in summer (18.2%) and only reported the adjusted OR for birth in winter was 4.07 (95% CI 1.01–4.84) in text without which season of birth is the reference or other season of birth effect sizes. Nabavi et al. (2013) provided the median IgE level for children born in each season of birth, highest for children born in winter, lowest in autumn, and reported the effect size based on linear regression analysis for winter compared to non-winter season of birth.

3.5. Meta - analysis

The forest plot has been grouped by the season: autumn, spring and winter. Within each season, we see the estimate from each study for the odds of being in the higher IgE level category compared to summer. The pooled estimates have been calculated using a random effects multivariate meta-analysis. When we pooled all of the studies that used a cut-off IgE level of ≥ 0.1 IU/ml, winter had the highest odds ratio (OR = 1.24, 95% CI = 1.01–1.52) (Fig. 2). There was evidence of heterogeneity between study estimates (restricted likelihood ratio test for heterogeneity p = 0.008). A similar trend was observed for winter when a cut-off of ≥ 0.5 IU/ml was used, although the 95% CI was wider and included the null (Fig. 3). We examined latitude as an effect modifier and found that the effect size (in terms of odds-ratio) of winter (vs summer) was close to 1 for higher latitudes (defined as being further away from the equator). However, this effect modification by latitude was not statistically significant for any combination of seasons (p > 0.2). As a sensitivity analysis, we removed (Hernandez et al., 2013) as this study with the largest OR was conducted closer to the equator, but the OR = 0.98 (95% CI 0.94-1.02) for the interaction term with winter did not change markedly and was still non-significant (p = 0.34).

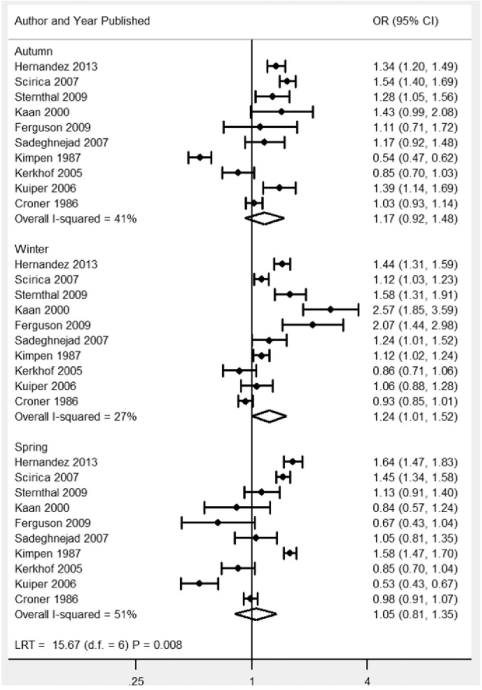

Fig. 2. Associations between season of birth and cord IgE, using cut-off level ≥ 0.1 IU/ml. Estimates are expressed as odds ratios (ORs) and 95% confidence intervals (CIs) pooled using a random-effects meta-analysis. LRT is restricted likelihood ratio test for heterogeneity between seasonal estimates. Studies ordered by latitude from nearest to farthest from equator.

4. Discussion

This systematic review identified evidence that birth during winter is associated with the greatest risk of elevated IgE levels in cord or neonatal blood, when compared to birth during summer. Elevated total IgE in cord blood maybe be an indicator of higher risk. As there is no specific cut-off value that is used to define “elevated” levels, as demonstrated by the range of values identified in our systematic review, we have elected to report associations for both ≥ 0.1 IU/ml and ≥ 0.5 IU/ml. While the estimates of the winter effect at both cut off points are quite similar, the confidence interval for our pooled estimate for > 0.5 IU/ml included the null. This appears likely to be due to there being more IgE values at ≥ 0.1 IU/ml threshold than ≥ 0.5 IU/ml, for any given population, so the effect estimate for ≥ 0.5 IU/ml has been less precisely estimated. Our qualitative synthesis showed that of all the studies that reported significant results with the season of birth as a risk factor for a high IgE level, there were significant results for different cut-off values for both categorical and continuous IgE measurements. Conventional meta-analysis methods could not be used in this setting because season of birth has four categories and different studies used different season as the reference category in assessment of associations. We chose summer as the reference category, because none of the studies reported summer as the highest risk season of birth for a high IgE level.

Exposure windows during pregnancy and the first year of life are important for immune development and subsequent allergic diseases in childhood (Kuzume and Kusu, 2007). Our review showed winter birth to be associated with the highest cord blood IgE levels. There are a number of potential explanations for these associations. A number of fungal spore taxa have been identified as aeroallergens (for example, *Alternaria, Cladosporium, Penicillium/Aspergillus, Coprinus*) (Green et al., 2003; Simon-Nobbe et al., 2008). They are ubiquitous and demonstrate varying seasonal peaks depending on their reproductive processes and the meteorological conditions (Burge, 2002). Pregnant women may be exposed to varying levels of different types of ambient fungal spores during the pollen season (late spring through summer) and through low pollen times (winter), potentially increasing maternal exposure to aeroallergens. In addition to outdoor environmental exposures, evidence suggests maternal exposures to indoor allergens such as dust

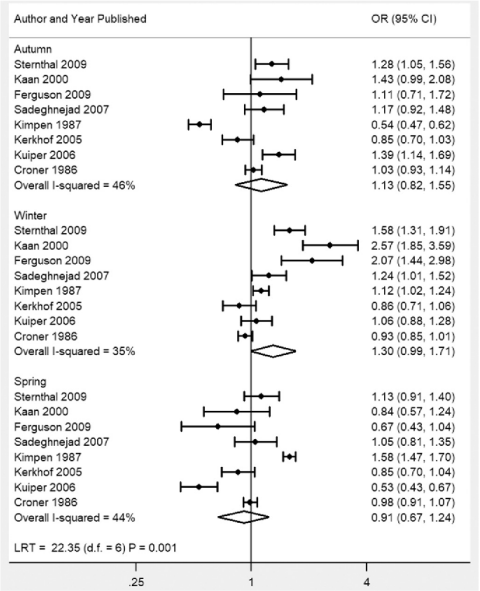

Fig. 3. Associations between season of birth and cord blood IgE, using cut-off level ≥ 0.5 IU/ml. Estimates are expressed as odds ratios (ORs) and 95% confidence intervals (CIs) pooled using a random-effects meta-analysis. LRT is restricted likelihood ratio test for heterogeneity between seasonal estimates. Studies ordered by latitude from nearest to farthest from equator.

mites are also associated with high IgE levels in cord blood of children born in late autumn and early winter (Bjerke et al., 1994). Lack of maternal sun exposure during autumn and early winter may also increase risk of vitamin D deficiency. An experimental study on cord blood found that vitamin D inhibits IL 4 that stimulates the production of IgE (Pichler et al., 2002). It might explain why children born in winter have high cord blood IgE, because they have lower vitamin D levels due to lower exposure to sun light and that leads to higher levels of IL 4 and IgE in cord blood. Our meta-analysis showed a weak but significant effect of winter birth on elevated cord blood IgE at Cut-off ≥ 0.1 IU/ml, and borderline at cut-off ≥ 0.5 IU/ml. The pooled effect size at both cut-offs were crude due to limitations with data availability.

Increasing evidence suggests cord blood IgE is a marker for children who may develop sensitization and wheeze. A study reported that cord blood IgE was a predictor of food allergy/urticaria at 12 months (Kaan et al., 2000). Numerous studies have shown associations between cord blood IgE and allergic sensitisation during childhood: at four (Tariq et al., 1999); five (Hansen et al., 1993); seven (Ferguson et al., 2009) and ten years of age (Sadeghnejad et al., 2004). In fact, the study that reported associations with allergic symptoms and positive skin prick tests at age 5 years also showed associations with allergic rhinoconjunctivitis at age 20 years (Pesonen et al., 2009).

It is possible that children with high cord blood IgE are more sensitive to allergens that make them more susceptible to allergic diseases in early life (Wen et al., 2011). And it is also possible that children have high cord blood IgE because of specific genetic polymorphisms, which may lead to higher risk of allergic diseases (Chen et al., 2009). Increased sensitivity to allergens and the existence of specific genetic polymorphisms in children who have high cord blood IgE may explain why cord blood IgE could predict allergic diseases in early life.

This systematic review has a number of strengths. Firstly, although the sample size varied among studies the majority were large, with a median sample size of 571 subjects and we were able to estimate effect sizes from the papers included in the meta-analysis. We extended the conventional meta-analysis to a different multivariate meta-analysis method to be able to combine the findings from multiple studies and resolve the uncertainty about which season poses greatest risk of high cord blood IgE. However, the review also has several issues that limit the value of its results. Firstly, the original studies considered used multiple methods of analysing the IgE level, treating it as both a continuous and a categorical measurement although all the studies included in meta-analysis used categorical measurement. There was also no consistent cut-off used for the categories. Few studies provided sufficient data to include in our meta-analysis. We were unable to conduct proper meta-regression analysis because studies that reported adjusted findings did not provide the necessary data to include in the meta-regression. Although we did include latitude as an effect modifier, these factors did not substantially alter the findings.

In conclusion, we have shown that a winter birth is associated with a weak increase in levels of cord blood IgE at cut-off ≥ 0.1 IU/ml, but borderline at cut-off ≥ 0.5 IU/ml. These findings, although moderate, provide further insights into the possible mechanisms that may contribute to early immune development. eason being a marker of high allergen environmental exposure during specific stages of pregnancy, combined with a genetic predisposition to allergic respiratory disease may adversely affect immune development. Further understanding the stage in pregnancy that is more vulnerable and to which season-related environmental exposure, in time may contribute to a reduction in the likelihood of allergic respiratory diseases later in childhood.

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Ethics

The study was a systematic review, as such no ethical approval was needed.

Competing interests

There are no competing interests to declare.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.envres.2017.05.026.

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Supplementary Table 1. Search terms used for the search strategy.

Search no.	Search terms
1	IgE
2	Cord blood
3	Newborn
4	Neonatal and neonate truncated.
5	Birth season
6	Birth month
Search terms 1	1 AND 2 AND (5 OR 6)
Search terms 2	1 AND 3 AND (5 OR 6)
Search terms 3	1 AND 4 AND (5 OR 6)

Chapter 5 Environmental grass pollen levels *in utero* and at birth and cord blood IgE: Analysis of three birth cohorts

This result chapter consists of a peer reviewed published article of original research to examine the association between grass pollen exposure during pregnancy and in early life with cord blood IgE. This article aimed to answer the second research question.

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Environmental grass pollen levels *in utero* and at birth and cord blood IgE: Analysis of three birth cohorts

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Grass pollen
In utero
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ABSTRACT

Background: Early life factors are associated with allergic respiratory diseases, but the role of high grass pollen concentrations during pregnancy and shortly after birth is not known.
Objective: To assess outdoor levels of grass pollen during the intrauterine period and at birth during peak pollen season on cord blood IgE in birth cohorts.
Methods: Three birth cohorts were included: MACS (n = 429), Australia; COPSAC$_{2000}$ (n = 200), Denmark; and LISA (n = 1968), Germany. Cord blood IgE was categorized (< 0.5 kU/L, 0.5–1 kU/L, > 1 kU/L) and dichotomized (high IgE ≥ 0.5 kU/L). Birth during the grass pollen season months and cumulative exposure to outdoor grass pollen counts during pregnancy with cord blood IgE were analysed using multinomial regression and analysed in meta-analysis using binomial regression adjusted for potential confounders.
Results: Birth during the grass pollen season had higher pooled odds of cord blood IgE > 0.5 kU/L 1.37 (95% CI 1.06, 1.77) in a meta-analysis with little heterogeneity between the three cohorts. Cumulative exposure to outdoor grass pollen counts during the entire pregnancy was associated with slightly lower pooled odds but significant (OR = 0.98, 95% CI: 0.96 to 0.99).
Conclusions: Birth during grass pollen seasons were associated with increased risk of high cord blood IgE in cities from both hemispheres, but high pollen loads in the environment during the entire pregnancy appeared protective. As IgE responses develop during the first months of life, our study findings provide new insights into the mechanisms of grass pollen exposure at birth and shortly after on possible allergic respiratory diseases.

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1. Introduction

Globally, especially in developed countries (Asher et al., 2006) allergic respiratory diseases in children are an important public health problem, and understanding the role of allergen exposure on IgE could help in managing this problem. Cord blood IgE may be important in identifying children at risk of developing allergic diseases. Although studies analysing cord blood IgE have been mixed, most of these studies found higher levels of IgE in cord blood in infants who go on to develop allergies in later childhood (Tariq et al., 1999; Ferguson et al., 2009). Understanding factors that may be associated with cord blood IgE, may help to understand the risk profile in the early life of children who are at risk of allergic diseases.

Season of birth as a possible marker of environmental allergen exposure has been consistently associated with cord blood IgE (Susanto et al., 2017). However season of birth may act as a proxy for other prenatal exposures such as ultraviolet-B (Camargo Jr et al., 2011), seasonal variations in maternal nutrient intake (Watson & McDonald, 2007) or viral infections during pregnancy (Sullivan Dillie et al., 2008). Little is known about cord blood IgE when exposed to high levels of pollen during pregnancy. To our knowledge, only two studies have reported the effect of exposures to pollen during pregnancy and risk of allergic disease in childhood. One study reported that exposure to high levels pollen in the last 12 weeks of pregnancy was associated with increased risk of asthma hospitalization during the first year of life (Lowe et al., 2012). Another reported a trend that high exposures to birch pollen during pregnancy increased the risk of sensitization to birch pollen at 5 years of age (Kihlström et al., 2003).

Studies including our own, have shown that outdoor pollen exposure during the first couple of months after birth is important in subsequent allergic respiratory diseases (Erbas et al., 2013; Kemp et al., 2009). We hypothesized that it may also be important during the pregnancy, especially during the 2nd and 3rd trimesters where the immune system develops (Holt & Jones, 2000) and among women with a history of allergic disease. In this analysis, using three cohorts from different countries with varying pollen seasons and distributions, we sought to investigate what role pollen concentrations in the outdoor environment have during pregnancy, at birth during months where outdoor levels of pollen peak (pollen seasons) on cord blood IgE. We specifically focussed on grass pollen as this is the predominant species with longer seasons and highest allergenic load and common to all regions included in this study.

2. Methods

2.1. Study design and population

This study was a cross-sectional analysis of data from the Melbourne Atopic Cohort Study (MACS) in Australia, Copenhagen Prospective Study of Asthma in Childhood ($COPSAC_{2000}$) cohort in Denmark, and Life-style Related Factors on the Immune System and the Development of Allergies in Childhood (LISA) cohort in Germany. Details of these studies, recruitment and data collection have been described previously (Bisgaard, 2004; Bisgaard et al., 2006; Bisgaard et al., 2007; Heinrich et al., 2002; Lowe et al., 2006). All studies required written informed consent from participating parents. In general, detailed demographic, environmental and familial allergy data were obtained at birth for all birth cohorts.

Briefly, 620 babies were enrolled into MACS by recruiting pregnant women living in Melbourne, Australia between March 1990 and November 1994. Infants were eligible to be enrolled if they had at least one-first-degree family member with a history of eczema and/or asthma and/or allergic rhinitis and/or severe food allergy. Ethical approval for MACS was given by the Mercy Maternity Hospital Ethics Committee.

$COPSAC_{2000}$ recruited 411 children between 1998 and 2001 from Copenhagen, Denmark, born to mothers with a verified history of asthma. The study was approved by Copenhagen Ethics Committee and the Danish Data Protection Agency.

LISA recruited a population based sample of 3097 healthy, term-born neonates between 1997 and 1999 from obstetric clinics in Munich, Leipzig, Wesel and Bad Honnef, Germany. Neonates displaying any of the following excluded from the study: preterm birth (maturity < 37 gestational weeks), low birth weight (< 2500 g), congenital malformation, symptomatic neonatal infection, antibiotic medication, hospitalization or intensive medical care during neonatal period. In addition, newborns from mothers with immune-related diseases (autoimmune disorders, diabetes, hepatitis B), on long-term medication or who abuse drugs and/or alcohol, and newborns from parents with a nationality other than German or who were not born in Germany, were excluded. Approval was given by the Ethics Committees of the Bavarian Board of Physicians, University of Leipzig and the Board of Physicians of North-Rhine-Westphalia. Written consent from the participant's families was obtained. A requirement for each cohort was that all children were born full-term.

2.2. Pollen collection and measurement

Pollen data of all three study areas (Australia, Denmark, Germany) were collected using a 7-day volumetric spore trap (Burkard) (Hirst, 1952). Burkard trap for MACS was located on the roof top of the Earth Sciences building, The University of Melbourne in Melbourne. For COPSAC, the pollen trap was placed at the Danish Meteorological Institute in Copenhagen. For LISA, volumetric spore traps were located within the city limits of Bochum, Dresden and Munich on the roofs of hospital buildings or university buildings. For each city, all traps were located > 15 m above the ground as per standard in pollen monitoring. Airborne grass pollen grains were trapped on an adhesive surface and identified under a light microscope. The daily average grass pollen counts are expressed as pollen grains/m^3 of air. High grass pollen concentrations in Melbourne appear usually between October and December. Daily pollen concentrations for grass were only available between October 1991 and January 1994 from one pollen trap located at the University of Melbourne. Grass pollen season in all study centers of LISA were detected usually between end of April and end of August with maximum concentrations between May to June. Daily average pollen concentrations of the three pollen monitoring stations in Bochum, Dresden and Munich were used for Germany, available for the whole period of birch and grass pollen season between 1997 and 1999. The grass pollen season in Copenhagen, Denmark, was from May to August with the peak between May to June. Daily pollen data in Copenhagen were available during the pollen season for all the year of births of $COPSAC_{2000}$. In both LISA and $COPSAC_{2000}$ the birch and grass pollen seasons overlap somewhat but here we define the season as the grass pollen season as our focus is on grass pollen.

2.3. Cord blood collection and measurement

In MACS, cord blood was collected from the umbilical vein at birth and is available for $n = 429$ of all participants. Total IgE was measured using solid-phase radio-immunoassay based on a sandwich technique, RIA Ultra kit (Pharmacia, Uppsala, Sweden), with a detection limit of 0.5 kU/L. Cord blood also measured for IgA using low-level IgA radial diffusion plates (Behring AG, Germany), samples with IgA levels > 32 μg/L were considered as contaminated by maternal IgE and removed from analysis (Ownby et al., 1996).

In $COPSAC_{2000}$, cord blood was collected by needle puncture of the umbilical cord vein. A total of $n = 200$ were available for analysis. Total IgE level was measured *via* ImmunoCAP assay (Phadia AB, Uppsala, Sweden) with a very low detection limit 0.1 kU/L. Total IgA was analysed using ELISA assay with a very low-level detection limit 0.1 μg/L (analysed by Phadia AB). Samples with IgA levels > 50 μg/L were considered as contaminated by maternal IgE and removed from

the analysis (Bonnelykke et al., 2010).

In LISA, cord blood samples were collected during recruitment in maternity wards as described previously (Heinrich et al., 2002). A total of $n = 1968$ cord blood samples were available for analysis.

Total-serum IgE was determined with the Pharmacia system (neonate IgE kit; Pharmacia, Freiburg, Germany). The limit of detection was 0.35 kU/L. IgA was also determined to exclude cord blood contaminated by the mothers' blood (Heinrich et al., 2002; Ownby et al., 1996). No single IgA level exceeded the cut-off point of 32 μg/L.

2.4. Definition of outcomes/exposures for statistical analysis

2.4.1. Outcome definitions

We defined IgE levels based on three cutoffs: low (< 0.5 kU/L), moderate (0.5 to 1 kU/L), and high (> 1 kU/L). We used 0.5 kU/L as the limit for the lowest group because this was a frequent cut-off level in previous studies to detect IgE in newborns (Kaan et al., 2000; Kerkhof et al., 2005; Kuiper et al., 2006; Sadeghnejad et al., 2007) and it was also the highest detection limit among all cohorts in this study. We used 1.0 kU/L as the limit for highest group as it was the highest IgE level used in previous studies to detect IgE in newborns (Kimpen et al., 1987; Sternthal et al., 2009).

We also dichotomized IgE levels into low (< 0.5 kU/L) and high (≥0.5 kU/L) to be used for the meta-analysis.

