Natural History and Consequences of Food Sensitisation: Results from Two Birth Cohort Studies

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Abstract

The prevalence of allergic diseases is increasing worldwide, particularly in Australia and other "westernised" countries. More recently, evidence suggests a second wave of the "allergy epidemic", with an increase in the prevalence of food allergies. Despite intense research in this area, there are numerous questions concerning the potential risk factors for the development of allergic diseases. It has been shown that sensitisation to aeroallergens is strongly associated with allergy progression. However the associations between food sensitisation and the development of allergic conditions remain unclear. Therefore, my doctoral work has investigated the natural history of food sensitisation from infancy up to adolescence in an allergy high-risk cohort. I have also investigated the associations between early life food sensitisation and subsequent probable food allergy, asthma, allergic rhinitis and lung function during later childhood and adolescence.

Data from an Australian allergy high-risk cohort (Melbourne Atopy Cohort Study (MACS)), and a German population-based cohort (Influence of Life-style related factors on the development of the Immune System and Allergies in East and West Germany PLUS the influence of traffic emissions and genetics (LISAplus)), were used to address the aims of this thesis. MACS recruited 620 infants with a family history of allergic disease prior to birth. The infants were followed from birth up to 18 years and skin prick test (SPT) was conducted at 6, 12 and 24 months, 12 and 18 years. LISAplus recruited 3,097 neonates from four German cities: Munich, Leipzig, Wesel and Bad Honnef. The participants were followed from birth up to 15 years and serum specific IgE was measured at age 2, 6, 10 and 15 years. In both cohorts, multiple surveys, that assessed the occurrence of allergic disease, were distributed throughout the follow-up period. Using these data along with sensitisation data, this thesis has contributed knowledge to address the following issues:

Natural History of food sensitisation from infancy up to 18 years

A better understanding of the natural history of food sensitisation provides insight, from a public health perspective, to appreciate the likely subsequent burden of food allergy and other allergic diseases over the life course. Longitudinal data on the natural history of food sensitisation beyond early childhood are scarce. Using MACS data, the prevalence of food sensitisation was found to be highest in infancy and declined after the age of 12 months.

In the first two years of life, egg white was the most common food sensitisation followed by peanut and cow's milk while peanut was the most prevalent food allergen at 18 years. Boys with eczema had the highest prevalences of egg and milk sensitisation throughout the follow-ups. A small proportion of children developed late onset food sensitisation (after the age of 2 years) which was unlikely to be clinically relevant.

Consequences of early life food sensitisation

Longitudinal birth cohort studies with prospective collection of data are the most appropriate design to evaluate the temporal association between early life food sensitisation and development of probable food allergy, asthma, allergic rhinitis and lung function during later childhood and adolescence. An association between food sensitisation at 12 months and the presence of adolescent food sensitisation and probable food allergy was noted in MACS, with sensitisation to more than one food allergen being the strongest predictor.

I also demonstrated that early life sensitisation to food without concurrent aeroallergen sensitisation was associated with increased risk of asthma and allergic rhinitis during later childhood (i.e. 10-12 years) in both the MACS and LISAplus studies. Stronger associations were observed for co-sensitisation to both food and aeroallergen. However, only co-sensitisation to both food and aeroallergens in early life was found to be associated with asthma and allergic rhinitis at 18 years in MACS. These findings support the concept of an "atopic march", in which early life food sensitisation progresses to later asthma and allergic rhinitis.

Moreover, I have demonstrated evidence that sensitisation to food allergens only at 6 or 12 months in MACS was associated with reduced FEV_1 in adolescence. Most of the observed effect was a direct association, although early life asthma but not aeroallergen sensitisation mediated these associations in part. However, these associations need to be confirmed in population-based studies as sensitisation was not assessed in the first year of life in the LISAplus study.

In conclusion, the results I present in this thesis increase our knowledge of the relationship between food sensitisation and allergic disease. Additionally, they suggest that further efforts to prevent the development of food sensitisation, and hence the progression from food

sensitisation to food allergy, asthma, allergic rhinitis and lung function impairment should be explored.

Declaration

This is to certify that:

i. The thesis comprises only my original work towards the PhD except where indicated in the Preface,

ii. Due acknowledgement has been made in the text to all other material used,

iii. The thesis is fewer than 100,000 words in length, exclusive of tables, maps, bibliographies and appendices as approved by the RHD Committee.

Signature..... Date.....

Shatha Aldurayiwsh

Preface

Under the guidance of my primary PhD supervisor **Professor Shyamali Dharmage** and supervisory panel (**Professor Katie Allen, Dr Adrian Lowe, Dr Caroline Lodge** and **Dr Bircan Erbas**), I was responsible for all analyses and presentation of data in this thesis, unless otherwise stated. The majority of the research questions presented in this thesis were investigated within the Melbourne Atopy Cohort Study (MACS), an Australian based longitudinal birth cohort. Two research questions were also investigated within the Influence of Life-style related factors on the development of the Immune System and Allergies in East and West Germany PLUS the influence of traffic emissions and genetics (LISAplus) study, a German population-based longitudinal birth cohort.

I was responsible for cleaning and analysing the MACS data that has been used within this thesis. The LISAplus data cleaning and analyses were performed by **Dr Marie Standl** (results presented in **Chapter 6**) and **Dr Agnes Luzak** (results presented in **Chapter 7**) with the guidance from the LISAplus investigators, particularly **Dr Joachim Heinrich** and **Professor Holger Schulz**. However, I was responsible for development of statistical methods and interpretation of the results.

Within my thesis, I have included two published and one accepted first author papers with multiple co-authors. All have been included as results chapters (**Chapter 4, 5 and 6**). I wrote the initial drafts of each of the first author publications, in addition to carrying out the literature searches, statistical analyses and interpretation of the results. My co-authors provided assistance with data collection, dataset maintenance, analytical design and editing of manuscript drafts. As such, I have provided authorisation forms from each co-author of published and accepted manuscripts, as well as a declaration of collaborative work from my principal supervisor. All work was carried out after my enrolment as a PhD research student.

This thesis contains no material which had been accepted for the award of any other degree or diploma in any university or any other institution. To the best of my knowledge, this thesis contains no material previously published or written by any other person, except where due reference is made in the text of the thesis, nor were other persons involved directly in analytical work, except as acknowledged. No third party was sought for editorial assistance.

Scholarship

I received a scholarship from my employer, King Saud University in Riyadh - Saudi Arabia, which is represented by the Saudi Arabian Cultural Mission (SACM) in Canberra - Australia, to undertake my PhD study.

Publications and presentations

First author publications arising from this thesis:

Alduraywish SA, Lodge CJ, Vicendese D, Lowe AJ, Erbas B, Matheson MC, Hopper J, Hill DJ, Axelrad C, Abramson MJ, Allen KJ, Dharmage SC. Sensitization to milk, egg and peanut from birth to 18 years: A longitudinal study of a cohort at risk of allergic disease. *Pediatr Allergy Immunol* 2016: **27**:83–91.

Alduraywish SA, Lodge CJ, Campbell B, Allen KJ, Erbas B, Lowe AJ, Dharmage SC. The march from early life food sensitization to allergic disease: a systematic review and meta-analyses of birth cohort studies. *Allergy* 2016; **71**:77–89

Alduraywish SA, Standl M, Lodge CJ, Abramson MJ, Allen KJ, Erbas B, Berg Av, Heinrich J, Lowe AJ, Dharmage SC. Is there a march from early food sensitization to later childhood allergic airway disease? Results from two prospective birth cohort studies. *Pediatr Allergy Immunol.* Accepted date 01 Sep 2016.

Publications under review:

Alduraywish SA, Luzak A, Lodge CJ, Allen KJ, Matheson MC, Gurrin LC, Erbas B, Heinrich J, Lehmann I, Berg Av, Standl M, Abramson MJ, Schulz H, Lowe AJ, Dharmage SC. Early life food sensitization is associated with adolescent lung function deficits: Results from two birth cohorts. *Allergy.*

Conference poster presentations

Alduraywish SA, Lodge CJ, Vicendese D, Lowe AJ, Erbas B, Matheson MC, Hopper J, Hill DJ, Axelrad C, Abramson MJ, Allen KJ, Dharmage SC. Food Sensitisation from Birth to 18 Years: A Longitudinal Analysis of a Cohort at Risk of Allergic Disease, ASCIA 2014 poster abstracts. *Internal Medicine Journal* 2014;**44**: 1-29.

Alduraywish SA, Lodge C, Lowe A, Erbas B, Abramson M, Svanes C, Hill D, Allen K, Dharmage S. Early Life Food Sensitisation is Associated with Lower Lung Function at 12 And 18 Years in a Cohort Risk of Allergic Disease. TSANZ Annual Scientific Meeting 2015. Asthma and Allergy – poster presentations. *Respirology* 2015;**20**: 63-69.

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I would also like to thank LISAplus investigators, particularly **Dr Joachim Heinrich, Professor Holger Schulz, Dr Marie Standl, and Dr Agnes Luzak** for their cooperation and patience in conducting the analyses related to LISAplus study as requested.

I also acknowledge all the people involved in the MACS and LISAplus studies, including the founders of both studies, those who coordinated the follow-ups, the participants and the funding bodies.

I would like to thank my husband Fahad Aldakheel for being my pillar of strength, even in the most stressful times and for encouraging me during my study. My little princess Layan and my son Faisal who always with their smiles, hugs and kisses fuelled my motivation to complete this work. I am also forever indebted to my father Professor Ahmed and my mother Meznah for dedicating their life to care for me and for their continuous support throughout my PhD. Words alone cannot express my gratitude. A big thank you is expressed to my sister Rana and to all my brothers and friends; with their encouragement my goals seemed a lot closer.

I would like to thank my sponsor, King Saud University, Riyadh - Saudi Arabia for their financial support. Without their funding, this work would not have been possible. Finally, I

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CHAPTER 1 - Introduction

1.1. Rationale

1.1.1. Overall aims and hypotheses of my thesis

The aims of my thesis are to examine the natural history of food sensitisation from infancy to adolescence and to investigate the associations between early life food sensitisation and subsequent probable food allergy, asthma, allergic rhinitis and lung function in late childhood and adolescence. I hypothesised that the prevalence of food sensitisation is comparable to that of other Westernised countries and early life food sensitisation is associated with later development of food allergy, asthma, allergic rhinitis and lung function.

1.1.2. What is food sensitisation?

Atopic sensitisation reflects the ability of a specific allergen to initiate an immunological response and to produce Immunoglobulin E (IgE) antibodies (Galli *et al.*, 2008). Food sensitisation is defined as the presence of a food specific IgE response occurring upon exposure of the immune system to certain food allergens (Asero *et al.*, 2007). The immunological mechanisms associated with atopic sensitisation and reactions are complex and involve several types of cells, cell receptors and mediators released by cells. Sensitisation arises when allergen-specific immunoglobulin E (s-IgE) antibodies are formed after exposure to a particular allergen through ingestion, inhalation or by contact with the skin. When an allergen enters the body, it is first processed by antigen presenting cells (APCs) (Sohi and Warner, 2008). The APCs activate the naive T cells to differentiate into CD4 T-helper cells (Th2). The Th2 cells produce several cytokines which stimulate the B-cells to produce IgE that is specific to that allergen (Jutel *et al.*, 2006; Sohi and Warner, 2008). The IgE sensitises basophils and mast cells by binding to IgE receptors (FcεRI) presented on their surface (Akdis, 2006). The IgE- FcεRI complexes that present on these cells facilitate antigen presentation (Larché *et al.*, 2006). The individual is therefore considered to be sensitised to that allergen. Sensitisation can be assessed by either skin prick test (SPT) or measurement of serum s-IgE antibodies. Individuals can have sensitisation to a food allergen without having clinical symptoms of an allergic reaction upon exposure. IgE-mediated allergic disorders require the existence of both sensitisation and the development of specific signs and symptoms on re-exposure to the specific allergen (Burks *et al.*, 2012). Food sensitisation usually begins in infancy and is closely associated with, and may be a preliminary step for,

IgE-mediated food allergy (Burks *et al.*, 2012). Additionally, food sensitisation can be associated with or predict the development of other allergic diseases, including eczema, asthma, and allergic rhinitis (Nickel *et al.*, 2002; Flohr *et al.*, 2004; Weinberg, 2005; Das, 2012).

1.1.3. What are allergic diseases and their public health implications?

Allergic diseases are a heterogeneous group of conditions including asthma, eczema (also known as atopic dermatitis), allergic rhinitis (also termed as hay fever) and food allergy. These disorders are likely to have a systemic involvement that can affect many organs and systems throughout the lifespan of allergic individuals (Spergel, 2010). While the term allergic disease is used to categorise these conditions, it should be noted that all these conditions can also be categorised as non-allergic, according to their aetiology and underlying pathophysiology (Novak and Bieber, 2003; Sin and Togias, 2011). Asthma is a chronic inflammatory condition of the airways, characterised by reversible airway obstruction and the presence of intermittent symptoms of wheezing, chest tightness, coughing and shortness of breath (Reddel *et al.*, 2015). Eczema (atopic dermatitis) is a common, chronic, relapsing, inflammatory skin disease characterised by rash that comes and goes, is usually itchy and is commonly present in skin folds (Kumar and Clark, 2002). Allergic rhinitis is characterised by the presence of one or more symptoms, including sneezing, nasal congestion, itching and rhinorrhea (Skoner, 2001). Food allergy is defined as “an adverse health effect arising from a specific immune response that occurs reproducibly on exposure to a given food” (Chafen *et al.*, 2010; Boyce *et al.*, 2011).

Over the past 50 years there has been a global epidemic of asthma, eczema and allergic rhinitis, especially in developed countries (Asher *et al.*, 2006; Mallol *et al.*, 2013). More recently, evidence suggests a second wave of the “allergy epidemic”, with an increase in the prevalence of food allergies (Prescott and Allen, 2011; Nwaru, Hickstein, Panesar, Muraro, *et al.*, 2014). Australia has one of the highest prevalences of childhood asthma, eczema and allergic rhinitis in the world (Asher *et al.*, 2006). Additionally, a population-based study from Melbourne, Australia, found that around 10% of one year old infants had challenge proven IgE-mediated food allergy (Osborne *et al.*, 2011). These allergic disorders pose a substantial

health burden on affected individuals, their families and healthcare resources (Meltzer, 2001; Lewis-Jones, 2006; Guilbert *et al.*, 2011).

1.1.4. Key issues in the epidemiology of food sensitisation

Despite the importance of early life sensitisation as a primary marker for allergy development, many important issues about the epidemiology of sensitisation, particularly food sensitisation, have not yet been resolved. In the following sections, I highlight some of these issues examined within my thesis.

1.1.4.1. Natural history of food sensitisation

Natural history refers to both the acquisition of a particular clinical condition and its resolution or persistence over time. Findings from clinical and epidemiological studies demonstrated that many food allergies, including milk, egg, peanut and soy allergies have their onset in early life and are usually outgrown later in childhood, although a minority, such as peanut allergy, persist into adolescence and even adulthood (Savage and Johns, 2015). However, longitudinal data on the natural history of food sensitisation from early life to beyond early childhood are scarce. Three studies have assessed food sensitisation up to adolescence and young adulthood (Rhodes *et al.*, 2002; Roberts *et al.*, 2012; Nissen *et al.*, 2013). Of these studies, two were limited by small sample sizes (n = 100 (Rhodes *et al.*, 2002) and n = 276 (Nissen *et al.*, 2013)) and the other did not describe the changes in food sensitisation from early infancy limiting ability to draw firm conclusions (Roberts *et al.*, 2012). Data on the natural history of food sensitisation may provide information concerning the persistence of food sensitisation, as well as the ability of food sensitisation to predict subsequent food allergy. It has been shown that chronic sensitisation is commonly associated with an increased risk of clinically relevant allergic disease (Kurukulaaratchy *et al.*, 2005). However, little is known about late onset food sensitisation (after 2 years of age) or how early life eczema or aeroallergen sensitisation may modify the persistence of food sensitisation. Determining which infants are likely to have persistent food sensitisation and/or allergy may guide both parents and clinicians by informing the discussion between clinicians and parents regarding the results of subsequent SPTs and the prognosis of late onset food sensitisation. Understanding the natural history of food sensitisation is important from a public health perspective in order to appreciate the likely subsequent burden of food allergy over the life

course. Longitudinal studies, where the same individuals are followed over time, are the appropriate study design in which to address questions related to natural history.

1.1.4.2. The consequences of food sensitisation

Another advantage of longitudinal studies is that they allow for examination of the temporal sequence between early life exposure and later development of the outcomes of interest. There is growing evidence that eczema and food allergy are the most common allergic diseases during the first two years of life, with some evidence that they are interrelated (Leung *et al.*, 2004; Martin *et al.*, 2014). While it is recognised that both early life eczema and food allergy may progress to asthma and allergic rhinitis, a concept known as the 'atopic march' (Zheng *et al.*, 2011), whether food sensitisation induces or is a consequence of eczema is controversial (Hill *et al.*, 2000; Lowe *et al.*, 2007). The progression from eczema to other allergic disease has been documented and systematically reviewed (Burgess *et al.*, 2009; Dharmage *et al.*, 2014). Few studies have assessed the evidence for the atopic march in relation to food allergy (Hill *et al.*, 1979; Bishop *et al.*, 1990; Høst, 1997; Tikkanen *et al.*, 2000). The overall findings from these studies showed that infants with cow's milk allergy had a higher risk for childhood asthma and allergic rhinitis. However, these studies were mainly based in a clinical setting and limited by a small sample size. Given that the prevalence of food allergy increased recently, it is important to evaluate whether early life food allergy "marches" towards other allergies. Although the gold standard method for diagnosis of food allergy is oral food challenge, there are relatively few epidemiological studies that measure food allergy in this way, as it is time consuming and requires specialised clinical management to ensure participant safety. Food sensitisation is one of the first steps in the pathogenesis of food allergy and is the measure most commonly used as an indirect marker for the presence of food allergy in epidemiological studies (Hill *et al.*, 2004; Rona *et al.*, 2007). The current evidence for the association between early life food sensitisation and subsequent development of other allergic diseases (asthma, eczema and allergic rhinitis) has not been systematically synthesised and evaluated.

There is increasing interest in the longitudinal relationships between allergic diseases, given the recent increase in the prevalence of these disorders worldwide (Mallol *et al.*, 2013). This information could contribute to identifying early interventions to prevent and reduce the burden of these disorders. Although several epidemiological studies have shown that early aeroallergen sensitisation is related to increased risk of allergic diseases in children (Kjaer *et*

al., 2009; Codispoti *et al.*, 2010; Lodge *et al.*, 2011) and adults (Schäfer *et al.*, 2007), the role of food sensitisation is less clear. There is growing evidence that allergic sensitisation starts during the first months of life, a period when maturation of the immune system is occurring in conjunction with exposure to allergens (Strobel, 2001). The initial sensitisation is generally related to food allergens, particularly cow's milk or hen's egg (Kulig *et al.*, 1999; Nissen *et al.*, 2013). Considering that food sensitisation tends to develop earlier than aeroallergen sensitisation, measuring food sensitisation in the early years of life may allow for earlier prediction of childhood and adolescent onset allergic airway disease and potentially target intervention strategies in early life.

Sensitisation to food allergens reflects the ability of those allergens to initiate immunological responses that results in activation of immune cells and release of inflammatory mediators (Galli *et al.*, 2008). The release of these mediators contributes to the acute symptoms and signs related to allergic reactions, including vasodilatation, increased vascular permeability and bronchial smooth muscle contraction (Hamelmann *et al.*, 1999; Galli *et al.*, 2008). In sensitised individuals, repeated exposure to allergens results in persistent inflammation that may lead to long term structural and functional changes of affected tissues such as lungs (Galli *et al.*, 2008). Despite the presence of several epidemiological studies that have examined the relationship between aeroallergen sensitisation and lung function measures (Sunyer *et al.*, 2000; Langley *et al.*, 2003; Illi *et al.*, 2006), the association between food sensitisation and subsequent lung function has not been evaluated. Moreover, it is not obvious whether food sensitisation influences lung function parameters directly or through other conditions such as asthma.

In this thesis, I examined the above issues within the Melbourne Atopy Cohort Study (MACS). MACS is an Australian prospective birth cohort study of infants with a family history of allergic diseases. This study was initially started as a randomised controlled trial (RCT) of the effect of three infant formulas (standard cow's milk, partially hydrolysed whey and standard soy formula) implemented at the time of weaning on the occurrence of allergic disease. Infants were recruited prior to birth from antenatal clinics and were followed frequently within the first two years of life, annually from 3-7 years and at 12 and 18 years. SPTs to common food allergens (cow's milk, egg white and peanut) and aeroallergens (dust mite, rye grass and cat dander) were performed at 6, 12 and 24 months and at 12 and 18 years.

The investigation of consequences of food sensitisation within the MACS study raises important issues. To address both the generalisability of findings to the general population (the MACS study population is an allergy high-risk cohort) and reproducibility of study findings, the consequences of food sensitisation were also examined within the Influence of Life-style related factors on the development of the Immune System and Allergies in East and West Germany PLUS the influence of traffic emissions and genetics (LISAplus) study. The LISAplus study is a longitudinal population-based birth cohort study from Germany that has followed the participants from birth up to 15 years. Data were collected frequently in the first two years then at 4, 6, 10 and 15 years. Blood samples for measurement of s-IgE were collected at 2, 6, 10 and 15 years. Further details of the methods used for these studies are presented in **Chapter 3**. The longitudinal nature of these cohorts, which extend from infancy up to adolescence, frequent assessment of food sensitisation as well as detailed collection of data throughout the follow-ups, make them suitable to address the above issues.

In this thesis, I cleaned the relevant data, generated the variables and performed the statistical analysis for the MACS birth cohort. Also, I interpreted the data from both MACS and LISAplus studies and incorporated the findings in the context of the current literature. I wrote the first draft of all included papers and revised with the input from my co-authors.

1.2. Objectives of this thesis

The objectives addressed in this thesis were:

- To examine the natural history of food sensitisation from infancy to late adolescence and to investigate if the natural history of food sensitisation is modified by sex, early life aeroallergen sensitisation and eczema.
- To examine the association between food sensitisation and subsequent probable food allergy.
- To synthesis the current evidence for the relationship between food sensitisation and subsequent development of allergic diseases including asthma, eczema and allergic rhinitis in birth cohort studies.
- To examine the association between food sensitisation in the first two years and development of allergic airway diseases (asthma and allergic rhinitis) during later

childhood and adolescence in the MACS cohort and to replicate the results related to 24 months sensitisation in the LISAplus study.

- To examine the association between food sensitisation in the first two years of life and lung function during adolescence in the MACS and to replicate the results related to 24 months sensitisation in the LISAplus study. Also, to investigate if these associations are mediated by aeroallergen sensitisation at 12 and 24 months or by early childhood asthma.

1.3. Overview of thesis

This thesis presents my original research undertaken during my PhD candidature. It is based mainly on data from the MACS study and also includes data from LISAplus study. This Introduction Chapter outlines the rationale and objectives for this thesis. A detailed literature review and background related to the research questions investigated within this thesis is presented in **Chapter 2**. The methods used to address the research aims of this thesis using the MACS and LISAplus studies, along with the general definitions and statistical methods are described in **Chapter 3**. **Chapters 4 to 7** present the results from this thesis. The natural history of egg, milk and peanut sensitisation and the association between food sensitisation at 12 months and probable food allergy at 12 and 18 years in MACS is presented in **Chapter 4**. The systematic review and meta-analysis related to the current evidence for the association between early food sensitisation and subsequent allergic disease is described in **Chapter 5**. Original analyses related to the relationship between food sensitisation in the first two years and subsequent asthma, allergic rhinitis and lung function, all of which were investigated within the MACS and LISAplus studies, are presented in **Chapters 6 and 7**. Finally, the overall discussion, conclusions and recommendations based on these results, along with the implications and suggestions for further research are presented in **Chapters 8 and 9**.

CHAPTER 2 - Literature Review

2.1. Overview

My doctoral work focuses on the natural history of food sensitisation and how food sensitisation is related to subsequent asthma, allergic rhinitis and lung function in later childhood and adolescence. Longitudinal studies, where the same individuals are followed over time, are the most appropriate design in which to address questions related to the natural history as well as the temporal sequence between early life exposure and later development of the outcomes of interest. For this reason, I used data from two birth cohort studies to address my aims.

In this chapter, I evaluate the evidence related to my research questions while highlighting the burden and public health implications of both food sensitisation and allergic diseases. Firstly, I discuss the current prevalence and the definitions of allergic diseases and the potential risk factors for these disorders. Secondly, I discuss food sensitisation in more detail, including its definition, mechanism for development, methods for clinical assessment and the key issues related to food sensitisation and its association with allergic diseases.

2.2. Burden of allergic disease

Allergic diseases are a heterogeneous group of conditions including asthma, eczema (also known as atopic dermatitis), allergic rhinitis (also termed as hay fever) and food allergy. They are common chronic disorders with substantial variation in their complexity and severity (Zheng *et al.*, 2011). These disorders are likely to have systemic involvement that can affect many organs and systems throughout the lifespan of allergic individuals (Spergel, 2010). Children and young adults bear the greatest burden of these conditions (Branum *et al.*, 2008; Akinbami *et al.*, 2011).

While the term allergic disease is used to categorise these conditions, it should be noted that all these conditions can also be categorised as non-allergic, according to their aetiology and underlying pathophysiology. Allergic diseases are grouped together under the presumption that they are, at least partially, mediated by an inappropriate immune response to environmental proteins (Barnes, 2011). A fundamental feature of allergic conditions is the ability of the involved organ to recognise common environmental allergens and to generate a

Th2 cytokine response to them, with subsequent production of immunoglobulin E (IgE) through activation of inflammatory cells (Galli and Tsai, 2012).

Although the majority of allergic diseases are associated with sensitisation (evidence of specific IgE production to an antigen as measured by SPT or serum s-IgE) and have their onset in early childhood across all ages, there are forms of these conditions that appear to be independent of atopy. These non-allergic forms of the disease (without evidence of sensitisation) have been subject to careful comparative investigation but so far no clear pathogenic pathways have been identified (Novak and Bieber, 2003; Sin and Togias, 2011).

It has been reported that at least half of the asthma cases in the community are attributable to sensitisation (Pearce *et al.*, 1999). Phase Two of the International Study of Asthma and Allergy in Childhood (ISAAC) showed that the fractions and prevalence of wheeze attributable to skin test reactivity in children differed strongly between populations, ranging from 0% to 94% and increased with economic development (Weinmayr *et al.*, 2007). The European Community Respiratory Health Survey (ECRHS) showed that the overall attributable fraction (AF) of asthma symptoms caused by atopy in adults was 30%. The AF varied widely between centres, ranging from 4% to 61% (Sunyer *et al.*, 2004). These variations could be related to the differences in the prevalence of sensitisation. Moreover, It has been demonstrated that around one third of children with eczema have evidence of sensitisation (Flohr *et al.*, 2004). A large community based study of adolescents and adults reported that approximately 77% of individuals with rhinitis had evidence of sensitisation (Mølgaard *et al.*, 2007). These observations have led to substantial controversy within the field concerning the exact definition of allergic conditions. In this thesis, I use the terms asthma, eczema and allergic rhinitis to describe the clinical manifestations of these conditions, irrespective of evidence of atopy. Allergic disorders are a major public health problem due to their high burden of disease in both children and adults. In the following section, I describe the prevalence and public health implications of allergic diseases.

2.2.1. Overall prevalence of allergic diseases

There has been a global epidemic of asthma, eczema and allergic rhinitis over the past forty years, while recent data suggests that food allergies are also increasing (Branum *et al.*, 2008; Mallol *et al.*, 2013; Prescott *et al.*, 2013; R. Mullins *et al.*, 2016). Multi-centre international projects have been established to provide insight into the prevalence and time

trends of asthma and/or other allergic diseases at different age groups using standardised questionnaires. These studies include the ISAAC (Asher *et al.*, 1995) and the ECRHS (Janson *et al.*, 2001). ISAAC Phase I, which was completed between 1994 and 1995, assessed the prevalence of symptoms of asthma, atopic eczema and allergic rhinoconjunctivitis in children. Stage I of ECRHS, which was conducted between 1991 and 1994, evaluated the variation in the prevalence of respiratory symptoms, asthma and allergic rhinitis in 20-44 year olds in 48 centres (Janson *et al.*, 2001). Overall, the burden of allergic disorders varies markedly within and across populations. Although they are highly prevalent in developed countries, rates of allergic diseases in developing countries have been observed to be rising (Zar *et al.*, 2007).