2.4.2. Exposure definitions

We defined grass pollen exposure in multiple ways: First, we defined pollen exposures as being born inside or outside pollen season where pollen season was defined as months where the grass pollination occurs in each region. For example, in Melbourne the main flowering period for the grasses is from October to December. Born inside refers to the child being born during this pollen season period. Second, we defined the variable, cumulative grass pollen measurements during entire pregnancy, as a continuous measurement where we summed the daily grass pollen measurements corresponding to the pregnancy months of the child's mother. We then divided the amount by 100 to get the effect of an increase of 100 grains/m^3 grass pollen exposure during the whole pregnancy. Cumulative grass pollen during the entire pregnancy were only available for $n = 179$ participants of the MACS cohort.

2.4.3. Potential confounders/effect modifiers

We used mother's age, history of hayfever and smoking during pregnancy, parent's socioeconomic status and infant's birth weight as adjustments in the regression analysis. For MACS, history of hayfever was defined as mother reporting currently or ever having hayfever, and smoking history was defined as currently smoking, or smoking in the last 3 months or in the last 6 months during pregnancy. Parent's socioeconomic status was comprised of mother's education, father's education, and father's occupation. Infant's birth weight data in MACS was not available instead we used infant's weight at 4 weeks age. In MACS, $n = 5$ observations were missing for fathers' occupation and $n = 21$ missing for weight at 4 weeks. In LISA, mother's history of allergy was defined as ever having asthma, eczema or hay fever. Smoking history was defined as ever smoking during the third trimester of pregnancy. Parent's socioeconomic status was represented by mother's education in LISA cohort. As LISA was a multi-center study, the study centers were considered as covariates in the models. In LISA, $n = 1$ observation was missing for maternal age, $n = 111$ missing for maternal smoking, $n = 50$ missing for maternal allergy and $n = 29$ missing for maternal education. Mother's history of hayfever in $COPSAC_{2000}$ was defined as history of doctor diagnosed hayfever. Symptoms were typically defined as significant problems with sneezing, blocked or runny nose in periods outside the common cold or flu and upon relevant exposure to a known allergen. Smoking history for $COPSAC_{2000}$ was defined as smoking ≥1 cigarette/week during the third trimester of pregnancy. Mother's education and father's education represented socioeconomic status in $COPSAC_{2000}$ with $n = 5$ missing for these variables.

2.5. Statistical analysis

We used multinomial logistic regression for the three-level cord blood IgE outcome. Results were presented as crude and adjusted relative-risk ratios (RRR) with corresponding 95% confidence intervals (CI). We used logistic regression for dichotomized cord blood IgE (> 0.5 kU/L) as estimates from these models were required for the pooled meta-analysis. These results were presented as crude and adjusted odds ratios (OR) with corresponding 95% CI.

All statistical analyses were performed locally in each study center. Cohort-specific effect sizes from a binary regression analysis were later meta-analysed for pollen season of birth and cumulative pollen exposures during pregnancy using inverse variance weighted random-effect models. I^2 statistics were calculated for statistical heterogeneity among studies. All analysis was performed in Stata™ version 14.2 (StataCorp, Texas, TX, USA). In LISA, analysis was performed in R version 3.3.2 (R Core Team (2016)) (R: A Language and Environment for Statistical Computing) using the function multinom from the package nnet for the multinomial regression models (Venables & Ripley, 2002).

3. Results

In MACS, cord blood samples were available for $n = 429$ participants. Although mothers with cord blood samples were slightly older than those without, there were no differences in the distribution of hayfever history, smoking history, or season of delivery between mothers with and without cord blood samples. Cord blood samples were available for $n = 1968$ participants in the LISA cohort. Those mothers without cord blood samples available were significantly older and more likely to ever have allergy (asthma, eczema or hay fever/rhinitis), but there was no difference in the prevalence of smoking during pregnancy. Cord blood samples were available for $n = 200$ of all $COPSAC_{2000}$ participants. There was no difference between the mothers with and without cord blood samples with regards to age, allergy (all mothers had asthma), smoking during pregnancy or season of delivery.

The characteristics of each study cohort with available cord blood data are detailed in Table 1. Maternal age was similar across the three

Table 1
Characteristics of study participants from each cohort with available cord blood data.

	No (%) or Mean (SD)		
	MACS ($n = 429$)	LISA ($n = 1968$)	$COPSAC_{2000}$ ($n = 200$)
Mother's age (mean (SD))	31 (4)	31 (5)	30 (4)
Mother's history of hayfever (No (%))	256 (60)	653 (34)	148 (74)
Mother's history of smoking during pregnancy (No (%))	39 (7)	205 (11)	25 (13)
Mother's education in year (mean (SD))	13 (2)		
Mother's education (No (%))			
• Low		196 (10)	
• Medium		805 (42)	
• High		938 (48)	
Mother's education			
• Elementary or college			220 (58)
• Medium			104 (27)
• University			58 (15)
High IgE			
• Low IgE (< 0.5 kU/L)	326 (76.0)	1528 (78)	160 (80)
• High IgE (≥0.5 kU/L)	103 (24.0)	440 (22)	40 (20)
IgE as three levels (No (%))			
• Low (< 0.5 kU/L)	326 (76.0)	1528 (78)	160 (80)
• Intermediate (0.5–1 kU/L)	49 (11)	196 (10)	17 (9)
• High (> 1 kU/L)	54 (13)	244 (12)	23 (11)

Table 2
The number of births during the pollen season and cumulative exposure to grass pollen exposure during the mother's entire pregnancy for each cohort.

	MACS (n = 429) No (%)	LISA (n = 1968) No (%)	$COPSAC_{2000}$ (n = 200) No (%)
Born in the pollen season	307 (71)	666 (34)	101 (51)
Months corresponding to birth during the pollen season			
• Month 1	51 (12)	136 (7)	9 (4)
• Month 2	39 (9)	149 (8)	11 (5)
• Month 3	32 (8)	156 (8)	21 (11)
• Month 4		225 (11)	21 (11)
• Month 5			8 (4)
• Month 6			29 (14)
Grass pollen during pregnancy, Mean (SD)	47.2 (26.1)	9.6 (5.9)	19.7 (10.4)

Notes: For MACS: month 1 was October, month 2 was November, and month 3 was December. For LISA: month 1 was April, month 2 was May, month 3 was June, month 4 was July. For $COPSAC_{2000}$: month 1 was March, month 2 was April, month 3 was May, month 4 was June, month 5 was July, month 6 was August.
Grass pollen measurement was in 100 grains/m^3.

cohorts. As high-risk cohorts, MACS and $COPSAC_{2000}$ had a high prevalence of maternal hayfever (60 and 74% respectively), while being population based LISA had the lowest prevalence (34%).

The prevalence of children born in the pollen season was highest in MACS, followed by LISA and $COPSAC_{2000}$ (Table 2). The mothers of children in MACS had the highest exposure of grass pollen during the entire pregnancy, almost five times that of children in LISA and more than double that of $COPSAC_{2000}$ (Table 2).

In MACS, birth during the grass pollen season was associated with high cord blood IgE > 1 kU/L in a multinomial analysis (RRR = 2.48, 95% CI: 1.38, 4.48) (Supplementary table 1). In particular, birth during October was associated with high cord blood IgE > 1 kU/L (3.03, 95% CI: 1.41, 6.52) and birth in December was associated with cord blood IgE > 1 kU/L (2.78, 95% CI: 1.09, 7.11). In LISA, birth in the first month of the pollen season was associated with increased risk of high cord blood IgE > 1 kU/L (2.05, 95% CI: 1.26, 3.33).

Adjusted analysis in MACS showed a stronger effect than in the crude analysis (Table 3), as birth in the grass pollen season was associated with IgE > 1 kU/L (RRR = 3.01, 95% CI: 1.58, 5.73). Birth in October and December was also associated with increased -risk of IgE > 1 kU/L (3.61, 95% CI: 1.59, 8.17, 4.10, 95% CI: 1.50, 11.24 respectively). In adjusted analysis, an April birth in LISA was associated with cord blood IgE > 1 kU/L (2.03, 95% CI: 1.24, 3.31). There were no significant associations in the adjusted analysis of the $COPSAC_{2000}$ cohort. Cumulative exposure to grass pollen in mothers during the entire pregnancy was associated with a slightly reduced risk for high cord blood IgE in MACS (0.97, 95% CI: 0.95, 0.99) and in LISA (0.96 [95% CI: 0.93, 0.99]) but not in the $COPSAC_{2000}$ cohort (Table 3).

A meta-analysis of all three cohorts and pollen season of birth indicated significantly higher odds of having high cord blood IgE compared to birth outside the grass pollen season (OR = 1.37, 95% CI: 1.06 to 1.77). There was no significant heterogeneity (I^2 = 14%, p = 0.31), suggesting little variation between the three studies (Fig. 1). The pooled odds of cumulative exposure to grass pollen in mothers during their entire pregnancy and cord blood IgE (≥0.5 kU/L) was slightly lower odds but significant (OR = 0.98, 95% CI: 0.96 to 0.99). The meta-analysis for this also found no significant heterogeneity (I^2 = 13.5%, p = 0.31) (Fig. 2).

As a sensitivity analysis, we redid the meta-analysis using only the MACS and $COPSAC_{2000}$ cohorts as both were high risk. We also redid the analysis only including mothers with a history of allergic disease in LISA. Results remained the same but became slightly stronger (Supplementary Figs. 1, 2, 3 and 4).

4. Discussion

This is the first study of three birth cohorts from three different countries (Australia and two in Europe) to show evidence of a pooled association between birth in grass pollen seasons and high cord blood IgE, with no evidence of heterogeneity between studies. Birth during the first month of the high grass pollen season seemed important for MACS and LISA. In Australia, cord blood IgE was higher in infants born in October (Southern spring). In Denmark and Germany cord blood IgE appeared to be higher in those born around April respectively. The estimated effect sizes were stronger in MACS, but the trends and significance level were consistent with LISA. $COPSAC_{2000}$ estimates also tended towards the same direction but not significant. Although birth in the entire pollen season was not significant, levels for individual months seemed to be significant. However, this needs to be interpreted with caution as power is much lower to detect associations and less reliable than the data sets as a whole.

There was some evidence that cumulative outdoor grass pollen exposure in mothers during their entire pregnancy maybe protective, but this was borderline significant in a pooled meta-analysis. It is possible that the third trimester could be the development of a sensitisation barrier to other environmental factors (sensitisation hypothesis). However, this is not the case when they are born during the high grass pollen seasons. This possible inverse effect indicates that season is independent of grass pollen suggesting exposure to other environmental factors may be important. It is also possible that infants born at the start of spring would have been carried through winter, and low maternal vitamin D was influencing the estimated effects. We do not have maternal vitamin D measurements to test this directly.

It is also possible that we are observing an effect of pollen exposure on sensitised mothers resulting in increased IgE at the start of the pollen season. As the season progresses, the immune response may lessen. Any possibility that there is a higher effect in MACS could be attributed to more pollen sensitised mothers, due to the location and sampling frame. Maternal pollen sensitization was not available in MACS. In the MACS analysis, birth in December (usually the last month of the pollen season) also had increased odds of > 1 kU/L which could partly be explained by the high pollen load in December during the study period (3 to 4 times the total pollen load occurred in December compared to November in 1992 and 1993).

No studies to date have investigated the association between birth during any pollen season and cord blood IgE level. Therefore, we compared our results with two previous studies that examined the distribution of high cord blood IgE level only in each month of birth with no data on pollen concentrations. A study of 1652 children in Sweden reported that children born in grass pollen season, around June to August, had a slightly higher cord blood IgE levels, but this association was not significant (OR 1.03, 95% CI: 0.74, 1.45) (Croner & Kjellman, 1986). Another study of 5353 children in Belgium showed that children born in grass pollen season, mid-May to mid-July, had higher odds of high cord blood IgE levels compared to children born in the rest of the year, although not significant (OR 1.21, 95% CI: 0.88, 1.66) (Sadeghnejad et al., 2007). These results are consistent with another study finding from LISA cohort on impact of prenatal exposure to indoor allergens derived from mites and cats (Heinrich et al., 2002). Increased maternal exposure to these indoor allergens during pregnancy showed increased odds for elevated total IgE in cord blood of offsprings. However, high pollen loads in the outdoor environment during pregnancy in this multicenter study were associated with lower cord blood IgE in both the Australian and the German cohorts but these were not significant in a pooled meta-analysis of cohorts.

There are several strengths in our analysis. First, we included cord blood IgE data from three birth cohorts in both hemispheres to investigate the consistency of the role of birth during a pollen season and high pollen loads in the outdoor environment *in utero*. Second, all three cohorts used the same assay manufactured by Pharmacia which

Table 3
Adjusted effects of birth in pollen seasons and grass pollen exposure on cord blood total IgE.

Cohort	n	Exposure	High IgE (< 0.5 kU/L as ref) OR (95% CI)	p	Multinomial IgE (< 0.5 kU/L as ref)			
					0.5–1 kU/L RRR (95% CI)	p	> 1 kU/L RRR (95% CI)	p
MACS	403	Born in grass pollen season	**1.91 (1.16, 3.15)**	**0.01**	1.19 (0.59, 2.42)	0.62	**3.01 (1.58, 5.73)**	**< 0.01**
	403	Born in grass pollen months						
		• Oct	**2.24 (1.16, 4.35)**	**0.01**	1.35 (0.52, 3.52)	0.54	**3.61 (1.59, 8.17)**	**< 0.01**
		• Nov	1.19 (0.51, 2.80)	0.68	0.82 (0.23, 2.89)	0.75	1.70 (0.58, 5.4.97)	0.33
		• Dec	**2.42 (1.06, 5.48)**	**0.03**	1.48 (0.46, 4.74)	0.51	**4.10 (1.50, 11.24)**	**< 0.01**
	169	Total grass pollen during pregnancy	**0.98 (0.97, 0.99)**	**0.02**	1.00 (0.98, 1.01)	0.64	**0.97 (0.95, 0.99)**	**< 0.01**
LISA	1797	Born in grass pollen seasons	1.25 (0.99, 1.58)	0.06	1.34 (0.97, 1.85)	0.07	1.18 (0.87, 1.59)	0.29
	1797	Grass pollen month birth						
		• April	**1.59 (1.05, 2.43)**	**0.03**	1.07 (0.55, 2.09)	0.83	**2.03 (1.24, 3.31)**	**< 0.01**
		• May	1.35 (0.89, 2.03)	0.15	1.55 (0.90, 2.66)	0.12	1.19 (0.7, 2.04)	0.52
		• June	1.16 (0.76, 1.76)	0.49	1.19 (0.66, 2.12)	0.56	1.14 (0.67, 1.94)	0.64
		• July	1.08 (0.75, 1.54)	0.68	1.45 (0.93, 2.28)	0.10	0.77 (0.46, 1.29)	0.32
	1797	Total grass pollen during pregnancy	**0.96 (0.94, 0.99)**	**< 0.01**	0.96 (0.93, 0.99)	0.03	**0.96 (0.93, 0.99)**	**0.02**
$COPSAC_{2000}$	195	Born in grass pollen seasons	1.21 (0.49, 2.80)	0.67	1.23 (0.31, 4.00)	0.76	1.11 (0.33, 3.28)	0.86
		Grass pollen month birth						
		• March	0.67 (0.03, 4.35)	0.72	N/A		1.74 (0.08, 13.4)	0.64
		• April	2.49 (0.58, 9.63)	0.19	2.44 (0.30, 14.0)	0.34	3.43 (0.44, 18.9)	0.18
		• May	0.92 (0.23, 3.00)	0.90	1.41 (0.19, 6.88)	0.69	0.64 (0.09, 3.07)	0.62
		• June	1.26 (0.36, 3.89)	0.70	0.78 (0.10, 3.92)	0.78	1.54 (0.30, 6.33)	0.57
		• July	0.70 (0.09, 3.81)	0.70	N/A		0.71 (0.07, 4.71)	0.74
		• August	0.47 (0.12, 1.44)	0.22	0.54 (0.07, 2.52)	0.47	0.35 (0.05, 1.59)	0.22
		Total grass pollen during pregnancy	0.97 (0.94; 1.00)	0.08	0.97 (0.92; 1.02)	0.23	0.97 (0.93; 1.01)	0.15

Model adjustments:
Mother's age, mother's education, father's education, father's occupation, mother's history of hayfever, mother's history of smoking during pregnancy and infant's weight at 4 weeks age for MACS.
Mother's age, mother's history of allergic disease, mother's history of smoking in pregnancy, mother's education, and study centers in LISA. In LISA models, study center was adjusted as a covariate as mixed effects models showed no difference in estimated effect sizes or *p* values.
Mother's age, mother's education, father's education, mother's history of hayfever, mother's history of smoking during pregnancy, and infant's birth weight in $COPSAC_{2000}$.

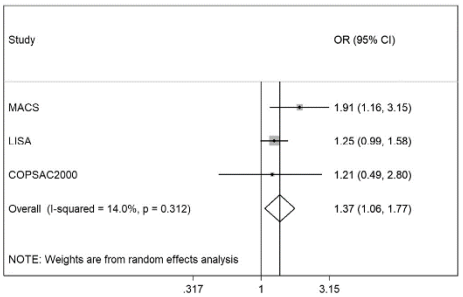

Fig. 1. Forest plot of associations between being born during grass pollen seasons and cord blood IgE (≥0.5 kU/L). Estimates are expressed as adjusted odds ratios (ORs) and 95% confidence intervals (CIs; pooled using a random-effects meta-analysis).

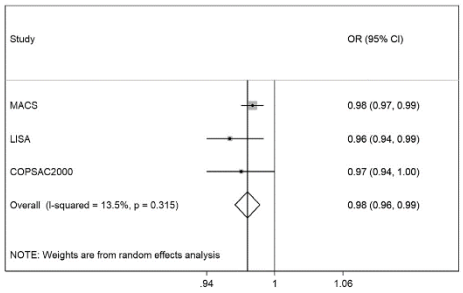

Fig. 2. Forest plot of associations between cumulative exposure to outdoor grass pollen in mothers during their entire pregnancy and cord blood IgE (≥0.5 kU/L). Estimates are expressed as adjusted odds ratios (ORs) and 95% confidence intervals (CIs; pooled using a random-effects meta-analysis).

increased the reliability of the cord blood IgE measurement although $COPSAC_{2000}$ used a more sensitive assay with a different cutoff for IgA. We used the same levels for IgE categorization. Third, we removed suspected contaminated samples from our analysis, so our results were specific for fetal IgE and minimized the possibility of maternal IgE contaminated cord blood.

Some limitations also need to be considered in interpreting our results. MACS and $COPSAC_{2000}$ recruited high-risk children (defined as having at least one family member with asthma and/or allergies), while LISA was a general population sample, it may not be representative and hence, generalisability may be limited. We did however conduct additional analysis by adjusting for maternal education and found that this adjustment did not substantially change the observed associations. Although there were different dominant pollens in each birth cohort, grass was still considered an important species in each country. However, we still cannot exclude overlapping of other aeroallergens in the atmosphere (such as other pollens including birch) which may be different across the study settings and therefore may play a role. Only MACS and LISA had pollen measurements for the entire year of birth (for a smaller sample of MACS), so children from $COPSAC_{2000}$ born outside of the pollen seasons did not contribute to the findings. In addition, we acknowledge the complexity in defining the pollen exposure, especially during the mother's entire pregnancy and interpreting these effects and birth during months where pollen peaked was somewhat challenging.

Although the cut points used for IgE levels are somewhat arbitrary the aim was to be consistent with other published studies assessing risk and cord blood IgE. We did not have other data on maternal environment, such as level of vitamin D during pregnancy, that might have affected cord blood IgE levels (Zittermann et al., 2004). While we have adjusted for a range of factors associated with cord IgE level, we cannot exclude the possibility of residual confounding, particularly by environmental exposures that vary across seasons, such as pollution. In MACS and $COPSAC_{2000}$ we did not have data on the time women spent indoors or outdoors during their pregnancy. Although some of the centers participating in the LISA cohort did collect some of this data it is incomplete and therefore adjustments for time spent outdoor could not be performed. The low sampling proportions from each cohort with available data on cord blood IgE makes our study vulnerable to sampling bias. However, there was little difference in characteristics between participants with/without cord blood data from each cohort.