Australia has one of the highest prevalences of reported allergic diseases (Asher *et al.*, 2006). A recent report from the Australasian Society of Clinical Immunology and Allergy (ASCIA) showed that the prevalence of asthma, allergic rhinitis, eczema and food allergy has increased in Australia, with around 4.1 million Australians (20%) having at least one allergic disease (ASCIA, 2013).

Accurately determining the prevalence of allergic diseases is challenging for several reasons. There are no standard definitions for allergic diseases, making the results from different studies non-comparable (as discuss in *Section 2.3*). Additionally, it is evident from several cross-sectional and longitudinal studies that the prevalence of allergic disorders is both sex and age dependant (Asher *et al.*, 2006; Möhrenschlager *et al.*, 2006). Finally, there are geographical differences in the prevalence of allergic disease (Asher *et al.*, 2006). For these reasons, interpretation of prevalence estimates necessitates description of the used definition of disease, the sex and age of individuals and the place of assessment in order to be compared with estimates from other studies. In the following section, I provide an overview of the prevalence, morbidity and mortality from common allergic disorders including asthma, eczema, allergic rhinitis and food allergy.

2.2.2. Prevalence of asthma

There is a substantial variation in the prevalence of asthma worldwide and across time. The global prevalence of asthma ranges from 1 to 18% of the total population in different countries (Bateman *et al.*, 2008). It is estimated by the World Health Organization (WHO) that the number of individuals with asthma is around 300 million, and if the rising

trends continue, it is expected to reach to 400 million by 2025 (Pawankar *et al.*, 2012). Asthma prevalence is increasing in low and middle income countries as they take on a Western-type lifestyle. On the other hand, it is plateauing in high income countries (Mallol *et al.*, 2013). The increase in asthma prevalence is paralleled by similar increases in other allergic disorders such as eczema and rhinitis (Mallol *et al.*, 2013).

The prevalence and trends in asthma should be evaluated while taking the definitions into account. The prevalence of asthma is commonly based on the symptom of "wheeze", which is the main symptom experienced by individuals with asthma. Results from Phase I of ISAAC, where a standard questionnaire and definitions were used across 56 countries, showed that the prevalence of childhood asthma symptoms varied more than 15-fold between countries, ranging from 2.1% to 32.2%. The highest prevalence of childhood asthma was observed in English speaking countries, such as Australia, the United Kingdom (UK) and New Zealand (Asher *et al.*, 2006). Findings from Phase III of ISAAC have shown a reduction in asthma prevalence in children aged 13-14 years between 1995 and 2002 in countries which formerly had had the highest baseline prevalence, such as Australia and Western Europe. In contrast, an increase in the frequency of asthma and asthma related symptoms was found in regions with low baseline prevalence (Pearce *et al.*). Similarly, results from Stage I of the ECRHS showed an eight-fold variation in the prevalence of wheeze between countries. Again, the prevalence of asthma symptoms was found to be highest in Australia, New Zealand, the UK and the United States (US) (Burney *et al.*, 1996).

Australia has one of the highest prevalences of asthma in the world. The prevalence of asthma in Australia has been estimated in a range of national health surveys. These surveys utilised self-reported measures without including objective measures. Overall, asthma affects one in ten Australians; this is equivalent to over 2 million people and is greater in the indigenous community. It is estimated that there were 2.4 million Australians with asthma in 2015 (9.9% of the population), of which 46% were male. The highest prevalence rate for asthma is reported to be in males aged 5-9 years at 14.6% (AsthmaAustralia, 2015).

2.2.3. Prevalence of eczema

Eczema affects around 20% of the paediatric population and up to 3% of adults in western societies (Darlenski *et al.*, 2014). One of the most valuable data sources on the prevalence of childhood eczema comes from the ISAAC study. There is striking worldwide

geographic variability in the prevalence of eczema symptoms (itchy skin rash that comes and goes). Findings from ISAAC Phase I showed that the highest prevalence of current eczema symptoms was reported in Nigeria, the United Kingdom, Finland, Sweden, the Republic of Ireland and New Zealand (Beasley, 1998). ISAAC Phase III reported that the prevalence of eczema symptoms ranged from 0.9% in India to 22.5% in Ecuador at ages 6 to 7 years and from 0.2% in China to 24.6% in Colombia at ages 13 to 14 years. Moreover, a prevalence over 15% was found in four of nine regions studied including Africa, Latin America, Europe (1 centre in Finland) and Oceania (Odhiambo *et al.*, 2009). Additionally, results from ISAAC Phase III showed that although eczema appears to have reached a plateau in the countries with the highest prevalence such as the New Zealand and the UK, eczema continues to increase in prevalence, specifically in young children (age 6–7 as compared to age 13–14 years) and in low income countries, such as Latin America or South East Asia (Mallol *et al.*, 2013).

Findings from ISAAC showed that the prevalence of eczema symptoms in Australia was within the top 12 of 56 countries surveyed. Australian girls were more likely to have eczema than boys, both at the ages of 6-7 and 13-14 years (Robertson *et al.*, 1998). The prevalence of eczema in 6-7 year old Australian children has increased from 11.1% in 1993 to 17.2% in 2002 (Robertson *et al.*, 2004). Despite the fact that eczema is common in infancy (Burr *et al.*, 2013), data from ISAAC may underestimate the actual prevalence of eczema as it was not assessed during this period. A recent Australian population-based study from Melbourne examined the prevalence of current eczema (based on clinical examination) in infants at the age of 12 months. Findings from this study showed that the population prevalence of observed eczema at 12 months was 20.3% (Martin *et al.*, 2013).

2.2.4. Prevalence of allergic rhinitis

The prevalence of allergic rhinitis in epidemiological studies ranges from 3% to 19% (Skoner, 2001). Data from ISAAC Phase I showed around 30-fold variation in the prevalence of allergic rhinitis symptoms between 155 centres in 56 countries (Beasley, 1998). The worldwide prevalence of rhinitis was 8.5% in 6-7 years and 14.6% in 13-14 years old (Aït-Khaled *et al.*, 2009). Results from ECRHS showed the prevalence of allergic rhinitis in adults was 21% (Janson *et al.*, 2001).

In Australia, allergic rhinitis is one of the most common chronic respiratory conditions. Results from ISAAC ranked Australia among the seven countries with the highest prevalences of allergic rhinitis (Beasley, 1998). It has been estimated from national data that allergic rhinitis affects around 16.1% of the Australian population, with a lower rate in children below the age of 14 years (7.7%) and a higher rate in adults aged 18 to 64 years (19.8%) (ABS, 2006). Findings from the 2007-08 National Health Survey showed that around 15% of the Australian population (about 3.1 million people) had self-reported allergic rhinitis (AIHW, 2011).

2.2.5. Prevalence of food allergy

The true prevalence of food allergy is difficult to establish for several reasons. The majority of prevalence studies have mainly focused on the most common food allergens, despite the presence of more than 170 foods that have been reported to cause IgE-mediated allergy (Burks *et al.*, 2012). Moreover, the prevalence of food allergies may have changed over time. Several studies have suggested a true increase in the prevalence over the past 10 to 20 years (Mullins, 2007; Branum *et al.*, 2008). Additionally, the variations and inconsistencies in study design and the used definitions for food allergy make it difficult to compare the food allergy prevalence between studies (Nwaru, Hickstein, Panesar, Muraro, *et al.*, 2014).

Food allergy has been reported to be more prevalent in young children and less common in adults (Sicherer and Sampson, 2010). In general, self-reported food allergy prevalence is higher than the prevalence figures based on medical history and clinical testing. Results from a meta-analysis found that food allergy prevalence was markedly heterogeneous between studies and the prevalence of self-reported food allergy to any tested food allergen varied from 3% to 35%, while the prevalence of oral challenge positivity ranged from 1% to 8% (Rona *et al.*, 2007). A recent systematic review and meta-analysis showed that the pooled point prevalence of self-reported food allergy was 5.9%, while the pooled point prevalence of food challenge positivity was 0.9% (Nwaru, Hickstein, Panesar, Muraro, *et al.*, 2014).

In Australia, a population-based study reported that the overall prevalence of challenge proven IgE-mediated food allergy among one year old children was around 10%. The prevalences of challenge proven allergy to peanut, raw egg and sesame were 3%, 8.9% and 0.8%, respectively (Osborne *et al.*, 2011). An earlier Australian study, investigated the

prevalence of food allergy in adults aged 26-50 years, found that around 1.3% suffered from food allergy, most commonly to peanut, followed by shrimp (prawn), cow's milk and egg, with the outcome based on a combination of medical history and diagnostic allergy testing (Woods, 2002).

Epidemiological surveys from Australia indicate that the incidence of all-cause anaphylaxis, particularly anaphylaxis to food, has increased over the past two decades. It has been shown that the prevalence rates of admissions for food induced anaphylaxis in Australia increased 350% between 1994 and 2005. Reported prevalence rates were higher for children less than 4 years of age and for peanut and tree nut anaphylaxis, with greater increases noted for older age groups and with other allergies, such as egg or cow's milk allergy (Liew *et al.*, 2009). A recent publication has assessed the hospital admission rates for food related anaphylaxis in Australia between 2005-2006 and 2011-2012, and compared these findings with those from the previous data (1998-1999 to 2004-2005). Results from this study showed that food related anaphylaxis has increased further in all age groups since 2004-2005. Despite that the major burden falls on those aged 0 to 4 years, there is primary evidence for a recent acceleration in incidence rates in those aged 5 to 14 years. This finding contrasts with the previous decade in which the highest increase was in those aged 0 to 4 years (Mullins *et al.*, 2015).

2.2.6. Public health implications of allergic diseases

2.2.6.1. Morbidity and mortality associated with allergic diseases

Allergic diseases are associated with an increased rate of healthcare utilisation, including emergency department visits and hospitalisations. With proper specialist care, mortality from asthma and food allergy is very rare. The mortality related to food allergy is mainly due to food induced anaphylaxis, which is a serious acute systemic allergic reaction that may cause death in otherwise healthy individuals (Cianferoni and Muraro, 2012). A recent Australian report showed that the food anaphylaxis fatality rates increased by 9.7% per year from 1997 to 2013 (R. J. Mullins *et al.*, 2016). Furthermore, it has been estimated that asthma is responsible for around 1 in every 250 deaths globally, many of which could be prevented by proper clinical management (Masoli *et al.*, 2004). In Australia, there were 411 deaths attributed to asthma in 2009 which represents 0.29% of all deaths in that year. Despite this, the mortality rate due to asthma in Australia remains high by international standards

(ACAM, 2011). In 2015, asthma was projected to be the underlying cause of 407 deaths in Australia (AsthmaAustralia, 2015).

2.2.6.2. Financial, social and psychological burden of allergic diseases

Allergic diseases can have adverse consequences on the quality of life for affected individuals and their families, influencing a range of factors including psychological and general wellbeing, as well as the ability to learn and process cognitive input particularly when symptoms are uncontrolled (Cummings *et al.*, 2010). Affected children may become distressed by their disease, with sleep disruptions, school absence and constraint in participation in family activities (Bollinger *et al.*, 2006; Pawankar *et al.*, 2012). Additionally, individuals with allergic diseases may experience tiredness, mood changes and reduced functional capability (Lewis-Jones, 2006).

Allergic diseases impose a substantial economic burden on individuals and the wider community (Bahadori *et al.*, 2009). Costs attributable to allergic diseases include both direct costs, such as physician visits, medications and hospital care, and indirect costs, such as lost work days due to illness or caring for a sick child. In Australia, the health system costs of asthma in 2015 were estimated to be $1.2 billion. In the year 2015, the average health system cost per person with asthma was $524 (AsthmaAustralia, 2015). Due to the high prevalence of asthma and its health and financial consequences, the Australian Health Minsters declared asthma a National Health Priority Area in 1999. It is still recognised as a National Health Priority Area in 2016. For eczema, the estimated direct costs to an Australian family were $330 to $1255 a year (Su *et al.*, 1997). The total wholesale cost of medications used in the treatment of allergic rhinitis has approximately doubled in the last 10 years, and in the year 2010 this cost amounted to around $226.8 million (AIHW, 2011).

In the following section, I discuss the various methods that have been used to define asthma, eczema, allergic rhinitis and food allergy in both clinical and research settings in more details.

2.3. Definitions of allergic diseases

It is difficult to define allergic disease for a number of reasons. Allergic disease definitions are based on the presence of several symptoms instead of a specific disease entity.

The symptoms of allergic reactions are not specific to allergic conditions and there are a range of other conditions that may simulate allergic reactions (Høst *et al.*, 2003). Finally, the symptoms of allergic disease usually follow a relapsing-remitting course and are varying over time within an individual. In this section, I describe how asthma, eczema, allergic rhinitis and food allergy have been defined in the literature.

2.3.1. Definition of asthma and lung function

Asthma is not a recent disease and its definition has been refined over years. There is no single test or pathognomonic characteristic that defines asthma (Kemp *et al.*, 1996). In the early 1960s, Scadding proposed that asthma should indicate an abnormality of function as "a disease characterised by wide variations over short periods of time in resistance to flow in intrapulmonary airways" (Hargreave and Nair, 2009). In clinical settings, asthma is identified by the presence of intermittent symptoms of wheezing, chest tightness, coughing and shortness of breath. Although wheeze is regarded as the main symptom of asthma, wheeze has a number of potential causes other than asthma (Kemp *et al.*, 1996).

Asthma is a chronic inflammatory disorder of the airways due to a complex interplay of genetics, the environment, and the immune system (Lee and Burks, 2006). Asthma was defined by the Global Initiative for Asthma (GINA) as "a chronic inflammatory disorder of the airways in which many cells and cellular elements play a role. The chronic inflammation is associated with airway hyperresponsiveness that leads to recurrent episodes of wheezing, breathlessness, chest tightness, and coughing, particularly at night or in the early morning. These episodes are usually associated with widespread, but variable, airflow obstruction within the lung that is often reversible either spontaneously or with treatment" (Bateman *et al.*, 2008)

The GINA definition of asthma, however, is of limited value in epidemiological studies. There is no gold standard or objective marker for the diagnosis of asthma, particularly between episodes of exacerbation. A definition of asthma in an epidemiological study should ideally be practicable, simple, standardised, highly sensitive and specific and have responses that are reproducible. Therefore, several methods have been used to define asthma in epidemiological studies and this has mainly involved the use of questionnaire data. In the following section, I discuss various methods that have been used to define asthma.

2.3.1.1. Definitions based on questionnaire

Self or parent reported doctor diagnosed asthma

The following question is used frequently in surveys to ascertain a diagnosis of asthma "Have you ever been told by a doctor that you have asthma?" This method for asthma diagnosis requires individuals to be seen by a medical practitioner as well as having clinical signs and symptoms of asthma at the time of examination. However, not all individuals with asthma symptoms will seek medical advice, specifically in those with mild disease or those who prefer to manage their symptoms with alternative medicines.

Use of doctor diagnosis for ascertainment of asthma also depends on individuals being able to accurately recall that the term "asthma" was used by the physician to describe their symptoms (Michel *et al.*, 2006). For these reasons, this particular definition of asthma has low sensitivity despite its high specificity (Kilpeläinen *et al.*, 2001). To overcome these limitations, an assigned physician needs to assess all the study participants for evidence of asthma. However, this is usually not feasible in epidemiological research.

Self or parental reported of asthma symptoms

Asthma symptom questionnaires have a wide application in epidemiological research, particularly in longitudinal surveys or large surveys, in which physiological tests and individual physician assessment may not be feasible to perform for all participants. A history of wheeze in the last 12 months is commonly used for defining asthma. This method for asthma definition has been used in both ISAAC (Asher *et al.*, 1995) and ECRHS (Burney *et al.*, 1994). In ISAAC, children were regarded as having asthma if they answered "Yes" to both following questions "Has your child ever had wheezing or whistling in the chest at any time in the past?" and "Has your child had wheezing or whistling in the chest in the last 12 months?" (Asher *et al.*, 1995). In ECRHS, asthma has been defined in different ways including "An attack of asthma within the last 12 months" (Burney *et al.*, 1996). Defining asthma by this means is quick, convenient and does not rely on access to health services. The validity of ISSAC and asthma symptoms questionnaires has been investigated in an Australian population-based study (Tasmanian Asthma Survey (TAS)) by comparing response to questionnaires with a physician assessment of asthma status in the past 12 months (Jenkins *et al.*, 1996). Findings from this study showed that these questionnaires are valid

instrument for determination of asthma symptoms in the past 12 months (sensitivity and specificity for TAS questionnaires were 80% and 97%, and for ISSAC questionnaires were 85% and 81%). Therefore, in this thesis I define asthma based on self-reported asthma in the last 12 months.

Nevertheless, the use of self or parental reports of asthma symptoms in defining asthma has limitations. The majority of questionnaire based definitions of asthma are dependent on a report of wheezing symptoms. Wheezing conditions can be induced by many disease manifestations such as viral infections, cystic fibrosis, vocal cord dysfunction syndrome (Weinberger and Abu-Hasan, 2007; Brand *et al.*, 2008) and COPD in adults (Abramson *et al.*, 2002). Therefore, the definition of asthma using reported symptoms is unable to exclude these different causes of wheeze. Additionally, the use of wheezing symptoms only for defining asthma may miss some individuals with asthma who presented without wheeze but with other symptoms such as cough. Due to these limitations, other objective clinical measures have been used for asthma definition in some epidemiological studies, including evaluation of lung function and assessment of bronchial hyperreactivity (BHR).

2.3.1.2. Definitions based on lung function measurement

Spirometry

Assessment of airflow obstruction along with its reversibility by using bronchodilators is considered the most useful confirmatory test for the diagnosis of asthma. Spirometry is a commonly used technique for assessment of airflow obstruction. It is a physiological test that measures how an individual inhales or exhales volumes of air as a function of time. The common indices of lung function that are evaluated in routine spirometry include: the forced vital capacity (FVC), which is defined as the volume delivered during expiration made as forcefully and completely as possible starting from full inspiration; the forced expiratory volume in one second (FEV_1), which is the maximal volume of air exhaled in the first second of a forced expiration from a position of full inspiration; and the FEV_1/FVC ratio which is the ratio of these two measures (Miller *et al.*, 2005).

Spirometry can be performed with various types of equipment and necessitates cooperation between the individual and the examiner. Spirometry should be carried out in a standardised

way, as recommended by the American Thoracic Society (ATS) (Crapo *et al.*, 1995) and the European Respiratory Society (ERS) (Miller *et al.*, 2005).

Lung function testing has been performed extensively in epidemiological studies to provide an objective measurement of adverse health effects attributed to certain environmental exposures (Chan-Yeung, 2000) and to predictor later development of respiratory diseases. Additionally, assessment of lung function can also be used in understanding the natural development of lung function over time. However, this method has limitations including an inability to measure underlying lung inflammation and its ability to diagnose asthma depends on the presence of asthma at the time of performing the test. In addition, there are practical and cost considerations. Spirometry is expensive, needing to be performed by trained personnel using specialised equipment and requires individuals to attend a respiratory laboratory. As it requires the participants cooperation, it is generally unsuitable for research in infants and young children (Gaffin *et al.*, 2010). The usefulness of spirometric measures in defining asthma also relies on the availability of an appropriate reference standard against which the indices can be measured. Such a reference standard will vary in different populations.

Bronchial hyperreactivity

Bronchial hyperreactivity or hyperresponsiveness (BHR) is defined as an exaggerated bronchoconstriction response to non-specific stimuli (Grootendorst and Rabe, 2004). It is recognised to be an indicator of inflammation in asthma and it is related to the severity of the disease (Peat *et al.*, 2001). There are several different agents used for assessment of BHR. BHR may be measured using pharmacological agents (such as histamine and methacholine) that act directly on the bronchial smooth muscle causing contraction. Another method is the use of compounds, such as hypertonic saline, mannitol and adenosine monophosphate that act indirectly by stimulating the release of contractile mediators from inflammatory cells, again causing contraction. An additional way involves the use of other stimuli, such as exercise or cold dry air (Brannan, 2010).

Despite the advantages of BHR assessment in epidemiological research, including objectivity and reliability, it has limitations. Measurement of BHR has high specificity (commonly over 80%) but low sensitivity (commonly below 50%) when compared with other clinical markers, which indicates that some individuals with asthma symptoms may be missed by

using this definition (Peat *et al.*, 2001). Additionally, BHR may be masked by the use of inhaled corticosteroids, which are commonly used for treating asthma (Currie *et al.*, 2003). Therefore, BHR is neither necessary nor a sufficient characteristic for asthma definition and dependence on BHR alone to define asthma in epidemiological studies will result in a degree of bias.

2.3.2. Definition of Eczema

Eczema is a common, chronic, relapsing, pruritic inflammatory skin disease that has typical distribution of rash. Eczema primarily affects young children and improves with age, but may continue into adulthood (Brown and Reynolds, 2006). Eczema usually manifests in particular patterns that are more common at certain ages. The first part to be affected is the face and then the body. The forearms, the flexures and extensor aspects of the knees are often the most affected areas in crawling infants. Conversely, the flexor surfaces of the elbows and the knees, as well as hands and feet, are frequently affected areas in older children (Barnetson and Rogers, 2002). Lesions of eczema typically spare the nappy area in infancy, which assist in making the diagnosis (Spergel and Paller, 2003).

Eczema is one of the most common skin conditions observed in infants and children and usually has its onset in the first 6 months of life. It has been reported that up to 80% of eczema cases are associated with atopy (Schmid *et al.*, 2001) and it has been argued to consider the presence of IgE sensitisation as an essential diagnostic criterion in eczema. The term “atopic dermatitis” implies the presence of eczematous skin manifestations which are accompanied by SPT positivity or presence of serum IgE antibodies. In this thesis, I use the term “eczema” to describe the clinical manifestation of rash, irrespective of evidence of atopy.

Eczema is generally difficult to define in both clinical and epidemiological settings because of its variable presentation and distribution as well as its intermittent course. Several diagnostic criteria have been developed to define eczema (Hanifin, 1980). Consensus criteria for the main clinical features of atopic dermatitis have led to a short list of reliable and valid discriminators that are used worldwide (Williams *et al.*, 1994). In the next section, I discuss how eczema has been defined in clinical and epidemiological settings.

2.3.2.1. Definitions based on clinical assessment

A number of clinical definitions of eczema based on physical examination have been suggested. The first diagnostic criteria were introduced in 1980 by Hanifin and Rajka and were based on several clinical signs and symptoms (Hanifin, 1980). An individual needs to have evidence of at least three of the four major features (including itchy, typical distribution and appearance, chronic relapsing condition and personal or family history of allergic disease) and at least three of the 23 minor features (Williams, 2000). These criteria are based on agreement between experienced dermatologists without objective clinical validation. The definition of eczema using these criteria is complex. Additionally, the reliability of assessment of some of these criteria is poor. It is therefore unsuitable to use in population-based epidemiological studies. However, the Hanifin and Rajka criteria are often used in clinical trials, most likely because of its high sensitivity (Brenninkmeijer *et al.*, 2008).

In 1994, the United Kingdom working-party diagnostic criteria were introduced by Williams et al. as an improvement of Hanifin and Rajka's diagnostic criteria for eczema. The UK working-party assessed the reliability of each of the features in the Hanifin and Rajka definition and the association between each of the features with dermatologist diagnosis of eczema (Williams *et al.*, 1994). Those criteria that showed the best reliability and validity against dermatologist assessment were used to form the UK working-party criteria (Williams *et al.*, 1994). These criteria include "itchy skin condition in the last 12 months plus three or more of (1) History of flexural involvement; (2) History of generally dry skin; (3) History of allergic disease in a first-degree relative and (4) Visible flexural dermatitis as per photographic protocol" in children under the age of two. For older children and adults, the UK working -party criteria is the same as above, but additionally includes onset under the age of two years and personal history instead of family history of allergic disease. The criteria are all non-invasive and suitable for clinical and epidemiological studies. A slight modification of the criteria is needed when infants are assessed.

The definition of eczema based on UK working -party requires a physical examination of the skin by a trained person to determine the presence of "visible flexural dermatitis", which is not always possible in epidemiological studies (Williams *et al.*, 1994). Relying on visible evidence of eczema may underestimate the cases as this condition follows a replacing-remitting course. Therefore, definitions based on physical features at the time of a single examination may lack sensitivity as a period prevalence estimate of eczema. Furthermore, it

is not always possible to perform physical examination in large epidemiological studies. For these reasons, eczema in epidemiological studies is often defined based on questionnaires.

2.3.2.2. Definitions based on questionnaire

Several questionnaire based definitions of eczema have been suggested, however only a small number of potential definitions have been validated. In ISAAC, current eczema is defined as a response of "Yes" to all of the following questions: "Has your child ever had an itchy rash which was coming and going for at least 6 months?", "Has your child had this itchy rash at any time in the last 12 months?" and "Has this itchy rash at any time affected any of the following places: the folds of the elbows, behind the knees, in front of the ankles, under the buttocks, or around the neck, ears or eyes?" (Asher *et al.*, 1995). This definition has been validated against dermatologist assessment in three studies (Chan *et al.*, 2001; Yamada *et al.*, 2001; Haileamlak *et al.*, 2005). The observed specificity and sensitivity from these studies varied between populations. Specificity was reported to be high (92%) in a study of Chinese children (Chan *et al.*, 2001) but low (66%) in a study of Ethiopian children (Haileamlak *et al.*, 2005). Similarly, the sensitivity of the ISAAC definition of eczema varied widely between these studies (50% versus 73%).

A number of other questionnaire based definitions of eczema have been suggested and have undertaken validation. A Danish group developed a questionnaire for eczema definition that relies on the UK working-party criteria (Olesen *et al.*, 2001). This definition was validated against a clinical assessment in a group of 61 children, and it showed good specificity (97%) and sensitivity (90%). Schultz Larsen and colleagues proposed a complex scoring system for eczema definition based on personal and family history of allergic disease and distribution of rash (Larsen *et al.*, 1996).

It has been proposed that a doctor diagnosis of eczema and/or use of topical steroid and other immunosuppressants could be a valid indicator for presence of eczema in infants (Hill *et al.*, 2000). Excluding treatment used on the nappy region or scalp should eliminate cases of nappy rash or seborrheic dermatitis that may be treated with topical immunosuppressants. The use of steroid treatment therefore could be a reasonable indicator of eczema in children under the age of two. This definition of eczema does not need physical examination and can be used in large epidemiological studies.

2.3.3. Definition of allergic rhinitis

Allergic rhinitis is a heterogeneous condition of the upper respiratory tract that is commonly underdiagnosed despite its high prevalence. Allergic rhinitis is an immune-mediated reaction that arises in the nose and is frequently characterised by the presence of one or more of the following symptoms: sneezing, nasal congestion, itching, and rhinorrhea (Skoner, 2001). Usually, these symptoms arise as a result of inflammation induced by an IgE-mediated immune response to specific allergens such as dust mites, pollens, moulds and animal dander. The immune response comprises the release of inflammatory mediators and the activation and recruitment of inflammatory cells to the nasal mucosa. In this thesis I have used the term "allergic rhinitis" to describe the clinical manifestations, irrespective of evidence of atopy. Many other non-allergic conditions, such as infectious rhinitis, can cause similar symptoms and are referred to as non-allergic rhinitis (Skoner, 2001). However, around 77% of individuals with rhinitis symptoms have evidence of elevated serum s-IgE (Mølgaard *et al.*, 2007).

"Hay fever" is a term commonly used to describe allergic rhinitis caused by pollen. Allergic rhinitis has been further classified as seasonal (associated with outdoor allergens such as pollen and moulds, and generally occurred during the seasons with high pollen count) or perennial (associated with indoor allergens such as dust mites, moulds, cockroaches and animal danders and could occur throughout the year at different seasons) (Bauchau and Durham, 2005). However, the classification of allergic rhinitis has been revised by the World Health Organization (WHO) and includes a measurement of the frequency and duration of the symptoms. Intermittent allergic rhinitis is termed as experiencing symptoms for <4 days/week or <4 consecutive weeks. Persistent allergic rhinitis is defined as symptoms occurring for > 4 days/week and > 4 consecutive weeks (Bauchau and Durham, 2005). The issues associated with defining allergic rhinitis in epidemiological studies are similar to those associated with defining asthma. In the following section, I discuss the various methods that have been used to define allergic rhinitis in epidemiological studies.

2.3.3.1. Self or parent reported doctor diagnosed allergic rhinitis

To meet this definition of allergic rhinitis, individuals are required to have sought medical assistance for symptoms, the medical practitioners must have noticed consistent signs and used the term "allergic rhinitis" or "hay fever", and the individual must have

recalled their medical practitioner using of this term. Requiring a medical practitioner to examine the participants of a study can overcome the majority of these issues. However this is often not practical.

2.3.3.2. Self or parental reported of allergic rhinitis symptoms

A number of symptom based questionnaires have been proposed for the assessment of allergic rhinitis. In ISAAC, allergic rhinitis is defined as a response of "Yes" to both of the following questions; "Has you child ever had a problem with sneezing, or a runny, or a blocked nose when he/she DID NOT have a cold or the flu?" and "In the past 12 months, has your child had a problem with sneezing, or a runny, or a blocked nose when he/she DID NOT have a cold or the flu?" (Asher *et al.*, 1995). This definition attempts to reduce false positive responses as a result of confusion between allergic rhinitis and upper respiratory tract infections.

Defining allergic rhinitis based on reported symptom questionnaires has a number of issues. There are many other conditions that can mimic the symptoms of allergic rhinitis such as viral infections, nasal polyps and ciliary defects (Skoner, 2001). Therefore, this definition is unlikely to distinguish between allergic rhinitis and these alternative diagnoses, which will lead to false positive responses. Individuals with symptoms of rhinitis without evidence of atopy have non-allergic rhinitis. Definitions of allergic rhinitis based on questionnaire response are not able to differentiate between the allergic and non-allergic forms of rhinitis. This is an important issue as these two distinct forms of rhinitis may have different aetiologies and prognoses.

2.3.4. Definition of food allergy

Food allergy is generally defined as "an adverse health effect arising from a specific immune response that occurs reproducibly on exposure to a given food" (Chafen *et al.*, 2010; Boyce *et al.*, 2011). This term therefore encompasses both IgE-mediated, non-IgE-mediated food allergies, or a combination of both. The majority of food allergic reactions are caused by IgE-mediated allergic reactions. IgE-mediated reactions involve the skin (angioedema and urticaria), gastrointestinal tract (nausea, vomiting, diarrhea, and abdominal pain) and respiratory tract (shortness of breath and wheezing) in the presence of food specific IgE. Additionally, IgE-mediated allergic reactions to foods are characterised by acute onset of

symptoms generally within minutes or hours after ingestion of or exposure to the trigger food (Burks *et al.*, 2012). Conversely, non–IgE-mediated reactions, which result from cell-mediated immune mechanisms, include: food protein-induced enterocolitis, proctocolitis and enteropathy syndromes (Burks *et al.*, 2012). Moreover, there are various adverse reactions to foods that do not comprise an immune response and therefore are not considered the result of food allergies. These include metabolic disorders, such as lactose intolerance, responses to pharmacologically active food components, or illness in response to toxins from microbial contamination. Certain psychological or neurological responses, such as food aversion, can also mimic food allergy but are not considered allergic disorders (Allen *et al.*, 2006). Unlike other food allergies, IgE-mediated food allergy is associated with a risk of anaphylaxis, defined in a consensus document as "a serious allergic reaction that is rapid in onset and may cause death" (Sampson *et al.*, 2006).

In this thesis, I focus on IgE-mediated food allergy only and no other forms of food reactions are discussed. In the following section, I discuss the methods used to define IgE-mediated food allergy in clinical and research settings.

2.3.4.1. Definitions based on clinical assessment

The diagnosis of food allergy requires a complete history and physical examination to determine possible causative foods and to ascertain the underlying pathophysiological basis, mainly whether the food induced reaction is likely to be an IgE-mediated or not, which direct the following assessment (Sicherer and Sampson, 2010). Clinically, IgE-mediated food allergies should be defined using oral food challenges (OFC), which are the gold standard for diagnosis of food allergy.

The OFC is involved of a gradual administration of a potential allergen under complete medical supervision to determine clinical reactivity or tolerance. Severe systemic allergic reaction can occur and for that reason the procedure should be undertaken by a trained person. Administration of the food is usually stopped when objective or subjective symptoms are initiated (Sicherer and Sampson, 2010). OFC can be either open or double-blind placebo-controlled food challenges (DBPCFC). Although the gold standard test to confirm the diagnosis of food allergy is DBPCFC, few studies to date have used this method in the diagnosis of food allergy in research setting. This is likely due to a number of factors. Performing a large number of food challenges is costly, time consuming and poses a risk

(although small) of anaphylaxis in food allergic individuals (Mehl *et al.*, 2012). Furthermore, clinical symptoms suggestive of IgE-mediated food allergy may be related to other non-IgE-mediated food disorders.

2.3.4.2. Definitions of food allergy in the research setting

Several methods have been used to define food allergy in large studies. Food allergies are commonly defined in epidemiological research according to sensitisation status to specific food allergens. Sensitisation can be assessed by either SPT or measurement of food s-IgE (both methods are discussed with more detail in *Section 2.5.4*). A positive skin test result may indicate food allergy (positive predictive value (PPV) ≥50%), but a negative skin test result almost rules out an IgE-mediated mechanism (negative predictive value (NPV) ≥95%) (Chapman *et al.*, 2006) (the PPV and NPV of SPT are described in detail in *Section 2.6.2.1*). Generally, larger wheal size reactions on SPT and higher concentrations of food s-IgE measured by in vitro tests correlate with a greater likelihood of allergic reactions. Although this method relies on an objective measure for the assessment of food allergy, a definition of food allergy based on sensitisation alone may result in high rate of false positivity and hence over diagnosis of food allergy. To overcome this issue, both sensitisation to certain food allergens and the presence of self-reported allergic symptoms after ingestion of such food may be used to define food allergy. However, self-reported symptoms of an allergic reaction to food are influenced by local culture and language background. Self-reporting of perceived food allergy reactions significantly overestimates allergies (Rona *et al.*, 2007).

In the previous sections, I described the burden and public health implications of allergic condition, along with their definitions in clinical and epidemiological settings. In the following sections, I briefly discuss the common potential risk factors for the development of these disorders.

2.4. Risk factors for the development of allergic disease

Allergic disorders share various risk factors such as a positive family history of atopic diseases, the production of IgE antibodies in response to allergens, an impaired balance

between cytokines of TH1- and TH2-type lymphocytes. Individuals commonly experience more than one form of allergic condition, either at the same time or over their life course.

Allergic diseases are complex multifactorial disorders whose aetiology is related to interaction between genetic factors and environmental exposures. Several factors have been identified to influence not only the development of IgE-mediated sensitivity but also the subsequent development of clinical symptoms of allergic disorders. Understanding factors related to the development of allergic disease is important in assessing an individual child's risk for atopy and identifying appropriate interventions. In the following sections, I discuss the potential genetic and environmental risk factors for the development of allergic diseases.

2.4.1. Genetic and heritable factors

The main independent risk factor for the development of atopic diseases is the presence of family history of allergic diseases (Wright, 2004). Maternal allergies have been shown to be associated with increasing risk of allergic diseases development more than paternal atopy (Ruiz *et al.*, 1992; Wright, 2004), but the evidence remains uncertain. It has been suggested that maternal and/or placental factors can considerably affect the development of atopy (Liu *et al.*, 2003).

Many studies have now conclusively shown that susceptibility to asthma and other allergic diseases has a heritable component. In children without atopic heredity, around 10% develop allergic disease, whereas 20–30% of children with single atopic heredity (parent or sibling) and around 40–50% of infants with double parental heredity have been shown to develop atopic disease (Bergmann *et al.*, 1997). It has been proposed recently that allergic disorders are caused by multiple genes, each contributing only a small effect to an individual’s risk of allergic diseases (Arruda *et al.*, 2005). Within the last few years, many gene polymorphisms have been identified that show an association to one or more manifestations of allergic disorders; however the majority of these polymorphisms induce only a small increase in the risk. These genetic polymorphisms have been reviewed in many studies (Blumenthal, 2005; Morar *et al.*, 2006; Ober and Hoffjan, 2006). The filaggrin gene located on human chromosome 1q21 within the epidermal differentiation complex (Brown and McLean, 2012). The key function of the protein filaggrin is regulating permeability of the skin to water and environmental agents such as allergens. Mutations in the filaggrin gene lead to loss of its function (Irvine *et al.*, 2011). Several studies have investigated the association between

filaggrin gene mutation and the risk of developing eczema and other allergic diseases (Bønnelykke *et al.*, 2010; Osawa *et al.*, 2011; Ponińska *et al.*, 2011). Findings from a systematic review and meta-analysis (van den Oord and Sheikh, 2009) showed that filaggrin gene defects increased risk of atopic sensitisation, atopic eczema, and allergic rhinitis. Additionally, filaggrin gene mutations increased the risk of having asthma in individuals with concomitant atopic eczema (van den Oord and Sheikh, 2009)."

However, it is unlikely that the change in genetic factors can explain the increased prevalence of allergic disorders seen over the last decades (see *Section 2.2.1* related to the prevalence of allergic diseases). It has been suggested that a complex interactions between genetic predisposition and life-long exposures to environmental factors leads to the development of sensitisation and allergic disease (Holloway, Holloway, *et al.*, 2010). Many of these environmental factors affect immune regulation through direct or indirect mechanisms (Martino *et al.*, 2014). Recently, the role of epigenetics in regulating the susceptibility to and the severity of allergic disease has drawn more attention. Epigenetic modifications involve biochemical reactions that modify gene expression in a given DNA sequence. These epigenetic modifications, involving both DNA methylation and histone acetylation, have a fundamental role in regulation of different cellular functions, including regulation of inflammatory responses, DNA repair, and cell proliferation (Prescott and Saffery, 2011). Better understanding of these early interactions could also provide opportunities for early intervention and disease prevention. In the next section, I describe the common potential environmental factors that could influence the occurrence of allergic diseases.

2.4.2. Environmental factors

In light of the fact that the risk of atopy and allergic manifestation is particularly high in industrialised countries with relatively high standards of living, it may be questioned what factors related to the Western lifestyle might be responsible for increasing the susceptibility to atopy and allergic conditions. It is evident from a number of studies that several environmental factors play a major role in the development of allergic diseases. The timing of exposure to these environmental influences may be of substantial importance. It has been proposed that the intrauterine and infancy periods, when the immune system is maturing in accordance with exposure to environmental factors, is the most influential time that is commonly associated with allergy development (Martino and Prescott, 2011). Most of these

factors have been reviewed in previous studies (von Mutius and von, 2000; Arruda *et al.*, 2005; Lack, 2012). In the following section, I provide a brief description of the main environmental factors proposed to influence the development of allergic conditions. In this thesis, I consider some of these environmental factors in my analyses.

2.4.2.1. Early life infection

Changes of lifestyle in industrialised countries have led to a reduction in the burden of infectious illnesses and are associated with the increase of allergic diseases, according to the "hygiene hypothesis". The hygiene hypothesis was first proposed by Strachan in 1989, who observed an inverse correlation between hay fever and the number of older siblings when following more than 17,000 British children born in 1958 (Strachan, 1989). The hygiene hypothesis states that current health care and hygiene practices have led to a relative sterilisation of the environment with condensed exposure to bacterial, viral or fungal components. This leads to an imbalance of the immune system which finally predisposes individuals to the development of allergic diseases (Schaub *et al.*, 2006).

A number of epidemiological studies support the theory of the hygiene hypothesis. Initial studies showed an association between family size (number of children) and the prevalence of atopic dermatitis and hay fever. The sibling effect was attributed to a higher infection rate of children with older siblings, providing evidence for the hygiene hypothesis (Strachan, 2000). In line with these findings, day care attendance in early life has been shown to be associated with reduced risk of asthma and recurrent wheezing among children with no maternal history of asthma (Celedón *et al.*, 2003). Additionally, major support for the hygiene hypothesis came from studies comparing the prevalence of allergic conditions of children growing up in rural environments with children born and growing up in urban areas. Several studies could demonstrate a strong association between growing up on a farm with frequent exposure to an environment rich in microbial components and a reduced risk to develop an allergic disease (Nicolaou *et al.*, 2005).

2.4.2.2. Breastfeeding and infant diet

There is an extensive body of literature assessing the association between breastfeeding and asthma and allergic disease. Some studies have supported a protective effect (Kull *et al.*, 2002; Lodge *et al.*, 2015), while a number of prospective longitudinal

studies have suggested that breastfeeding might increase the risk of development of atopic diseases, mainly after about 7 years of age (Bergmann *et al.*, 2002; Matheson *et al.*, 2007). Thus, it is still uncertain whether the development of allergic disease can be prevented by breastfeeding (Matheson *et al.*, 2012; Lodge *et al.*, 2015).

The duration of exclusive breastfeeding that confers a protective effect is not well defined. Result from a Swedish birth cohort showed that exclusive breastfeeding for four months or more seemed to reduce the risk of asthma during the first 8 years of life. At this age, a reduced risk was also observed particularly for asthma combined with sensitisation to common food or inhalant allergens. Furthermore, breastfeeding appears to have a beneficial effect on lung function (Kull *et al.*, 2010; Waidyatillake *et al.*, 2013).

The evidence related to the association between the timing of first introduction of solid foods, particularly the introduction of common allergenic foods including cow's milk, egg and peanut, and the risk of childhood allergic diseases is controversial. Until very recently, early introduction of food was thought to increase the risk of allergic disease and delayed introduction until 6 months of age was recommended. More recently, there has been evidence to support a "critical window" for food allergen introduction in which early introduction (4-6months) of foods may indeed confer protection against allergic conditions (Prescott *et al.*, 2008; Nwaru *et al.*, 2013). A systematic review for the relationship between early introduction of solid foods to infants (before age 4 months) and the development of allergic disease found that early solid feeding may increase the risk of eczema. However, there are little data supporting an association between early solid feeding and other allergic conditions (Tarini *et al.*, 2006).

2.4.2.3. Parental smoking

It is evident that there is a link between maternal smoking during pregnancy and adverse health outcomes in infants. A meta-analysis showed that the exposure to prenatal maternal smoking was associated with 40% increase in risk of wheeze in children aged ≤2 years (Burke *et al.*, 2012). This meta-analysis further observed a similar magnitude of effect for the relation between prenatal maternal smoking and incidence of wheeze and asthma between ages 3 and 4 years. Additionally, maternal smoking during pregnancy is considerably associated with reduced respiratory function in early infancy and recurrent wheezing during infancy and early childhood (Carlsen and Carlsen, 2001). It has been also

shown that parental smoking is strongly associated with increased risk of allergic disorders (Pattenden *et al.*, 2006; Thacher *et al.*, 2014).

2.4.2.4. Allergen exposure

Allergic diseases are frequently associated with sensitisation to one or more indoor allergens and occasionally to outdoor allergens (Sharpe *et al.*, 2015). The development of sensitisation to an allergen requires exposure to that allergen. Once sensitisation has occurred, repeated exposure to that allergen is likely to trigger symptoms. Evidence for the association between allergen exposure and the risk of allergic diseases has shown contradictory results. The different results between studies can be related to the type and dose of the allergen, the timing of exposure and characteristics of the specific allergen, as well as susceptibility of the child. Although a dose–response relationship between exposure to house dust mites and the development and severity of asthma was demonstrated in a number of studies (Custovic *et al.*, 1996; Peat *et al.*, 1996), no direct significant association between early exposure to indoor allergens (house dust mite and cat allergens) and asthma up to the age of 7 years was shown in another studies (Lau *et al.*, 2000).

There is growing body of evidence that sensitisation to indoor and outdoor allergens are related to increase risk of asthma and other allergic manifestations (Boulet *et al.*, 1997; Bush, 2008). Moreover, results from a German birth cohort study showed a strong association between sensitisation to mite and/or cat allergens and wheezing, which became significant around the third year of life. Furthermore, children with sensitisation to indoor allergens showed higher bronchial responsiveness (Lau *et al.*, 2002).

Unlike evidence related to aeroallergen sensitisation, the associations between food sensitisations and other allergic diseases have not been extensively investigated. The concurrent increase in the prevalence of allergic disorders, particularly food allergies (as described in *Section 2.2.5*), along with atopic sensitisation has raised questions concerning the role of food sensitisation in the pathogenesis of these conditions (J. Heinrich *et al.*, 2002).

Based on the concept of the “atopic march” (as describe in *Section 2.6.3.1*) individuals with early life food sensitisation could be at potential risk not only for subsequent development of food allergy, but also for asthma and allergic rhinitis (Spergel, 2010; Zheng *et al.*, 2011; Saunes *et al.*, 2012; Nissen *et al.*, 2013). As such, it is possible that food sensitisation is a

critical factor contributing to the burden of allergic diseases. As the main focus of my thesis is food sensitisation, I describe food sensitisation in more detail focusing on its definition, pathophysiology, methods of assessments and the key issues related to the epidemiology of food sensitisation in the following sections of this Chapter.

2.5. Food sensitisation

2.5.1. Definition of food sensitisation

The terms “allergy” and “atopy” are commonly used loosely and interchangeably. Allergy is defined as an abnormal immunological reaction that is initiated by exposure to a substance at a dose tolerated by normal persons. Allergy can be either antibody-mediated or cell-mediated. In the majority of individuals with allergic symptoms originating from the mucosal membrane in the airways and gastrointestinal tract, the produced antibodies belong to the IgE isotype and these individuals are said to have an IgE-mediated allergy (Johansson *et al.*, 2004).

Sensitisation arises when individuals are first exposed to potential allergens. Sensitisation reflects the ability of a specific allergen to initiate the immunological response and to produce IgE antibodies (Galli *et al.*, 2008). Accordingly, food sensitisation is defined as the presence of a food specific IgE response occurring upon exposure of the immune system to a certain food allergen (Asero *et al.*, 2007). Individuals can have allergic sensitisation to an allergen without having clinical symptoms of an allergic reaction upon exposure. Therefore, sensitisation itself is not adequate to define allergic manifestations. IgE-mediated allergic disorders require both the existence of sensitisation and the development of specific signs and symptoms on re-exposure to a specific allergen (Burks *et al.*, 2012).

The term “allergen” refers to any substance that has the ability to stimulate the production of IgE secretion and hence induce an allergic reaction in susceptible individuals after re-exposure (Johansson *et al.*, 2004; Galli *et al.*, 2008). Allergens come from different sources such as pollen, animals or their products, fungi, food, or dust (Platts-Mills and Woodfolk, 2011) and are categorised according to the method of penetration into the body: by ingestion, inhalation or direct contact (Baldacci *et al.*, 2001). The majority of allergens that stimulate

IgE antibodies are proteins (Johansson *et al.*, 2001). The characteristics of allergenic foods are discussed in the next section.

2.5.2. Allergenic foods

Food allergens are defined as "those specific components of food or ingredients within food (typically proteins, but sometimes also chemical haptens) that are recognised by allergen-specific immune cells and elicit specific immunologic reactions, resulting in characteristic symptoms" (Boyce *et al.*, 2011). Although any food can initiate sensitisation and an allergic reaction, only about 5% of the 170 foods identified to cause IgE-mediated responses are responsible for the most significant allergic reactions. These common food allergens include egg, peanut, tree nuts, milk, wheat, fish, shellfish and soy (Allen *et al.*, 2006; Burks *et al.*, 2012). Milk, peanut, egg, wheat and soy account for 90% of allergic reactions in children, while fish, shellfish, peanut and tree nuts account for 85% of food reactions in adolescent and adults (Lee and Burks, 2006). Allergenic food proteins ingested either in the raw or cooked forms stimulate the formation of IgE antibodies when introduced into a genetically predisposed host, often resulting in sensitisation and allergic reactions. Food proteins with more than 62% similarity to human proteins are unlikely to be allergenic (Berin and Sampson, 2013). Interestingly, other non-oral routes for food sensitisation, such as respiratory sensitisation and skin exposure to environmental food allergens, have been demonstrated particularly in situations when there is epithelial barrier dysfunction (Burks *et al.*, 2012)

The immunological phenomenon of cross-reactivity has consequences for both the diagnosis and treatment of certain food allergies. Cross-reactivity occurs when a food allergen has a structural or sequence similarity with a different food allergen or aeroallergen. The development of an allergic reaction to cross-reactive allergens is variable and determined by the type of food allergen. For example, it is common to have clinical cross-reactivity between different types of shellfish, while cross-reactivity among legumes is uncommon (Traidl Hoffmann *et al.*, 2009; Burks *et al.*, 2012).

The analysis of allergenic foods has identified common molecular characteristics that promote allergenicity. In general, food allergens are glycoproteins with molecular sizes ranging from 10 to 70 kDa, they are water-soluble, stable to heat and digestive enzymes, have greater stability during digestion than other molecules in food (Valenta *et al.*, 2015) thus

enabling them to sensitise the gastrointestinal tract of the host (Lee and Burks, 2006; Berin and Sampson, 2013) and have multiple IgE binding epitopes (van Wijk and Knippels, 2007; Berin and Sampson, 2013). Processes such as heating may alter the allergenicity of food proteins in different ways. For example, high temperatures decrease the allergenicity of egg and milk whereas high roasting temperatures increase the allergenicity of peanut (Berin and Sampson, 2013). In the next section, I discuss how these allergenic foods induce the immune response in genetic susceptible individuals.

2.5.3. Mechanism for the development of food sensitisation and reactions

The immune system is constantly exposed to proteins from different sources, such as bacteria and food. The challenge for the immune system is to avoid developing inappropriate immune responses to harmless environmental antigens, whilst maintaining the ability to respond to harmful pathogens (Kumar and Clark, 2002). Allergic reactions develop when the immune system responds to foreign proteins inappropriately (Galli *et al.*, 2008).

The immunological mechanisms associated with allergic sensitisation and reactions are complex and involve several types of cells, cell receptors and mediators released by cells. Sensitisation arises when allergen specific IgE (s-IgE) antibodies are formed after the first exposure to a specific allergen through ingestion, inhalation or by contact with the skin. When an allergen enters the body, it is first processed by antigen presenting cells (APCs) (Sohi and Warner, 2008). The APCs activate the naive T cells to differentiate into CD4 T-helper cells (Th2) under the influence of the cytokine interleukin 4 (IL-4) (Jutel *et al.*, 2006). Once generated, the Th2 cells produce cytokines (IL-5 and IL-13), which stimulate the B-cells to produce IgE that is specific to that allergen (Jutel *et al.*, 2006; Sohi and Warner, 2008). The IgE sensitises basophils and mast cells by binding to the high affinity receptor for IgE (FcεRI) presented on their surface (Akdis, 2006). The IgE- FcεRI complexes that present on the B-cells, mast cells and basophils facilitates antigen presentation (Larché *et al.*, 2006; Tan *et al.*, 2012). The individual is thus considered to be sensitised to that allergen (**Figure 2.1**).

On subsequent exposure to the allergen, s-IgE bound to FcεRI on the surface of sensitised mast cells and basophils is crosslinked by the allergen, resulting in degranulation and release

of inflammatory mediators (**Figure 2.1**). The released mediators include vasoactive amines (mainly histamine), chemokines, lipid mediators and other cytokines (Larché *et al.*, 2006; Galli *et al.*, 2008; Sohi and Warner, 2008). These mediators cause a variety of clinical symptoms of allergy that vary according to the site of the reaction. These symptoms can include respiratory (wheeze, cough), cutaneous (eczema, urticaria, angioedema), gastrointestinal (e.g. nausea, vomiting, abdominal pain, diarrhoea), or systemic manifestations (e.g. collapse due to hypotension) (Galli *et al.*, 2008).

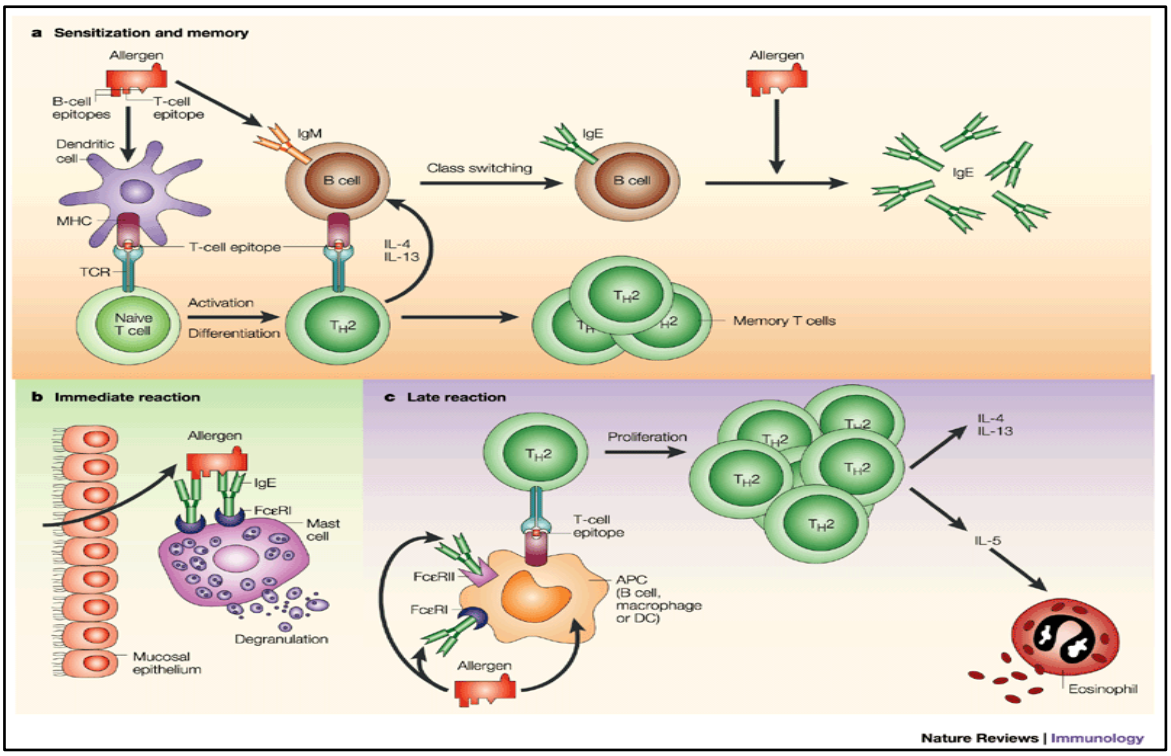

Figure 2.1 - Mechanism for the development of atopic sensitisation.

(a) **Sensitisation and memory**. Initial contact with minute amounts of intact, soluble allergen at mucosal surfaces, particularly of the respiratory tract, might favour allergen uptake by potent antigen-presenting cells (for example, dendritic cells) and/or immunoglobulin-mediated capture by specific B cells. If T helper 2 (TH2)-cell help is acquired, cytokines such as interleukin-4 (IL-4) and IL-13 will be produced that favour immunoglobulin-class switching of specific B cells to immunoglobulin E (that is, sensitisation). Sensitisation leads to the establishment of IgE+ memory B cells and allergen-specific memory T cells. Subsequent repeated allergen contact will boost IgE+ memory B cells that receive T-cell help to produce increased levels of allergen-specific IgE antibodies. These are loaded by means of specific receptors (Fcalt epsilonRI, high-affinity IgE receptor; Fcalt epsilonRII, low-affinity IgE receptor) onto mast cells, basophils, monocytes, dendritic cells and B cells.

(b) **Immediate reaction**. The crosslinking of effector-cell-bound IgE by allergens leads to the release of biologically active mediators (histamine, leukotrienes) by means of degranulation and, so, to the immediate symptoms of allergy.

(c) **Late reaction**. This is caused by the presentation of allergens to T cells, which become activated, proliferate and release pro-inflammatory cytokines (for example, IL-4, IL-5 and IL-13).

Source: adapted from (Valenta *et al.*, 2015).