In summary, this is the first analysis of three birth cohorts from both hemispheres to show that a pooled effect of birth during high grass pollen seasons were associated with increased risk of high cord blood IgE. On the other hand, exposure to high pollen loads in women during their entire pregnancy seemed protective. Although the link between cord blood IgE and subsequent allergic respiratory diseases is still unproven, IgE responses develop during the first months of life. Therefore, our study findings provide new insights into the mechanisms of exposure during the first year of life and possible subsequent allergic respiratory diseases.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2018.06.036.

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Supplementary Table 1. Crude effect of birth in grass pollen seasons and grass pollen exposure on cord blood total IgE

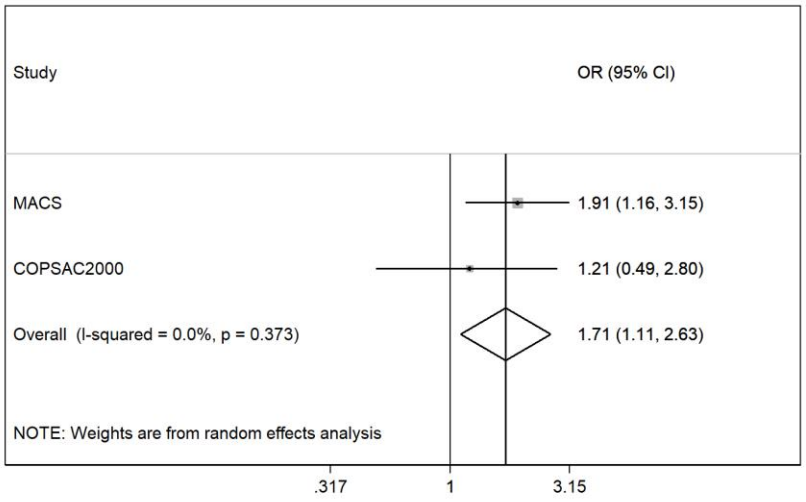
Cohort	Exposure	High IgE (<0.5 kU/L as ref) OR (95% CI)	p	Multinomial IgE (<0.5 kU/L as ref) 0.5-1 kU/L *RRR (95% CI)*	p	>1 kU/L *RRR (95% CI)*	p
MACS (n=429)	Born in grass pollen seasons	**1.68 (1.05, 2.70)**	**0.03**	1.04 (0.53, 2.06)	0.91	**2.48 (1.38, 4.48)**	**<0.01**
	Grass pollen month of birth						
	• Oct	**2.03 (1.07, 3.84)**	**0.03**	1.22 (0.48, 3.12)	0.68	**3.03 (1.41, 6.52)**	**<0.01**
	• Nov	1.12 (0.51, 2.47)	0.79	0.67 (0.20, 2.32)	0.53	1.67 (0.64, 4.35)	0.29
	• Dec	1.95 (0.89, 4.25)	0.09	1.28 (0.42, 3.94)	0.67	**2.78 (1.09, 7.11)**	**0.03**
	Total grass pollen during pregnancy[a]	0.99 (0.98, 1.00)	0.06	1.00 (0.98, 1.02)	0.94	**0.98 (0.96, 0.99)**	**0.01**
LISA (n=1968)	Born in grass pollen seasons	1.24 (0.99, 1.55)	0.06	**1.37 (1.01, 1.86)**	**0.04**	1.15 (0.86, 1.52)	0.35
	Grass pollen month birth						
	• April	**1.52 (1.02, 2.26)**	**0.04**	1.09 (0.58, 2.05)	0.80	**1.84 (1.16, 2.93)**	**0.01**
	• May	1.32 (0.89, 1.95)	0.17	1.69 (1.02, 2.81)	**0.04**	1.04 (0.61, 1.77)	0.88
	• June	1.16 (0.78, 1.72)	0.47	1.19 (0.68, 2.08)	0.54	1.13 (0.69, 1.86)	0.63
	• July	1.10 (0.79, 1.55)	0.57	1.44 (0.93, 2.23)	0.10	0.85 (0.53, 1.36)	0.49
	Total grass pollen during pregnancy	**0.96 (0.94,0.99)**	**<0.01**	**0.96 (0.93, 0.99)**	**0.01**	**0.97 (0.94, 1.00)**	**0.03**
COPSAC$_{2000}$ (n=200)	Born in grass pollen seasons	1.12 (0.46, 2.50)	0.80	1.18 (0.32, 3.30)	0.78	1.07 (0.33, 2.91)	0.90
	Grass pollen month birth						
	• March	0.51 (0.03, 3.00)	0.53	N/A		0.92 (0.05, 5.76)	0.94
	• April	2.31 (0.56, 8.47)	0.21	2.57 (0.35, 12.8)	0.28	2.10 (0.29, 10.1)	0.39
	• May	0.95 (0.25, 2.92)	0.94	1.06 (0.15, 4.59)	0.95	0.87 (0.13, 3.62)	0.86
	• June	1.27 (0.38, 3.68)	0.68	1.13 (0.16, 4.90)	0.89	1.38 (0.29, 5.04)	0.65
	• July	1.35 (0.19, 6.38)	0.73	N/A		2.45 (0.33, 12.3)	0.31
	• August	0.65 (0.18, 1.91)	0.47	0.72 (0.11, 3.03)	0.69	0.59 (0.09, 2.39)	0.51
	Total grass pollen during pregnancy	0.97 (0.94, 1.01)	0.411	0.97 (0.93, 1.02)	0.25	0.97 (0.94, 1.02)	0.21

a : Only available for n=179 for MACS. LISA is adjusted for study center as it is a multi-center study (Munich, Wesel, Leipzig, and Bad Honnef).

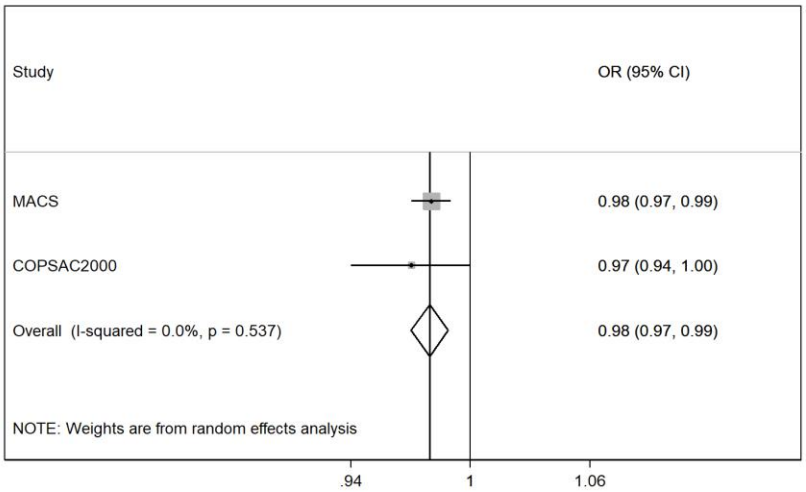
Supplementary Table 2. Crude and adjusted effects of birth in the pollen seasons and grass pollen exposure during pregnancy on cord blood total IgE in LISA infants whose mother had a history of allergic disease

	Exposure	High IgE (<0.5 kU/L as ref) OR (95% CI)	p	Multinomial IgE (<0.5 kU/L as ref) 0.5-1 kU/L *RRR (95% CI)*	p	>1 kU/L *RRR (95% CI)*	p
Crude (n=653)	Born in pollen seasons	1.12 (0.78, 1.61)	0.55	0.86 (0.51, 1.45)	0.57	1.36 (0.87, 2.13)	0.18
	Grass pollen month birth						
	• April	1.49 (0.81, 2.74)	0.20	0.34 (0.08, 1.48)	0.15	**2.53 (1.31, 4.31)**	**0.01**
	• May	0.84 (0.43, 1.66)	0.62	0.73 (0.28, 1.96)	0.54	0.93 (0.40, 2.20)	0.88
	• June	1.11 (0.57, 2.13)	0.76	0.83 (0.31, 2.22)	0.71	1.36 (0.62, 2.98)	0.45
	• July	1.10 (0.63, 1.91)	0.74	1.27 (0.63, 2.56)	0.50	0.93 (0.43, 2.00)	0.85
	Total grass pollen during pregnancy	**0.96 (0.93, 1.00)**	**0.06**	0.99 (0.94, 1.05)	0.72	**0.95 (0.90, 0.99)**	**0.02**
Adjusted (n=615)	Born in pollen seasons	1.21 (0.83, 1.75)	0.33	0.86 (0.50, 1.48)	0.59	1.56 (0.87, 1.59)	0.29
	Grass pollen month birth						
	• April	**1.94 (1.03, 3.68)**	**0.04**	0.42 (0.10, 1.82)	0.24	**3.62 (1.79, 7.31)**	**<0.01**
	• May	0.84 (0.41, 1.72)	0.64	0.59 (0.20, 1.75)	0.34	1.09 (0.46, 2.61)	0.84
	• June	1.16 (0.6, 2.25)	0.66	0.82 (0.31, 2.22)	0.70	1.54 (0.69, 2.43)	0.29
	• July	1.12 (0.64, 1.98)	0.70	1.31 (0.64, 2.66)	0.46	0.91 (0.40, 2.05)	0.81
	Total grass pollen during pregnancy	**0.95 (0.92, 0.99)**	**0.01**	0.99 (0.93, 1.05)	0.68	**0.92 (0.88, 0.97)**	**<0.01**

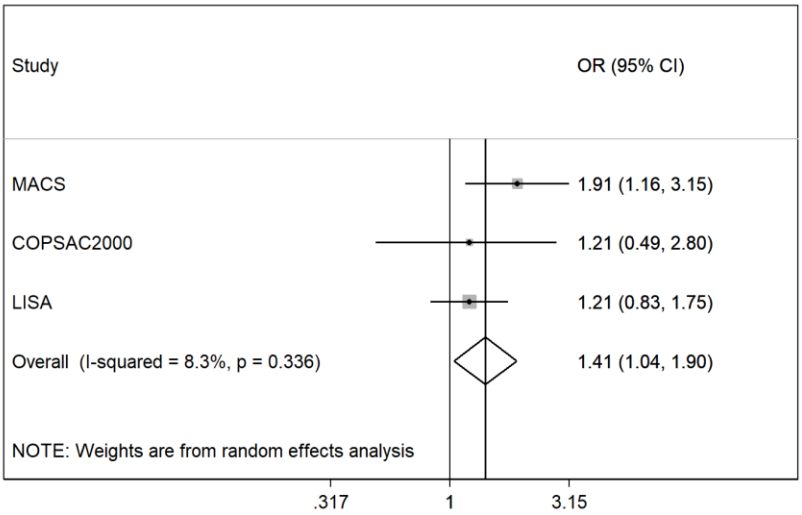
LISA is adjusted for study center as it is a multi-center study (Munich, Wesel, Leipzig, and Bad Honnef) for crude effect. Adjusted for mother's age, mother's history of smoking in pregnancy, mother's education, and study centers in LISA. In LISA models, study center was adjusted as a covariate as mixed effects models showed no difference in estimated effect sizes or p values for adjusted effect.



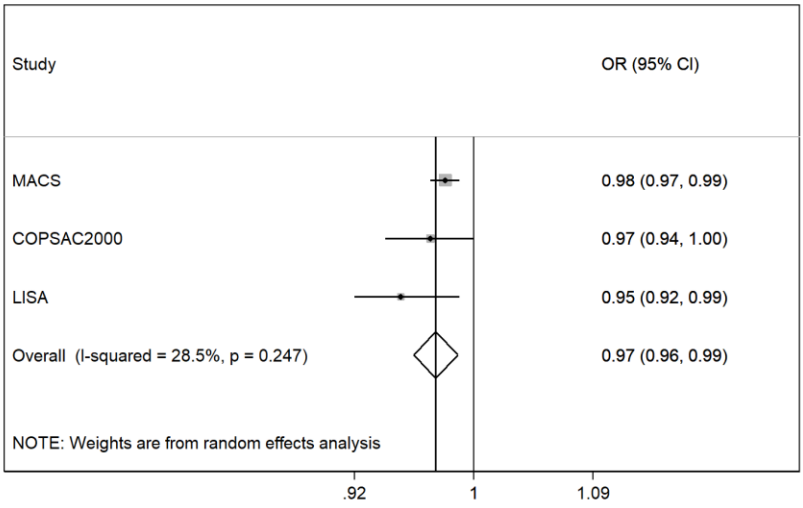
Supplementary Figure 1. Forest plot of born in pollen season effect on cord blood IgE (MACS and $COPSAC_{2000}$ only)



Supplementary Figure 2. Forest plot of pollen exposure during pregnancy effect on cord blood IgE (MACS and $COPSAC_{2000}$ only)



Supplementary Figure 3. Forest plot of born in pollen season effect on cord blood IgE (LISA only included mother with allergy history)



Supplementary Figure 4. Forest plot of pollen exposure during pregnancy effect on cord blood IgE (LISA only included mother with history allergy)

Chapter 6 Associations between grass pollen exposure *in utero* and in early life with food allergy in 12-month-old infants

This result chapter consists of an accepted for publication article of original research to assess the associations between grass pollen exposure during pregnancy and in early life with food allergy in infants. This original research article addressed the research questions number three and four

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Associations between grass pollen exposure *in utero* and in early life with food allergy in 12-month-old infants

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Disclosure of potential conflict of interest

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ABSTRACT

Background: Birth during pollen seasons seem to influence food allergy risk, however no study has assessed pollen exposure. We assessed the role of pollen exposure, *in utero* and in the first months of life, on risk of food allergy in infancy.

Methods: The HealthNuts cohort, a population-based cohort of 5,276 infants was used. Using multinomial regression, we assessed grass pollen exposure, *in utero* and up to the first 6 months of life, on hen's egg, sesame and peanut allergy outcomes at 12 months.

Results: Of those exposed to grass pollen season during the 10^{th} to 12^{th} weeks of gestation, 28% were not sensitized to food, 34% were sensitized but tolerant and 30% were allergic to food. Gestation at 10^{th} to 12^{th} week during grass pollen season showed increased risk to hen's egg sensitization adjusted multinomial odds ratio (aMOR)=1.39 (95% CI: 0.99, 1.96) and peanut sensitization aMOR=1.30 (95% CI: 0.98, 1.73). Cumulative pollen exposure in the first seven days of life also increased risk of peanut sensitization aMOR=1.21 (95% CI: 1.01, 1.44). Exposure between the first 4 to 6 months of life increased risk of hen's egg sensitization aMOR=1.02 (95% CI: 1.004, 1.04) and sensitization to all foods aMOR=1.02 (95% CI: 1.003, 1.04). There was evidence that grass pollen exposure was associated with food challenge diagnosed food allergy, but only among infants with a maternal history of food allergy.

Conclusion: Exposure to grass pollen in the *intrauterine* period and infancy may be important in the sensitization to food allergic pathways but more studies are needed to replicate these findings.

Keywords: grass pollen, *in utero,* food allergy, sensitization

INTRODUCTION

The prevalence of food allergy in children varies substantially between countries and is known to be high in affluent countries (33, 227). Our studies have reported that in Melbourne (Australia), the prevalence of challenge-confirmed food allergy at age one year was 11% and at age four years was 3.8% (33). Food allergy manifests in the first years of life and although family history of food allergy is a key factor, pathogenesis is multifactorial with many familial and environmental factors playing a part (33, 37, 40, 77, 228). Studies to date suggest factors at birth or in the first months of life are associated with the development of food allergy. Being born in autumn and winter has consistently been associated with higher odds of food allergy at one year, in children aged 0 to 4 years and in older children aged 7-15 years (40-42).

A study in Finland found that children who had their 11th gestational week from April to May (high birch and alder pollen seasons in Finland) had higher odds of food sensitization up to 4 years of age (5). It is understood that the liver produces IgE around this period and the lung and spleen contribute thereafter (211). The same study in Finland also reported that pollen season and food sensitization associations were modified by parental history of allergic manifestation such as hay fever or pollen allergy (5). Although the study in Finland showed that exposure during the pollen season was important, they did not have actual pollen measurements to assess actual pollen exposure effects, nor did they assess food challenge diagnosed food allergy.

We know that the phenotypes and pathophysiology of food allergy are varied. Although there seems to be some interest in the pollen/food allergy syndrome, gaps in understanding the likely link remains (98, 229). It is possible that season of birth may be a marker of pollen exposure. Exposure to consecutive high pollen days (defined as days with at least 50 pollen/m3) during grass pollen season may promote pollen sensitivity, sensitization and allergy, even in the absence of ingestion of foods. These effects vary by geographic regions, but grass sensitizers are common globally (230). Melbourne, Australia, has days with extremely high levels of grass pollen during grass pollen season. Our studies have consistently shown that high levels of outdoor grass pollen during grass pollen season are associated with emergency department presentations, admissions and lung function outcomes in children and adolescents (231, 232).

Our own work on grass pollen exposure during the entire pregnancy, high cord blood IgE, pollen exposure up to the first six months, and asthma and hay fever in young children suggest that grass pollen exposure may be an important factor in the etiology of allergic diseases (3, 233). Effect modification by maternal history of allergic respiratory disease, especially history of food allergy, may also be important. To explore this possible early-life pollen exposure and food allergy pathway further, we used a large population-based sample of 12-month-old infants (n=5,276) enrolled in the HealthNuts cohort in Melbourne, Australia, which has data on timing of birth, sensitization and food challenge diagnosed food allergy.

METHODS

Study Design and Population

HealthNuts study is a population-based cohort study in Melbourne. It is designed to study the prevalence, determinants, natural history, and burden of allergic diseases with a focus on food allergy in children. The recruitment process has been described in detail in a previous article (234). Briefly, recruitment occurred between September 2007 and August 2011 during immunization sessions and enrolled 5,276 infants aged 11 to 15 months. All infants participating in this study were tested for four common food allergens (egg, peanut, sesame and either cow's milk or shrimp) by the skin prick test (SPT). Infants with any detectable SPT wheal (size greater or equal to one milimeter (mm) diameter greater than the negative control for a particular allergen) for any of the allergens tested were offered a repeat SPT and an oral food challenge (OFC). We used a two mm wheal on the repeat SPT as the definition for food sensitized and a combination of SPT and OFC for food allergy (33). A randomly selected sample of infants from the entire sample with a 0 mm SPT wheal to all foods were also invited for oral food challenge as negative controls.

Food sensitization and food allergy definitions

The outcome variables were:

Not sensitized: Negative (<2 mm) SPT to all foods,

Food-sensitized tolerant: Negative OFC but positive sensitization test (SPT wheal ≥ 2 mm and/or specific IgE to food ≥ 0.35 kU/L), and Food allergic: Positive OFC and positive sensitization test (SPT wheal ≥ 2mm and/or specific IgE to food ≥ 0.35 kU/L). The food-sensitized tolerant and food allergic outcomes were further analyzed as follows:

Single food allergy to hen's egg or peanut, which are the two most prevalent food allergies (9.5% and 3.1%, respectively, supplementary table 1), and allergy to any one of hen's egg, peanut, or sesame. We did not assess for cow's milk and shrimp allergy.

Pollen collection and measurement

Pollen data were collected using a volumetric spore trap (Burkard) during a predetermined grass pollen season each year which begins on 1 October and ends on 31 December. Pollen data are not available outside this period. The Burkard trap was located on the roof top, 15 meters above ground, of the Earth Science Building inside The University of Melbourne. Airborne grass pollen grains were trapped on an adhesive surface to be identified under a light microscope, with the daily average grass pollen concentrations being expressed as pollen grains/m3 of air. Although one trap is used for the collection of grass pollen, this is a representation of wind-borne pollen which travels long distances. The concentration derived from one trap has shown to be a good proxy for exposure among those living up to 50km from the monitoring station in Melbourne (235).

Exposure definitions

We used multiple definitions for pollen exposure. To be consistent with the study by Pyrhonen and colleagues (2012), we developed a variable for exposure at the 11th week and between 10th – 12th week of gestation (5). We also considered at birth, first seven days, first three months, between first four to six months, and the first six months.

We calculated the gestational week based on the gestational age at birth. Then we grouped them, for each time point in utero and at birth, as inside the peak grass pollen season (October to December) or outside the peak grass pollen season (January to September).