Of all individuals exposed to an allergen, the majority will develop tolerance. Only a proportion will experience clinically significant sensitisation. Inter-individual differences in susceptibility to the development of sensitisation and allergic reaction responses are complex and still not fully understood. Several heritable and environmental factors associated with the development of sensitisation versus tolerance have been identified (van Wijk and Knippels, 2007; Galli *et al.*, 2008). The most important determinant that can enhance the sensitisation process is genetic predisposition, which is most likely due to the inheritance of several mutant genes (Holloway, Yang, *et al.*, 2010). For instance, IgE-mediated allergy occurs in 40–60% of children if both parents are atopic, and in 5–10% of children if neither parent is atopic (Johansson *et al.*, 2001).

Other potential factors that influence the risk of developing sensitisation include the type of allergen and its physiochemical properties, allergen concentration, route of exposure and age at exposure (Galli *et al.*, 2008; Traidl Hoffmann *et al.*, 2009). The physiochemical characteristics of the allergen, such as molecular weight, hydrophobicity and stability appear to be related to the development of sensitisation. Allergens can enter the body through different routes such as the gastrointestinal tract, the airways and skin. The route of allergen exposure and dose appear to be significant factors for the development of sensitisation (Strid and Strobel, 2005). Exposure to low doses of allergen can stimulate sensitisation and allergic reactions, while exposure to high doses of allergen can induce tolerance through different pathways (Traidl Hoffmann *et al.*, 2009). In the next section, I discuss the common methods used for the assessment of sensitisation.

2.5.4. Clinical assessment of food sensitisation

Sensitisation to a food allergen can occur without any clinical manifestation of an allergic reaction. Accordingly, identification of sensitisation to a food allergen should be related to an individual's medical history and physical examination (Sicherer *et al.*, 2012). Assessment of sensitisation is commonly conducted to evaluate the presence of IgE antibodies after exposure to certain allergens (Baldacci *et al.*, 2001). Additionally, assessment of sensitisation is a very important process for early identification of children at increased risk for the development of later allergic disease. For these purposes, different confirmatory tests can be conducted to detect allergen specific IgE antibodies in an individual's skin or

blood. The most commonly used tests in clinical practice and epidemiological studies are skin prick tests (SPT) and serum specific IgE assays.

2.5.4.1. Skin prick test (SPT)

Skin prick test is the most convenient method for identifying IgE-mediated allergic reactions and has the advantage of being easy to perform, inexpensive, reliable and providing a quick result. SPT is used to test adults and children from birth onwards (Heinzerling *et al.*, 2013).

SPT is indicated if an IgE-mediated allergic reaction is suspected, based on medical history and clinical symptoms. Thus, SPT provides objective confirmation of sensitisation to a specific allergen (Bernstein *et al.*, 2008). Another indication of SPT is to screen individuals with a predisposition for allergic diseases, such as food allergy, eczema, asthma and allergic rhinitis. SPT also can be used to identify sensitised individuals in certain populations and can be performed in epidemiological studies to determine trends in the rate of sensitisation or differences in sensitisation between regions (Heinzerling *et al.*, 2013). Repeated testing may be required to identify new sensitisations, particularly in young children, or if the presence of new environmental allergens are suspected (Heinzerling *et al.*, 2013).

SPT should be performed by a physician or a healthcare professional. Emergency equipment should be available at the time of testing due to the potential risk of systemic allergic reactions (Liccardi *et al.*, 2006). SPT is commonly performed on the volar surface of the forearm or back. The skin is first cleaned with alcohol and may be marked with numbers corresponding to the allergens. Using a sterile lancet, a small prick is made into the skin through a drop of allergen extract (**Figure 2.2**). This allows a small amount of allergen to enter the skin. After 15 to 20 minutes, the test area is examined for wheal and flare reactions (Heinzerling *et al.*, 2013).

A positive control skin test (histamine) and negative control skin test (diluent) are necessary for accurate interpretation of SPT results (Bernstein *et al.*, 2008). The positive control should be positive to ensure that the test tools are applied correctly and to exclude negative results, possibly caused by interfering medications. The negative control excludes the existence of dermographism (an exaggerated wealing tendency when the skin is stroked) (Taşkapan *et al.*, 2006) which, when present, makes the tests difficult to interpret (Heinzerling *et al.*, 2013).

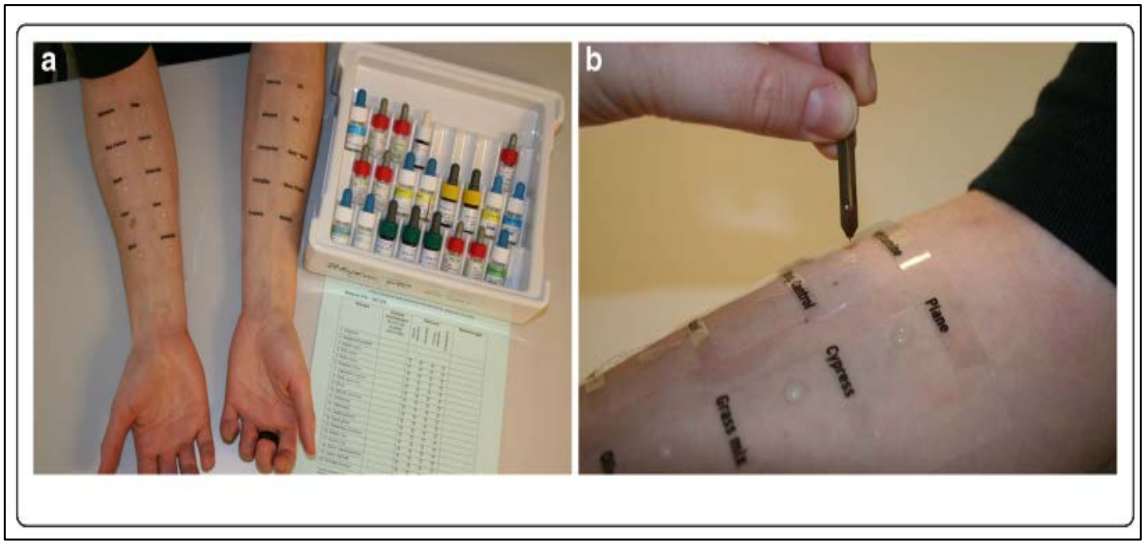

Figure 2.2 - SPT procedures.

(a) Preparation for skin prick test on the forearm.

(b) Prick testing with lancet through a drop of allergen extract.

Source: adapted from (Heinzerling *et al.*, 2013).

A positive SPT reflects the presence of mast cell-bound IgE specific to the tested allergen (Plebani, 2003). Although different thresholds are used to define a positive SPT reaction, the most widely accepted cut-off point is a wheal diameter of 3 mm or more than the negative control (P. A. Eigenmann *et al.*, 2013). Positive SPT reactions are likely to be smaller in infants and children younger than 2 years (Bernstein *et al.*, 2008). Therefore, results of SPT should be interpreted with caution in this age group (P. A. Eigenmann *et al.*, 2013).

SPT is highly specific and sensitive to diagnose inhalant allergens, 70-95% and 80-97%, respectively, but lower for food allergens, ranging from 30-90% and 20-60%, depending on the type of allergen and methods utilised (i.e. pricking with extracts vs. prick-to-prick (pricking the fresh food, commonly fresh fruits or vegetables, with the lancet and then pricking the skin, to test for sensitisation) techniques) (Heinzerling *et al.*, 2013).

There are a number of limitations related to skin testing. SPT cannot be performed in individuals who have extensive dermographism, urticaria or eczema (Bernstein *et al.*, 2008) and current use of some medications may affect the validity of SPT results. For example, antihistamine medications may considerably supress wheal and flare responses and thus

interfere with the accurate interpretation of the test results. Therefore, first and second generation antihistamine medications should be discontinued at least two to three days prior to SPT (Bernstein *et al.*, 2008). The reliability of skin testing is based on several factors including the skills of the examiner, the test instrument used and the potency and stability of test reagents (Bernstein *et al.*, 2008). Due to the above limitations, measurement of serum specific IgE can be used to identify sensitisation. I discuss this method in the next section.

2.5.4.2. Serum specific immunoglobulin E (s-IgE)

The measurement of s-IgE is an essential complementary test for the diagnosis of IgE mediated allergic reactions and is conducted mainly in individuals who have contraindications to perform SPT (Asero *et al.*, 2007). Testing of s-IgE can be performed for individuals at any age (P. A. Eigenmann *et al.*, 2013). Several commercially available immunoassays are used to detect s-IgE in patient's blood. The majority of these tests are based on radioallergosorbent tests (RAST) or enzyme-linked immunosorbent assays (ELISA) techniques that provide quantitative results (P. Eigenmann *et al.*, 2013). However, RAST technique is no longer used. A modified assay, CAP system fluorescent enzyme immunoassay (FEIA) (Pharmacia Diagnostics, Uppsala, Sweden), has the advantage of increasing the allergen-binding capacity compared to previous techniques and also quantitates the results as kilounits of allergen-specific IgE per litre (kUA/L) (Lee and Burks, 2006). These tests are more sensitive in detecting low levels of allergen-specific IgE (Gerez *et al.*, 2010). Multi-allergen IgE screening can measure IgE binding to a panel of allergens in a single test (Bernstein *et al.*, 2008). The multi-allergen IgE antibody screen is a qualitative test that evaluates a patient's serum for the presence of IgE antibodies specific to a mixture of common food or aeroallergens (Hamilton and Adkinson, 2003). These screening assays can decrease the need for multiple allergen specific IgE measurements in individuals with a low clinical probability of atopic disease.

A positive test is defined when s-IgE levels reach >0.1 kU/ml or >0.35 kU/ml. However, there are significant inter-assay variations, mainly due to methodological variations (P. A. Eigenmann *et al.*, 2013). The results of s-IgE measurement tests are not affected by the current use of medications. Therefore, discontinuation of medication is not required for performing the tests (Plebani, 2003). Another advantage of s-IgE tests is that there is no risk for systemic reaction. On the other hand, disadvantages of s-IgE assays include the

requirement for blood samples, delayed availability of the results (from hours to days) and high cost (Sicherer *et al.*, 2012).

A range of allergen components tests is currently available for use in clinical practice. These tests help differentiating between primary sensitisation and immunological cross-reactivity to proteins with similar protein structures (Borres *et al.*, 2011). For example, 13 peanut allergen components have been identified; five of them are clinically relevant. These components include Ara h 1, Ara h 2, Ara h 3, Ara h 8 and Ara h 9 (Asarnoj *et al.*, 2010).

The advantages and disadvantages of SPT and s-IgE for assessment of IgE antibodies are outlined in the following section.

2.5.4.3. Comparison between SPT and s-IgE

Since the discovery of IgE antibodies, controversy remains concerning the advantages and limitations of skin testing and blood testing for the assessment of IgE antibodies, with studies demonstrating both agreement and disagreement between these techniques (Dolen, 2001). Skin testing is the most commonly used method for determining IgE antibody activity in clinical practice (Heinzerling *et al.*, 2013). The main advantage of SPT compared to the measurement of s-IgE antibodies is that the results can be interpreted in a short time (within 15 to 20 minutes) after applying the allergen extract to the skin. Moreover, SPT can be used to assess sensitisation to less common allergens, such as fresh vegetables and fruits in which s-IgE antibodies measurements are not available. An additional advantage of SPT is that the results are apparent to doubtful patients as they can see the wheal and observe the reaction (Plebani, 2003). On the other hand, the measurement of s-IgE provides better quantitative results and hence it may be more beneficial for monitoring specific IgE levels over time (Gerez *et al.*, 2010). In addition, s-IgE assays lack of interference from antihistamine medications or extensive dermatitis. Therefore, specific IgE immunoassays may be preferable to SPT in the following conditions: (1) widespread skin disease such as severe dermographism and active dermatitis; (2) greater risk of anaphylaxis from SPT; (3) current use of medications that interfere with skin reactivity such as antihistamines; and (4) uncooperative individuals (Bernstein *et al.*, 2008).

In the previous sections, I described in detail the definition and mechanism for development of food sensitisation along with clinical methods used for the assessment of food

sensitisation. In the next sections, I discuss the key epidemiological issues related to food sensitisation

2.6. Key issues related to food sensitisation and its association with allergic disease and lung function

2.6.1. Natural history of food sensitisation

Food sensitisation is regarded as a condition that generally develops early in life (Kulig, 1999; Roberts *et al.*, 2012; Nissen *et al.*, 2013), but it is not a static phenomenon. Rather, it is a dynamic process often characterised by a sequence of new sensitisation and remission events that occurs mainly during childhood (Roberts *et al.*, 2012; Depner *et al.*, 2013). Studies examining the time course of allergic sensitisation to food and inhalant allergens have shown that food sensitisation develops before respiratory sensitisation. Food sensitisation are often outgrown in later life during which there is an increase in the prevalence of respiratory sensitisation (Valenta *et al.*, 2015).

The presence of food sensitisation has a major influences on the development and course of common atopic disorders such as food allergy (Sicherer and Sampson, 2009; Schnabel *et al.*, 2010), eczema (Bergmann *et al.*, 1994), asthma (Michael Kulig *et al.*, 1998; Nickel *et al.*, 2002), and allergic rhinitis (Kulig *et al.*, 2000). Atopic sensitisation is considered to be fundamental in the pathogenesis of such allergic diseases. Longitudinal studies are able to examine the natural history of food sensitisation over time. Understanding the natural course of sensitisation to common food allergens has the potential to identify causal factors associated with the development or loss of sensitisation and can identify specific sensitisation phenotypes that better predict the development and prognosis of clinical allergic conditions thereby, enhancing our understanding regarding the relationship between sensitisation and clinical allergic disease. In addition, such studies provide a rich resource of data on the incidence and prevalence of the different phenotypes, which are important for examining temporal trends across time and populations. Progress in our understanding of the natural history of food sensitisation is dependent on the availability of evidence from such studies for the development of more effective treatment options and ultimately, preventative strategies that will alleviate the escalating disease burden associated with such allergic reactions.

Therefore, I describe the current evidence on the prevalence of food sensitisation as well as up to date knowledge on the natural history of food sensitisation from longitudinal studies in the next section.

2.6.1.1. Prevalence of food sensitisation

Information related to the prevalence and distribution of sensitisation to food allergens is limited. Reports related to the prevalence of food sensitisation have generally relied on data from cross-sectional studies. Several systematic reviews of the literature have been conducted to assess the prevalence of food sensitisation (Rona *et al.*, 2007; Zuidmeer *et al.*, 2008; Nwaru, Hickstein, Panesar, Muraro, *et al.*, 2014; Nwaru, Hickstein, Panesar, Roberts, *et al.*, 2014). Results from these reviews are heterogeneous and the circumstances under which these studies have been conducted not often standardised. Some of this heterogeneity could be explained by differences in geographical region, genetic predisposition, the rate of exposure to allergens and methods used for sensitisation assessment. However, sensitisation to food allergens may be relatively common and appears to be increasing (Venter *et al.*, 2010), as is true for sensitisation to inhalant allergens (Jarvis *et al.*, 2005; Asarnoj *et al.*, 2008).

Recent European systematic reviews and meta-analyses on the prevalence of food allergy reported the overall prevalence of sensitisation to at least one food allergen was 10.1% and 2.7% as assessed by serum s-IgE and SPT, respectively. Additionally, a higher prevalence of positive s-IgE to food allergens was observed in children (<18 years) than adults (Nwaru, Hickstein, Panesar, Muraro, *et al.*, 2014). The pooled prevalence of sensitisation to cow's milk was 0.3% and 4.7% when assessed by SPT and serum s-IgE, respectively. The point prevalence of egg sensitisation was 0.8% for SPT positivity and 3.6% for s-IgE positivity. More commonly, the prevalence of sensitisation to peanut was 1.7% and 8.6% when assessed by SPT and serum s-IgE, respectively (Nwaru, Hickstein, Panesar, Roberts, *et al.*, 2014).

In the US, data from the National Health and Nutrition Examination Survey (NHANES) 2005–2006 (Liu *et al.*, 2010) showed that the estimated overall prevalence of food sensitisation, as assessed by measurement of serum s-IgE concentration, was 16.8%. The highest prevalence was observed in children aged 1 to 5 years in which 28.1% were sensitised, then declined gradually with age to reach 13% in adults aged ≥60 years. In 1 to 5 year old children, the most common food allergens were milk (22%) and egg (13.9%), while in older children (6 to 19 year) sensitisation to peanut was more prevalent (10.7%).

2.6.1.2. Longitudinal studies on the natural history of food sensitisation

A number of longitudinal studies have assessed the natural history of food sensitisation from early life (Bergmann *et al.*, 1994; Kulig *et al.*, 1999; Rhodes *et al.*, 2002; Dean *et al.*, 2007; Dubakiene *et al.*, 2012; Roberts *et al.*, 2012; Nissen *et al.*, 2013). The overall findings from these studies demonstrated that sensitisation to food allergens was mainly observed during infancy and early childhood, whereas sensitisation to aeroallergens was not prominent during this period and steadily increased in later ages. The natural course of food and aeroallergen sensitisation during the first six years of life has been examined in a German study (Kulig *et al.*, 1999). Results from this study showed that the prevalence of food sensitisation remained constant during the study period (around 10%) despite a decrease in the annual incidence rate of food sensitisation from 10% at 1 year to 3% at 6 years. On the other hand, the prevalence of aeroallergen sensitisation increased from 1.5% at 1 year to 26% at 6 years. A study from the UK showed the prevalence of food sensitisation was 2.8%, 3.9% and 3.7% at 1, 2 and 3 years, respectively. The equivalent figures for aeroallergen sensitisation was 1.3%, 6.4% and 10.7% (Dean *et al.*, 2007). However, differences in study design, study area and subjects have to be taken into consideration when comparing the results.

The most frequent food allergens in the first two years of life are hen's egg, cow's milk and to lesser extent peanut (Kulig *et al.*, 1999; Burks *et al.*, 2001; Dean *et al.*, 2007). On the contrary, the most common food allergens in adulthood are fish, shellfish, peanuts and tree nuts (Burks *et al.*, 2001). However, there are some variations in the type of allergenic foods in different countries mainly due to the exposure to food allergens (Burks *et al.*, 2001).

Elevated prevalence of sensitisation to food allergens in early life and aeroallergens later could be explained by the maturation of immune system and development of oral tolerance to food allergens, as well as an increased exposure to aeroallergens with increasing age. This is in accordance with the course of clinical allergic disease, in which eczema and food allergy are mainly diseases of early childhood while asthma and allergic rhinitis commonly develop in later childhood and adulthood (Bantz *et al.*, 2014).

The presence of IgE antibodies to specific food allergens varies from time to time and does not depend on age. The occurrence of transient sensitisation to food allergens is a common phenomenon in childhood (Kulig *et al.*, 1999; Rhodes *et al.*, 2002; Depner *et al.*, 2013). One

study reported that around 81% of children sensitised to hen's egg and 72% sensitised to cow's milk had transient sensitisation (Kulig *et al.*, 1999). Children with transient food sensitisation have lower risk of subsequent allergic disease compared to children with long lasting sensitisation (Michael Kulig *et al.*, 1998).

Although a number of longitudinal studies investigated the course of food sensitisation over time (Bergmann *et al.*, 1994; Kulig *et al.*, 1999; Rhodes *et al.*, 2002; Dean *et al.*, 2007; Dubakiene *et al.*, 2012; Roberts *et al.*, 2012; Nissen *et al.*, 2013), the majority were performed during early childhood. Only three studies have investigated this issue into late adolescence and young adulthood (Rhodes *et al.*, 2002; Roberts *et al.*, 2012; Nissen *et al.*, 2013). Out of these three studies, two were restricted by small sample sizes (n=100 and 276) and did not assess peanut sensitisation (Rhodes *et al.*, 2002; Nissen *et al.*, 2013). Peanut is an important allergen because peanut sensitisation is more likely to persist with age (Ho *et al.*, 2008) and is more often related to severe reactions and anaphylaxis (Burks *et al.*, 1999).

Given that there is a geographical difference in the prevalence of sensitisation to common food allergens, local assessment of sensitisation pattern is needed. (Bousquet *et al.*, 2007). Although a high prevalence of allergic diseases has been reported in Australia, no Australian study has investigated the longitudinal changes in sensitisation to food allergens.

The Australian longitudinal study (MACS) that I use in my doctoral work, followed children from birth up to 18 years and assessed the sensitisation to common food allergens (hen's egg, cow's milk and peanut) starting from the age of 6 months. These characteristics allowed for detailed examination of the natural course of food sensitisation from infancy to late adolescence. Therefore, due to all above issues, the following research question was examined (results are presented in **Chapter 4)**:

What is the natural history of skin prick test positivity to common food allergens (egg white, cow's milk and peanut) from 6 months to 18 years?

2.6.1.3. Potential factors that could modify the natural history of food sensitisation

There are several factors that could potentially influence the occurrence and the course of food sensitisation. These include genetic factors (Tsai *et al.*, 2009), environmental factors and the interaction between them. A detailed explanation of the role of these factors on food sensitisation is beyond the scope of this thesis. However, for further exploration of the natural history of food sensitisation, in the following section, I discuss the potential risk factors that have been proposed to influence the course of food sensitisation based on previous literature. These include sex (Roberts *et al.*, 2012), eczema (Gustafsson *et al.*, 2003) and sensitisation to aeroallergens (Roberts *et al.*, 2005).

Sex

Several clinical and epidemiologic studies have indicated sex differences in the prevalence of allergic diseases. Previous literature suggested that male sex was an important risk factor for development of sensitisation (Tariq *et al.*, 1998). In addition, males usually have a higher prevalence of sensitisation, especially polysensitisation (sensitisation to more than one allergen) than females (Pereira *et al.*, 2005; Dean *et al.*, 2007; Baatenburg de Jong *et al.*, 2011; Roberts *et al.*, 2012). Therefore, I investigated the following research question within this thesis:

Does sex modify the natural history of food sensitisation?

Eczema

Eczema is frequently related to atopic sensitisation particularly in young children (Leung *et al.*, 2004; Hill *et al.*, 2008) but not all children with eczema have evidence of sensitisation (Williams and Flohr, 2006). There is a growing body of evidence that eczema can induce atopic sensitisation through different mechanisms. Atopic eczema is associated with dry and damaged skin that facilitates the absorption of allergens and induction of sensitisation (Sator *et al.*, 2003). Another proposed mechanism that eczema is likely to

stimulate atopic sensitisation is through the impairment of skin and gut barrier function. In animal models, it has been shown that epicutaneous exposure to peanut and egg proteins following the removal of the outer layer of epidermis (stratum corneum) stimulates sensitisation (Heratizadeh *et al.*, 2011). Several studies have repeatedly shown that mutations in filaggrin gene are associated with impaired skin barrier function and increased risk of sensitisation and allergic disease (van den Oord and Sheikh, 2009).

The association between food sensitisation and eczema has been investigated in several epidemiological studies. Data from previous studies has demonstrated that sensitisation, particularly to hen's egg in infancy, was associated with presence of eczema in childhood (Hill *et al.*, 2000). Additionally, the proportion of infants sensitised to food allergens, mainly to cow's milk and hen's egg, was increased with the severity and duration of eczema in infants (Bergmann *et al.*, 1994; Hill *et al.*, 2007). Several studies have evaluated the course of sensitisation to food allergens in children with eczema (Gustafsson *et al.*, 2003; Hon *et al.*, 2008). The majority of children with early onset eczema had sensitisation to at least one food allergen (Gustafsson *et al.*, 2003; Hill *et al.*, 2008). This evidence raises the potential for eczema to modify the natural history of food sensitisation. For that reason, the following research question was addressed in this thesis:

Does early life eczema modify the natural history of food sensitisation?

Aeroallergen sensitisation

Most food sensitised individuals have sensitisation to aeroallergens (Dean *et al.*, 2007). Co-sensitisation may be related to the underlying atopic potential of the individual and can occur to allergens found in the same environment. A large population-based study investigated the prevalence and relationship between a large panel of food and aeroallergens skin prick test reactivity among 7 year old children (Roberts *et al.*, 2005). Results from this study demonstrated a strong association between sensitisation to allergens within biologically similar groups as well as between allergens from different groups. Another study showed that around 56% of children sensitised to cow's milk and 88% sensitised to soy were co-sensitised to aeroallergens (Baatenburg de Jong *et al.*, 2011). Therefore, in this thesis I investigated the following research question:

Does aeroallergen sensitisation at 6 months modify the natural history of food sensitisation?

All the above research questions related to the natural history of food sensitisation are presented in the results **Chapter 4** of this thesis. In the following sections of my literature review, I discuss the current evidence related to the associations between food sensitisation and allergic diseases and lung function.

2.6.2. Association between food sensitisation and food allergy

Food sensitisation is one of the first steps in the defining the pathogenesis of food allergy (Berin, 2015; Valenta *et al.*, 2015). Detection of food specific IgE antibodies is considered as an indirect marker for the presence of food allergy. Additionally, sensitisation to more than one food allergen is frequently observed in symptomatic individuals (Dubakiene *et al.*, 2012). However, sensitisation alone is not enough for the diagnosis of clinical food allergy and the presence of clinical symptoms after ingestion of the food is required (Sicherer and Sampson, 2010). In a longitudinal population-based cohort, Schnabel et al. (2010) showed that early onset (at the age of 2 years) food sensitisation was a stronger risk for doctor diagnosed food allergy at the age of 6 years (OR= 4.7; 95% CI 2.0–11.2) (Schnabel *et al.*, 2010).

Even though food sensitisation is frequently an early life event, it has been proposed that exposure to the appropriate dose of food allergens during this critical period is important for proper immune response to foods. A number of studies have suggested that delayed weaning patterns influence the increased prevalence of peanut allergy (Du Toit *et al.*, 2008; Fox *et al.*, 2009). Likewise, there is evidence that delayed introduction of cereals is associated with a higher risk of wheat allergy (Poole *et al.*, 2006).

Although OFC is the gold standard test for diagnosis of food allergy, relatively few epidemiological studies have utilised this measure for defining food allergy (as discussed in *Section 2.3.4*) (Osborne *et al.*, 2011; Sicherer, 2011). Conducting population-based studies using the gold standard method for diagnosis of food allergy is difficult due to the cost, risk to participants and issues with compliance (Mehl *et al.*, 2012). Therefore, 95% positive predictive values (PPVs) have been recognised as an alternate to OFC to minimise over

diagnosis of food allergy. Many epidemiological studies have depended on the detection of food specific IgE antibodies, either by SPT or serum s-IgE measurement, in diagnosing food allergy. Larger wheal sizes of skin prick testing or greater measured IgE levels have been shown to be associated with an increased likelihood that individuals are truly food allergic (Hill *et al.*, 2004; Celik-Bilgili *et al.*, 2005). In this thesis, I defined food allergy based on the SPT wheal size in addition to reported history of allergic reaction. Hence, in the following section, I discuss the predictive values of SPT and the levels of serum s-IgE antibodies in the diagnosis of IgE-mediated food allergy.

2.6.2.1. Predictive value of serum s-IgE levels and SPT in defining IgE-mediated food allergy

The levels of serum s-IgE cannot always reflect the presence of underlying allergic reaction on an individual basis (P. A. Eigenmann *et al.*, 2013). Undetectable serum food-specific IgE might be associated with clinical reactions in 10% to 25% of people (Sicherer and Sampson, 2010). A number of studies have identified the threshold for levels of specific IgE to egg, milk and peanut allergens, as assessed by ImmunoCAP, that are predictive of clinical allergic reaction (Sampson and Ho, 1997). The 95% PPV thresholds have been reported to vary between different study populations and across different age groups (Komata *et al.*, 2007). It has been shown that the diagnostic levels of IgE, which could predict clinical reactivity in children and adolescents who had food allergy with 95% predictive value, were 15 kUA/L for peanut, 6 kUA/L for egg and 32 kUA/L for milk (Sampson and Ho, 1997). Recently, an Australian population-based study (Peters *et al.*, 2013) found that peanut s-IgE levels of ≥34 kUA/L and egg sIgE levels of ≥1.7 kUA/L had 95% PPVs for challenge proven food allergy in infants.

In respect to SPT, different diagnostic cut-off values for SPT wheal sizes have been proposed. However, these cut-offs may be population specific and thus not necessarily applicable to each population. Generally, negative SPT reactions confirm the absence of IgE mediated allergy (with >90% negative predictive value), while a positive test does not necessarily confirm the diagnosis of food allergy (Sicherer and Sampson, 2010). A number of studies have assessed the predictive values of SPT wheals diameter of various food allergens in predicting the results of food challenge test (Roberts and Lack, 2005; Johannsen *et al.*, 2011; Peters *et al.*, 2013). The majority of these studies were conducted in hospital based

settings. The summary of the SPT wheal sizes for egg, milk and peanut food allergens along with their specificities, sensitivities, positive and negative predictive values is presented in **Table 2.1**. Overall, a wheal size of ≥5mm for egg white, ≥8mm for milk and peanut has high specificity for predicting positive results in a food challenge test.

Double-blind placebo-controlled challenge studies in children demonstrated that SPT has a PPV of 76% and 89% for clinical reactions to cow's milk and hen's egg, respectively (Heinzerling *et al.*, 2013). A study by Hill et al. (2004) investigated the association between SPT weal sizes to egg, cow milk, and peanut, and challenge outcomes. Results showed consistent associations between the wheal diameter of SPT to egg ≥7 mm, milk ≥8 mm and peanut ≥8 mm and observed adverse reaction on OFC. In children aged 2 years or younger, lower SPT wheal diameters were reported (≥5 mm for egg, ≥6 mm for milk and ≥4 mm for peanut). A more recent Australian population-based study (Peters *et al.*, 2013) showed that peanut SPT responses of ≥8 mm and egg SPT responses of ≥4 mm had 95% PPVs for challenge proven food allergy in infants.

Considering the PPV of SPT in the literature, I investigated the following question within this thesis (results are presented in **Chapter 4):**

Does food sensitisation at 1 or 2 years predict probable food allergy at 12 and 18 years?

Table 2.1 - Sensitivity, specificity, positive and negative predictive values of SPT that predict positive food challenge tests for egg, milk and peanut allergen.

Country (reference)	Study population Age of participants (sample size)	Food allergen	SPT weal size (mm)	Sensitivity	Specificity	PPV	NPV
Australia (Sporik, 2000)	Clinic based median age of 3 years (555)	Peanut	≥8 (>2 years)	-	100%	95%	-
			≥4 (<2 years)				
		Egg white	≥7 (>2 years)	-	100%	95%	-
			≥5 (<2 years)				
		Cow's milk	≥8 (>2 years)	-	100%	95%	-
			≥6 (<2 years)				
France (Rancé *et al.*, 2002)	Hospital based (363)	Peanut	≥3	100%	66.1%	73.3%	100%
			≥16	14.7%	100%	100%	55.2%
Italy (Monti, 2002)	Clinic based aged 1-19 months (107)	Egg white	≥3	87.5%	85.7%	92.6%	77%
			≥4	62.5%	91.4%	93.7%	54.2%
			≥5	18%	100%	100%	37.2%
UK (Roberts and Lack, 2005)	Hospital based (135)	Peanut	≥8	25.4%.	98.5%	94.4%	57.3%
Germany (Verstege *et al.*, 2005)	Clinic based (385)	Egg white	11.2 (<1 year)	-	-	95%	-
			13.3 (≥1 year)				
		Cow's milk	9.7 (<1 year)	-	-	95%	-

			15.7 (≥1 year)				
Australia (Nolan *et al.*, 2007)	Clinic based (51)	Peanut	≥7	-	97%	93%	-
Italy (Mauro, 2007)	Clinic based (104)	Cow's milk (fresh milk)	>8	-	-	83.3%	-
Australia (Wainstein *et al.*, 2007)	Clinic based (84)	Peanut	≥8	75%	66.7%	78%	62.9%
			≥15	5.8%	100%	100%	40.2%
Australia (Johannsen *et al.*, 2011)	Clinic based Children aged <5 years (49)	Peanut	>7	83%	84%	83%	84%
Australia (Peters *et al.*, 2013)	Population-based Infants aged 11 – 15 months (5276)	Peanut	≥8	54%	98%	-	80%
		Egg white	≥4	46 %	93%	-	44 %

2.6.3. Associations between food sensitisation and other allergic disease

Several studies have demonstrated that early sensitisation to food and inhalant allergens could be regarded as a risk factor for the development of subsequent allergic disease (Sigurs *et al.*, 1994; Zeiger and Heller, 1995; Nickel *et al.*, 1997). Allergic sensitisation is frequently asymptomatic but sensitised children are more prone to develop disease in later years (Hattevig *et al.*, 1987; Zeiger and Heller, 1995). Early identification of children at risk is crucial for initiating primary and secondary preventive measures. It is also of interest to determine risk groups in which various preventive strategies can be evaluated in interventional studies. In this section, I describe the current evidence related to food sensitisation as a predictor for the development of symptomatic allergic diseases.

2.6.3.1. Atopic march

Allergic diseases may manifest at different ages starting from early life and these conditions are interrelated. In infancy, the main atopic symptoms are eczema, gastrointestinal symptoms and recurrent wheezing, whereas bronchial asthma and allergic rhinitis are the main problems later in childhood. Adverse reactions to foods, mainly cow's milk proteins, are most common in the first year of life, whereas allergy to inhalant allergens mostly occurs in later childhood.(Halken, 2003).

There is a growing body of evidence, especially from longitudinal studies, that the clinical manifestation of atopy as well as IgE antibodies against food and aeroallergen show a systematic sequence of events. The progression of atopic manifestations from eczema and food allergy in early life to asthma and allergic rhinitis is known as "atopic march" (**Figure 2.3**) (Spergel, 2010). The prominent characteristic of the clinical signs is that some features become more obvious with time, whereas others diminish or disappear completely. Detectable food antibodies against food allergens, particularly egg and milk, commonly precede or accompany early allergic symptoms and signs. Similarly, sensitisation to aeroallergens, first to indoor allergens then to outdoor allergens, precedes the clinical manifestation of allergic respiratory diseases (Kulig *et al.*, 1999).

Although various birth cohort studies describe this phenomenon, the mechanism underlying this progression is not fully understood. It is also not yet known whether the progression from

one allergic outcome to another is causal or a result of shared environment and/or shared genetics (Burgess *et al.*, 2009).

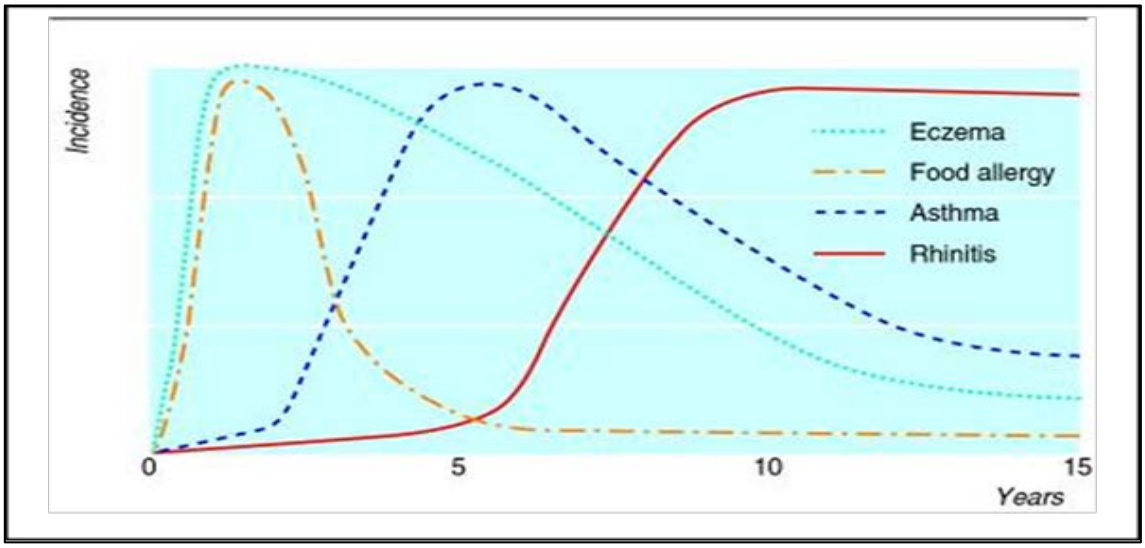

Figure 2.3 - Progression of atopic manifestations by age (atopic march).

Source: adapted from (Barnetson and Rogers, 2002).

Role of food sensitisation and allergy in the atopic march

Eczema and food allergy frequently co-exist, mostly in those with early onset, severe and persistent atopic eczema. Food allergy is a known aggravating cause of eczema and the prevalence of IgE-mediated food allergy among children with eczema is about 35% (Eigenmann *et al.*, 1998). Whether children with IgE-mediated food allergy are at increased risk of developing subsequent other allergic manifestations, such as asthma and allergic rhinitis, is uncertain. Recently, it has been shown that challenge proven cow's milk allergy in infancy increased the risk of bronchial hyperresponsiveness and airway inflammation (as surrogate markers for asthma) at school age (Malmberg *et al.*, 2010). However, it is unclear whether the observed association is related to co-manifestation of other allergic conditions, such as eczema and allergic rhinitis, that predict asthma or is a consequence of cow's milk allergy itself.

The average age of sensitisation to various allergens varies substantially and this has an effect on which allergens are more likely to trigger the allergic march. Egg and milk allergies are among the very first allergies to appear, often before the age of 12 months (Kulig *et al.*,

1999). As a result, both egg and milk allergies have been examined closely for their possible roles in allergic march.

Several studies have demonstrated that sensitisation to food allergen is related to the expression of different atopic diseases and could be a risk factor for their development. Thus, early identification and quantification of specific allergic sensitisation, together with accurate assessment of the severity and stability of sensitisation, is an important step in early identification of children at high-risk of allergic disease. However, the question of whether early life food sensitisation, a primary step in food allergies, marches to other allergic disease is a controversial but important issue considering the observed rise in the prevalence of food allergy. To date, the evidence for the association between sensitisation to food allergens in early life and subsequent development of allergic disease including asthma, eczema and allergic rhinitis has not been systematically investigated and synthesised. Prospective birth cohorts are ideal design to answer this question as they provide an opportunity to investigate early life determinants as well as to assess the temporality. In this thesis, therefore, I performed a systematic review and meta-analysis to investigate the following question (results are presented in **Chapter 5):**

What is the current evidence for the association between food sensitisation in the first two years and subsequent development of asthma, eczema and allergic rhinitis?

2.6.3.2. Food sensitisation in relation to allergic airway diseases

Although sensitisation to aeroallergens has been shown to be associated with asthma and allergic rhinitis, the relationship between food specific IgE antibodies and these allergic airway conditions remains incompletely explored. A number of epidemiological studies have investigated the relationship between food sensitisation and respiratory allergies. Rhodes and colleagues studied 100 infants from atopic families during a 22 year period in the United Kingdom and found the major risk factor for adult asthma was sensitisation to foods in the first year (odds ratio, 10.7; 95% CI, 2.1 to 55) (Rhodes *et al.*, 2001). The pattern of sensitisation to food allergens in relation to allergic airway diseases was also been examined in 1,314 infants from Germany (Michael Kulig *et al.*, 1998). Results showed that persistent

sensitisation to food allergens increased risk for developing allergic rhinitis and asthma at 5 years by 3.4 folds and 5.5 folds, respectively, when compared to transient food sensitisation.

Sensitisation to hen's egg in early life has been proposed as a predictor for allergic respiratory disease during childhood (Hattevig *et al.*, 1987; Burr *et al.*, 1997; Nickel *et al.*, 1997; Arshad *et al.*, 2001; Kotaniemi-Syrjänen *et al.*, 2003). Additionally, in a population-based study of 1,218 children, symptomatic egg allergy (defined based on the presence of history of skin rash, respiratory or abdominal symptoms within four hours of ingestion of a particular food on two separate occasions) at the age of 2 years was associated with increased risk of asthma and allergic rhinitis at the age of 4 years (OR= 5.0; 95%CI 1.1 to 22.3) (Tariq *et al.*, 2000).

Eczema in early life should be considered when assessing the predictive role of food s-IgE antibodies on later development of allergic airway diseases. It has been shown that eczema is associated with increased risk of developing respiratory allergy. This risk is especially high in children with an early onset food sensitisation (Gustafsson *et al.*, 2000; A. Lowe *et al.*, 2007; Marenholz *et al.*, 2009).

Although a number of epidemiological studies have assessed the association between food sensitisation and subsequent asthma and/or allergic rhinitis up to 7 years (Burr *et al.*, 1997; Brockow *et al.*, 2009; Kjaer *et al.*, 2009), only a few cohorts have assessed these associations beyond this age (Rhodes *et al.*, 2001; Bekkers *et al.*, 2013; Garden *et al.*, 2013). However, concomitant early life eczema and/or wheeze have not been considered in most studies. In this thesis, I investigated the association between food sensitisation, with or without aeroallergen sensitisation, in the first two years of life and subsequent development of asthma and allergic rhinitis, whilst taking into account various confounding factors. These associations were examined within two birth cohort studies, the allergy high-risk (MACS) and the population-based (LISAplus). The following research question was examined (results are presented in **Chapter 6):**

Are there associations between early life food sensitisation and asthma and allergic rhinitis in later childhood and adolescence?

Are the associations between food sensitisation at 2 years and asthma and allergic rhinitis in later childhood consistent across the allergy high-risk study (MACS) and the population-based study (LISAplus)?

2.6.4. Association between food sensitisation and lung function

Lung development and growth commences during the gestational period and continues after birth, with almost 85% of alveoli developing postnatally. By the age of 2 years, the growth in alveolar numbers is almost complete. Further increases in lung volume occur up to the age of 20-25 years (Merkus *et al.*, 1996). Since lung growth and maturation is continuous from embryonic life to adolescence, factors that interfere with this process may affect lung function in later life and increase the risk of respiratory disease. It is therefore important to understand potential risk factors during this critical period of lung development.

Spirometry is the diagnostic test that is often used to assist with the diagnosis of obstructive and restrictive lung conditions (as discussed in *Section 2.3.1.2***)**. Spirometric indices that are commonly used include FVC, FEV_1 and the ratio between these two. FEV_1 is usually abnormal in obstructive diseases such as asthma and chronic obstructive pulmonary disease (Miller *et al.*, 2005).

Epidemiological studies have shown that sensitisation to aeroallergens in early life is associated with accelerated loss of lung function during childhood (Lowe *et al.*, 2004; Illi *et al.*, 2006). The mechanisms underlying the relationship between allergen sensitisation and lung function remain unclear. It has been suggested that allergen sensitisation could induce inflammatory processes that lead to airflow limitation (Gottlieb *et al.*, 1996). This inflammatory process includes release of mediators and cytokines from activated mast cells. The release of mediators contributes to the acute symptoms and signs related to allergic reactions, including vasodilatation, increased vascular permeability and bronchial smooth muscle contraction (Hamelmann *et al.*, 1999; Galli *et al.*, 2008). After repeated exposure to allergens, persistent inflammation occurs that may lead to long term structural and functional changes of affected tissues (Galli *et al.*, 2008).

Although several epidemiological studies have examined the relationship between aeroallergen sensitisation and lung function, the association between food sensitisation and subsequent lung function has not been evaluated in longitudinal studies. This is an important research question in the context of the recently observed rise in food allergies. Therefore, in this thesis, I examined the following questions (results are presented in **Chapter 7):**

Are there associations between early life food sensitisation and lung function measures in adolescence?

Are the associations between food sensitisation at 2 years and lung function measures at adolescence consistence across the high-risk MACS study and the population-based LISAplus study?

For further investigation of the association between food sensitisation and lung function, the role of asthma and early life aeroallergen sensitisation was explored.

2.6.4.1. The role of asthma in the association between food sensitisation and lung function

It has been proposed in previous studies that sensitisation to food allergens is associated with allergic respiratory diseases such as asthma (as discussed in *Section 2.6.3.2*) (Nickel *et al.*, 1997; Michael Kulig *et al.*, 1998; Brockow *et al.*, 2009). However, sensitisation alone does not independently predict the development of this allergic condition (Bousquet *et al.*, 2006).

The majority of asthma that originates in early life is associated with impaired lung function which may lead to persistent disease in adult life (Phelan *et al.*, 2002). Airway inflammation, airflow obstruction and bronchial hyperresponsiveness are characteristic phenotypic features of asthma. Clinically, airflow obstruction in asthma is not fully reversible, and many asthmatic subjects experience an accelerated and progressive loss of lung function over time. The concept of airway remodelling in asthma proposes that the alteration of the structure and function of main airway constituents (including epithelium, mucus glands, airway smooth muscle and blood vessels) might explain, at least in part, the progressive loss of lung function that is observed clinically in asthmatic individuals. Asthma in early childhood has been

shown to be associated with airway remodelling which may result in fixed airflow obstruction (Ulrik and Backer, 1999) and subsequently a decrease in lung function mainly in individuals with moderate to severe asthma (James *et al.*, 2005). Nevertheless, to what extent asthma could influence this decrease in allergen a sensitised individual is less obvious. In this thesis therefore, I examined the following question (results are presented in **Chapter 7):**

Does asthma mediate the association between food sensitisation in the first year of life and lung function measures at adolescence?

2.6.4.2. The role of early life aeroallergen sensitisation in the association between food sensitisation and lung function

Food sensitisation has been demonstrated to be associated with increasing risk for subsequent aeroallergens sensitisation (M Kulig *et al.*, 1998). A previous study found that a marked IgE response to hen's egg at the age of 12 months was predictive of subsequent sensitisation to inhalant allergens (Nickel *et al.*, 1997). Another study showed that sensitisation to food allergens during infancy was a predictor of sensitisation to inhalant allergens (M Kulig *et al.*, 1998).

The association between aeroallergen sensitisation and lung function deficit has been demonstrated in a number of studies (Omenaas *et al.*, 1996; Sunyer *et al.*, 2000; Langley *et al.*, 2003). A direct correlation between the numbers of allergens a child is sensitised to and the impaired lung function was observed. A study by Schwartz and Weiss (1995) showed that sensitisation to indoor allergens among asthmatic children aged 6 to 12 years was associated with decline in the level of FEV_1.

It is unclear whether the effect of food sensitisation on lung function is due to sensitisation to aeroallergens or not. Therefore, I examined the following research question within this thesis (results are presented in **Chapter 7):**

Does aeroallergen sensitisation mediate the association between food sensitisation in the first year of life and lung function measures at adolescence?

2.7. Summary

In summary, allergic disorders (other than food allergies) have increased over the last few decades, whilst food allergies have increased only in the recent past. The significance of an increase in food allergy may not only be restricted to the burden of food allergy but also to other allergic diseases, as food allergy appears very early in childhood and is considered to increase the risk for developing other allergic disorders. Food sensitisation is the preliminary step in the food allergy pathway and therefore, an important biomarker for the risk and associated burden of food allergy and other allergic diseases. However, there is a paucity of data on the consequences of food sensitisation.

The overall aims of my PhD are to understand the natural history of food sensitisation and to examine the associations between early life food sensitisation and subsequent development of probable food allergy, asthma, allergic rhinitis and lung function measures.

CHAPTER 3 - General Methods

3.1. Overview

My overall aims were to examine the changes in the prevalence of food sensitisation from infancy to adolescence and to investigate the consequences of early life food sensitisation. My hypotheses were that the prevalence of food sensitisation is comparable to that of other Westernised countries and early life food sensitisation is associated with increased risk of later food allergy, asthma, allergic rhinitis and reduced lung function. These questions were examined within the Melbourne Atopy Cohort Study (MACS), an Australian based longitudinal birth cohort study investigating the development of allergic disease in high-risk infants. The main advantage of utilising MACS for these analyses is the long period of follow-up that extends from infancy to adolescence, including frequent assessment of food sensitisation throughout the follow-up period. The MACS study methods are described in *Section 3.2.*

As MACS is a high-risk cohort recruited on the basis of a family history of allergic disease, the results generated may not be directly applicable to the general population. The consequences of food sensitisation were also therefore examined within the Influence of Life-style related factors on the development of the Immune System and Allergies in East and West Germany PLUS the influence of traffic emissions and genetics (LISAplus) study, a population-based longitudinal birth cohort study. The German based LISAplus study methods are described in *Section 3.3*.

3.2. The Melbourne Atopy Cohort Study (MACS)

3.2.1. Study overview

MACS is a longitudinal birth cohort study conducted in Melbourne, Australia. The MACS project began in 1989 as a randomised controlled trial (RCT) investigating the effect of three different infant formulas introduced after cessation of exclusive breastfeeding on the occurrence of allergic diseases. The participants were followed from birth up to 18 years. The initial protocol and execution of this study was undertaken by Dr David Hill, Dr Cliff Hosking and Dr John Thorburn. The 18-year follow-up has been directed and carried out by Prof Shyamali Dharmage.

3.2.2. Recruitment of participants

Pregnant women attending antenatal clinics affiliated to Mercy Hospital for Women, one of the main metropolitan birthing centres in Melbourne and a tertiary referral centre for the state of Victoria in Australia, were invited to participate if at least one parent or sibling of the unborn infants had a history of asthma and/or food allergy and/or hay fever and/or eczema.

Specific information related to the project was positioned within the outpatient antenatal clinics at the Mercy Hospital for Women. Additionally, posters and leaflets were distributed to obstetricians who worked within the Mercy Hospital and who were encouraged to forward this information to women whose infants may be eligible for the study. The study information concisely outlined the project aims (mainly to reduce the risk of allergic diseases by modification of infant diet) and the eligibility criteria (history of allergic diseases in first degree relatives). Expectant mothers were asked to contact the research staff if they were interested in enrolling their infants into this study. A total of 620 infants born between 24 March 1990 and 1 November 1994 were recruited.

3.2.3. Randomly allocated study formulas

Randomisation occurred prior to birth, assigning each infant to either: a standard cow's milk based formula (NAN^{TM},Nestle, Vevey Switzerland); a partially hydrolysed whey formula (NAN^{TM}HA); or a soy milk formula (ProSobee^{TM}, Mead Johnson, U.S.). An independent statistician created computer generated random allocation schedules containing coded and blinded allocations of formula. Both staff and parents were blind to the contents of the formula tins which were labelled at an independent location. As participants were enrolled in the study, they were issued with the next allocation schedule number. The trial was registered retrospectively with Australian and New Zealand Clinical Trials Registry (ACTRN 12609000734268).

The results presented in my thesis use MACS as an observational prospective birth cohort. Using data from randomised controlled trials to test additional hypotheses about the association between non-randomised exposures and outcomes determined during

long term follow-up is a well-established method. It is based on the testable assumption that the randomised intervention does not influence the associations of interest (Howard and Howard, 2012). A previous MACS publication showed that the randomisation status (infant formula allocation) was not associated with the outcome of interest up to 7 years of age (Lowe *et al.*, 2011), therefore MACS continues as an observational study. Despite this, the effect of an intervention formula (by intention to treat at baseline) was considered in statistical methods as a potential confounder in all analyses included in this thesis.

3.2.4. Data collection

The overview of collected MACS information, samples and tests in each follow-up is shown in **Figure 3.1**. The following section describes the data and clinical testing at each follow-up in more detail.

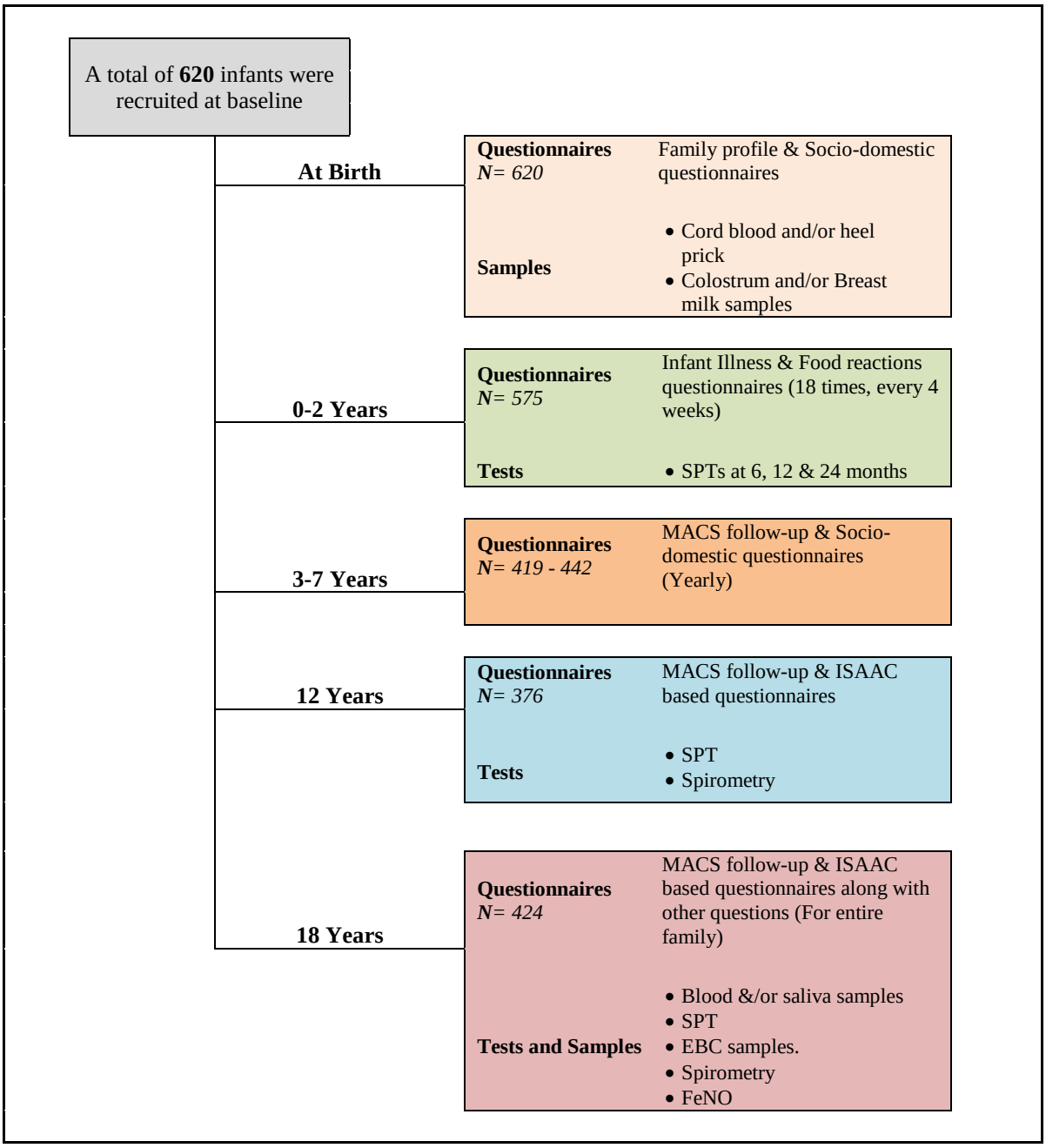

Figure 3.1 - Collected MACS questionnaires, samples and tests at each follow-up.

Abbreviations: **SPT**: Skin Prick Test; **EBC**: Exhaled breath condensate; **FeNO**: Expired Nitric Oxide.

3.2.4.1. Questionnaires

Data collection in the first two years of life

Baseline data were collected during initial recruitment when mothers consented to participate in the study. Two questionnaires were administered at that time including "Family data ante-natal profile" and "Initial Socio-Domestic Questionnaire". The "Family data ante-natal profile" (**Appendix 1**) questionnaire addressed information concerning parental age, marital status, birth place, smoking history, education level, occupation, place of residence, social security benefits, parental and older sibling history of allergic diseases (asthma, eczema, hay fever and food and drug allergies). The "Initial Socio-Domestic Questionnaire" (**Appendix 2**) collected information concerning environmental exposures at home such as floor coverings (presence of carpet in the living room, infant's bedroom and parents' bedroom), type of cooking and heating facilities (gas, electric or other) and the presence of animals in the home (gathered separately for cat, dog, birds, horse, Guinea pig and other).