Daily grass pollen levels outside the grass pollen season were considered as zero because grass pollen levels outside the grass pollen season are usually very low and therefore exposures will be near null. We summed all daily grass pollen measurements at each time point (11th week and between 10th – 12th weeks of gestation, at birth, first seven days, first three months, between four to six months, and first six months). We report associations as the effect of an increase of 100 grains/m3 cumulative grass pollen exposure.

Potential adjustments, confounders or effect modifiers

We used maternal age in years, maternal history of smoking during pregnancy, and ultraviolet exposure during pregnancy as potential adjustments for the association between pollen exposure and the outcome. Ultraviolet exposure was defined by cumulative measurement of daily ultraviolet level in Melbourne. We considered maternal history of hayfever and food allergy as effect modifiers for the association, based on results from other studies and our own, when we considered early-life pollen exposure (37, 122). Maternal history of hay fever and food allergy based on maternal response to the question “does anyone in your family suffer asthma, eczema, hay fever, food allergy (please specify food)” (37).

Statistical analysis

We used multinomial logistic regression to analyze the association between maternal pollen exposure and the three categories outcome variable (not sensitized, food-sensitized tolerant and food allergic). Results were presented as crude and adjusted multinomial odds ratios (MOR) with corresponding 95% confidence intervals (CI). We adjusted the models for maternal age in years, maternal history of smoking during pregnancy or ultraviolet exposure during pregnancy, if they were significant at

5% level in adjusted models. For some models, we mutually adjusted for born in grass pollen season and exposure in first seven days. We considered maternal history of hayfever and maternal history of food allergy as possible effect modifiers. Strata specific results are presented with the p value from the interaction term in the regression model. We use $p<0.1$ for the interaction term because the power to test for interactions is lower than the test for main effects in the regression models (236). Both variables were considered as effect modifiers in separate regression models. All analysis was performed by Stata™ version 14.2 (StataCorp, College Station, TX, USA).

Ethical approval

HealthNuts study had ethical approval from the Victorian State Government Office for Children (reference no. CDF/07/492), the Victorian State Government Department of Human Services (reference no. 10/07), and the Royal Children's Hospital Human Research Ethics Committee (reference no. 27047).

RESULTS

Characteristics of the participants

There were 4,728 out of 5,276 infants with complete data for the analysis, 84.7% (4,005) of the participants were not sensitized to foods tested, 4.9% (231) were food-sensitized tolerant, and 10.4% (492) allergic to one of hen's egg, peanut or sesame (Table 1). The proportion of male and female infants in the Health Nuts cohort were equal and a small proportion (5.3%) had low birth weight (<2500 grams). More than half of the mothers were aged <34 years at delivery and about a quarter of them were born outside Australia (237). More infants were allergic to hen's egg compared to peanut or sesame (9.5%, 3.1% and 0.6% respectively) while more infants were sensitized tolerant to peanut compared to hen's egg or sesame (4.7%, 3.2%, and 2.9% respectively) (Supplementary Table 1). Cumulative pollen exposure during each time point was greater among food-sensitized tolerant infants and less so among infants allergic to any foods (Table 2).

Table 1. HealthNuts participants' characteristics.

Characteristics	All infants n = 4 728	Not sensitized* n = 4 005	Food-sensitized tolerant* n = 231	Food allergic* n = 492
Maternal age (year), mean (SD)	33 (4.8)	32.9 (4.8)	33.5 (4.7)	33 (4.2)
Maternal history of smoking during pregnancy, n (%)	219 (4.6)	200 (5)	7 (3)	12 (2.4)
Maternal history of hay fever, n (%)	1 455 (30.8)	1 201 (30)	74 (32)	180 (36.6)
Maternal history of food allergy, n (%)	273 (5.8)	232 (5.8)	14 (6.1)	27 (5.5)
Child gestational age at delivery, n (%)				
• ≤36 weeks	276 (6.2)	245 (6.5)	15 (6.8)	16 (3.5)
• 37 – 42 weeks	4 138 (93)	3 489 (92.8)	206 (92.8)	443 (96.1)
• ≥43 weeks	34 (0.8)	31 (0.8)	1 (0.4)	2 (0.4)
UV exposures in the first trimester (J/m^2), mean (SD)	26.12 (16.57)	25.91 (16.58)	27.72 (16.24)	27.09 (16.64)
UV exposures in all trimester (J/m^2), mean (SD)	25.09 (16.24)	25.3 (16.27)	23.41 (16.19)	24.2 (15.93)

* Hen's egg, peanut, or sesame.

Table 2. HealthNuts participants' pollen exposure.

Exposures	All infants n = 4 728	Not sensitized* n = 4 005	Food-sensitized tolerant* n = 231	Food allergic* n = 492
Child 11th week gestation in peak pollen season, n (%)	1 091 (24.5)	907 (24.1)	64 (28.8)	120 (26)
Pollen exposure at 11th week gestation, mean (SD)	0.32 (0.87)	0.32 (0.87)	0.41 (0.93)	0.32 (0.87)
Child 10-12th week gestation in peak pollen season, n (%)	1 278 (28.7)	1 063 (28.2)	76 (34.2)	139 (30.2)
Pollen exposure at 10-12th week gestation, mean (SD)	0.96 (2.34)	0.96 (2.35)	1.19 (2.45)	0.89 (2.14)
Child born in peak pollen season, n (%)	1 240 (26.2)	1 073 (26.8)	56 (24.2)	111 (22.6)
Pollen exposure in the first 7 days, mean (SD)	0.37 (0.92)	0.37 (0.92)	0.38 (0.98)	0.34 (0.94)
Pollen exposure in the first 3 months, mean (SD)	4.53 (7.27)	4.52 (7.26)	4.82 (7.38)	4.52 (7.33)
Pollen exposure between months 4-6, mean (SD)	4.22 (7.13)	4.17 (7.1)	5.29 (7.86)	4.19 (7.01)
Pollen exposure in the first 6 months, mean (SD)	8.81 (9.13)	8.73 (9.12)	10.15 (9.47)	8.78 (9.07)

* Hen's egg, peanut, or sesame.
Grass pollen exposure was measured in 100 pollen grains/m3.

Main effects analysis

All unadjusted results are presented in the online Supplementary Table 2. In the adjusted analysis only, maternal history of smoking during pregnancy was included as an adjustment variable as it was statistically significant. None of the other variables, including ultraviolet exposure during pregnancy, was either not significantly associated with the outcomes or didn't change the estimated effect sizes of the pollen exposure by more than 10%.

The occurrence of the 10th to 12th week gestation during the grass pollen season showed a moderate trend towards increase odds to hen's egg sensitized tolerant aMOR= 1.39 (95% CI: 0.99, 1.96 p=0.057), peanut aMOR=1.30 (95% CI: 0.98, 1.73 p=0.073) and when all outcomes were combined aMOR=1.32 (95% CI: 0.99, 1.76 p=0.057). Exposure to pollen during the 11th week gestation moderate trend towards increase odds for peanut sensitized tolerant aMOR= 1.34 (95% CI: 0.99, 1.8 p=0.056). None of the exposures during in utero were associated with oral food challenge diagnosed allergy other than born in the grass pollen season which was protective. In contrast, cumulative pollen exposure in the first seven days of life (and being born in the grass pollen season) increased risk of peanut sensitized tolerant aMOR= 1.21 (95% CI: 1.01, 1.44). Increasing pollen exposure between the first four to six months of life increased odds for hen's egg sensitized tolerant aMOR = 1.02 (95% CI: 1.004, 1.04) and sensitized tolerant to all foods when combined aMOR= 1.02 (95% CI: 1.003, 1.04) (Table 3).

Table 3. Adjusted effects of pollen exposure *in utero* on food allergy outcomes at 12 months

	Food-sensitized tolerant MOR (95% CI)	p	Food allergic MOR (95% CI)	p
Hen's egg				
Gestation week 11 in peak pollen season	1.27 (0.89, 1.83)	0.19	1.15 (0.91, 1.44)	0.24
Gestation week 10-12 in peak pollen season	1.39 (0.99, 1.96)	0.057	1.07 (0.86, 1.34)	0.53
Born in peak pollen season	0.9 (0.62, 1.3)	0.57	0.798 (0.634, 1.004)	0.054
• Pollen exposure in first 7 days	1.1 (0.88, 1.37)	0.39	1.06 (0.92, 1.22)	0.42
Pollen exposure in first 3 months	1.00 (0.98, 1.02)	0.95	1.00 (0.98, 1.01)	0.50
Pollen exposure in between first 4-6 months	1.02 (1.004, 1.04)	0.01	1.00 (0.99, 1.01)	0.98
Pollen exposure in first 6 months	1.01 (1.00, 1.03)	0.09	1.00 (0.99, 1.01)	0.64
Peanut				
Gestation week 11 in peak pollen season	1.34 (0.99, 1.8)	0.056	1.21 (0.83, 1.77)	0.31
Gestation week 10-12 in peak pollen season	1.30 (0.98, 1.73)	0.073	1.24 (0.87, 1.77)	0.24
Born in peak pollen season	0.76 (0.55, 1.05)	0.09	0.97 (0.67, 1.4)	0.86
• Pollen exposure in first 7 days	1.21 (1.01, 1.44)	0.03	0.98 (0.77, 1.25)	0.86
Pollen exposure in first 3 months	1.00 (0.99, 1.02)	0.74	1.00 (0.98, 1.03)	0.71
Pollen exposure in between first 4-6 months	1.01 (0.99, 1.03)	0.23	1.01 (0.99, 1.03)	0.42
Pollen exposure in first 6 months	1.01 (0.99, 1.02)	0.22	1.01 (0.99, 1.03)	0.30
Hen's egg, peanut, or sesame				
Gestation week 11 in peak pollen season	1.27 (0.94, 1.72)	0.11	1.11 (0.89, 1.38)	0.37
Gestation week 10-12 in peak pollen season	1.32 (0.99, 1.76)	0.057	1.09 (0.89, 1.35)	0.40
Born in peak pollen season	0.87 (0.64, 1.19)	0.39	0.80 (0.64, 0.99)	0.04
• Pollen exposure in first 7 days	1.09 (0.91, 1.31)	0.35	1.07 (0.93, 1.23)	0.34
Pollen exposure in first 3 months	1.01 (0.99, 1.02)	0.50	1.00 (0.99, 1.01)	0.92
Pollen exposure in between first 4-6 months	1.02 (1.003, 1.04)	0.02	1.00 (0.99, 1.01)	0.93
Pollen exposure in first 6 months	1.02 (1.003, 1.03)	0.02	1.00 (0.99, 1.01)	0.83

- Mutually adjusted to each other born in peak pollen season and pollen exposure in first 7 days.
- Adjusted for maternal smoking during pregnancy. Food sensitization was defined as positive if SPT ≥2 mm or sIgE ≥0.35 kU/L. Associations expressed as effect per 100 pollen grains/m3 increase.

Interaction analysis

Maternal history of food allergy was an important effect modifier of grass pollen exposure almost at all time points in utero, at birth, and in early life for food challenge diagnosed allergy. Specifically, cumulative grass pollen exposure during the 10th to 12th week gestation and cumulative exposure to pollen between the 4 to 6-month period increased the risk of hen's egg allergy among infants of mothers with a history of food allergy. Increased risk of peanut allergy was observed when exposed to pollen during

the 10th to 12th week of gestation and between the 4 to 6-month period among infants with maternal history of food allergy. Infants born in grass pollen seasons had lower risk for allergy to hen's egg and lower risk for allergy to any of hen's egg, peanut, or sesame among mothers who reported a history of food allergy (Table 4).

Table 4. Interaction results of pollen exposure and maternal history of food allergy on food sensitization and allergic outcomes at age 12 month. A multinomial regression with not sensitized as the reference category.

	Maternal history of food allergy		
	No (n = 4 455)	Yes (n = 273)	p value for interaction
Hen's egg	MOR (95%CI)	MOR (95%CI)	
Food sensitized tolerant			
Gestation week 11 in peak pollen season	1.23 (0.84, 1.79)	2.02 (0.57, 7.2)	0.45
Gestation week 10-12 in peak pollen season	1.38 (0.0.97, 1.97)	1.67 (0.47, 5.9)	0.42
Born in peak pollen season	0.96 (0.66, 1.4)	0.26 (0.03, 2.08)	0.22
• Pollen exposure in first 7 days	1.12 (0.9, 1.39)	0.54 (0.09, 3.02)	0.40
Pollen exposure in first 3 months	1.00 (0.98, 1.03)	0.8 (0.58, 1.1)	0.16
Pollen exposure in between first 4-6 months	1.02 (1.003, 1.05)	1.02 (0.94, 1.1)	0.89
Pollen exposure in first 6 months	1.02 (1.00, 1.03)	0.96 (0.89, 1.05)	0.23
Food allergic			
Gestation week 11 in peak pollen season	1.08 (0.85, 1.37)	2.65 (1.11, 6.33)	0.05
Gestation week 10-12 in peak pollen season	1.00 (0.8, 1.26)	3.02 (1.28, 7.1)	0.01
Born in peak pollen season	0.84 (0.66, 1.06)	0.23 (0.05, 0.99)	0.08
• Pollen exposure in first 7 days	1.07 (0.92, 1.23)	0.88 (0.47, 1.64)	0.53
Pollen exposure in first 3 months	0.99 (0.98, 1.01)	1.01 (0.95, 1.07)	0.63
Pollen exposure in between first 4-6 months	1.00 (0.98, 1.01)	1.06 (1.01, 1.11)	0.01
Pollen exposure in first 6 months	0.99 (0.98, 1.01)	1.05 (1.01, 1.1)	0.01
Peanut			
Food sensitized tolerant			
Gestation week 11 in peak pollen season	1.26 (0.93, 1.72)	3.02 (0.97, 9.35)	0.14
Gestation week 10-12 in peak pollen season	1.25 (0.93, 1.68)	2.4 (0.78, 7.42)	0.27
Born in peak pollen season	0.78 (0.56, 1.09)	0.47 (0.1, 2.15)	0.51
• Pollen exposure in first 7 days	1.21 (1.01, 1.44)	1.17 (0.67, 2.05)	0.91
Pollen exposure in first 3 months	1.00 (0.99, 1.02)	0.99 (0.91, 1.08)	0.78
Pollen exposure in between first 4-6 months	1.01 (0.99, 1.03)	1.05 (0.99, 1.11)	0.20
Pollen exposure in first 6 months	1.01 (0.99, 1.02)	1.04 (0.98, 1.1)	0.30
Food allergic			
Gestation week 11 in peak pollen season	1.09 (0.74, 1.62)	13.96 (1.53, 127)	0.02
Gestation week 10-12 in peak pollen season	1.14 (0.78, 1.65)	11.14 (1.22, 102)	0.04
Born in peak pollen season	1.01 (0.7, 1.47)	2.65e-06 (0, .)	0.97
• Pollen exposure in first 7 days	0.98 (0.76, 1.25)	N/A	0.99
Pollen exposure in first 3 months	1.00 (0.98, 1.03)	0.94 (0.77, 1.15)	0.51
Pollen exposure in between first 4-6 months	1.00 (0.98, 1.03)	1.09 (1.004, 1.18)	0.06
Pollen exposure in first 6 months	1.01 (0.99, 1.03)	1.06 (0.97, 1.16)	0.24
Hen's egg, peanut, or sesame			
Food sensitized tolerant			
Gestation week 11 in peak pollen season	1.23 (0.9, 1.68)	2.14 (0.68, 6.69)	0.36
Gestation week 10-12 in peak pollen season	1.3 (0.97, 1.75)	1.72 (0.55, 5.37)	0.63
Born in peak pollen season	0.91 (0.66, 1.24)	0.44 (0.09, 2.00)	0.35
• Pollen exposure in first 7 days	1.11 (0.92, 1.33)	0.6 (0.22, 2.16)	0.41
Pollen exposure in first 3 months	1.01 (0.99, 1.03)	0.9 (0.77, 1.06)	0.16
Pollen exposure in between first 4-6 months	1.02 (1.00, 1.03)	1.06 (1.00, 1.13)	0.15
Pollen exposure in first 6 months	1.02 (1.00, 1.03)	1.02 (0.96, 1.08)	0.88
Food allergic			
Gestation week 11 in peak pollen season	1.02 (0.81, 1.29)	3.34 (1.44, 7.73)	0.008

Gestation week 10-12 in peak pollen season	1.01 (0.81, 1.26)	3.66 (1.59, 8.41)	0.003
Born in peak pollen season	0.84 (0.67, 1.05)	0.21 (0.05, 0.91)	0.06
• Pollen exposure in first 7 days	1.08 (0.94, 1.24)	0.84 (0.45, 1.59)	0.44
Pollen exposure in first 3 months	1.00 (0.99, 1.01)	1.00 (0.94, 1.06)	0.96
Pollen exposure in between first 4-6 months	1.00 (0.98, 1.01)	1.07 (1.02, 1.12)	0.004
Pollen exposure in first 6 months	1.00 (0.99, 1.01)	1.05 (1.01, 1.1)	0.01

- Mutually adjusted to each other: born in peak pollen season and pollen exposure in first 7 days.

Adjusted for maternal smoking during pregnancy. Food sensitization was defined as positive if SPT ≥2 mm or sIgE ≥0.35 kU/L. N/A – insufficient data for computation of effects. Associations expressed as effect per 100 pollen grains/m^3 increase.

DISCUSSION

Our study identified several periods during pregnancy and the first year of life when increased pollen exposure was associated with a moderate increase in risk of food sensitization and food challenge diagnosed food allergy but only among infants with mothers' history of food allergy.

There was an increase in infants' risk of hen's egg and peanut sensitization at 12 months with cumulative pollen exposure between the 10th and 12th week gestation period. Exposure to pollen in the first seven days increased risk of peanut but not hen's egg sensitization. When exposed to increase pollen during the first four to six months of life, there was an increased risk of hen's egg and any food sensitization. Exposure to grass pollen season during the 10th and 12th week gestation period and increase pollen between 4 to 6 months increased risk of hen's egg and peanut food challenge diagnosed food allergy among infants with maternal history of food allergy.

Although several analyses were carried out with no adjustment for multiple comparisons, we have observed a range of moderately statistically significant and consistent results for a number of outcomes assessed, making these findings important. The direction of these results is consistent with the hypothesis that exposure to grass pollen during grass pollen season may increase the risk of sensitization to foods in the absence of ingestion of allergic foods. It is possible that infants who exhibit sensitization to certain foods but are not allergic when tested with an oral food challenge are in fact the group of children that have multiple sensitizations including to other aeroallergens, particularly to ryegrass. Although we do not have data on aeroallergen sensitization in this cohort, one of our earlier studies assessing the natural history of food allergy reported that at 12 months, sensitization to egg and peanut was highly

prevalent, and in almost all of these, they were sensitized to at least one aeroallergen (238). Other studies too have shown similar findings (5, 239).

Our findings extend on the findings reported by Pyrhonen and colleagues (2012) who reported higher incidence of food sensitization in children up to four years of age whose 11th gestational week occurred during the months when high concentrations of alder and birch pollen were observed (5). Here, we have shown that these adverse effects of high grass pollen exposure impact on food sensitization outcomes much earlier at 12 months. They did not assess history of maternal food allergy as an effect modifier. To our knowledge, the only other study that analyzed the association between grass pollen exposure in early life and food sensitization in children was our earlier study that reported no association between grass pollen exposure in the first three months and food sensitization assessed by SPT in children aged two years (3). In this current population-based study of infants in the same city in Australia we observed similar results and found no associations with 3-month pollen exposure. Although there is a growing interest in exposure to allergens in the first four to six months and the development of food allergies in children, our findings are not directly comparable because the introduction of food during this period is a separate topic to our current study (240, 241).

Environmental exposures such as grass pollen periods may promote sensitization to foods. We only focused on grass pollen exposure as clinically it is a major trigger of allergies and asthma exacerbations requiring hospitalisations in children and adolescents. Moreover, grass pollen sensitisation in Melbourne is high (242) and the grass pollen season seems to coincide with the timing of allergic disease (243). Multiple studies in children have reported increased risk of peanut sensitization and pollen sensitization. The strongest hypothesis to explain this result is the cross-reactivity sensitization between pollen and peanut due to structural similarities between the proteins in pollen and peanut. Asarnoj and colleagues (2011) reported this association with birch pollen in a birth cohort in Stockholm, Sweden and Niggeman and colleagues (2011) reported the same association with grass pollen in Berlin, Germany. Although these studies did not assess pollen exposure nor sensitization, in utero or during the first year of life, it is possible that exposure to high pollen further exacerbates this risk. Further studies are needed to assess this exposure pathway.

To our knowledge, our study is the first to report an association between hen's egg sensitization and pollen exposure. Our reported findings for elevated odds of hen's egg sensitisation and pollen exposure in utero are modest and it is possible that pollen exposure acted as a proxy for another seasonal factor, such as Vitamin D level. Vitamin D deficiency has been proposed to be a potential cause of food allergy (130, 244). Offspring of women who are vitamin D insufficient during pregnancy have an elevated risk of food sensitisation (245). Moreover, some studies also support a role of current vitamin D levels and food allergy outcomes, particularly in children with specific polymorphisms in vitamin D binding receptors (246).

Therefore, the observed associations may be due to pollen exposure during pregnancy being correlated (due to shared seasonality of exposure), to the true causal effect, with the effect of vitamin D levels in pregnancy. But without the vitamin D measures we were unable to adjust for this variable in the regression model to assess this possibility. As an alternative to measured Vitamin D, we assessed if UVR and pollen are correlated. It should be noted that these correlations are not causal in nature (e.g. UVR does not "cause" pollen exposure) so does not fit the definition of a shared cause confounder but could generate a spurious association. A study of medically confirmed food allergy in 835 children aged 0 to 4 years living in the Australian Capital Territory (ACT) reported high rates of peanut allergy among infants born in winter and as UVR exposure increased they reported a decline in the relative rate of food allergy (42). Similarly, our own earlier study using the HealthNuts cohort identified associations between infant postnatal serum Vitamin D, UVR exposure for the month the child was born and food allergy outcomes (130). Here, we used a composite measure of indirect UVR exposure at the end of the first trimester of pregnancy, developed for an earlier HealthNuts study (247). When we adjusted for this measure of UVR, none of the estimated pollen exposure effects changed greatly to warrant further investigation. Nevertheless, without data on maternal Vitamin D levels during pregnancy, we cannot discount the effects not captured here.

Interestingly, maternal history of food allergy seems to be important but only for oral challenge diagnosed food allergy, suggesting alternative pathways that needs to be explored further for strata-specific analysis with larger sample sizes than reported here. Maternal history of allergy, either food allergy or other allergy, is a very important risk factor for food allergy in children (77, 228). It is possible that mothers with food

allergies have been consuming healthier plant-based foods such as fruits, nuts and vegetables compared to mothers without a history of food allergy. The proteins that induce IgE response in some of these feeds are also present in pollen including the grasses (230). We don't really know if this reactivity causes clinical symptoms, but it is possible that if cumulative exposure occurs during grass pollen seasons, the effects would be greater. Although other factors have been reported to be associated with food allergy such as father's history of food allergy, Vitamin D status, ethnicity, and eczema, we hypothesize that familial effects such as maternal history of food allergy plays an important role in the environmental causative pathways to allergic diseases in early life (37, 42, 201). This needs to be investigated further as power may be an issue when strata-specific results are presented.

The use of oral food challenge (OFC) to diagnose food allergy, instead of just food sensitization by SPT or specific IgE, is the major strength of this study. Rather than just using born in grass pollen season as a proxy for pollen exposure, we used daily pollen measurement during grass pollen season as a more precise measurement for pollen exposure in utero and in early life. As HealthNuts is a population-based study, the findings maybe applicable to the general population of families with similar demographics and circumstance. There are several limitations to be considered in this study. We only conducted oral food challenge on infants with positive SPT because it was assumed that infants with negative SPT did not have IgE-mediated food allergy. This assumption was tested by performing OFC on 200 randomly selected children with SPT negative and they showed no reaction to OFC (33).

A potential limitation is that we did not have pollen measurement outside the predefined pollen season. Although the start and end timing slightly vary, grass pollen season seems to occur during October to early-January with about 90% of the annual pollen load occurring during this period. Studies have shown that low or clinically irrelevant levels outside this period (44, 248). Therefore, the impacts on the outcome are likely to be negligible. We had no data on time spent outdoors; it is possible that mothers reporting hay fever symptoms may remain indoors during high pollen days. Therefore, the effects are likely to be stronger than what s reported. We also only used maternal report to define maternal history of hayfever and food allergy, which might have led to misclassification. This misclassification might contribute to decreasing the effect of maternal history of hayfever and food allergy to null. We do not have age at

exposure to first respiratory virus season as this is a plausible confounder. Although this study is exploratory, it is important to consider the issue of multiple comparisons. Several analyses have been undertaken and there seems to be a consistent finding for grass pollen exposure in utero and some food allergy outcomes.

In summary, our findings suggest that there is an important period during the intrauterine stage and the first six months of life, where environmental exposures such as high grass pollen may increase risk of food allergies in infants. Whether it is pollen and food cross-reactivity or multiple sensitizations to food and aeroallergens, more studies are needed to replicate these findings in children.

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Supplementary Table 1. HealthNuts participants' distribution for each food outcome.

Food	Not sensitized	Food-sensitized tolerant	Food allergic
Hen's egg, n (%) (n=4,840)	4 229 (87.4)	154 (3.2)	457 (9.5)
Peanut, n (%) (n=4,935)	4 552 (92.2)	232 (4.7)	151 (3.1)
Sesame, n (%) (n=4,946)	4 769 (96.4)	144 (2.9)	33 (0.6)
Hen's egg, peanut or sesame, n (%) (n=4,728)	4 005 (84.7)	231 (4.9)	492 (10.4)

Supplementary Table 2. Unadjusted effects of pollen exposure on food allergy outcomes at 12 months.

	Food-sensitized tolerant MOR (95% CI)	p	Food allergic MOR (95% CI)	p
Hen's egg				
Gestation week 11 in peak pollen season	1.27 (0.89, 1.83)	0.19	1.15 (0.92, 1.44)	0.22
Gestation week 10-12 in peak pollen season	1.39 (0.99, 1.96)	0.058	1.08 (0.87, 1.34)	0.50
Born in peak pollen season	0.9 (0.62, 1.30)	0.57	0.8 (0.63, 1.00)	0.05
• Pollen exposure in first 7 days	1. 1 (0.88, 1.37)	0.39	1.06 (0.92, 1.23)	0.40
Pollen exposure in first 3 months	1.00 (0.98, 1.02)	0.95	0.99 (0.98, 1.01)	0.44
Pollen exposure in between first 4-6 months	1.02 (1.004, 1.04)	0.01	1.00 (0.99, 1.01)	>0.99
Pollen exposure in first 6 months	1.01 (1.00, 1.03)	0.09	1.00 (0.99, 1.01)	0.57
Peanut				
Gestation week 11 in peak pollen season	1.335 (1.00, 1.80)	0.053	1.21 (0.83, 1.77)	0.31
Gestation week 10-12 in peak pollen season	1.30 (0.98, 1.74)	0.069	1.24 (0.87, 1.78)	0.23
Born in peak pollen season	0.76 (0.55, 1.05)	0.09	0.97 (0.67, 1.4)	0.85
• Pollen exposure in first 7 days	1.21 (1.01, 1.44)	0.03	0.98 (0.77, 1.25)	0.88
Pollen exposure in first 3 months	1.00 (0.98, 1.02)	0.79	1.00 (0.98, 1.03)	0.74
Pollen exposure in between first 4-6 months	1.01 (0.99, 1.03)	0.24	1.01 (0.99, 1.03)	0.43
Pollen exposure in first 6 months	1.01 (0.99, 1.02)	0.25	1.01 (0.99, 1.02)	0.32
Hen's egg, peanut, or sesame				
Gestation week 11 in peak pollen season	1.28 (0.95, 1.72)	0.11	1.11 (0.89, 1.38)	0.36
Gestation week 10-12 in peak pollen season	1.32 (0.99, 1.76)	0.055	1.1 (0.89, 1.35)	0.39
Born in peak pollen season	0.87 (0.64, 1.19)	0.39	0.8 (0.64, 0.99)	0.04
• Pollen exposure in first 7 days	1.09 (0.91, 1.31)	0.34	1.07 (0.93, 1.23)	0.32
Pollen exposure in first 3 months	1.00 (0.99, 1.02)	0.53	1.00 (0.99, 1.01)	0.98
Pollen exposure in between first 4-6 months	1.02 (1.003, 1.04)	0.02	1.00 (0.99, 1.01)	0.95
Pollen exposure in first 6 months	1.02 (1.002, 1.03)	0.02	1.00 (0.99, 1.01)	0.90

- Mutually adjusted to each other: born in peak pollen season and pollen exposure in first 7 days.

Food sensitization was defined as positive if SPT ≥2 mm or sIgE ≥0.35 kU/L.

Associations expressed as effect per 100 pollen grains/m^3 increase.

Chapter 7 Association between grass pollen exposure *in utero* and early life is associated with asthma and hay fever in children and adolescence

This final result chapter consists of an original research thesis chapter. This chapter addressed research questions number 5 and 6. This chapter will be published after submission of my thesis.

Introduction

In previous chapters, this doctoral study analysed the association of pollen exposure with level of IgE in cord blood and food allergy in 12-month old infants. In this chapter, it extended the age window for outcome measures in children and adolescents and focus on allergic respiratory disease outcomes. Several studies including previous published work had reported association between pollen exposure *in utero*, at birth, and infancy to early life allergic respiratory diseases (3-5, 47, 209, 233, 249, 250). A study from Stockholm, Sweden found that high level pollen exposure in the last 12 weeks of pregnancy was associated with increase odds for hospital admission due to asthma in the first year of life 1 year (4). A different study in the same city, Stockholm, Sweden showed that high pollen exposure during pregnancy had a trend of increase prevalence for asthma, hay fever, and atopic dermatitis in children age 4.5 years (209). A study in Melbourne, Australia reported that born in pollen season and pollen exposure up to first 6 months of life increased the odds for hay fever at the age of 6 years (3).

To the best of my knowledge, no study has reported any association between pollen exposure *in utero*, at birth, and in the first 6 months of life with asthma and hayfever in adolescence. Thus, it is hypothesized that the effect of pollen exposure was not just in early life but carried through to adolescence.

In this chapter, this doctoral study intended to further analyse the effect of pollen exposure to allergic respiratory diseases, focusing on asthma and hay fever, in children and adolescents. Just like in previous chapters, it would like to examine which period of life that pollen exposure associated with allergic respiratory diseases and whether maternal history of asthma or hay fever modified these association. By gaining a better understanding of the risk window of the pollen exposure to allergic respiratory diseases,

we can target interventions to better manage allergic respiratory diseases and reduce the burden of disease on individuals.

Methods

Study design and population

The Melbourne Atopy Cohort Study (MACS) started as randomized clinical trial, enrolling infants who were born between 24th March 1990 and 1st November 1994. Expectant mothers were approached during antenatal care visits at the former Mercy Hospital for Women (East Melbourne) and other private consulting suites. Mothers were included in the study if they had at least one-first-degree family member (father, mother, or older sibling) with a history of eczema, and/or asthma, and/or allergic rhinitis, and/or severe food allergy.

The trial objective was to assess the effect of three infant formulae on the incidence of allergic disease in childhood. Before birth, unborn infants were randomized into cow's milk, soy milk, or a partially hydrolyzed whey formula. The study continues as observational study about the natural history of allergic disease and theirs associated factors. Child/children were followed up multiple times until the age of 18 years. In my study I considered the asthma and hay fever outcome measurements collected by survey at age 6-7 years, 12 years, and 18 years, with additional ISAAC questionnaire at age 12 years (251).

Data collection

Data were collected on the family on child's birth: family history of allergy, parent education and employment status, and tobacco smoking in the family. Data on the child's health (i.e. asthma, eczema, hay fever, diarrhoea) were collected on multiple timepoints from birth: 18 times in the first 2 years, (16 times every 4 weeks from 4 weeks until 64 weeks of age, then at 18 months and 2 years of age), by a telephone survey. Followed by annual telephone survey from 3 to 7 years of age and additional clinical examination at 12 and 18 years of age (Lowe et al., 2017). This analysis use data at timepoint 6/7 years (later called as 6 years), 12 years, and 18 years as hay-fever is difficult to diagnose prior to this age.

The study response rates varied at each timepoint in this analysis (Figure 1). At timepoint 6 years, the response rate was still high, 495 children (80%) were able to be contacted for survey. The response rate decreased at timepoint 12 years, with only 360 children (58%) underwent the clinical examination and survey. Due to additional effort from the study team to trace lost children at timepoint 12 years, the response rate at 18 years increased to 68% (423 children).

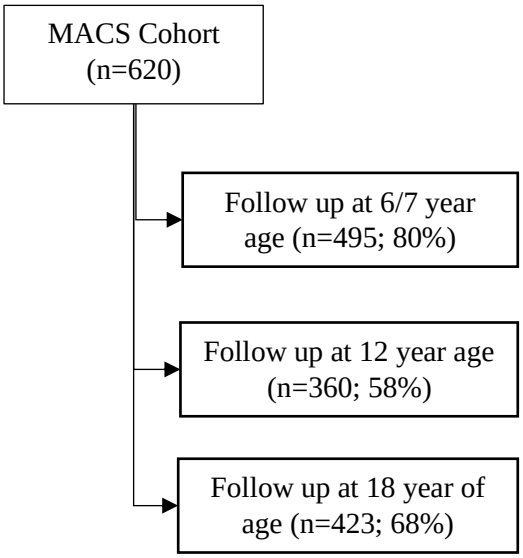

Figure 1. Response rate of study participants at age 6 to 7 years, age 12 year, age 18 year.

Pollen exposure data was measured using a volumetric spore trap (Burkard) daily between October 1991 and January 1994 from one pollen trap located at Earth Sciences building inside the complex of the University of Melbourne. The pollen trap was located on the roof top, 15 metres above ground, as per standard in pollen monitoring. Using the pollen trap, airborne grass pollen was trapped on an adhesive surface, then identified and measured under a light microscope every day. The daily average grass pollen counts were expressed as number of pollen grains per m3 of air. Although, pollen in Melbourne is usually detectible from August to January, the peak pollen season occurs between October to December (44).

Definitions

Exposure

Similar to previous chapters, this chapter defined pollen exposure in multiple ways. First as a binary measurement, that is, inside peak pollen season (October to December) or outside peak pollen season (January to September) for birth and particular weeks of gestation. Also, as a continuous measurement where daily grass pollen measured during each period. This chapter defined exposure in multiple time periods including *in utero* and in early life. It used the end of first trimester, specifically 11th week gestation, as this was reported to be associated with food allergies (5) and it was also believed this *in utero* period might be associated with asthma and hay fever. However, this doctoral study considered gestational age as an approximate measure, therefore it used the period between 10th to 12th week gestation as a broad range for the 11th week gestation. The gestation week was calculated based on the assumption that all infants' gestational age at birth was 40 weeks. Early life period that had been reported for pollen exposure to be associated with allergic respiratory diseases in children and used in this analysis were day of birth, first 7 days, first 3 months, between first 4 to 6 months, and the first 6 months (3-5, 47).

Outcome

Parents were asked how many episodes of asthma and hay fever their child had in the last 12 months at child's ages 6 years, 12 years, and 18 years. In this analysis, asthma was defined as having at least one episode of asthma in the last 12 months at each timepoint. Hay fever as having at least one episode of hay fever in the last 12 months and a separate category for "asthma only", defined as having at least one episode of asthma and no episode of hay fever in the last 12 months.

Potential confounders or effect modifiers

Maternal age in years at child's birth, maternal history of smoking during pregnancy, and maternal history of asthma were considered as potential confounders for the association between pollen exposure and asthma/hay fever in children. Maternal history of asthma and hay fever were also considered as potential effect modifiers for the association because previous studies had reported that history of asthma or even allergic diseases in mother were associated with asthma in children (3, 37, 223). This analysis only considered maternal history of asthma and hay fever, instead of paternal,

based on a meta-analysis results that showed maternal history of asthma is a bigger risk factor for asthma in children (224).

Statistical analysis

Logistic regression was used to analyse the association between pollen exposure at 11th week gestation, 10th to 12th gestation age, at birth, first 7 days, first 3 months between first 4 to 6 months and first 6 months with asthma, asthma only, and hay fever at 6 years age, 12 years age, and 18 years age. Because pollen season might be a proxy for multiple *in utero* environmental factors (ultra violet exposures, respiratory infections, cold weather) exposures (252, 253), not just grass pollen exposure, the analysis on the periods in 11th week gestation, 10th to 12th week gestation, and first 7 days together was performed in two ways. First, each period was analysed as inside or outside peak pollen season. Second, each period was analysed for inside or outside peak pollen season together with the summed grass pollen exposure. However, summed grass pollen exposure was analysed only for the first 3 months, between first 4 to 6 months, and first 6 months periods because the interest was in the early life exposures, not *in utero* exposures. The total amount of pollen was divided by 100 to get the effect size of an increase of 100 grains/m^3 for the cumulative pollen exposure.

The model was adjusted for maternal age at child's birth, maternal history of smoking during pregnancy, and maternal history of asthma as potential confounders. Results were presented as crude and adjusted odds ratios (OR) along the corresponding 95% confidence intervals (CI). All the exposures and confounders were statistically significant if the p-value was less than 0.05 and change the estimated effect size of the pollen exposure by at least 10%. Interaction analysis was conducted with maternal history of asthma and maternal history of hay fever and the strata specific results were presented. Interaction analysis will be deemed as significant if p-value for interaction was less than 0.1 because the power for interaction analysis is considered lower than non-interaction analysis (236). All analysis in this chapter was performed in Stata™ version 14.2 (StataCorp, Texas, TX, USA).

Ethical approval

All mothers gave written informed consent for the participation of their children in MACS. Ethical approval for MACS was granted by the Mercy Maternity Hospital Ethics Committee (R07/20 and R88/06) and by the Royal Children's Hospital Ethics committee for the 18-year follow-up.

Results

With increasing age, the proportion of children with hay fever episodes increased. On the contrary, the proportion of children with asthma decreased so that the number of children (Table 1). The characteristics of the mothers are shown in table 2. The mean age of mothers was 31.2 years (SD 4.4 years) and the history of hay fever was reported to be higher than asthma (61% vs 43%).

Table 1. Characteristics of MACS cohort study participants mothers

Variable	No (%)	Mean (SD)
Maternal age (n = 620)		31.2 (4.4)
Maternal history of smoking during pregnancy (n = 620)	47 (7.6)	
Maternal history of asthma (n = 619) • No • Yes	 • 351 (57) • 268 (43)	
Maternal history of hay fever (n = 619) • No • Yes	 • 244 (39) • 375 (61)	

Table 2. Children's asthma-hay fever status measured at each follow up visit

	Age at Measurement (No, %)		
	6 years	12 years	18 years
Asthma	148/495 (30)	84/359 (23)	98/408 (24)
Asthma only	86//495 (17)	30/359 (8)	27/408 (7)
Hay fever	117/495 (24)	134/360 (37)	209/423 (49)

Exposures

Of the MACS participants, one in four children had their 11th week gestation age during the peak pollen season. A higher proportion of children were born in the peak pollen season (28%) and a slightly even higher proportion of children had 10th to 12th week gestation in the peak pollen season (29%). Median pollen exposure in 11th week gestation and in the first seven days of life were equal but the third quartile was higher in 11th week gestation (1.68 vs 1.54, respectively). Children in MACS were exposed to somewhat higher levels of pollen in the first 3 months compared to the next 3 months of life (median =3.14 vs 2.82, respectively) (Table 3).

Association between pollen exposure and asthma

At the age of six, children who had their 11th week or between the 10th and 12th week of gestation in peak pollen season or who had higher pollen exposure in the 11th week of gestation showed increased odds of asthma only (no hayfever) but not age 12 or 18 years (table 4). Born in peak pollen season was not significant for asthma in any follow up. Pollen exposure at 3, 4-6 or up to first 6 months of life was not significant for asthma at 6, 12 or 18 years (Table 4). When adjusted for maternal age and history of asthma estimated pollen effects were similar. Both gestation during peak pollen seasons around week 10 and 12 increased odds of asthma only at 6 years (Table 6).