Following birth, an allergy trained research nurse conducted telephone surveys with mothers every four weeks until the age of 15 months, and then at 18 months, and 2 years with a total of 18 surveys in the first two years of life. There were two questionnaires administered at each interview including "The food reaction questionnaire" and "The Infant Illness Questionnaire". The "Food reaction questionnaire" (**Appendix 3**) recorded information related to oral exposures for the infant including feeding behaviour (duration of breast feeding, the age of introduction and type of formula to which the infant was exposed, the age of introduction of 23 different solid foods and possible reactions to the foods for all liquid and solid food exposures). The "Infant Illness Questionnaire" (**Appendix 4**) documented symptoms related to the introduction of food (colicky abdominal pain, vomiting and diarrhoea) and development of allergic/atopic diseases (location of skin rash and treatment used for it, doctor diagnosis of asthma and duration of symptoms, blocked/runny nose), contact with health care professionals, use of any medications (such as antibiotics, antihistamines, nasal sprays, cough mixture and asthma medications). Immunisation

status was also recorded as well as current length/height and weight. Information on the home environment was also collected again during this follow-up.

Data collection from three to seven years of life

From 3 to 7 years of age, an annual telephone based follow-up survey was administrated for each child (**Appendix 5**). These surveys involved information concerning the diagnosis, frequency of symptoms, medical consultation and treatment of the allergy related childhood diseases including asthma, eczema, hay fever, urticaria and hives and food and drug reactions. Information on the occurrence and treatment for upper and lower respiratory tract infections, otitis media, gastroenteritis and other infectious diseases such as chicken pox, measles, whooping cough and mumps was also collected. Additionally, information on growth and development (height and weight), health care utilisation (GP consultations, hospitalisation and medications), childcare and school attendance and immunisation status was recorded. Moreover, information on the home environment was updated.

Data collection at 12-year follow-up

After the 7-year follow-up, a further follow-up was carried out and was termed the "12-year follow-up". A total of 376 children completed this follow-up and their ages ranged from 7 to 15 years. In this follow-up, two questionnaires were administered including the "MACS Follow-up survey", which has been administrated from 3 to 7 years, and the "ISAAC Questionnaire". The "MACS Follow-up survey" was similar to the previous follow-up survey. The "ISAAC Questionnaire" (**Appendix 6**) recorded comprehensive information in relation to the symptoms of asthma, rhinoconjunctivitis and atopic eczema. The collected information related to asthma symptoms involved the average number of wheezing attacks, the presence of sleep disturbance or limited speech due to wheezing, dry cough at night and how these symptoms affected the child's life. Additionally, information concerning the use of asthma medications, medical consultation and emergency department visit due to asthma was also collected. The gathered information related to rhinoconjunctivitis included the presence and frequency of sneezing, runny or blocked nose which was not related to cold or flu, the occurrence of associated symptoms such as itchy-watery eyes and how these symptoms

affected the child's daily activities. The information related to eczema symptoms involved the presence, frequency and distribution of itchy rash and how this rash disturbed the child's sleeping.

Data collection at 18-year follow-up

The 18-year follow-up carried out between 2009 and 2012. A total of 424 children completed this follow-up and their ages ranged from 15 to 21 years. In the 18-year follow-up, the survey (**Appendix 7**) included more extensive information related to participants' allergies and other diseases, general health and activity, education level, smoking history and quality of life. Additionally, information concerning home environment and exposures was recorded. The questions related to allergic diseases were a follow-up of both the initial MACS questionnaire and the ISAAC questionnaire administrated at the 12-year follow-up. All immediate family members (biological parents and siblings) along with the originally enrolled child (proband) were invited to participate in this follow-up.

3.2.4.2. Samples and clinical testing

Along with the collected survey data, several specimens were collected during the follow-up period such as blood, cord blood, colostrum and breast milk samples. Additionally, some clinical testing was performed such as skin prick testing (SPT), exhaled nitric oxide and spirometry (**Figure 3.1**). In my thesis, I have used data from SPTs and spirometry, therefore detailed description of these two methods is provided in the following sections.

Skin Prick Testing

Skin Prick Testing was performed at 6, 12 and 24 months and then at 12 and at 18 years following a standard technique (Aas and Belin, 1973). A trained allergy nurse performed the test by placing a drop of each allergen extract on infants' back, or on the volar surface of the forearm in older children, then the skin area was pricked using a lancet. Allergens tested up to the 12-year follow-up were egg white, cow's milk, peanut, cat dander, *Dermatophagoides pteronyssinus* (HDM) and *Lolium perenne* (rye grass)

(Bayer, Spokane, WA, USA; ALK-Abello, Hollister-Stier). At 18 years, extra allergens were added to the existing battery: *Alternaria tenuis*, *Penicillium notatum*, *Hormodendrum cladosporiodes*, mixed grass, cashew and shrimp. A positive control (histamine acid phosphate 1mg/ml) was also used. However no negative saline control was done up to 12-year follow-up (see *Section 8.5.1.4*). The SPTs were read at 15-20 minutes using a ruler. The wheal size was measured directly in mm by calculating the mean length of longest wheal diameter and the diameter perpendicular to it. An average of these two measurements was calculated. The total number of participants with SPT data for each follow-up is presented in **Figure 3.2**.

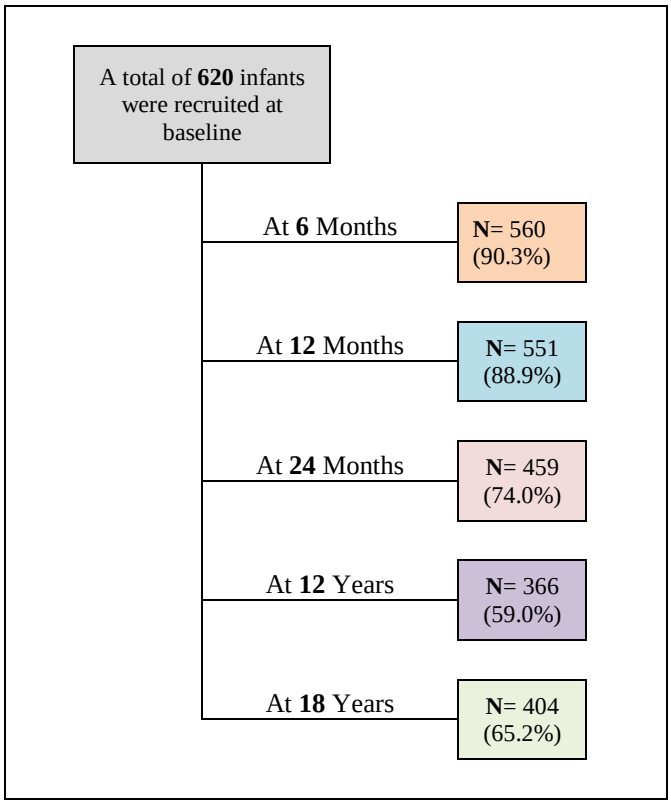

Figure 3.2 - Flow chart of collected MACS SPTs data along with their proportions from the original cohort.

The parents of the children were also skin prick tested to the same allergens within the first 2 years of life. Additionally, all immediate family members were skin prick tested at the 18-year follow-up. The protocol for SPT at 18 years is presented in **Appendix 8**.

Spirometry

The spirometry was conducted at both 12 and 18-year follow-ups following standardised techniques based on American Thoracic Society (ATS) guidelines (Crapo *et al.*, 1995) and European Respiratory Society (ERS) guidelines (Miller *et al.*, 2005). A three litre syringe was used for calibration, and biological controls were used.

At the 12-year follow-up, respiratory function assessment was performed by trained research nurses using the Spirocard system (SpiroCard™ PC Spirometer, QRS Diagnostic. Plymouth, Minnesota, USA). Repeatability of the reading was checked according to ATS 1994 criteria (Crapo *et al.*, 1995). The total number of participants with spirometric data at the 12-year follow-up was 366.

At the 18-year follow-up, pre- and post-bronchodilator (Salbutamol) spirometry was conducted by trained respiratory scientists according to a standard protocol using the EasyOne™ Spirometer system (NDD Medical Technologies Inc, Andover MA). There was a two stage method of selection of tests for study inclusion. Initially, flow-volume loops were marked for inclusion/exclusion by respiratory scientists. Then each test was reviewed for flow-volume loop selection and inclusion/exclusion. ATS/ERS 2005 criteria were used for test acceptance. The spirometry protocol and the data collection sheet at the 18-year follow-up are shown in **Appendix 9**. Participants were asked to discontinue long acting bronchodilators 12 hours and short acting bronchodilators 4 hours prior to the test. The height of participants was recorded at the time of testing, without shoes to the nearest 0.1cm.

The total number of participants with spirometric data at the 18-year follow-up was 411 pre-bronchodilator and 401 post-bronchodilator.

3.2.5. Ethics

The study was approved initially, up to 7 years of age, by the Human Research Ethics committee of the Mercy Maternity Hospital. At the 12-year follow-up, the study was additionally approved by the University of Melbourne Ethics Committee. At the

18-year follow-up, the study was approved by both the Royal Children's Hospital and The University of Melbourne Ethics Committees. Written informed consent was obtained from all mothers up to the 12-year follow-up and then from all the participants in the 18-year follow-up.

3.2.6. Use of MACS data within my thesis

Not all data from the questionnaires described above and outlined in **Appendices 1 to 7** are relevant to my thesis. Data from some questionnaire responses and SPT and spirometric results were used to create relevant variables in Stata statistical software to examine specific associations related to my research questions.

3.2.6.1. List of used questionnaires

- Family Data Ante-Natal Profile
  - Parental age
  - Parental education
  - Parental smoking history
  - Sibling data
- The Infant Illness Questionnaire
  - Sex
  - Age of child in weeks
  - Skin symptoms
  - Respiratory symptoms
- MACS Follow-up Questionnaire
  - Asthma
  - Hay fever/runny nose/rhinitis

- Eczema
- Adverse reactions due to food

- 18-year Follow-up questionnaire
  - Respiratory health
  - Nasal symptoms
  - Food allergies
  - Eczema and rash
  - Personal smoking

3.2.6.2. Data cleaning and variables generation

All data related to this thesis were explored for missing data and potential categorisation.

Cleaning SPT results data

Skin prick test results were entered into the dataset as a numerical variable (beginning from 0) indicating the observed wheal size of the reaction to specific allergen as described in *Section 3.5.4.1*.

For the purpose of this thesis, I generated a new variable for each tested food and aeroallergen at each time point as binary negative (if the wheal size < 2mm at 6, 12 and 24 months or < 3mm at 12 and 18-year follow-ups) and positive (if the wheal size more than or equal to these cut-off points). Then, I generated other binary variables related to sensitisation to any food (when the participant had at least one positive SPT to any tested food allergen) and any aeroallergen (when the participant had at least one positive SPT to any tested aeroallergen) at each tested time point (i.e. 6, 12 and 24 months, 12 and 18 years).

Additional variables were generated from the SPT results at 6, 12 and 24 months named "sensitisation to food only", "sensitisation to aero only" and "co-sensitisation to both

food and aeroallergen". "Sensitisation to food only" was defined when the participant had a positive SPT to any food allergen and a negative SPT to all tested aeroallergens at the time of measurement. "Sensitisation to aero only" was defined when the participant had a positive SPT to any aeroallergen and negative SPT to all tested food allergens at a specific time point. "Co-sensitisation to both food and aeroallergen" was defined when the participant had a positive SPT to at least one tested food and one tested aeroallergen at the time of measurement.

Furthermore, "mono" and "poly" food sensitisation variables were generated by using SPT results at 6, 12 and 24 months and were defined as follows: "mono food sensitisation" when the participant had a positive SPT to only one tested food allergen at a given time point and "poly food sensitisation" when the participant had a positive SPT to at least two tested food allergen at a given time point.

Cleaning food allergy questionnaires at 12 and 18 years and generation of "persistent sensitisation" and "probable food allergy" variables

Definitions of "persistent sensitisation" and "probable food allergy" were based on reported history of allergic reaction to a specific food allergen by the participant and the SPT wheal size to that food allergen as shown by SPT results at the 12-year and 18-year follow-ups. I cleaned participants' responses to "adverse reactions due to food" questionnaires at 12 years and their responses to "food and other allergies questionnaires" at 18 years to generate a "reported history of allergic reaction to food allergen" variable. This new variable was binary: "yes" (when the participant responded ≥1 to the following question at the 12-year follow-up "How many bouts of adverse reaction due to food in the past year" and determined the type of food allergen; or at the 18-year follow-up they responded "yes" to the following question "Have you ever had food allergies?" and also they indicated the type of food allergen) and "No" (when the participant's response to the previous questions were "0" and "no" at 12 and 18-year follow-ups, respectively). Then I generated the "Probable food allergy" and the "Persistent sensitisation" variables based on the wheal size of a specific food allergen and the reported history of food reaction. The definition of these two variables is described in *Section 3.4.*

Cleaning and generation of an eczema variable

For the purpose of this thesis, eczema was defined as the parental report of doctor diagnosed eczema or treatment of skin rash with topical steroid (excluding nappy or scalp areas) (Lowe *et al.*, 2007). In the MACS dataset, the eczema variable represents the age of the child (in weeks) when first eczema was reported by the parents. I generated new variables named "eczema in the first 6 months" (if the age of child when first eczema was reported was ≤ 26 weeks), "eczema in the first year" (if the age of child when first eczema was reported was ≤ 52 weeks) and "eczema in the first two years of life" (if the age of child when first eczema was reported was ≤ 104 weeks).

3.3. The LISAplus study

3.3.1. Study population

The LISAplus study is a population-based birth cohort study conducted in Germany. The participants were not preselected based on family history of allergic diseases. A total of 3,097 healthy, full term neonates were recruited from four German cities: Munich, Leipzig, Wesel and Bad Honnef between December 1997 and January 1999. Neonates were excluded from the study if they met at least one of the following criteria: (1) preterm birth (maturity <37 gestational weeks), (2) low birth weight (< 2500 g), (3) congenital malformation, (4) symptomatic neonatal infection, (5) antibiotic medication, (6) hospitalisation or intensive medical care during neonatal period. Moreover, newborns from mothers with immune related diseases, such as 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 also excluded (J Heinrich *et al.*, 2002). The participants have been followed from birth up to 15 years.

3.3.2. Data collection

The overview of collected LISAplus questionnaires and measures and/or samples in each follow-up is shown in **Figure 3.3**. The following section describes in details the data that have been included in this thesis.

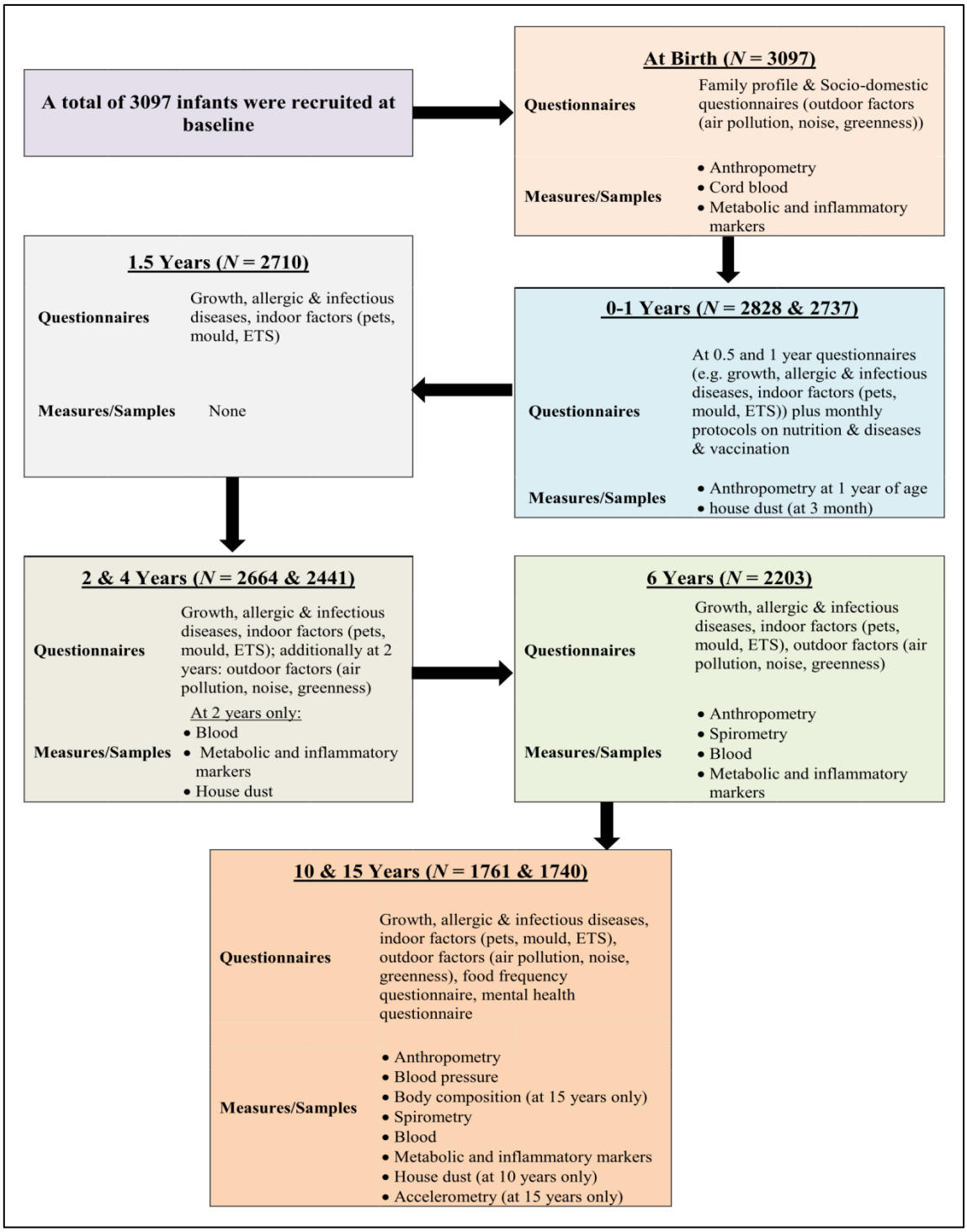

Figure 3.3 - Collected LISAplus questionnaires and measures/samples at each follow-up.

3.3.2.1. Questionnaires

Questionnaires on family history of atopy, parental education, health problems during pregnancy, smoking during pregnancy and exposure to environmental tobacco smoke during pregnancy at home and other lifestyle factors were completed by parents shortly after delivery. Additionally, repeated parental completed questionnaires, as shown in **Figure 3.3**, were collected at regular time intervals during the first 15 years (0.5, 1, 1.5, 2, 4, 6, 10 and 15 years of age).

3.3.2.2. Samples and clinical testing

Several specimens were collected during the follow-up period including blood, cord blood and sampling of house dust from the mothers' mattresses. Additionally, some specific clinical tests were undertaken such as spirometry. In this thesis, data from s-IgE and spirometry were involved, therefore detailed description of these two methods is provided in the following sections.

Measurement of serum specific IgE

Blood was collected at ages 2, 6, 10 and 15 years. Specific serum immunoglobulin E (s-IgE) concentrations were assayed by the CAP-RAST FEIA system (Pharmacia Diagnostics, Freiburg, Germany) according to the manufacturer's instructions. Screening tests were used to test allergic sensitisation against food (FX5: egg, cow milk, peanut, wheat, soybean and cod fish) and inhalant allergens (at 2 years: HX2 (mite), E1 (cat), MX1 (mold), RX1 (pollen); and at 6, 10 and 15 years: SX1 (*Dermatophagoides pteronyssinus*, cat, dog, rye, *timothy grass*, *Cladosporium herbarum*, birch and mugwort)). Children were assigned as s-IgE positive if their sIgE concentrations of any of the tested allergens exceeded 0.35kU/l, otherwise as s-IgE negative. For food sensitisation, the single allergens were tested separately if the s-IgE concentrations more than or equal 0.35kU/l (at 2 years only egg, cow milk and peanut).

Spirometry

Spirometry was performed at the 6, 10 and 15-year follow-ups before and after bronchodilation with salbutamol (at 15 years only). Participants were in a sitting position and wearing nose clips. Flow-volume curves were obtained using a pneumotachograph-type

spirometer (EasyOne Worldspirometer, ndd, Zurich, Switzerland), which was calibrated daily using a three litre calibration pump supplied by the manufacturer. The procedure, all measurements as well as the evaluation of the results were in line with ATS/ERS recommendations (Miller *et al.*, 2005).

In order to obtain optimal flow-volume curves, participants performed at least three and up to eight trials per test, under the guidance of trained examiners. In participants without contraindications, a bronchodilator response with ß-agonist was performed with a 200 µg (2 x 1 puff 100 µg) salbutamol dose. The participant was subsequently asked to take five slow and deep breaths from the spacer (Volumatic) with a breath-hold time of five to ten seconds to allow optimal particle deposition. The second spirometry test was performed 15 minutes later.

3.3.3. Ethics

The LISAplus study was approval by the local Ethics Committees (Bavarian Board of Physicians, University of Leipzig, and Board of Physicians of North-Rhine-Westphalia) and written parental consent was obtained.

3.3.4. Data cleaning and variables generation

The data cleaning and analyses related to LISAplus were performed by Marie Standl (results presented in **Chapter 6**) and Agnes Luzak (results presented in **Chapter 7**) with guidance from the LISAplus investigators, particularly Joachim Heinrich and Holger Schulz.

3.4. Summary of the definitions used within this thesis

Unless otherwise stated, the following definitions for the MACS and LISAplus data have been used throughout this thesis:

3.4.1. Definition of primary exposure variables (Sensitisation)

In ***MACS***, cleaning and generation of sensitisation variable has been described previously in *Section 3.2.6.2* in more detail.

Sensitisation or positive SPT

"Sensitisation or positive SPT" was defined if the SPT wheal size was ≥ 2 mm in the first two years or ≥ 3 mm at 12 and at 18 years. Although the most widely accepted cut-off point that describes the positive SPT reaction is a wheal diameter of ≥3 mm, 2 mm was used in this thesis to define sensitisation in children under the age of 2 years, as SPT reactions are likely to be smaller in children of this age. The 2 mm cut-off point has been selected to define the positive SPT in this age group previously (Bernstein *et al.*, 2008).

Food sensitisation

"Food sensitisation" was defined as a positive SPT to any tested food allergen (including egg white, cow's milk and peanut).

In ***LISAplus***, children were determined to be s-IgE positive if the s-IgE concentration of any of the tested allergens was more than or equal 0.35kU/l, otherwise as s-IgE negative. "Food sensitisation" was defined if food specific IgE concentrations exceeded 0.35kU/l.

3.4.2. Definition of the main outcomes

Persistent sensitisation

"Persistent sensitisation" was defined if SPT wheal size to a specific food allergen of ≥3 mm and <8 mm at 12 or 18 years without a reported history of food reaction to the same food.

Probable food allergy

Larger SPT wheal sizes indicate a greater likelihood of clinical food allergy (Hill *et al.*, 2004). Thus in this thesis, due to lack of challenge proven food allergy data in MACS, **"probable food allergy"** was defined as either (1) SPT wheal size to a specific food allergen ≥3 mm and <8 mm at 12 or 18 years with a reported history of allergic reaction to the same food; or (2) SPT wheal size to a specific food allergen ≥8 mm at 12 or 18 years (Sporik, 2000).

Current asthma and allergic rhinitis

Current asthma and allergic rhinitis were defined according to participants' responses to questionnaires at age 10 years in LISAplus and at 12 and 18 years in MACS.

In ***MACS***, **"current asthma"** was defined as one or more episodes of asthma and/or the use of any asthma medications in the last 12 months. **"Current allergic rhinitis"** was defined as one or more episodes of allergic rhinitis and/or the use of any treatment for allergic rhinitis in the last 12 months.

In ***LISAplus***, **"current asthma"** was defined as doctor diagnosis of asthma at the age of 10 years or intake of asthma medication during the past 12 months. **"Current allergic rhinitis"** was defined as doctor diagnosis of seasonal and/or perennial rhinitis at the age of 10 years.

Lung function outcomes

For each lung function measure including pre or post bronchodilator (BD) FEV_1, FVC or FEV_1/FVC ratios, a statistically significant change when compared with the reference group was considered as evidence for change in lung function.

3.4.3. Definition of potential confounders

Eczema in the first two years

In ***MACS***, **"eczema"** was defined as parental report of doctor diagnosis of eczema or treatment of rash with topical steroid (excluding nappy or scalp areas) in the first two years.

In ***LISAplus***, **"eczema"** was defined as doctor diagnosis of eczema in the past 6 months and/or rash in the past 6 months asked at the age of 2 years.

Early life wheeze

In ***MACS***, **"early life wheeze"** was defined if the response to the following question was >5 days "How many days of cough and/or chest rattle and/or wheeze has your child had in the past 4 weeks?"

In ***LISAplus***, **"early life wheeze"** was defined according to the response to the following question "In the past 6 months, has your child had whistling or wheezy sound of breathing in the chest?" asked during the follow-up at age 2 years in LISAplus.

Exclusive breastfeeding

Defined if the infant only received breast milk without any additional food or drink, not even water except for drops or syrups consisting of vitamins, minerals, or medicines (Geneva, 1991).

3.5. General Statistical Methods

This section describes the general statistical methods that have been used throughout this thesis for the investigation of the natural history of food sensitisation and examining the associations between atopic sensitisation in the first two years and probable food allergy, allergic airway diseases and lung function in adolescence. More detailed methods are presented within each chapter.

The natural history of food sensitisation was investigated by calculating the point prevalence of sensitisation to any food or aeroallergens and to specific food allergen including egg white, cow's milk and peanut at 6, 12, 24 months then at 12 and 18 years. The point prevalence for

sensitisation at each tested time point was calculated as the proportion of children with a positive SPT from the total number of analysed children at that time point.

The association between food sensitisation in early life and probable food allergy was examined using multiple logistic regressions to allow adjustment for confounding variables. The estimates were expressed as odds ratios with 95% confidence intervals (95% CI). A similar approach was used for examining the association between food sensitisation and subsequent allergic airway disease. For lung function outcomes measured on a continuous scale, linear regression models were used and the estimates were expressed as beta regression coefficients along with their 95% CI. All statistical tests were two sided with a *p* value of <0.05 considered as statistically significant.

Potential confounding variables are described in the methods section of the relevant chapters. These variables were included in the final model if there was evidence that they caused confounding, defined as 10% change in the odds ratio for the association of interest. Variables were also included if there was evidence in the published literature that these variables were important confounders of the association of interest.

Potential effect modification between each of the examined exposure variables and other variables was assessed using interaction terms in the corresponding models. Models with and without interactions were compared using likelihood ratio tests. The interaction term was retained in the final model if it had a p-value of <0.1 in that model.

Stata software (release13.0; Stata Corp, College Station, TX, USA) was used for all analyses of the MACS data and R version 3.1.0 was used for all analyses of the LISAplus data (Statistical Package, 2009) within this thesis.

CHAPTER 4 - The natural history of food sensitisation from birth to 18 years

4.1. Chapter introduction

This chapter includes a published paper in the *Pediatric Allergy and Immunology Journal* entitled "**Sensitisation to milk, egg and peanut from birth to 18 years: A longitudinal study of a cohort at risk of allergic disease**" (with data supplement). This paper describes the natural course of skin prick test positivity to three common food allergens; egg white, cow's milk and peanut and investigates if this course is modified by sex, eczema in the first six months of life and aeroallergen sensitisation at 6 months in MACS, the allergy high-risk cohort. Furthermore, the association between food sensitisation at 1 and 2 years and probable food allergy at 12 and 18 years was also examined.

The findings add to the body of evidence that supports high prevalence of food sensitisation during infancy. Additionally, they provide valuable information for clinicians to predict the risk of food allergy during adolescence in individuals who had early life sensitisation to common food allergen and at high-risk of allergy.

4.2. Publications

4.2.1. Abstracts

Alduraywish SA, Lodge CJ, Vicendese D, Lowe AJ, Erbas B, Matheson MC, Hopper J, Hill DJ, Axelrad C, Abramson MJ, Allen KJ, Dharmage SC. Food Sensitisation from Birth to 18 Years: A Longitudinal Analysis of a Cohort at Risk of Allergic Disease, Ascia 2014 poster abstracts. *Internal Medicine Journal* 2014;**44**: 1-29.

4.2.2. Main document and data supplement

Alduraywish SA, Lodge CJ, Vicendese D, Lowe AJ, Erbas B, Matheson MC, Hopper J, Hill DJ, Axelrad C, Abramson MJ, Allen KJ, Dharmage SC. Sensitization to milk, egg and peanut from birth to 18 years: A longitudinal study of a cohort at risk of allergic disease. *Pediatric Allergy and Immunology* 2016;**27**: 83-91.