Association between pollen exposure and hay fever

11th week gestation during the peak pollen season increased odds of hay fever at 18 years in an unadjusted analysis but actual exposure to pollen levels during the week of the 11th gestation period was protective when mutually adjusted. Born in pollen season at six years of age was associated with hay fever at 6 years of age (OR 2.32, 95% CI: 1.19, 4.54), while pollen exposure in the first seven days was protective (OR 0.88, 95% CI: 0.77, 0.99) when mutually adjusted for pollen season. None of the exposures at 3, 4-6 months or up to 6 months were associated with hay fever at age 6, 12 or 18 years (Table 5). Little change in the estimated pollen effects were observed when I adjusted for mothers age and history of asthma. (Table 7).

Table 3. Children's pollen exposures measurement available for MACS cohort study participants

Variable	No (%)	Continuous measurement*				
		Min	Median	P75	P95	Max
11^{th} week gestation (n = 620) • Outside peak pollen • In peak pollen	 • 465 (75) • 155 (25)					
Pollen exposures in 11^{th} week gestation (n=319)		0	0.17	1.68	7.28	12.82
10^{th} -12^{th} week gestation (n = 620) • Outside peak pollen • In peak pollen	 • 441 (71) • 179 (29)					
Pollen exposures in 10^{th} -12^{th} week gestation (n=319)		0	0.56	7.14	21.5	29.29
Born in peak pollen season (n = 620) • Outside peak pollen • In peak pollen	 • 447 (72) • 173 (28)					
Pollen exposure in first 7 days (n = 411)		0	0.17	1.54	9.05	18.39
Pollen exposures in the first 3 months (n = 364)		0.07	3.14	29.3	66.06	73.66
Pollen exposures in between first 4-6 months		0.07	2.82	29.71	68.22	73.66
Pollen exposures in first 6 months		0.43	27.66	68.41	78.88	79.86

* pollen exposures in 100 grain/m^3

P75: percentile 75

P95: percentile 95

Table 4. Crude association between Pollen exposure and Asthma

Exposure	Asthma at 6 years		Asthma only at 6 years		Asthma at 12 years		Asthma at 18 years	
	OR (95% CI)	P	OR (95% CI)	p	OR (95% CI)	p	OR (95% CI)	p
Gestation week 11 in peak pollen season	1.26 (0.82, 1.94)	0.29	1.70 (1.03, 2.8)	0.03	1.15 (0.67, 1.97)	0.60	1.00 (0.59, 1.69)	0.99
• Gestation week 11 in peak pollen season • Pollen exposure in gestation week 11	• 1.82 (0.9, 3.69) • 0.95 (0.82, 1.1)	0.09 0.47	• 2.01 (0.86, 4.71) • 0.98 (0.83, 1.16)	0.10 0.82	• 1.15 (0.64, 3.74) • 0.95 (0.79, 1.15)	0.32 0.61	• 1.46 (0.64, 3.34) • 0.98 (0.84, 1.15)	0.37 0.80
Gestation week 10-12 in peak pollen season	1.31 (0.87, 1.99)	0.19	1.51 (0.93, 2.47)	0.09	1.22 (0.73, 2.05)	0.45	1.03 (0.63, 1.69)	0.90
• Gestation week 10-12 in peak pollen season • Pollen exposure in gestation week 10-12	• 1.34 (0.62, 2.89) • 1.01 (0.96, 1.07)	0.45 0.71	• 2.81 (1.13, 7.00) • 0.97 (0.9, 1.04)	0.02 0.33	• 1.04 (0.4, 2.69) • 1.03 (0.96, 1.1)	0.93 0.46	• 0.97 (0.39, 2.41) • 1.01 (0.95, 1.08)	0.95 0.74
Born in peak pollen season	0.84 (0.54, 1.30)	0.43	0.68 (0.39, 1.19)	0.17	0.79 (0.45, 1.39)	0.41	0.96 (0.58, 1.59)	0.87
• Born in peak pollen season • Pollen exposure in first 7 days	• 1.14 (0.6, 2.19) • 0.96 (0.87, 1.07)	0.68 0.45	• 0.61 (0.25, 1.48) • 1.04 (0.92, 1.17)	0.27 0.54	• 0.72 (0.29, 1.79) • 0.98 (0.86, 1.13)	0.47 0.80	• 0.87 (0.39, 1.93) • 1.03 (0.93, 1.15)	0.73 0.56
Pollen exposure in first 3 months	1.00 (0.99, 1.01)	0.97	0.99 (0.98, 1.01)	0.25	1.00 (0.98, 1.01)	0.73	1.00 (0.99, 1.01)	0.89
Pollen exposure in between first 4-6 months	1.00 (0.99, 1.01)	0.48	1.00 (0.99, 1.01)	0.90	1.01 (0.99, 1.02)	0.30	1.00 (0.99, 1.01)	0.57
Pollen exposure in first 6 months	1.00 (0.99, 1.01)	0.55	0.99 (0.98, 1.01)	0.32	1.00 (0.99, 1.01)	0.56	1.00 (0.99, 1.01)	0.71

- Mutually adjusted to each other

Table 5. Crude association between Pollen exposure and Hay fever

Exposure	Hay fever at 6 years		Hay fever at 12 years		Hay fever at 18 years	
	OR (95% CI)	p	OR (95% CI)	p	OR (95% CI)	p
Gestation week 11 in peak pollen season	0.77 (0.47, 1.26)	0.30	0.84 (0.52, 1.37)	0.48	1.2 (0.77, 1.85)	0.41
• Gestation week 11 in peak pollen season	• 0.98 (0.45, 2.15)	0.97	• 1.24 (0.55, 2.76)	0.60	• 2.35 (1.12, 4.9)	0.02
• Pollen exposure in gestation week 11	• 0.93 (0.78, 1.11)	0.40	• 1.00 (0.85, 1.17)	0.95	• 0.83 (0.71, 0.97)	0.01
Gestation week 10-12 in peak pollen season	0.86 (0.54, 1.37)	0.52	0.86 (0.54, 1.37)	0.52	1.32 (0.87, 2.00)	0.18
• Gestation week 10-12 in peak pollen season	• 0.61 (0.25, 1.45)	0.26	• 0.86 (0.36, 2.04)	0.72	• 2.14 (0.99, 4.6)	0.05
• Pollen exposure in gestation week 10-12	• 1.02 (0.96, 1.09)	0.52	• 1.03 (0.97, 1.1)	0.29	• 0.96 (0.9, 1.01)	0.11
Born in peak pollen season	1.25 (0.8, 1.96)	0.33	0.81 (0.5, 1.32)	0.39	0.96 (0.63, 1.47)	0.86
• Born in peak pollen season	• 2.32 (1.19, 4.54)	0.01	• 1.61 (0.75, 3.46)	0.22	• 1.02 (0.54, 1.95)	0.94
• Pollen exposure in first 7 days	• 0.88 (0.77, 0.997)	0.04	• 0.87 (0.76, 1.00)	0.05	• 1.00 (0.91, 1.09)	0.98
Pollen exposure in first 3 months	1.01 (1.00, 1.02)	0.09	1.00 (0.98, 1.01)	0.53	1.00 (0.99, 1.01)	0.50
Pollen exposure in between first 4-6 months	1.00 (0.99, 1.01)	0.47	1.01 (1.00, 1.02)	0.20	1.00 (0.99, 1.01)	0.84
Pollen exposure in first 6 months	1.01 (1.00, 1.02)	0.05	1.00 (0.99, 1.01)	0.57	1.00 (0.99, 1.01)	0.72

- Mutually adjusted to each other

Table 6. Adjusted association between Pollen exposure and Asthma

Exposure	Asthma at 6 years		Asthma only at 6 years		Asthma at 12 years		Asthma at 18 years	
	aOR (95% CI)	p	aOR (95% CI)	p	aOR (95% CI)	p	aOR (95% CI)	P
Gestation week 11 in peak pollen season	1.27 (0.82, 1.96)	0.28	1.74 (1.05, 2.86)	0.03	1.17 (0.68, 2.01)	0.57	0.99 (0.59, 1.68)	0.98
• Gestation week 11 in peak pollen season • Pollen exposure in gestation week 11	• 1.83 (0.9, 3.72) • 0.95 (0.82, 1.1)	0.09 0.45	• 2.2 (0.93, 5.2) • 0.98 (0.83, 1.16)	0.07 0.81	• 1.56 (0.64, 3.8) • 0.95 (0.79, 1.15)	0.32 0.62	• 1.4 (0.61, 3.25) • 0.98 (0.84, 1.15)	0.43 0.82
Gestation week 10-12 in peak pollen season	1.31 (0.86, 1.98)	0.20	1.55 (0.95, 2.52)	0.08	1.21 (0.72, 2.04)	0.47	1.01 (0.61, 1.66)	0.97
• Gestation week 10-12 in peak pollen season • Pollen exposure in gestation week 10-12	• 1.35 (0.62, 2.93) • 1.01 (0.95, 1.07)	0.45 0.74	• 3.16 (1.25, 7.97) • 0.96 (0.9, 1.04)	0.01 0.32	• 1.03 (0.39, 2.7) • 1.03 (0.96, 1.10)	0.95 0.45	• 0.92 (0.36, 2.33) • 1.01 (0.95, 1.08)	0.86 0.70
Born in peak pollen season	0.87 (0.56, 1.34)	0.52	0.69 (0.4, 1.2)	0.19	0.81 (0.46, 1.43)	0.46	0.99 (0.59, 1.65)	0.95
• Born in peak pollen season • Pollen exposure in first 7 days	• 1.15 (0.6, 2.21) • 0.97 (0.87, 1.07)	0.68 0.51	• 0.62 (0.26, 1.52) • 1.03 (0.92, 1.16)	0.29 0.59	• 0.69 (0.28, 1.72) • 0.99 (0.86, 1.14)	0.42 0.92	• 0.82 (0.36, 1.86) • 1.05 (0.94, 1.17)	0.64 0.38
Pollen exposure in first 3 months	1.00 (0.99, 1.01)	0.95	0.99 (0.98, 1.01)	0.22	1.00 (0.98, 1.01)	0.75	1.00 (0.99, 1.01)	0.95
Pollen exposure in between first 4-6 months	1.00 (0.99, 1.01)	0.60	1.00 (0.98, 1.01)	0.71	1.01 (0.99, 1.02)	0.40	1.00 (0.99, 1.01)	0.65
Pollen exposure in first 6 months	1.00 (0.99, 1.01)	0.64	0.99 (0.98, 1.00)	0.21	1.00 (0.99, 1.01)	0.65	1.00 (0.99, 1.01)	0.73

Notes: adjusted for maternal age and maternal history of asthma

- Mutually adjusted to each other

Table 7. Adjusted association between Pollen exposure and Hay fever

Exposure	Hay fever at 6 years		Hay fever at 12 years		Hay fever at 18 years	
	aOR (95% CI)	p	aOR (95% CI)	p	aOR (95% CI)	p
Gestation week 11 in peak pollen season	0.76 (0.47, 1.25)	0.28	0.84 (0.51, 1.36)	0.46	1.2 (0.78, 1.86)	0.41
• Gestation week 11 in peak pollen season	• 0.9 (0.41, 1.99)	0.80	• 1.19 (0.53, 2.7)	0.67	• 2.39 (1.13, 5.02)	0.02
• Pollen exposure in gestation week 11	• 0.93 (0.78, 1.11)	0.42	• 0.99 (0.84, 1.17)	0.95	• 0.83 (0.71, 0.97)	0.02
Gestation week 10-12 in peak pollen season	0.83 (0.52, 1.33)	0.44	0.84 (0.52, 1.34)	0.46	1.33 (0.87, 2.01)	0.18
• Gestation week 10-12 in peak pollen season	• 0.54 (0.22, 1.32)	0.17	• 0.8 (0.33, 1.94)	0.62	• 2.22 (1.02, 4.81)	0.04
• Pollen exposure in gestation week 10-12	• 1.02 (0.96, 1.09)	0.48	• 1.04 (0.97, 1.1)	0.29	• 0.96 (0.9, 1.01)	0.11
Born in peak pollen season	1.29 (0.82, 2.03)	0.27	0.81 (0.49, 1.32)	0.39	0.97 (0.64, 1.48)	0.89
• Born in peak pollen season	• 2.28 (1.16, 4.48)	0.01	• 1.54 (0.71, 3.35)	0.27	• 1.01 (0.53, 1.93)	0.98
• Pollen exposure in first 7 days	• 0.88 (0.78, 1.01)	0.06	• 0.88 (0.76, 1.01)	0.06	• 1.00 (0.92, 1.1)	0.96
Pollen exposure in first 3 months	1.01 (1.00, 1.02)	0.07	1.00 (0.98, 1.01)	0.56	1.00 (0.99, 1.01)	0.49
Pollen exposure in between first 4-6 months	1.00 (0.99, 1.01)	0.45	1.01 (1.00, 1.02)	0.16	1.00 (0.99, 1.01)	0.77

Interaction between pollen exposure and maternal history of asthma

Nearly all the exposures for asthma or hay fever symptoms had no interaction effect with maternal history of asthma at any follow up. The only significant interaction results with significant stratified association were the interaction between 11th week of gestation and 10th to 12th week of gestation in peak pollen season with maternal history of asthma for hay fever symptoms at 6 years age. Having an 11th week of gestation and 10th to 12th week of gestation in peak pollen season was a protective factor for children whose mother had no history of asthma (OR 0.43, 95% CI: 0.19, 0.97; OR 0.23, 95% CI: 0.06, 0.86, respectively), but not for children whose mother with history of asthma (Table 8). Several exposures had significant interaction (p for interaction <0.1) but non-significant stratified association.

Interaction between pollen exposure and maternal history of hay fever

In table 9, there were several significant interaction results for asthma at 6 years, 12 years, and 18 years. Period 11th week of gestation in peak pollen season and higher pollen exposure in 11th week of gestation showed a trend of protective effect for asthma in children whose mother had no history of hay fever and a trend for risk effect in children whose mother had history of hay fever. While born in peak pollen season and higher pollen exposure in the first 7 days showed opposite direction of effect.

Like interaction with maternal history of asthma, of all significant interaction effect, only one had significant association in the stratified analysis. Born in peak pollen season had a protective effect for asthma at 12 years for children whose mother had history of hay fever (OR 0.42, 95% CI: 0.18, 0.95). When the same exposure being adjusted for pollen exposure in first 7 days, the stratified association effect was still the same with wider confidence interval and no longer significant. In follow up at 18 years of age, interaction between born in peak pollen season and maternal history of hay fever was significant but the stratified association was weaker and no longer significant.

Table 8. Interaction between Pollen exposure and Maternal History of Asthma

Asthma at 6 years	No asthma history OR (95% CI)	Asthma history OR (95% CI)	p-value for interaction
Gestation week 11 in peak pollen season*	1.62 (0.66, 4.00)	2.08 (0.83, 5.17)	0.67
Pollen exposure in gestation week 11*	1.00 (0.83, 1.2)	0.89 (0.72, 1.09)	0.32
Gestation week 10-12 in peak pollen season**	1.2 (0.47, 3.07)	1.54 (0.57, 4.14)	0.66
Pollen exposure in gestation week 10-12**	1.03 (0.97, 1.1)	0.98 (0.91, 1.06)	0.25
Born in peak pollen season***	1.43 (0.62, 3.29)	0.92 (0.39, 2.14)	0.40
Pollen exposure in first 7 days***	1.00 (0.9, 1.11)	0.82 (0.64, 1.04)	0.10
Pollen exposure in first 3 months	1.00 (0.98, 1.01)	1.00 (0.99, 1.02)	0.51
Pollen exposure in between first 4-6 months	1.01 (0.99, 1.02)	1.00 (0.98, 1.01)	0.52
Pollen exposure in first 6 months	1.00 (0.99, 1.01)	1.00 (0.99, 1.01)	0.73

Asthma only at 6 years	No asthma history OR (95% CI)	Asthma history OR (95% CI)	p-value for interaction
Gestation week 11 in peak pollen season*	2.86 (1.00, 8.14)	1.53 (0.47, 5.04)	0.38
Pollen exposure in gestation week 11*	1.08 (0.89, 1.31)	0.77 (0.53, 1.13)	0.09
Gestation week 10-12 in peak pollen season**	3.48 (1.18, 10.3)	2.71 (0.75, 9.76)	0.73
Pollen exposure in gestation week 10-12**	1.00 (0.93, 1.08)	0.87 (0.75, 1.00)	0.05
Born in peak pollen season***	0.57 (0.19, 1.72)	0.7 (0.22, 2.24)	0.78
Pollen exposure in first 7 days***	1.05 (0.94, 1.19)	0.84 (0.58, 1.23)	0.23
Pollen exposure in first 3 months	0.98 (0.97, 1.00)	1.00 (0.98, 1.02)	0.32
Pollen exposure in between first 4-6 months	1.01 (0.99, 1.02)	0.98 (0.95, 1.01)	0.07
Pollen exposure in first 6 months	1.00 (0.98, 1.01)	0.99 (0.97, 1.01)	0.43

Asthma at 12 years	No asthma history OR (95% CI)	Asthma history OR (95% CI)	p-value for interaction
Gestation week 11 in peak pollen season*	0.97 (0.29, 3.2)	2.39 (0.78, 7.34)	0.22
Pollen exposure in gestation week 11*	0.89 (0.67, 1.18)	1.00 (0.8, 1.23)	0.49
Gestation week 10-12 in peak pollen season**	0.67 (0.2, 2.32)	1.55 (0.47, 5.13)	0.25
Pollen exposure in gestation week 10-12**	1.02 (0.93, 1.12)	1.03 (0.95, 1.12)	0.73
Born in peak pollen season***	0.57 (0.16, 2.05)	0.78 (0.27, 2.27)	0.66
Pollen exposure in first 7 days***	0.98 (0.84, 1.16)	1.01 (0.83, 1.22)	0.81
Pollen exposure in first 3 months	1.00 (0.98, 1.02)	1.00 (0.98, 1.02)	0.77
Pollen exposure in between first 4-6 months	1.01 (0.99, 1.02)	1.00 (0.99, 1.02)	0.87
Pollen exposure in first 6 months	1.00 (0.99, 1.02)	1.00 (0.99, 1.02)	0.74

Asthma at 18 years	No asthma history OR (95% CI)	Asthma history OR (95% CI)	p-value for interaction
Gestation week 11 in peak pollen season*	1.23 (0.41, 3.71)	1.58 (0.55, 4.58)	0.71
Pollen exposure in gestation week 11*	0.97 (0.79, 1.2)	0.99 (0.82, 1.2)	0.89
Gestation week 10-12 in peak pollen season**	0.95 (0.3, 2.98)	0.89 (0.28, 2.85)	0.92
Pollen exposure in gestation week 10-12**	1.00 (0.93, 1.09)	1.02 (0.94, 1.11)	0.75
Born in peak pollen season***	1.09 (0.38, 3.15)	0.66 (0.24, 1.78)	0.43
Pollen exposure in first 7 days***	1.08 (0.97, 1.21)	0.93 (0.74, 1.16)	0.17
Pollen exposure in first 3 months	1.00 (0.99, 1.02)	1.00 (0.98, 1.01)	0.55
Pollen exposure in between first 4-6 months	1.00 (0.98, 1.02)	1.01 (0.99, 1.02)	0.43
Pollen exposure in first 6 months	1.00 (0.99, 1.01)	1.00 (0.99, 1.02)	0.84

Hay fever at 6 years	No asthma history OR (95% CI)	Asthma history OR (95% CI)	p-value for interaction
Gestation week 11 in peak pollen season*	0.36 (0.11, 1.26)	1.62 (0.62, 4.2)	0.03
Pollen exposure in gestation week 11*	0.77 (0.54, 1.1)	1.00 (0.83, 1.2)	0.16
Gestation week 10-12 in peak pollen season**	0.23 (0.06, 0.86)	0.94 (0.33, 2.71)	0.04
Pollen exposure in gestation week 10-12**	0.99 (0.9, 1.08)	1.05 (0.97, 1.13)	0.25
Born in peak pollen season***	3.5 (1.46, 8.39)	1.53 (0.65, 3.61)	0.13
Pollen exposure in first 7 days***	0.92 (0.81, 1.05)	0.76 (0.59, 0.98)	0.14
Pollen exposure in first 3 months	1.01 (1.00, 1.03)	1.00 (0.99, 1.02)	0.30
Pollen exposure in between first 4-6 months	0.99 (0.98, 1.01)	1.01 (1.00, 1.03)	0.11
Pollen exposure in first 6 months	1.01 (0.99, 1.02)	1.01 (1.00, 1.02)	0.67

Hay fever at 12 years	No asthma history OR (95% CI)	Asthma history OR (95% CI)	p-value for interaction
Gestation week 11 in peak pollen season*	1.00 (0.35, 2.83)	1.43 (0.51, 4.03)	0.58
Pollen exposure in gestation week 11*	0.95 (0.88, 1.02)	1.07 (0.87, 1.31)	0.21
Gestation week 10-12 in peak pollen season**	0.72 (0.25, 2.13)	0.9 (0.3, 2.71)	0.73
Pollen exposure in gestation week 10-12**	1.02 (0.93, 1.1)	1.05 (0.97, 1.14)	0.47
Born in peak pollen season***	2.37 (0.88, 6.42)	1.05 (0.4, 2.76)	0.18
Pollen exposure in first 7 days***	0.9 (0.78, 1.05)	0.81 (0.64, 1.03)	0.39
Pollen exposure in first 3 months	1.00 (0.98, 1.02)	0.99 (0.97, 1.01)	0.49
Pollen exposure in between first 4-6 months	1.00 (0.98, 1.02)	1.02 (1.00, 1.03)	0.16
Pollen exposure in first 6 months	1.00 (0.99, 1.01)	1.01 (0.99, 1.02)	0.49

Hay fever at 18 years	No asthma history OR (95% CI)	Asthma history OR (95% CI)	p-value for interaction
Gestation week 11 in peak pollen season*	2.21 (0.9, 5.46)	2.63 (0.97, 7.1)	0.77
Pollen exposure in gestation week 11*	0.77 (0.63, 0.95)	0.89 (0.74, 1.08)	0.24
Gestation week 10-12 in peak pollen season**	2.11 (0.85, 5.21)	2.39 (0.85, 6.7)	0.83
Pollen exposure in gestation week 10-12**	0.94 (0.88, 1.00)	0.98 (0.91, 1.06)	0.32
Born in peak pollen season***	1.24 (0.55, 2.79)	0.79 (0.33, 1.86)	0.38
Pollen exposure in first 7 days***	1.00 (0.91, 1.1)	1.01 (0.85, 1.19)	0.95
Pollen exposure in first 3 months	1.01 (0.99, 1.02)	1.00 (0.98, 1.02)	0.51
Pollen exposure in between first 4-6 months	1.00 (0.99, 1.01)	1.00 (0.98, 1.02)	0.81
Pollen exposure in first 6 months	1.00 (0.99, 1.01)	1.00 (0.99, 1.01)	0.75

Notes: adjusted for maternal age

*, **, *** Mutually adjusted to each other

Table 9. Interaction between Pollen exposure and Maternal History of Hay fever

Asthma at 6 years	No hay fever history OR (95% CI)	Hay fever history OR (95% CI)	p-value for interaction
Gestation week 11 in peak pollen season*	1.36 (0.48, 3.84)	2.24 (0.98, 5.14)	0.40
Pollen exposure in gestation week 11*	0.91 (0.73, 1.14)	0.96 (0.81, 1.15)	0.64
Gestation week 10-12 in peak pollen season**	0.98 (0.33, 2.89)	1.67 (0.69, 4.06)	0.37
Pollen exposure in gestation week 10-12**	0.98 (0.9, 1.07)	1.02 (0.96, 1.09)	0.31
Born in peak pollen season***	1.94 (0.78, 4.82)	0.82 (0.37, 1.82)	0.11
Pollen exposure in first 7 days***	1.01 (0.9, 1.13)	0.85 (0.7, 1.04)	0.09
Pollen exposure in first 3 months	1.01 (0.99, 1.02)	0.99 (0.98, 1.01)	0.18
Pollen exposure in between first 4-6 months	1.00 (0.98, 1.01)	1.01 (0.99, 1.02)	0.48
Pollen exposure in first 6 months	1.00 (0.99, 1.02)	1.00 (0.99, 1.01)	0.63

Asthma only at 6 years	No hay fever history OR (95% CI)	Hay fever history OR (95% CI)	p-value for interaction
Gestation week 11 in peak pollen season*	1.41 (0.41, 4.8)	2.85 (1.01, 8.02)	0.33
Pollen exposure in gestation week 11*	0.96 (0.75, 1.23)	0.99 (0.81, 1.22)	0.83
Gestation week 10-12 in peak pollen season**	1.8 (0.5, 6.48)	4.23 (1.41, 12.68)	0.25
Pollen exposure in gestation week 10-12**	0.92 (0.81, 1.04)	0.98 (0.91, 1.06)	0.28
Born in peak pollen season***	0.91 (0.29, 2.9)	0.45 (0.15, 1.4)	0.33
Pollen exposure in first 7 days***	1.08 (0.95, 1.23)	0.86 (0.63, 1.18)	0.14
Pollen exposure in first 3 months	1.00 (0.98, 1.02)	0.99 (0.96, 1.01)	0.38
Pollen exposure in between first 4-6 months	0.99 (0.97, 1.01)	1.00 (0.99, 1.02)	0.34
Pollen exposure in first 6 months	0.99 (0.98, 1.01)	0.99 (0.98, 1.01)	0.86

Asthma at 12 years	No hay fever history OR (95% CI)	Hay fever history OR (95% CI)	p-value for interaction
Gestation week 11 in peak pollen season*	0.6 (0.15, 2.39)	2.75 (0.98, 7.74)	0.05
Pollen exposure in gestation week 11*	0.7 (0.43, 1.13)	1.04 (0.85, 1.29)	0.09
Gestation week 10-12 in peak pollen season**	0.54 (0.14, 2.12)	1.64 (0.55, 4.9)	0.14
Pollen exposure in gestation week 10-12**	0.94 (0.84, 1.06)	1.07 (0.99, 1.15)	0.04
Born in peak pollen season***	1.55 (0.47, 5.13)	0.42 (0.13, 1.34)	0.07
Pollen exposure in first 7 days***	1.04 (0.91, 1.21)	0.77 (0.54, 1.1)	0.08
Pollen exposure in first 3 months	1.01 (0.99, 1.03)	0.99 (0.97, 1.01)	0.15
Pollen exposure in between first 4-6 months	0.99 (0.97, 1.02)	1.01 (1.00, 1.03)	0.21
Pollen exposure in first 6 months	1.00 (0.99, 1.02)	1.00 (0.99, 1.02)	0.97

Asthma at 18 years	No hay fever history OR (95% CI)	Hay fever history OR (95% CI)	p-value for interaction
Gestation week 11 in peak pollen season*	0.86 (0.25, 3.05)	2.03 (0.76, 5.43)	0.23
Pollen exposure in gestation week 11*	0.74 (0.49, 1.12)	1.06 (0.89, 1.26)	0.08
Gestation week 10-12 in peak pollen season**	0.47 (0.12, 1.88)	1.43 (0.51, 4.04)	0.14
Pollen exposure in gestation week 10-12**	0.92 (0.81, 1.04)	1.05 (0.98, 1.13)	0.04
Born in peak pollen season***	1.95 (0.61, 6.24)	0.58 (0.23, 1.46)	0.06
Pollen exposure in first 7 days***	1.11 (0.98, 1.25)	0.88 (0.7, 1.11)	0.05
Pollen exposure in first 3 months	1.01 (0.99, 1.03)	0.99 (0.97, 1.01)	0.12
Pollen exposure in between first 4-6 months	0.98 (0.96, 1.00)	1.01 (1.00, 1.03)	0.02
Pollen exposure in first 6 months	0.99 (0.98, 1.01)	1.00 (0.99, 1.02)	0.35

Hay fever at 6 years	No hay fever history OR (95% CI)	Hay fever history OR (95% CI)	p-value for interaction
Gestation week 11 in peak pollen season*	0.6 (0.17, 2.18)	1.15 (0.47, 2.8)	0.37
Pollen exposure in gestation week 11*	0.9 (0.68, 1.2)	0.94 (0.77, 1.15)	0.80
Gestation week 10-12 in peak pollen season**	0.49 (0.14, 1.77)	0.63 (0.24, 1.68)	0.72
Pollen exposure in gestation week 10-12**	1.01 (0.92, 1.11)	1.03 (0.96, 1.1)	0.77
Born in peak pollen season***	4.24 (1.57, 11.42)	1.67 (0.77, 3.64)	0.10
Pollen exposure in first 7 days***	0.93 (0.81, 1.07)	0.8 (0.65, 0.99)	0.18
Pollen exposure in first 3 months	1.02 (1.00, 1.03)	1.01 (0.99, 1.02)	0.38
Pollen exposure in between first 4-6 months	1.00 (0.98, 1.02)	1.01 (0.99, 1.02)	0.54
Pollen exposure in first 6 months	1.01 (1.00, 1.02)	1.01 (1.00, 1.02)	0.78

Hay fever at 12 years	No hay fever history OR (95% CI)	Hay fever history OR (95% CI)	p-value for interaction
Gestation week 11 in peak pollen season*	1.12 (0.33, 3.82)	1.23 (0.49, 3.1)	0.88
Pollen exposure in gestation week 11*	1.07 (0.87, 1.32)	0.94 (0.76, 1.15)	0.30
Gestation week 10-12 in peak pollen season**	0.72 (0.2, 2.53)	0.87 (0.33, 2.32)	0.77
Pollen exposure in gestation week 10-12**	1.06 (0.97, 1.15)	1.02 (0.95, 1.1)	0.46
Born in peak pollen season***	2.12 (0.7, 6.39)	1.32 (0.55, 3.21)	0.45
Pollen exposure in first 7 days***	0.93 (0.79, 1.08)	0.8 (0.65, 1.00)	0.22
Pollen exposure in first 3 months	1.00 (0.97, 1.02)	1.00 (0.98, 1.01)	0.96
Pollen exposure in between first 4-6 months	1.01 (0.99, 1.03)	1.01 (0.99, 1.02)	0.75
Pollen exposure in first 6 months	1.00 (0.99, 1.02)	1.00 (0.99, 1.01)	0.80

Hay fever at 18 years	No hay fever history OR (95% CI)	Hay fever history OR (95% CI)	p-value for interaction
Gestation week 11 in peak pollen season*	2.7 (0.98, 7.46)	2.15 (0.89, 5.18)	0.70
Pollen exposure in gestation week 11*	0.79 (0.62, 1.01)	0.85 (0.72, 1.02)	0.58
Gestation week 10-12 in peak pollen season**	2.47 (0.87, 7.01)	1.98 (0.81, 4.84)	0.70
Pollen exposure in gestation week 10-12**	0.94 (0.87, 1.02)	0.97 (0.91, 1.03)	0.53
Born in peak pollen season***	1.09 (0.42, 2.81)	0.94 (0.45, 1.98)	0.79
Pollen exposure in first 7 days***	0.99 (0.89, 1.11)	1.02 (0.9, 1.15)	0.74
Pollen exposure in first 3 months	1.00 (0.99, 1.02)	1.00 (0.99, 1.02)	0.90
Pollen exposure in between first 4-6 months	1.00 (0.99, 1.02)	1.00 (0.98, 1.01)	0.51
Pollen exposure in first 6 months	1.00 (0.99, 1.02)	1.00 (0.99, 1.01)	0.68

Notes: adjusted for maternal age

*, **, *** Mutually adjusted to each other

Discussion

To my knowledge, the findings from this chapter is the first to identify several periods starting from *in utero*, at birth and at early life with an evidence of an association between pollen exposure and allergic respiratory diseases in high risk children and adolescence. *In utero* exposures were strong for both asthma and hay fever but periods after birth and up to and including 6 months cumulative pollen exposure showed little effects. Maternal history of asthma and maternal history of hay fever modified some of these exposure at different time points.

Pollen exposure in utero and at birth

Together with cumulative pollen exposure in the same period, gestation at week 11 during peak pollen season showed a trend of higher risk to asthma and hay fever at any time point. When I increased the period of observation to gestation week 10 till 12, it was important only for asthma (without hay fever) in children at 6 years of age and hay fever at age 18 years. Pollen exposure at birth did not show a pattern at all but had a significant risk for hay fever at 6 years. Only a few studies have reported on the association between pollen exposure *in utero* or at birth with asthma or hay fever. Lowe et al. found no association between pollen exposure in the first 12 weeks of pregnancy and risk of hospital admission for asthma, but this was for infants at 12 months (4). Kihlström et al analysed the association between high dose pollen exposure *in utero* and asthma, hay fever, and atopic dermatitis in children 4 to 5 years of age (209). They found that high dose pollen exposure *in utero* had a trend of protective effect for asthma and hay fever when compared to low dose pollen exposure *in utero*. There were several reasons that might explain the difference between findings from my study and previous studies. First, my study had different proportion of hereditary allergic disease than the other two studies. Second, I measured the asthma and hay fever at different age, where my study measured the respiratory allergic diseases at multiple age of year and all of them at older age than the previous studies.

Pollen exposure from birth up to 6 months

None of the periods from birth up to 6 months had any significant or any trend of an association between with asthma, hay fever, or even asthma only at 6, 12 or 18 years. I did find some protective effect of pollen exposure in the first 7 days of life with hay

fever at 6 and 12 years which seems consistent with the study by Lowe and colleagues where they found that higher pollen exposure in the last trimester were associated with protective effect for risk of hospital admission for asthma at age of 1 year (4).

Maternal history of asthma and hay fever as effect modifiers

Interaction analysis showed that maternal history of asthma was an effect modifier for pollen exposure *in utero* and early life for children. Children whose mothers had history of asthma had higher odds for hay fever at age 6 years. Interestingly, maternal history of hay fever was an effect modifier for pollen exposure at birth and asthma at age 12 years. The effect was protective in children whose mother had history of hay fever, while the effect was in different direction in children whose mother did not have history of hay fever. My results were not in line with Kihlström et al, which showed that maternal history of pollen allergy had a stronger influence than the pollen exposure itself on hay fever in children (223). Few have studied actual pollen exposure in early life and although the sample at each exposure window was small findings from my study still underlined the importance of pollen exposure *in utero* and at birth, even after I adjusted the effect size with maternal history of asthma and performed an interaction analysis with maternal history of asthma or hay fever.

Strengths and Limitations

There were several strengths from this study. I analysed associations in multiple follow ups as they gave a broader spectrum of the association between pollen exposure and allergic respiratory diseases in children until adolescents. Second, I used actual measured pollen exposure, instead of using born in pollen season as a proxy. This way, a direct analyses of pollen exposure were possible.

My results however must be interpreted with caution. One, the population is high risk children because one of the eligibility criteria was having a first-degree family history of allergic diseases. These results are therefore only generalizable to high risk populations. Two, the power for some of the analysis was low because of the low response rate for follow up at age 12 and 18 years, especially the strata specific analysis needs to be interpreted with caution due the low numbers. Multiple comparisons are

also an issue, but we did set out with a priori hypothesis to test these associations at various exposure windows. I could not make use of the entire available data in the MACS cohort because daily pollen exposure data were only available between October 1991 and January 1994, so I was unable to calculate the pollen exposure for any timepoint before October 1991 or after January 1994, therefore some of the children had to be excluded from the analysis. The possible response rate lead to possible different characteristics between children who attended and those who did not attend the follow up visit. However, there were no big differences of several factors such as gender or family history of allergic disease between children who lost to follow up and children who still in study. Thus it was safe to assume that these results will apply for the children who missed the follow up visit (251). There are several factors other than maternal history that have been reported to be associated with respiratory allergic diseases in children that follow a cyclical pattern, i.e. vitamin D levels, respiratory viral infection, other air pollutants, non-optimal weather factors, ambient temperature, and humidity (47, 219, 254-257). I did adjust for viral infections and an index for high levels of air pollution, but the estimated effects did not change. Unfortunately, I did not have data on maternal vitamin D status during pregnancy and could not test this in my study.

Conclusion

I have shown evidence that pollen exposure is an important environmental exposure that may have impacts from *in utero* and at birth for respiratory allergic diseases in children and adolescence. Maternal history of allergic respiratory diseases played an important role in modifying some of these observed associations.

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Chapter 8. Discussion

The adverse effects of pollen continue to be a major problem globally. Grass pollen is a trigger of asthma and other allergies during peak pollen seasons in sensitive individuals globally. Grass and other pollen are prevalent in Australia, Europe and other regions. It triggers asthma exacerbations on days when pollen is high. Birth during pollen seasons also seems important. However, we know little about early life exposures, especially *in utero*, and subsequent exposure in first months of life or in childhood. Thus, gaps remain in our understanding of these exposure periods and allergic respiratory outcomes in children and adolescents.

My thesis aimed to address current gaps and provide additional evidence on associations between pollen exposure and the development of food allergy and allergic respiratory disease in children and adolescents. I wanted to determine if a particular exposure period during early life is associated with the development of food allergy and allergic respiratory disease. By better understanding the role of these exposure periods in early life it would be possible to develop intervention to modify behaviours and reduce exposure thereby potentially reducing the long-term burden of disease. I explored the early-life time period in two categories: before birth (during pregnancy) and after birth (up to the first six months of life). The specific objectives of my thesis were:

1. To systematically synthesise the evidence as to whether season of birth, which is a crude marker of pollen seasons, is associated with IgE in cord blood at birth. (Research Question 1).
2. To investigate whether actual pollen counts during pregnancy and at birth is associated with IgE in cord blood at birth, with comparison of results from cohorts in both the southern and northern hemispheres (Research Question 2).
3. To investigate whether actual pollen count during pregnancy and in early life is associated with food allergy in infancy and whether the association is modified by maternal history of allergic disease (Research Question 3 and 4).

4. To investigate whether pollen count during pregnancy, and in early life, is associated with allergic respiratory diseases in children and adolescents, and if these associations are modified by maternal history of allergic diseases (Research Question 5 and 6).

In this final chapter of my thesis, I summarize the findings, which I have discussed in detail in each results chapter (chapter 4 to 7). Following this, I outline the strengths and limitations of my doctoral research and the impact of the findings for public health practice and clinical research. Finally, I developed a brief conclusion of my thesis and suggest future directions.

8.1. Thesis findings

8.1.1. Synthesis on the evidence of the association between season of birth and IgE at birth

To answer the first research question, I performed a systematic review and meta-analysis of studies with any results on the association between season of birth and IgE at birth. I wanted to understand current evidence on the association between season of birth which can be considered as a proxy for various environmental exposures, especially grass pollen and cord blood IgE, which could be used as the first step to identify risk of food allergy and allergic respiratory disease in the child. A better understanding about this enabled me to further refine research questions for my thesis.

In this research question I used season of birth as a proxy for the environmental exposure in early life and I used IgE in cord blood or at birth as a first step for the development of food allergy and allergic respiratory disease.

The systematic review revealed the reasons why it was difficult to come to a conclusion on whether infants who were born in specific birth seasons will be at risk for higher level of cord IgE compared to the ones who were born in other seasons. First, the definition of outcome and exposure variables across these studies are quite heterogeneous. For the outcome, most analysed the IgE level as categorical, but some analysed them as continuous. Even among studies that used categorical IgE level, they

used different cut-off level for the categories. For the exposure, there was no consensus on which season should be used as the reference. Each study had their own reasoning for choosing the reference season of birth based on what they assumed as the season of birth with the lowest risk of elevated cord IgE levels. However, the majority used summer as the reference, which is what I also used in the meta-analysis.

My systematic review found 20 studies that met the eligibility criteria and most of them were of high quality except for a number of studies. These studies were considered of poor quality because of limited reporting of the sample, using only correlation instead of regression to address their research questions, and because they did not attempt to identify potential confounders or effect modifiers. Only 9 studies reported the effect size of the association between season of birth and IgE levels. To get a pooled effect size, I managed to get summary statistics from half of the 20 included studies for inclusion in the meta-analysis. The pooled effect size showed that children born in winter (compared to those born in summer) were at significantly greater risk of having elevated IgE level when I used a lower cut-off level (≥0.1 IU/ml) but borderline when I used a higher cut-off level (≥0.5 IU/ml).