ORIGINAL ARTICLE

Sensitization to milk, egg and peanut from birth to 18 years: A longitudinal study of a cohort at risk of allergic disease

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Keywords
birth cohort; egg; food sensitization; food allergy; milk; peanut

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Abstract

Background: Longitudinal data on the natural history of food sensitization beyond early childhood are scarce. We aimed to investigate the natural history of milk, egg and peanut sensitization from infancy to 18 years and assess whether early food sensitization predicted adolescent food allergy.

Methods: Sensitization to cow's milk, hen's egg and peanut was measured by skin prick testing at ages 6 months, 1, 2, 12 and 18 years in a high-risk allergy birth cohort (n = 620). Generalized additive models investigated interactions with sex, eczema and aeroallergen sensitization in infancy. Logistic regression assessed the relationships between early food sensitization and adolescent sensitization and probable food allergy up to 18 years.

Results: The prevalence of egg and peanut sensitization peaked at 12 months, while milk sensitization peaked at both 1 and 12 years. Boys with early eczema had the highest prevalences of milk and egg sensitization throughout follow-ups. However, neither sex nor eczema influenced the prevalence of peanut sensitization over time. New onset food sensitization beyond the age of 2 was observed in 7% of participants.Food sensitization at 12 months was associated with increased risk of adolescent food sensitization and adolescent probable food allergy, with sensitization to more than one food allergen had the strongest predictor.

Conclusions: Food sensitization prevalence is highest in infancy and declines after 12 months of age. Boys with early-life eczema have the highest prevalence of milk and egg sensitization. Food sensitization at 12 months can predict children at greater risk of adolescent sensitization and probable food allergy at 12 and 18 years.

During the past 10–20 years, it has been suggested that the global prevalence of food allergy has increased (1). The food allergy epidemic lags behind epidemics of asthma, eczema and allergic rhinitis (2). The reasons for this lag are unknown, but it may be due to aetiological differences (3).

Food sensitization usually begins in infancy, and is closely associated with, and may be a preliminary step for IgE-mediated food allergy (4), but the natural history of food sensitization has not been well characterized. Such data may provide information concerning the persistence of food sensitization, as well as the ability of food sensitization to predict subsequent food allergy. Furthermore, little is known about late-onset food sensitization (after 2 years of age) or the way that early-life eczema or aeroallergen sensitization may modify the persistence of food sensitization. Determining which infants are likely to have persistent food sensitization and/or allergy may guide both parents and clinicians. In addition, understanding the natural history of food sensitization is important from a public health perspective to appreciate the likely subsequent burden of food allergy over the life course.

Several studies have examined the patterns of skin reactivity to food allergens in childhood (5, 6). However, only three

studies have assessed food sensitization up to adolescence and young adulthood (7–9). Two of these (8, 9) were limited by small sample sizes (n = 100 and n = 276, respectively), and the other did not describe the changes in food sensitization from early infancy limiting ability to draw firm conclusions (7).

We examined the natural history of milk, egg and peanut sensitization using data from the Melbourne Atopic Cohort Study (MACS) which conducted frequent skin prick testing (SPTs) to these food allergens from 6 months to 18 years of age.

Methods

Study design and study sample

Melbourne Atopic Cohort Study is a prospective birth cohort from Melbourne, Australia, which recruited a total of 620 infants born between 1990 and 1994 initially started as a randomized controlled trial (RCT) investigating the effects of three infant formulas implemented on weaning on allergic disease In RCTs where the randomization factor (in this case infant formula allocation) does not affect the association between the exposures and the outcomes of interest, it is possible to perform observational analyses of the data (10). Previous published work from MACS using an intention-to-treat analysis showed no difference in allergic disease outcomes among the three groups (11). Details of recruitment and data collection have already been described (12, 13). Briefly, pregnant women attending antenatal clinics at the Mercy Hospital were invited to participate if at least one parent or sibling of the unborn infant had a history of asthma, food allergy, hay fever or eczema.

Data collection

Participants were followed from birth until 18 years of age. Baseline data were collected during pregnancy. After birth, telephone interviews were conducted by an allergy-trained nurse every 4 weeks until 15 months, at 18 months, at 2 years and then annually until 7 years of age. Further surveys were conducted at 12 and 18 years.

Skin prick tests

Skin Prick tests (SPTs) were conducted at 6 months, 1, 2, 12 and 18 years according to standard techniques (14). Allergens tested in all follow-ups included egg white, cow's milk, peanut, *Dermataphagoides pteronyssinus*, *Lolium perenne* and cat dander (Bayer, Spokane, WA, USA; ALK-Abello, Round Rock, Texas, USA; Hollister-Stier, Spokane, WA, USA). SPTs were conducted by an allergy nurse on infants' backs or flexor surface of forearm in older children and then pricked using a lancet. SPTs were read at 15–20 min (13).

Ethics

This study was approved by Human Research Ethics Committees of the Mercy Maternity Hospital, Royal Children's Hospital and University of Melbourne.

Definitions

Sensitization (positive SPT): average wheal diameter of ≥2 mm in children aged ≤2 years (15) and ≥3 mm in older children (8).

New sensitization: development of a positive SPT in participants who had negative results in all previous follow-ups.

Early sensitization: positive SPT in first 2 years.

Late sensitization: positive SPT after the age of 2 years.

Mono-food sensitization: sensitization to one tested food allergen only.

Poly-food sensitization: sensitization to ≥2 food allergens at any particular SPT point.

Adolescent food sensitization: SPT wheal size to a specific food allergen of ≥3 mm and <8 mm at 12 or 18 years without a known history of food reaction (16).

Adolescent probable food allergy: Larger SPT wheals sizes indicate a greater likelihood of clinical food allergy (17). Thus, probable food allergy was defined as either: (i) SPT wheal size to a specific food allergen ≥3 mm and <8 mm at 12 or 18 years with a reported history of allergic reaction to the same food, or (ii) SPT wheal size to a specific food allergen ≥8 mm at 12 or 18 years (16).

Eczema <2 years: doctor diagnosis or treatment of rash with topical steroid (excluding nappy or scalp areas) (18).

Statistical analysis

Multinomial logistic regression was used to estimate odds ratios for the association between early food sensitization and adolescent sensitization or probable food allergy at 12 and 18 years. Potential confounders considered were age, sex, eczema, aeroallergen sensitization and RCT allocation arm. Factors that changed the estimates by at least 10% were included in the final model. Generalized additive models (GAMs) were used to examine potential effect modification of the prevalence of sensitization over time by sex, eczema by 6 months and aeroallergen sensitization at 6 months. Stratified results were presented when the p value of 3-way interactions (time, sex and eczema × time, sex and aeroallergen sensitization separately) was significant ($p < 0.05$). Analyses were performed in STATA 12 (StataCorp, College Station TX, USA) and the MGCV package in R version 3.1.0.

Results

Study sample

Baseline demographics have been published elsewhere (13). In brief, parents of MACS probands were mainly Australian born (83% of fathers and 87% of mothers) and well educated (61% of fathers and 59% of mothers attended university). SPT results were available for 90.3%, 88.9%, 74.0%, 59.0% and 65.2% of the MACS cohort at 6 months, 1, 2, 12 and 18 years, respectively. The only differences in participants who did not attend the 12- and 18-year follow-ups were that their parents were younger, less educated and more likely to smoke (Table S1).

Sensitization prevalence

The prevalence of food sensitization was highest in infancy (26.3% at 12 months) and then declined to 18 years (8.4%) (Fig. 1). Conversely, the prevalence of inhalant allergen sensitization was lowest in infancy (18.5% at 12 months) and increased gradually in subsequent follow-ups (63.6% at 18 years). Almost all food-sensitized participants at 18 years (97.1%) were sensitized to at least one aeroallergen.

At 12 months, egg white was the most common food sensitization (19.2%) followed by peanut (14.7%) and then milk (8.7%). At 18 years, peanut was the most common sensitization (6.7%), while the prevalences of egg and milk sensitization were both below 5% (Fig. 2). The proportion of food-sensitized participants who had probable food allergy at 18 years were 77.8% (7/9) for egg, 50% (3/6) for milk and 40.7% (11/27) for peanut. 50% the participants with probable peanut allergy, 70% with probable egg allergy and all with probable milk allergy had been prescribed an adrenaline auto-injector.

Sex, eczema in the first 6 months and aeroallergen sensitization at 6 months modified the natural history of milk sensitization over time ($p < 0.001$ and $p = 0.02$ for eczema and aeroallergen sensitization, respectively) (Fig. 3a,b). Boys with eczema had the highest prevalence of milk sensitization at all follow-ups with two peaks at 1 and 12 years. In girls with eczema, there was a gradual decline in the prevalence of milk sensitization from 6 months to 18 years. Among non-eczematous children, with the exception of slightly higher prevalence in boys at 12 years, boys and girls exhibited similar patterns of milk sensitization (Fig. 3a).

In girls with aeroallergen sensitization at 6 months, a peak in the prevalence of milk sensitization at 12 months was observed, which was followed by a sharp decline in subsequent follow-ups. In boys with aeroallergen sensitization, there was nonlinear variation in milk sensitization over the follow-up period (Fig. 3b).

The natural history of egg sensitization was modified by sex and eczema ($p = 0.01$). Boys with eczema during the first 6 months were more likely to be sensitized to egg compared to girls with eczema. In non-eczematous children, boys and girls had a similar prevalence of sensitization up to 24 months, after which age a higher prevalence of egg sensitization was observed in boys (Fig. 3c).

The natural history of peanut sensitization was not modified by sex, eczema or aeroallergen sensitization at 6 months.

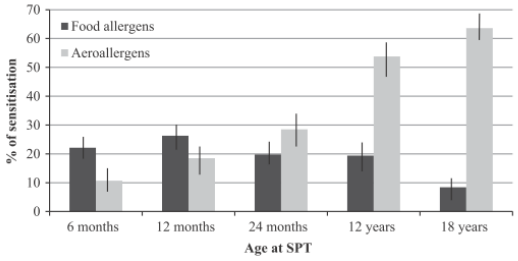


Figure 1 Prevalence of food (egg white, cow's milk and peanut) and aeroallergen (house dust mite, rye grass and cat dander) sensitization from 6 months up to 18 years in allergy high-risk cohort.

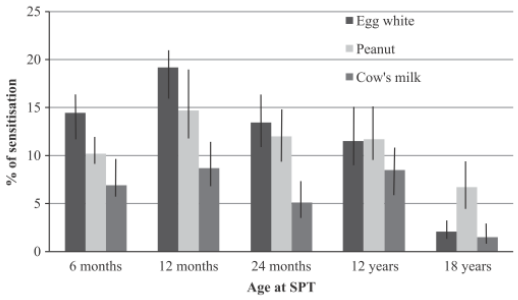


Figure 2 The prevalence of specific food allergen sensitization based on skin prick testing from 6 months up to 18 years.

Incidence and changes in food sensitization from 6 months to 18 years

Children (n = 357, 57.6% of the initial cohort) who underwent SPT at 6, 12 and 24 months in addition to 12 and/or 18 years were analysed to investigate how sensitization changed over time. Those included were similar except that those attending all follow-ups were more likely to have older and more educated parents, and food sensitization at 24 months (Table S2) than who did not.

Early- and late-onset food sensitization

The risk of developing new sensitization was greatest from birth to 6 months (21.8%, 95% CI 17.7% to 26.5%) and declined after this age. Late-onset food sensitization developed in 24 (6.7%) participants. Of those, 9 (37.5%), 7 (29.2%) and 2 (8.3%) developed mono-sensitization to peanut, egg white and cow's milk, respectively. Compared to those who developed sensitization during infancy, the late-onset group had smaller wheal sizes to any specific food allergen (3 mm vs. 5 mm) and fewer reported allergic reactions to food to which they were sensitized (8.3% vs. 25%).

Changes in food sensitization status

Between all follow-ups, resolution of sensitization was higher than development of sensitization (Fig. 4). Dynamic transition in food sensitization status was highest between 6 and 12 months. The rate of losing sensitization was 4.5% per year from 2 to 10 years and this increased to 9.3% per year from 12 to 18 years while the rate of developing new sensitization during the same periods was 1.2% per year and 0.7% per year, respectively.

A similar pattern of dynamic change was observed for each specific food allergen (milk, egg white, peanut) (Figures S1, S2 and S3).

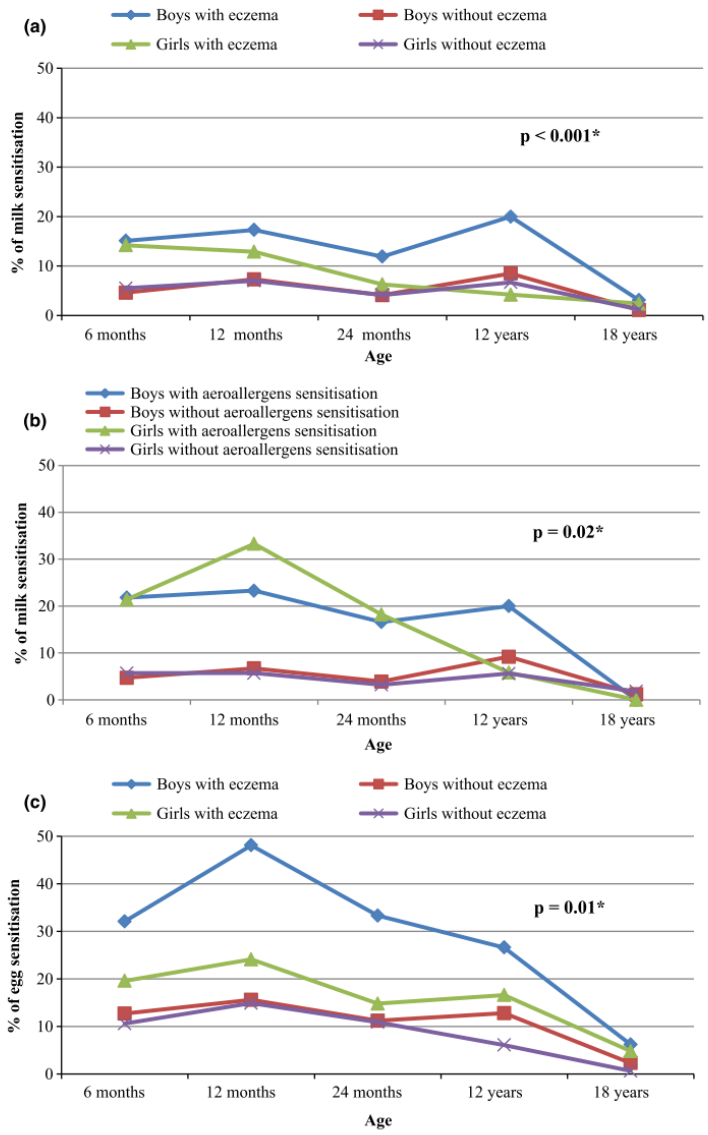


Figure 3 The prevalence of milk and egg sensitization stratified by sex and eczema or aeroallergen sensitization in a high-risk allergy cohort. (a) Milk sensitization stratified by sex and eczema in the first 6 months; (b) milk sensitization stratified by sex and aeroallergen sensitization at 6 months; and (c) egg sensitization stratified by sex and eczema in the first 6 months. *p values in figure 3 related to interaction of the prevalence of specific food allergen sensitization over time by sex, eczema by the age of 6 month or aeroallergen sensitization at 6 months.

Early mono- vs. poly-food sensitization and subsequent outcomes

At 6, 12 and 24 months, more than 50% of food-sensitized children were mono-food sensitized (Fig. 5a–c). Only a small proportion was poly-sensitized to all three food allergens (9.7%, 15.2% and 11%). Among children who had any food sensitization by 2 years (Fig. 5d), 51.3% were poly-sensitized, 17.7% of those to all three food allergens.

Although both mono- and poly-food sensitization at 12 months were associated with an increased risk of having adolescent sensitization or probable food allergy at 12 and 18 years, a stronger association was observed with poly-food sensitization (Table 1). After adjustment for early-life eczema and aeroallergen sensitization, odds ratios were attenuated, but mostly remained statistically significant. Eczema and aeroallergen sensitization did not modify these associations. A similar pattern was observed for sensitization at 24 months. We had

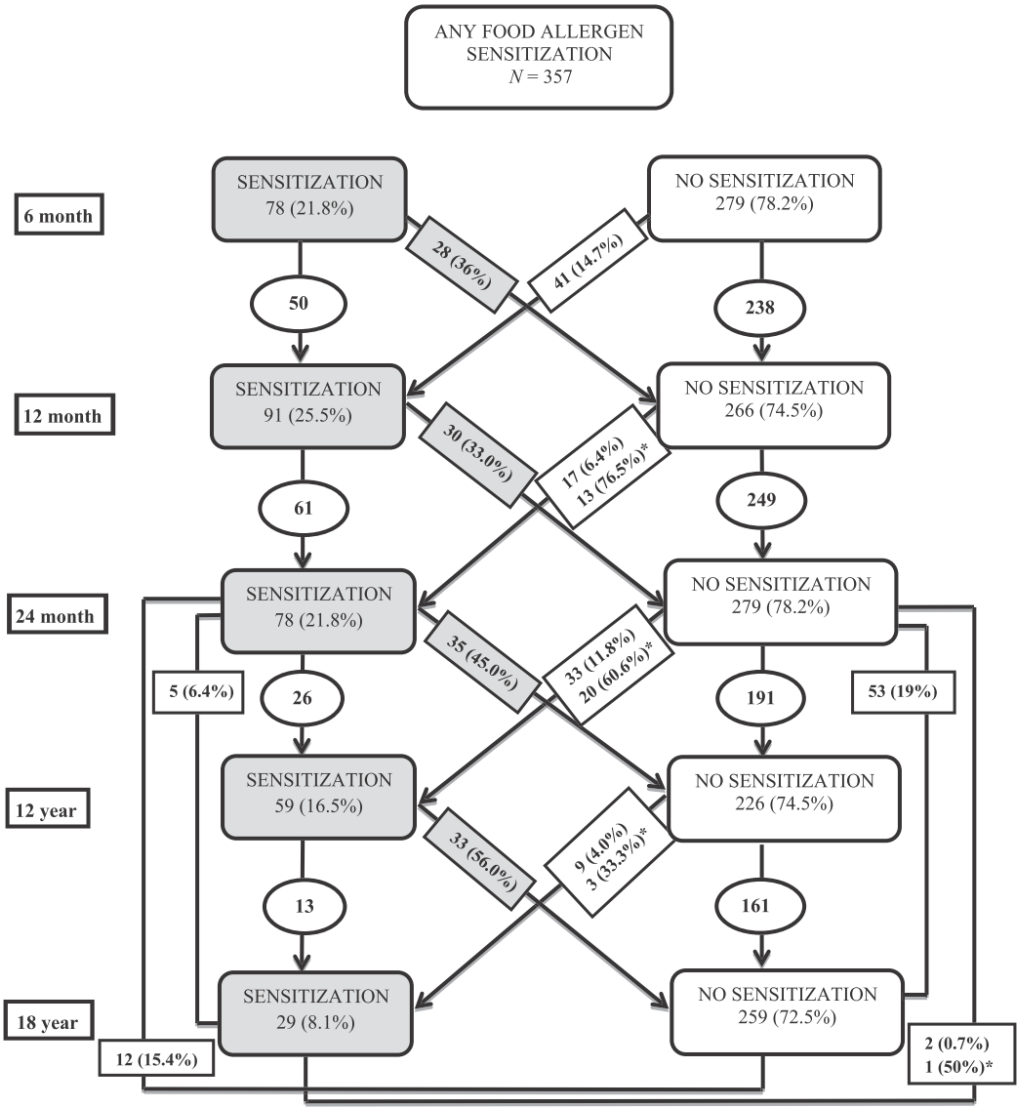
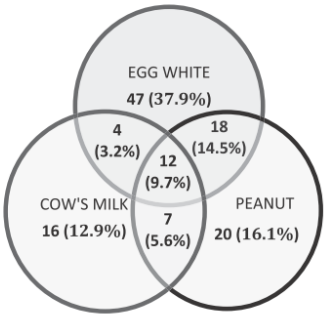


Figure 4 Changes in sensitization to any tested food allergen from 6 months to 18 years in high-risk allergy cohort. (*the number of participants who were never sensitized before and the proportion of those who were not sensitized in the previous follow-up).

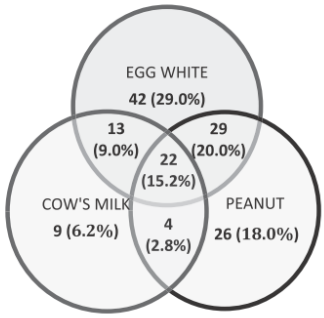
little power to conduct strata-specific analysis of individual food allergens and adolescent sensitization/probable food allergy (cell size n < 4).

Discussion

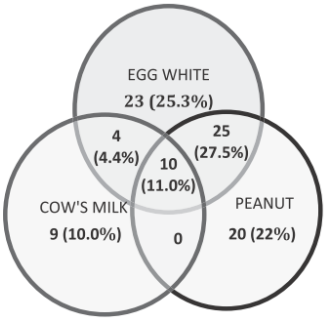
This is the first study to explore the natural history of sensitization patterns for egg, milk and peanut from 6 months to 18 years. We confirmed that food sensitization was more prevalent in early life, and the most common food allergens in the first 2 years were egg white and peanut. The transition rate between sensitization and non-sensitization status varied from infancy to adolescence. Food sensitization patterns in early life predicted persistence of sensitization and probable food allergy in adolescence. While some children developed new food sensitization after 24 months, this 'late'-onset sensitization



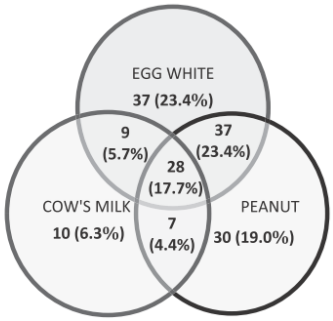
(a) Mono and poly food sensitization pattern at 6 months.
Total number of food-sensitized subjects at 6 months = 124 (22.14%)



(b) Mono and poly food sensitization pattern at 12 months.
Total number of food-sensitized subjects at 12 months = 145 (26.3%)



(c) Mono and poly food sensitization pattern at 24 months.
Total number of food-sensitized subjects at 24 months = 91 (19.8%)



(d) Mono and poly food sensitization pattern during the first 2 years of life.
Total number of food-sensitized subjects in the first 2 years = 158 (38.0%)

Figure 5 Mono- and poly-food sensitization pattern in a high-risk allergy cohort at (a) 6 months, (b) 12 months, (c) 24 months and (d) in the first 2 years.

appeared to be less clinically important. Sex, early-life eczema and/or aeroallergen sensitization modified the natural history of milk and egg, but not peanut sensitization.

Although the natural history of food sensitization has been described in early childhood (5, 6), only three studies have investigated this question into late adolescence (7–9). The Poole and Odense birth cohorts were restricted by small sample sizes (n = 100 and n = 276, respectively) and did not assess peanut sensitization. Peanut is an important allergen given that sensitization is less likely to resolve with age (19) and more often related to severe reactions and anaphylaxis (20). Although the Isle of Wight birth cohort had greater numbers (n = 1456) and included peanut sensitization, analysis was restricted to 543 children in the first 3 years (21).

A higher prevalence of food sensitization was observed in our cohort than in European birth cohorts (5). Around 19.2% of MACS children had egg sensitization at 12 months compared to 6% in the German Multicenter Allergy Study (22). Although sensitization to cow's milk is known as the most common food allergen during infancy (6), we found that egg white and peanut were the most common sensitized foods in infancy. Australia has one of the highest prevalences of reported allergic diseases (23). Results from an international study, Early Prevention of Asthma in Atopic Children, for children aged 12–24 months with a history of eczema, showed that Australia had one of the highest prevalences of egg and peanut sensitization (24). Additionally, findings from an Australian population-based study have shown that the

Table 1 The association between food sensitization at 12 months and adolescent sensitization/probable food allergy at 12 and 18 years

			Unadjusted		Adjusted*	
Food sensitization status at 12 Months:	Adolescent sensitization N (%)	Probable food allergy N (%)	Adolescent sensitization ORs (95% CI)	Probable food allergy ORs (95% CI)	Adolescent sensitization ORs (95% CI)	Probable food allergy ORs (95% CI)
At 12 years (N = 349)						
No sensitization N = 265 (75.9%)	30 (11.3)	6 (2.3)	Baseline			
Mono-sensitization N = 44 (12.6%)	10 (22.7)	4 (9.1)	2.5 (1.1–5.7)	5.1 (1.4–19.0)	1.9 (0.8–4.6)	3.5 (0.9–14)
Poly-sensitization N = 40 (11.5%)	8 (20)	11 (27.5)	2.9 (1.2–7.1)	19.9 (6.7–59.5)	2.01 (0.8–5.3)	12.2 (3.7–40.5)
At 18 years (N = 370)						
No sensitization N = 273 (73.8%)	5 (1.8)	3 (1.1)	Baseline			
Mono-sensitization N = 50 (13.5%)	6 (12)	2 (4)	7.6 (2.2–25.9)	4.2 (0.68–25.9)	6.2 (1.7–22.1)	3.4 (0.5–21.8)
Poly-sensitization N = 47 (12.7%)	5 (10.6)	11 (23.4)	8.5 (2.3–31.2)	31.3 (8.3–118.5)	5.6 (1.3–23.7)	21.01 (4.8–91.4)

*Adjusted for eczema in the first year and aeroallergen sensitization at 12 months.

prevalence of sensitization (SPT ≥3 mm) to egg and peanut allergens (11.8% and 6.4%, respectively) was higher than sensitization to cow's milk (5.6%) at the age of 12 months (25). These differences may be related to variations in genetic backgrounds, geographical and environmental exposures and infant feeding practice between different populations.

We did not observe any gender change in the prevalence of food sensitization at adolescence, as is seen in other allergies, such as asthma (26). However, the gender switch in case of food sensitization/allergy has not been fully explored previously. Interestingly, aeroallergen sensitization and eczema modified the natural history of milk and/or egg, but not peanut sensitization. These findings suggest that environment may play a significant role in changing the sensitization pattern over time. Nevertheless, lack of frequent assessment between the ages of 2 and 12 years in most participants makes it difficult to interpret the pattern of food sensitization during this period.

While food sensitization is common in early life, our results also showed that around 7% of participants developed new food sensitization after the age of 2 years. A birth cohort study in the UK also reported new food sensitization at the age of 11 years (8). This may be due to the lack of early exposure to specific food allergens, age-related maturation of the immune system, increasing exposure to chemicals that challenge the immune system or increasing responses to food allergens. However, we found this late-onset sensitization less likely to be clinically relevant.

Our results showed that food sensitization status changed throughout the follow-up period. Therefore, individual skin test reactivity at one time point does not necessarily reflect the sensitization pattern at all times. However, SPT results in early life could predict later sensitization and/or food allergy. We found that food sensitization at 12 months was associated with increased risk of sensitization and probable food allergy in later life, especially poly-food sensitization. Therefore, early food sensitization could be a useful long-term prognostic marker to advise parents with sensitized children who may be apprehensive given implications for quality of life (27). Nevertheless, these results should be interpreted cautiously as they were based on multiple comparisons and small numbers of individuals with food sensitization and food allergy in later follow-ups. This has resulted in wide confidence intervals in the statistical analyses. Studies with larger sample sizes are needed to confirm these associations.

The major strengths of MACS are the long period of follow-up, relatively large sample size and frequent assessment of sensitization to common food allergens (especially in the first 2 years of life). Loss to follow-up is a potential source of bias in any cohort study. However, this is unlikely to have influenced our results given the good participation rate (65%) at the 18-year follow-up. Furthermore, parental education and smoking status were the only baseline factors that influenced the participation rate. Although both lower educational level and smoking are proxy measures for lower socio-economic class, the relationship between socio-economic status and atopic sensitization is controversial. However, recent studies suggest a higher risk of atopic sensitization in individuals from lower socio-economic classes (28). Therefore, the results presented may be an underestimate due to the limited number of children from lower socio-economic groups included. Another limitation is that the study was restricted to children who had first-degree relatives with allergic diseases. However, a recent population-based study showed that 68.9% of participants have a first-degree relative with allergic disease (25). This suggests that our findings are likely to be generalizable to the Australian population. Nevertheless, the generalizability of findings to the children from low-risk communities is limited. Lack of food challenge testing in MACS is a substantial limitation since the diagnosis of food allergy cannot be made by SPT results alone (29).