The meta-analysis provided some evidence that children born in certain seasons may have a biomarker for increased risk after birth. This exposure occurs before any direct exposure with the child. I hypothesized that a winter birth could act as a proxy for pollen exposure during the early phase of pregnancy. Although there were conflicting results, the IgE level itself could act as a biomarker for the development of allergic respiratory diseases in later life, as infants with higher level of IgE level at birth are at an increased risk of allergic respiratory diseases. This chapter in my thesis was published in Environmental Research journal in June 2017 (DOI: 10.1016/j.envres.2017.05.026).

The next step was to investigate whether these findings for winter season of birth was a proxy for pollen exposure in early life. Infants born in winter season are mostly exposed to summer and autumn seasons *in utero* where the pollen count is much higher compared to the winter season. In contrast infants born in the summer season are mostly exposed to winter and spring season *in utero*. Although the pollen count in the spring season is very high, it is compensated by the very low level of pollen in the winter season. Whether there is an association between pollen count during pregnancy and at birth with IgE in cord blood is answered in the next research question.

8.1.2. Association between pollen count during pregnancy and at birth with IgE in cord blood

In this analysis, I determined whether the pollen count during pregnancy and at birth is associated with IgE in cord blood. I used data from three birth cohorts: MACS in Melbourne, Australia; COPSAC2000 in Copenhagen, Denmark; and LISA in Germany. I measured associations with pollen counts using two methods: cumulative exposure of outdoor pollen count *in utero* and birth during grass pollen season. I categorized the cord blood IgE level into (< 0.5 kU/L, 0.5–1 kU/L,>1 kU/L) and dichotomized (high IgE ≥0.5 kU/L) to be consistent with the current literature. Each region was analysed separately and then I meta-analysed the association of being born in grass pollen season with elevated cord blood IgE level (≥0.5 kU/L).

My analysis revealed a complex set of findings. The meta-analysis showed that infants born in the grass pollen season had higher pooled odds of cord blood IgE ≥0.5 kU/L. However, infants with higher cumulative exposure to outdoor grass pollen *in utero* had lower pooled odds for cord blood IgE ≥0.5 kU/L. This means that infants born in grass pollen season had higher odds for high cord blood IgE level if they were not exposed to high cumulative grass pollen count *in utero*. These results were consistent between studies, with no significant heterogeneity between the three birth cohorts being observed.

My results were among the first to study the association of grass pollen exposure in early life with high cord blood IgE level at birth. The two earlier studies showed a trend that infants born in the pollen season was associated with IgE level at birth although not significant (27, 69). The non-significant results in Crone & Kjellman (1986) could be explained because they used higher cut-off level for high IgE (0.9 kU/L). In Sadeghnejad and colleagues (2007), the lack of observed association could be because many infants had complications at birth and others unmeasured factors could contribute to these findings. My results on actual pollen exposure were novel. To my knowledge, no study explored the association between cumulative pollen exposure *in utero* with high levels of cord blood IgE at birth so I could not directly compare my results with previous studies. These results shed new insights into the early life exposures prior to birth and support the theory that pollen exposure during pregnancy and at birth maybe

involved with the development of high cord blood IgE. This chapter was published in Environment International journal in July 2018 (DOI: 10.1016/j.envint.2018.06.036).

These results became the foundation for my next analysis that pollen exposure during pregnancy and early life could also be associated with food allergy in infants.

8.1.3. Association between pollen count during pregnancy and at birth with food allergy in infancy

In this analysis, I explored whether pollen count during pregnancy and in early life is associated with food allergy in infancy and whether the association itself is modified by maternal history of allergic disease. I used data from HealthNuts cohort, a population-based cohort in Melbourne, Australia to answer these research questions. I explored the association of 11th week and between 10th to 12th week of gestation during peak grass pollen season because a previous study reported that children with higher exposure during 11th week gestation had higher proportion of positive food allergy in the first 4 years of life. For the exposure in early life, I explored the association of born in peak grass pollen season, cumulative pollen exposure in the first 7 days, first 4 to 6 months, and first 6 months of life. Two outcomes were assessed. The first was food sensitization to hen's egg, peanut, or to any of hen's egg, peanut, or sesame at age 12 months and oral food challenge diagnosed food allergy to hen's egg, peanut or to any of hen's egg, peanut, or sesame. Although sensitization and oral food challenge diagnosed food allergy are related, they are considered as two separate outcomes.

My analysis showed increased risk for infant hen's egg and peanut sensitization at 12 months with increasing cumulative pollen exposure between the 10th and 12th week gestation period. Being exposed to higher pollen count during the first 4 to 6 months of life was associated with increased risk of hen's egg sensitization and sensitization to hen's egg, peanut, or sesame. The maternal history of food allergy is also associated with pollen exposure that exposure to peak pollen season during the 10th and 12th week gestation period and increase pollen between 4 to 6 months increased risk of hen's egg and peanut food challenge diagnosed food allergy among infants with maternal history of food allergy.

Although several analyses were carried out with no adjustment for multiple comparisons, I observed a range of statistically significant but some borderline and consistent results for a number of the outcomes assessed. The direction of these results is consistent with the hypothesis that exposure to grass pollen during peak grass pollen seasons may also increase the risk of sensitization to foods in the absence of ingestion of allergic foods. It is possible that infants who exhibit sensitization to certain foods but are not allergic when tested with an oral food challenge, are in fact the group of children that have multiple sensitizations including to other aeroallergens, particularly to ryegrass. I do not have the data on aeroallergen from this cohort, however a previous study in Melbourne reported that in 12 months infants, sensitization to egg and peanut were highly prevalent and almost in all of these they were sensitized to at least one aeroallergen (238).

To my knowledge, my findings were the first to report the association between grass pollen exposure during pregnancy and early life with both food sensitization and oral food challenge diagnosed allergy in infants. The only study that assessed different types of pollen *in utero* was from Pyrhonen and colleagues (2012) but they reported the association with different type of pollens and in children up to 4 years of age using specific IgE for food, skin prick test, and open food challenge for outcomes (5). They also adjusted their analysis for maternal history of only pollen allergy compared to my findings that adjusted the results for maternal history of food allergy.

My study identified several periods during pregnancy and the first year of life when increased pollen count was associated with a moderate increase of risk for food sensitization and oral food challenge diagnosed food allergy although only among infants with maternal history of food allergy. My study suggests that there is an important period during intrauterine stage and the first 6 months of life, where environmental exposures to high grass pollen count may increase risk of food allergies in infants. Moreover, maternal history of allergies modifies these associations. I recommend more studies to replicate these findings in children to find whether the results because of grass pollen and food cross-reactivity or because of multiple sensitization to food and aeroallergens. Findings from this chapter are accepted for publication in *International Journal of Environmental Health Research* journal.

8.1.4. Association between pollen count during pregnancy and at birth with allergic respiratory disease in children and adolescents

My earlier chapters suggested that early life pollen exposure, especially in pregnancy maybe important in the allergic respiratory disease pathway so my final analysis was to explore the association between pollen exposure during pregnancy and in early life with allergic respiratory disease in children and adolescents. For this final analysis, I used the MACS birth cohort data. For the pollen exposure during pregnancy, I again analysed the 11th week gestation and 10th to 12th week gestation in peak grass pollen seasons. Also, cumulative pollen exposure during 11th week and 10th to 12th week gestation. For the pollen exposure in early life, I analysed birth in peak grass pollen season and cumulative pollen exposure in the first 7 days, first 3 months, between first 4 to 6 months, and first 6 months of life. I analysed the allergic respiratory disease specifically asthma and hay fever at age 6 years, 12 years, and 18 years.

My analysis showed that pollen exposure during pregnancy was associated with asthma in childhood and hay fever in childhood and adolescence. Children who had gestation week 11 and gestation week 10 to 12 in peak grass pollen seasons had higher odds for having asthma at age 6 years. Adolescents who had gestation week 11 and gestation week 10 to 12 in peak grass pollen season had higher odds for having at least an episode of hay fever at age 18 years.

My interaction analysis between pollen exposure during pregnancy till early life with maternal history of asthma revealed several interesting results but because of low numbers when I conducted the stratified analysis needs to be interpreted with caution. Children with maternal history of asthma had lower odds for having at least an episode of asthma at age 6 years with non-significant stratified analysis result. In contrast, children with maternal history of asthma had higher odds of having at least an episode of hay fever at age 6 years and this was the only significant interaction in the stratified analysis. Children with maternal history of hay fever had higher odds of having at least an episode of asthma at age 12 years and higher odds of having at least an episode of hay fever at age 18 years, none of these had significant stratified results.

More studies are needed to replicate my findings. Only two other studies have assessed such associations. Lowe et al. found no association between pollen exposure in the first 12 weeks of pregnancy, but they assessed risk of hospital admission for asthma in the first year of life, an age when asthma is difficult to distinguish from other

acute triggers (4). The other study found that high pollen count exposures during the entire pregnancy had a protective effect for asthma and hay fever at age 4-5 years, although the adjusted analysis results were not significant (209). None of these two previous studies adjusted their analysis for maternal history of asthma or hay fever, thus I could not directly compare my interaction analysis results with these studies.

As the two other studies and my own analyses report mixed findings, more studies are needed. My findings strengthen the possible theory that exposure to pollen counts during pregnancy and in the first months of life is involved with the development of allergic respiratory disease in childhood and adolescents. My interaction analysis was the first one that provided evidence on the possible modification of the association between pollen exposure during pregnancy and in early life with asthma and hay fever in childhood and adolescence, by maternal history of asthma and hay fever.

8.2. Strengths and limitations

The strengths and limitations for each analysis has already been discussed in detail in each of the result chapters (Chapter 4, 5, 6, and 7). In this section, I briefly describe the strengths and limitations of my whole thesis in general.

8.2.1. Strengths

Study design: My study is nested within several well-established birth cohorts MACS, COPSAC2000, LISAplus, and HealthNuts. The children in these birth cohorts have been followed up periodically and comprehensibly. Although the data I used for my research was not purely longitudinal, the data I used from these multiple birth cohorts allowed me to have a greater picture of the pollen exposure association with allergy starting from newborn till adolescents. I also used reliable definitions for the outcome in my study, which was similar with the outcome in other studies, to allow for comparison with prior research. For the high IgE level in chapter 5, I excluded IgE measurement that were suspected of contamination from maternal IgE. For the food allergy definition in chapter 6, I used the gold standard definition of food allergy, oral food challenge. For the asthma and hay fever in chapter 6, I used children aged 6 years and above because diagnosing asthma in children below 6 years is not reliable.

Pollen exposure measurement: my thesis used outdoor pollen measurement to support the analysis instead of just using season of birth or pollen season as a proxy for pollen exposure. The pollen measurement in my study is similar with the pollen

measurements used in almost all studies, using a trap pollen measurement device. This pollen measurement met the guidelines from World Allergy Association and the Burkard spore trap for the pollen measurement is certified by American Academy of Allergy, Asthma, and Immunology. The availability of daily pollen count, at least during peak pollen season, for the analysis allowed me to analyse the pollen exposure in more detail.

Confounding and interaction analysis: As much as possible, I adjusted my analyses for variables considered as confounders or adjustments from previous studies. Although I did not have all the data that others included, I adjusted the analysis for maternal smoking history, maternal history of allergic disease, maternal age, and ultraviolet exposures. No previous studies had done interaction analysis of mother history of food allergy for the association between pollen exposure and food sensitization and allergy, or mother history of asthma and hay fever for the association between pollen exposure and asthma and hay fever in childhood and adolescence. My interaction analysis showed that it is important to consider the interaction effect of maternal history of allergic disease instead of including it as a confounder. Including it as an interaction term allowed me to identify that the association between pollen exposure in early life was modified by mothers' history of allergic disease. This suggest that reporting maternal allergy strata-specific analysis is important if there is sufficient power to do so.

8.2.2. Limitations

There are several limitations for my thesis. One of the main limitations being that the pollen count was not available daily throughout the year. For example, I was only able to include 69% of MACS participants because the daily pollen data measurement was only available between October 1991 and January 1994 but the infants in MACS were born between March 1990 and November 1994. I believed the smaller sample in MACS did not affect the analysis because the lack of pollen measurement data did not systematically influence the inclusion of subjects in my thesis. Nevertheless, the small sample size means we may have limited power to detect any associations. Similarly, for the food allergy chapter, as pollen measurements were only available daily during peak pollen season when infants in HealthNuts cohort were born, I made the assumption

that the pollen count outside peak pollen season was very low and the effect of pollen exposure outside peak pollen season would be negligible.

Measurement of some of the outcomes in my thesis could be considered as limitations. In the analysis of cord blood IgE, the cord blood IgE could be contaminated by maternal IgE as mentioned by several previous studies (95, 210, 258). I addressed this problem by using IgA level in cord blood to exclude several subjects. The basis for this method is that IgA is not normally produced by foetal *in utero*, hence detectable IgA in cord blood means contamination by maternal blood and should be excluded from analysis (258). In the analysis of food allergy outcomes, the measurement of food allergy was limited as not all infants in HealthNuts was tested by oral food challenge. However, some of HealthNuts infants with negative IgE or skin prick test were randomly selected for oral food challenge and the results were negative.

Although I considered possible confounders, the possibility of residual confounding of variables I did not have remains. Previous studies adjusted their analysis of food allergy, asthma, and hay fever with several factors such as air pollution, respiratory viral infections, or vitamin D levels (47, 130, 219, 244). Other studies reported associations between non-optimal weather factors, ambient temperature, and humidity with asthma (254-256). All these factors could be considered as potential confounders/adjustments. As these variables were not available in the studies that I used for my research in this thesis, I was unable to adjust for these in my analyses.

8.2.3. Generalisability of the results

MACS and COPSAC2000 are high-risk children cohorts because one of the inclusions criteria is that at least one of the parents have a history of allergic disease at recruitment. Therefore, the results from these cohorts are only applicable to high-risk populations. LISAplus and HealthNuts are both population-based cohorts. The results from the two cohorts could be generalized to a wider population, after taking into account the other characteristics of these cohorts such as the cohort location as they primarily reside in urban areas.

My thesis analysed the association with grass pollen exposure. Grass pollen is the major pollen in southern hemisphere. In the northern hemisphere, grass is still important

but so is birch and weed. Therefore, it is possible that the effects of pollen exposure differ and depends on the region.

8.3. Implications and recommendations

8.3.1. Public Health Implications and recommendations

The importance of pollen exposure as an acute trigger for allergic respiratory disease is already established. In Melbourne Australia, high asthma days occur during peak grass pollen seasons. The catastrophic thunderstorm asthma event that occurred in Melbourne in November 2016 triggered thousands of asthma related hospital admission and caused mortality (43). This event led National Asthma Council Australia to develop a public health campaign for persons with allergic rhinitis, asthma, and for general practitioners to implement programs to work closely with people with these conditions. This campaign emphasizes the use of hay fever preventer, asthma preventer, or both during the grass pollen season in Australia.

In contrast, there is a lack of data on the association of pollen exposure in early life with allergic respiratory disease outcomes. In my thesis, I found that only a handful of studies addressed these associations. My thesis contributed to filling this gap by providing additional results on the association between pollen exposure during pregnancy and in early life with allergic respiratory disease in childhood and adolescence. Although findings from my thesis varied, in general the findings showed that there was an association.

My thesis results suggest the need for public health campaigns to not only focusing on interventions during pollen seasons but also increasing awareness of the potential harmful effects during pregnancy and early life. Additional studies are needed to replicate these findings. For example, simple avoidance strategies during pregnancy (if occurs in pollen seasons) could be implemented to minimize exposure among high risk families and IgE in cord blood could be assessed. These studies could replicate my thesis results and measure the feasibility of avoidance strategies.

8.3.2. Clinical implications and recommendations

In my thesis I have shown evidence that pollen exposure during pregnancy and early life is associated with higher risk of developing allergic respiratory disease in later life, and that children of mothers with allergic disease maybe a particularly susceptible sub-group. Although it is impossible for pregnant mothers to entirely avoid pollen exposure, it is possible that they can modify their behaviour to reduce pollen exposure such as by remain indoors on extremely high pollen days. Pollen applications on smart devices are already in use. However, community wide implementation of this technology for pollen avoidance strategies could help to alert at risk families about incoming high pollen days. A wider recognition of the importance of these smart device applications through the clinical community such as general practitioners (GPs) and maternal health services may support uptake in usage by families.

If the findings of my research are confirmed, practitioners involved with caring for expectant mothers could provide material on the detrimental effects of pollen, especially mothers who already have a history of allergies. Those clinicians could educate their patients about the risk for the mothers' children to develop allergic respiratory disease later in life may increase with higher exposure to outdoor pollen.

8.3.3. Research implications and recommendations

The main findings of my thesis provided a foundation for further research on the effects of pollen exposure during pregnancy and early life and the development of allergic respiratory disease. Although more studies are needed to replicate my findings perhaps it is important that there is more focus on avoidance messages to modify behaviours during peak pollen seasons

Although I found that the 10 to 12 week period of gestation is important, larger studies with daily pollen count data throughout the entire pregnancy for all participants is needed to better detect a critical exposure window. If we could find the critical time period, we could focus avoidance to pollen exposure during that time period. Avoidance of pollen during a specific time period is more feasible for pregnant mothers instead of during the entire pregnancy. Additional studies are needed to replicate my findings.

One possible study is to conduct a clinical trial to determine the feasibility of pollen avoidance strategies during pregnancy on outcomes such as cord blood IgE is a possible

extension to this research. This is just one possible example of future research to replicate and extend the findings from my thesis.

8.4. Conclusion

This thesis adds to the current scientific knowledge on the detrimental effects of environmental exposures. Specifically, it addressed the gaps in knowledge about pollen exposure involvement and allergic respiratory disease. Born in winter season was associated with higher odds for high IgE level in cord blood and at birth. There is inverse association between born in high grass pollen season and cumulative grass pollen level during pregnancy. Born in high grass pollen season is a risk factor for elevated level of cord blood IgE, but cumulative grass pollen level during pregnancy is a protective factor. This inverse association showed an effect of pollen exposure on sensitized mothers. It led to increased IgE for infants born at the start of pollen season. As the season progresses, the sensitized mothers were exposed to higher cumulative grass pollen, the immune response may lessen. Thus, infants born outside high grass pollen season, but exposed to whole season of high grass pollen, had lower IgE. Pollen exposure during pregnancy and in early life was associated with food sensitization and allergy, although there were no consistent results on the association. Maternal history of food allergy was an effect modifier for these associations. Pollen exposure during gestation week 10 to 12 was a risk factor for asthma and hay fever at different time points in childhood and adolescents.

These findings highlight the importance of early life pollen exposure in allergic respiratory disease. Future larger cohorts with both pregnancy data and acute exposure data are recommended to replicate these unique findings documented in my PhD thesis. Expectant mothers could be an important but urgent extension of research to determine the critical time period when pollen exposure affects the development of allergic respiratory disease. Finding the critical time period could help to decrease the effect of pollen exposure as it is not feasible to completely eliminate exposure to outdoor pollen during the entire pregnancy. Moreover, as my thesis showed that maternal history of allergy diseases is an effect modifier, it is therefore important that new studies should also include interacting effects of maternal allergy status. Both family planning clinics and GPs could also provide additional information on the increased risk and support

mothers with a history of allergic disease during their pregnancy and subsequent first months of life of the offspring.

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Appendix: Publication for Examination

This appendix includes the following manuscripts for examination purposes – copyright is retained by the publisher.

- Susanto, N. H., Vicendese, D., Salim, A., Lowe, A. J., Dharmage, S. C., Tham, R., . . . Erbas, B. (2017). Effect of season of birth on cord blood IgE and IgE at birth: A systematic review and meta-analysis. Environ Res, 157, 198-205. doi:10.1016/j.envres.2017.05.026
- Susanto, N. H., Schoos, A. M., Standl, M., Lowe, A. J., Dharmage, S. C., Svanes, C., . . . Erbas, B. (2018). Environmental grass pollen levels *in utero* and at birth and cord blood ige: Analysis of three birth cohorts. Environ Int, 119, 295-301. doi:10.1016/j.envint.2018.06.036