In the current analysis, three food allergens were tested: egg, milk and peanut. Many other common food allergens (e.g. tree nuts, wheat, soya, fish) were not assessed in MACS follow-ups. This, in turn, may underestimate the actual figure of the prevalence of food sensitization. Although egg, milk and peanut are the most common food allergens in Westernized countries (30), the present analysis would be of limited value in populations with sensitization to other food allergens.

Conclusion

Food sensitization in this high-risk allergy cohort is prevalent in early life, and sensitization status changes throughout

childhood and adolescence. Although a small proportion of children may develop late-onset food sensitization, it is unlikely to be clinically relevant. Early-life SPT to common food allergens can provide valuable information for physicians in predicting the risk of sensitization and food allergy into adolescence in individuals at high risk of allergy.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. The dynamic changes of sensitization to cow's milk allergen from 6 months to 18 years.

Figure S2. The dynamic changes of sensitization to egg white allergen from 6 months to 18 years.

Figure S3. The dynamic changes of sensitization to peanut allergen from 6 months to 18 years.

Table S1. Comparison of baseline characteristics between following and missing participants at 12 years and at 18 years.

Table S2. Characteristics of analysed and non-analyzed participants.

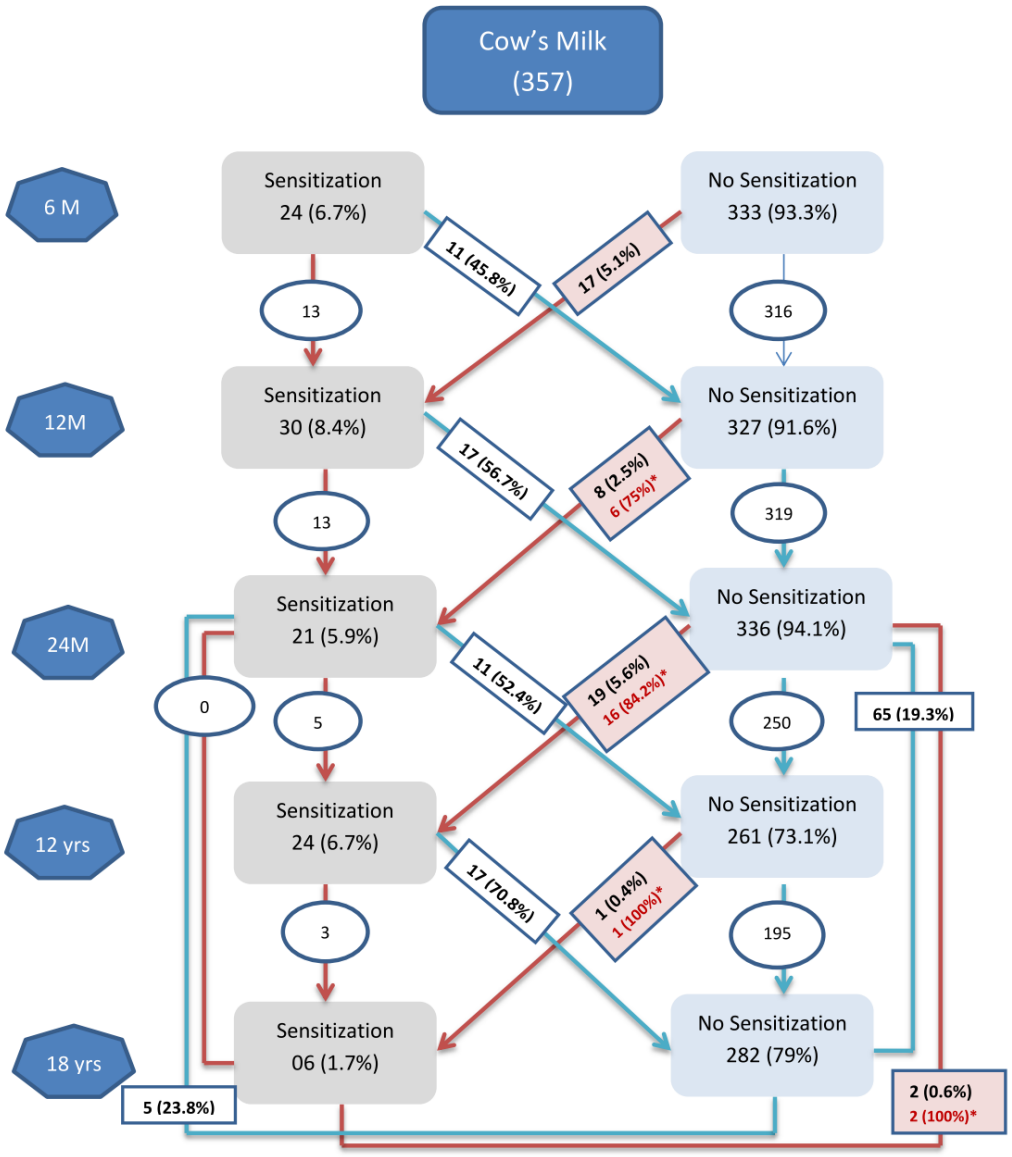

Figure S1: The dynamic changes of sensitization to cow's milk allergen from 6 months to 18 years. *(*) The number of participants who never been sensitized in previous follow-ups and their proportion from those who developed sensitization.*

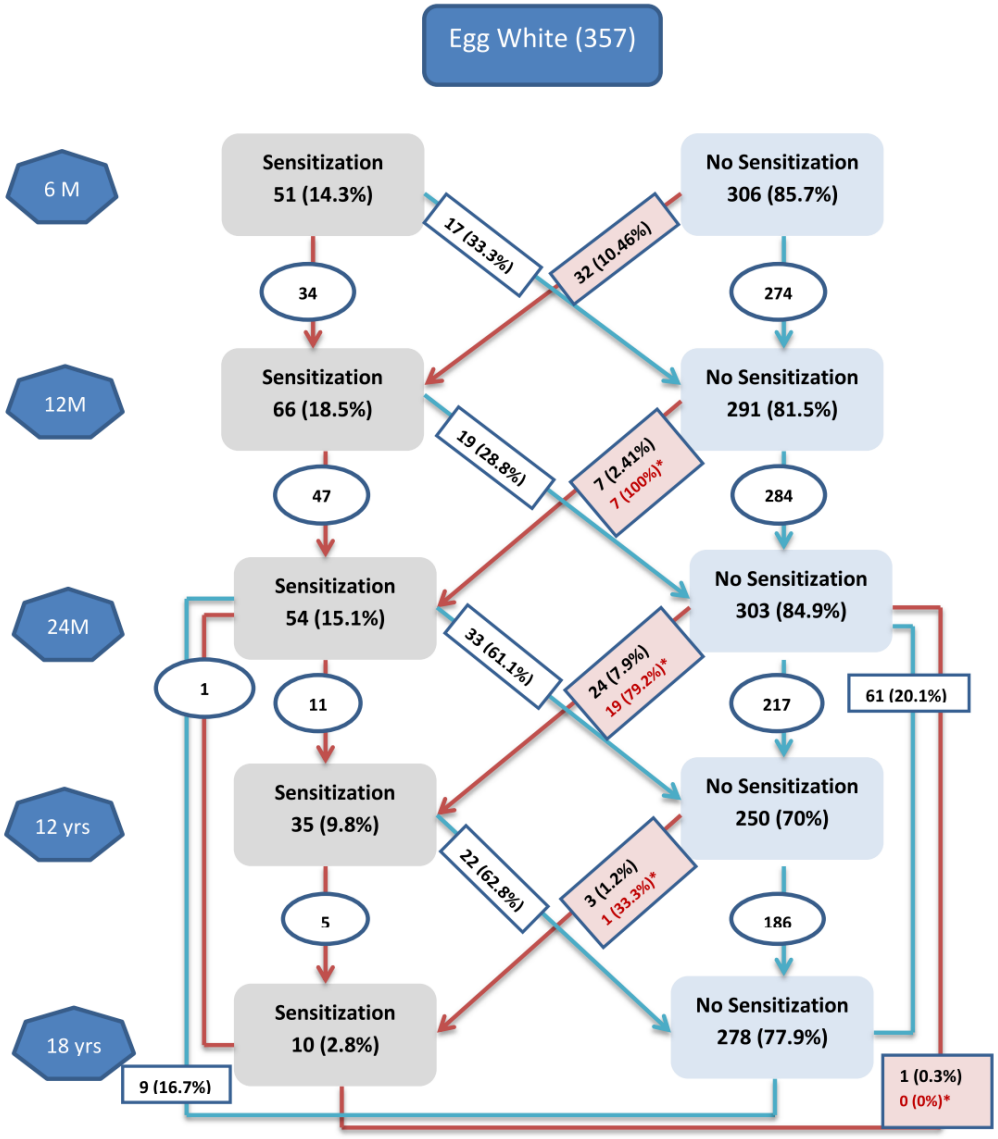

Figure S2: The dynamic changes of sensitization to egg white allergen from 6 months to 18 years. *(*) The number of participants who never been sensitized in previous follow-ups and their proportion from those who developed sensitization.*

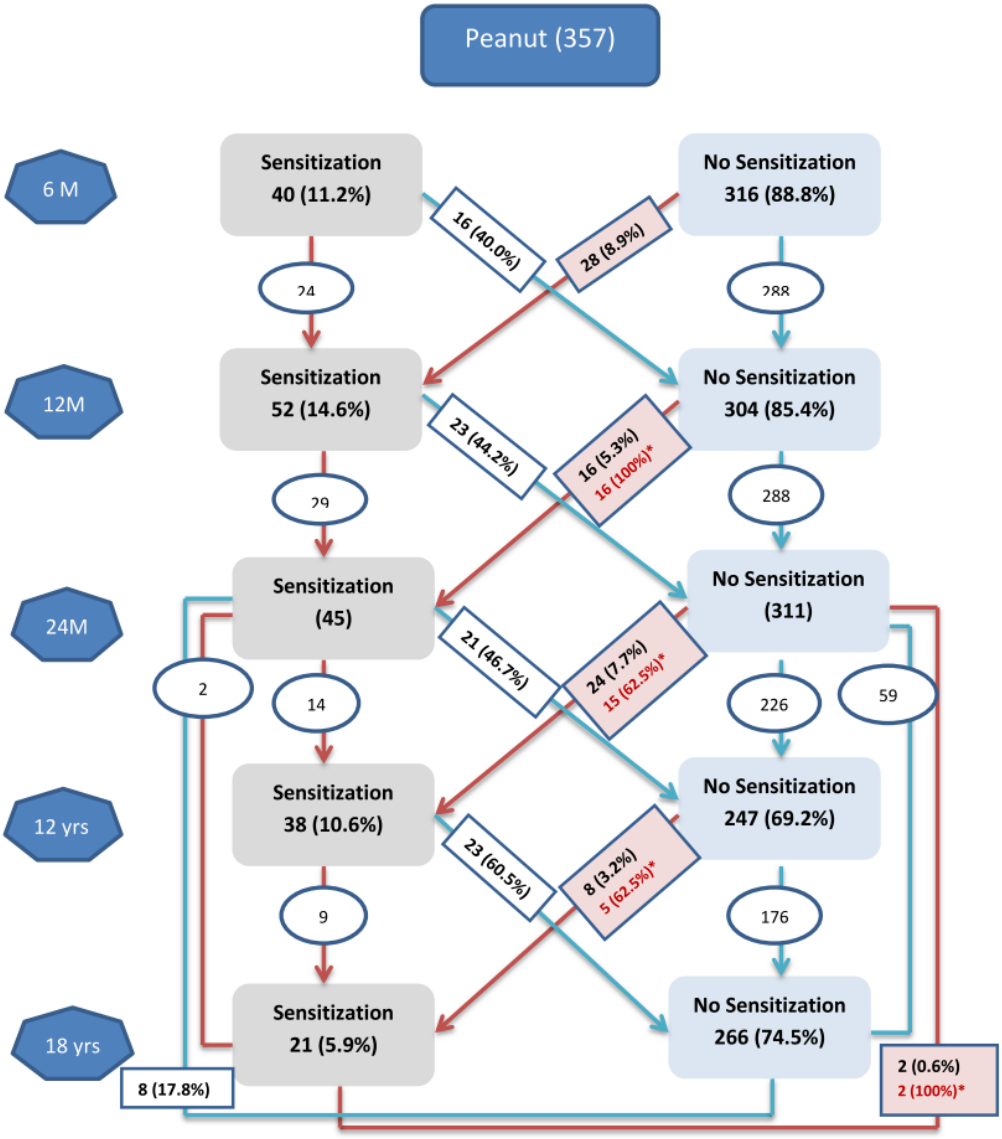

Figure S3: The dynamic changes of sensitization to peanut allergen from 6 months to 18 years. *(*) The number of participants who never been sensitized in previous follow-ups and their proportion from those who developed sensitization.*

Table S1: *Comparison of baseline characteristics between following and missing participants at 12 years and at 18 years*

Characteristics	*At 12 years*			*At 18 years*		
	Attending N=366 (59.0%)	**Missing N=254 (41.0%)**	**P-value (for the difference)**	**Attending N=404 (65.2%)**	**Missing N=216 (34.8%)**	**P-value (for the difference)**
Sex: Male (%) Female (%)	194 (53.0%) 172 (47.0%)	123 (48.4%) 131 (51.6%)	0.26	206 (51.0%) 198 (49.0%)	111 (51.4%) 105 (48.6%)	0.9
Mother's education: ▪ Higher education (%)	228 (62.3%)	137 (53.9%)	**0.03**	260 (64.4%)	105 (48.6%)	**0.0001**
Father's education: ▪ Higher education (%)	225 (61.5%)	153 (60.2%)	0.7	265 (65.6%)	113 (52.3%)	**0.001**
Maternal smoking	20 (5.5%)	15 (5.9%)	0.8	17 (4.2%)	18 (8.3%)	**0.03**
Paternal smoking	55 (15.0%)	55 (21.6%)	**0.03**	59 (14.6%)	51 (23.6%)	**0.005**
Number of siblings: ▪ No siblings (%) ▪ 1 sibling (%) ▪ 2 or more (%)	 139 (38.0%) 118 (32.2%) 109 (30.0%)	 110 (43.3%) 82 (32.3%) 62 (24.4%)	 0.18 0.99 0.14	 163 (40.3%) 129 (31.9%) 112 (27.7%)	 86 (39.8%) 71 (32.9%) 59 (27.3%)	 0.89 0.8 0.9
Atopic diseases in siblings:						
▪ Food allergy: 1. Milk allergy 2. Egg allergy 3. Other food	140 (38.3%) • 100 (27.3%) • 60 (16.4%) • 112 (30.6%)	92 (36.2%) • 63 (24.8%) • 44 (17.3%) • 74 (29.1%)	0.6 0.48 0.76 0.69	155 (38.4%) • 113 (28.0%) • 73 (18.1%) • 125 (30.9%)	77 (35.6%) • 50 (23.1%) • 31 (14.4%) • 61 (28.2%)	0.5 0.19 0.23 0.4
▪ Eczema	144 (39.3%)	94 (37.0%)	0.55	155 (38.4%)	83 (38.4%)	0.98
▪ Asthma	135 (36.9%)	84 (33.1%)	0.3	141 (34.9%)	78 (36.1%)	0.76
▪ Hay fever	84 (23.0%)	51 (20.1%)	0.39	90 (22.3%)	45 (20.8%)	0.67
Maternal atopic diseases:						
▪ Food allergy: 4. Milk allergy 5. Egg allergy 6. Other food	137 (37.4%) • 73 (19.9%) • 28 (7.7%) • 110 (30.1%)	102 (40.2%) • 50 (19.7%) • 25 (9.8%) • 80 (31.5%)	0.49 0.9 0.3 0.7	155 (38.4%) • 81 (20.0%) • 28 (6.9%) • 117 (28.9%)	84 (38.9%) • 42 (19.4%) • 25 (11.6%) • 73 (33.8%)	0.89 0.85 **0.05** 0.21
▪ Eczema	150 (41.0%)	91 (35.8%)	0.19	158 (39.1%)	83 (38.4%)	0.86
▪ Asthma	159 (43.4%)	109 (42.9%)	0.89	169 (41.8%)	99 (45.8%)	0.33
▪ Hay fever	212 (57.9%)	163 (64.2%)	0.11	242 (59.9%)	133 (61.6%)	0.6
Paternal atopic diseases:						
▪ Food allergy: 7. Milk allergy 8. Egg allergy 9. Other food	79 (21.6%) • 38 (10.4%) • 17 (4.6%) • 57 (15.6%)	52 (20.5%) • 23 (9.1%) • 13 (5.1%) • 37 (14.6%)	0.73 0.58 0.78 0.73	78 (19.3%) • 35 (8.7%) • 18 (4.5%) • 58 (14.4%)	53 (24.5%) • 26 (12.0%) • 12 (5.6%) • 36 (16.7%)	0.12 0.17 0.5 0.44
▪ Eczema	72 (19.7%)	54 (21.3%)	0.6	85 (21.0%)	41 (19.0%)	0.54
▪ Asthma	99 (27.0%)	59 (23.2%)	0.28	110 (27.5%)	48 (22.2%)	0.17
▪ Hay fever	172 (47.0%)	112 (44.1%)	0.47	188 (46.5%)	96 (44.4%)	0.61
Early Food and aeroallergen sensitization						
Food sensitization at 6 months (%)	71 / 342= 20.8%	53 / 218 = 24.3%	0.32	79/ 366 = 21.6%	45/ 194= 23.2%	0.66
Aeroallergen sensitization at 6	37 / 342= 10.8%	23 / 218= 10.6%	0.92	37 / 366 = 10.1%	23 / 194 = 11.9%	0.52

months (%)						
Food sensitization at 12 months (%)	84 / 349= 24.1%	61 / 202= 30.2%	0.11	97/ 374= 25.9%	48 / 177= 27.1%	0.76
Aeroallergen sensitization at 12 months (%)	64/ 349= 18.3%	38/ 202= 18.8%	0.89	69 / 374 = 18.4%	33/ 177 = 18.6%	0.95
Food sensitization at 24 months (%)	61 / 313= 19.5%	30 / 146 = 20.5%	0.79	71/ 322= 22.0%	20 / 137 = 14.6%	0.06
Aeroallergen sensitization at 24 months (%)	93/ 313= 29.7%	38 / 146= 26.0%	0.41	96 / 322 = 29.8%	35 / 137 = 25.5%	0.35

Table S2: *Characteristics of analysed and non-analyzed participants*

Participants characteristics	**Analyzed N= 357 (57.6%) (%)**	**Non-analyzed N= 263 (42.4%) (%)**	***P-values***
Sex			0.93
Male	51.3	51	
Female	48.7	49	
Mothers' age (mean/years)	31.6	30.7	**0.01**
Fathers' age (mean/years)	34	32.6	**0.001**
Mothers' higher education	66.7	48.3	**< .005**
Fathers' higher education	64.1	56.7	**0.06**
Smoking status			
Mother	5.04	6.5	0.4
Father	12.9	24.3	**< .005**
Siblings number			0.61
No sib	40.6	39.5	
1 sib	32.2	32.3	
≥ 2 sib	27.2	28.1	
Siblings atopy			
Food allergy	36.7	38.4	0.7
Eczema	37.8	39.2	0.7
Asthma	33.1	38.4	0.2
Hay fever	20.7	23.2	0.3
Maternal atopy			
Food allergy	38.9	38	0.8
Eczema	40.3	37	0.4
Asthma	43.4	43	0.9
Hay fever	58	63.9	0.13
Paternal atopy			
Food allergy	20.2	22.4	0.5
Eczema	21	19.4	0.6
Asthma	26.3	24.3	0.6
Hay fever	46.2	45.2	0.8
Food sensitisation			
6 M	21.8	22.7	0.8
12 M	25.5	27.8	0.5
24 M	21.8	12.7	**0.04**
12 Y	20.7	14.8	0.2
18 Y	10.1	6.5	0.3
Aeroallergen sensitisation			
6 M	10.1	11.8	0.5
12 M	18.2	19.1	0.8
24 M	29.4	25.5	0.4
12 Y	53.7	54.3	0.9
18 Y	67.4	66.4	0.8

CHAPTER 5 - Association between food sensitisation and subsequent allergic diseases: a systematic review and meta-analyses

5.1. Chapter introduction

This chapter includes a published paper in the *Allergy Journal*, which ranked as the second top journal in the field of allergy, entitled "**The march from early life food sensitisation to allergic disease: a systematic review and meta-analyses of birth cohort studies**" (with data supplement). This paper describes the current evidence for the associations between food sensitisation in early life and subsequent development of wheezing/asthma, eczema and allergic rhinitis from birth cohort studies.

This was the first systematic review and meta-analyses that assessed the associations between early life food sensitisation and subsequent development of allergic diseases. Findings from this review showed that early life food sensitisation was associated with an increased risk of infantile eczema, childhood wheeze/asthma, eczema and allergic rhinitis and young adulthood asthma. Meta-analyses demonstrated that food sensitisation in the first two years of life was related to an increased risk of wheeze/asthma, eczema and allergic rhinitis from 4 to 8 years.

5.2. Publications

5.2.1. Main document and data supplement

Alduraywish S, Lodge C, Campbell B, Allen K, Erbas B, Lowe A, Dharmage S. The march from early life food sensitization to allergic disease: A systematic review and meta-analyses of birth cohort studies. *Allergy* 2016;**71**: 77-89.

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The march from early life food sensitization to allergic disease: a systematic review and meta-analyses of birth cohort studies

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Keywords
allergic rhinitis; asthma; atopic march; eczema; food sensitization.

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Abstract

Background: There is growing evidence for an increase in food allergies. The question of whether early life food sensitization, a primary step in food allergies, leads to other allergic disease is a controversial but important issue. Birth cohorts are an ideal design to answer this question.

Objectives: We aimed to systematically investigate and meta-analyse the evidence for associations between early food sensitization and allergic disease in birth cohorts.

Methods: MEDLINE and SCOPUS databases were searched for birth cohorts that have investigated the association between food sensitization in the first 2 years and subsequent wheeze/asthma, eczema and/or allergic rhinitis. We performed meta-analyses using random-effects models to obtain pooled estimates, stratified by age group.

Results: The search yielded fifteen original articles representing thirteen cohorts. Early life food sensitization was associated with an increased risk of infantile eczema, childhood wheeze/asthma, eczema and allergic rhinitis and young adult asthma. Meta-analyses demonstrated that early life food sensitization is related to an increased risk of wheeze/asthma (pooled OR 2.9; 95% CI 2.0–4.0), eczema (pooled OR 2.7; 95% CI 1.7–4.4) and allergic rhinitis (pooled OR 3.1; 95% CI 1.9–4.9) from 4 to 8 years.

Conclusion: Food sensitization in the first 2 years of life can identify children at high risk of subsequent allergic disease who may benefit from early life preventive strategies. However, due to potential residual confounding in the majority of studies combined with lack of follow-up into adolescence and adulthood, further research is needed.

Over the past 50 years, there has been a global epidemic of asthma, eczema and allergic rhinitis (1) with an exponential increase since early 1990. This global epidemic of allergies has posed a substantial public health burden on affected individuals, their families and healthcare resources (2–4). Recent evidence suggests a 'second wave' of the allergy epidemic, which is related to an increase in food allergies (5–7). This is supported by a recent European systematic review of three UK-based studies which found weak evidence for an increasing prevalence of food allergy since the 1990s (6). Additionally, Branum et al. (8) recently showed an increase in self-reported food allergy and healthcare utilization during the period 1997–2007 among US children.

Atopic dermatitis (eczema) and food allergy are the most common allergic diseases during the first 2 years of life, with some evidence that they are temporally interrelated (9, 10). While it is acknowledged that both early life eczema and food allergy may progress into asthma and allergic rhinitis, a

concept known as the 'atopic march'(11), whether food sensitization induces or is a consequence of eczema is controversial (12, 13). The progression from eczema to other allergic disease has been documented and systematically synthesized (14). However, few studies have assessed the evidence for the atopic march in relation to cow's milk allergy (15–18). Given the increase in childhood food allergy, it is important to evaluate whether food allergy marches towards other allergies.

Although the gold standard for diagnosis of food allergy is oral food challenge, there are relatively few epidemiological studies that measure food allergy in this way. Food sensitization is one of the first steps in the defining the pathogenesis of food allergy and is the measure most commonly used in allergy birth cohorts. The aim of this systematic review and meta-analyses was to assess and quantify the role of early food sensitization on risk of allergic disease from infancy to young adulthood. We limited the review to birth cohort studies only, as this design provides the ideal opportunity to investigate early life determinants.

Methods

Search strategy

The protocol of this systematic review was developed and approved by all co-authors prior to commencement of the search. We systematically searched Medline (Web of Knowledge) and Scopus databases for English language peer-reviewed original articles, published from 1950 to 2014 by linking three groups of keywords (MeSH HEADING in Medline and TITLE-ABS-KEY in Scopus). The first group of keywords was related to food sensitization (the exposure), the second group to allergic diseases outcomes and the third group to birth cohorts (Table S1). We also searched the reference lists of all included articles to find potentially other relevant articles.

Inclusion criteria and definitions

Birth cohort studies were considered eligible for inclusion if they met the following criteria: (i) presented the association between food sensitization, as defined by skin prick testing (SPT) or serum-specific immunoglobulin E (s-IgE), in early life (i.e. in the first 2 years) and the subsequent development of asthma, eczema and/or allergic rhinitis outcomes during late infancy (1–2 years), childhood (>2–12 years), adolescence (>12–18 years) or adulthood (>18 years); (ii) defined asthma as either doctor diagnosed or parent or self-reported wheeze (1–5 years) or asthma (>5 years); (iii) defined eczema as either doctor diagnosed or parent or self-reported eczema or 'atopic dermatitis'; and (iv) defined allergic rhinitis as either doctor diagnosed or parent or self-reported allergic rhinitis or hay fever.

Selection of included articles

Abstracts of all articles were assessed for eligibility by two authors independently (SA, BC). The same authors then further assessed the full-text articles of selected abstracts to determine article inclusion.

Data extraction and assessment

A standardized form was used for data extraction. The following information was extracted from each included article: author, year of publication, country/study location, name of the cohort, years of enrolment, original sample size, number included in the analysis, study population, food allergens tested and methods used to define food sensitization. Findings from eligible articles were tabulated according to the outcome assessed and included the following data: timing of sensitization and allergic outcomes assessment, risk estimates along with 95% CI/p value and assessed confounders. Corresponding authors of the included articles were contacted for further information if the reported data were incomplete.

The quality of each included article was assessed using Newcastle–Ottawa Scale (NOS) criteria (19). This scale assesses evidence of study quality based on selection of sample, comparability and the quality of outcome assessment. Each article was scored using a standard 'starring (*)'system. In addition, three confounders were predetermined as being necessary for inclusion in adjusted models. These were family history of atopic disease (in population-based studies), concurrent eczema and/or wheezing and centre (in multicentre studies). Concurrent eczema and wheeze were considered essential confounders to control for co-manifestation of the relevant phenotypes.

Statistical methods

Meta-analysis methods

We computed separate forest plots for all three allergic outcomes during infancy, childhood and adulthood. We conducted meta-analyses when effect estimates from three or more studies were available and when the heterogeneity across the studies, as assessed by I^2 (20), was less than 80%. To evaluate conflicting results, more studies are needed for a meta-analysis. We chose a minimum of three as this allowed us to evaluate the robustness due to studies from different populations, regions, follow-ups and co-varying factors. If we were to include only two studies, it would be challenging to estimate any between-study variations, as we assume a moderate degree of heterogeneity. We used random-effects model in all meta-analysis. All analyses were performed using Stata 13 (StataCrop, College Station, TX, USA).

Results

The electronic search yielded 1225 articles. After abstract review, the full texts of 106 articles were retrieved for assessment, from which 15 articles were eligible for inclusion (Fig. 1).

Study characteristics

The 15 included articles represented 13 birth cohort studies, of which seven cohorts were conducted in Europe (21–29),

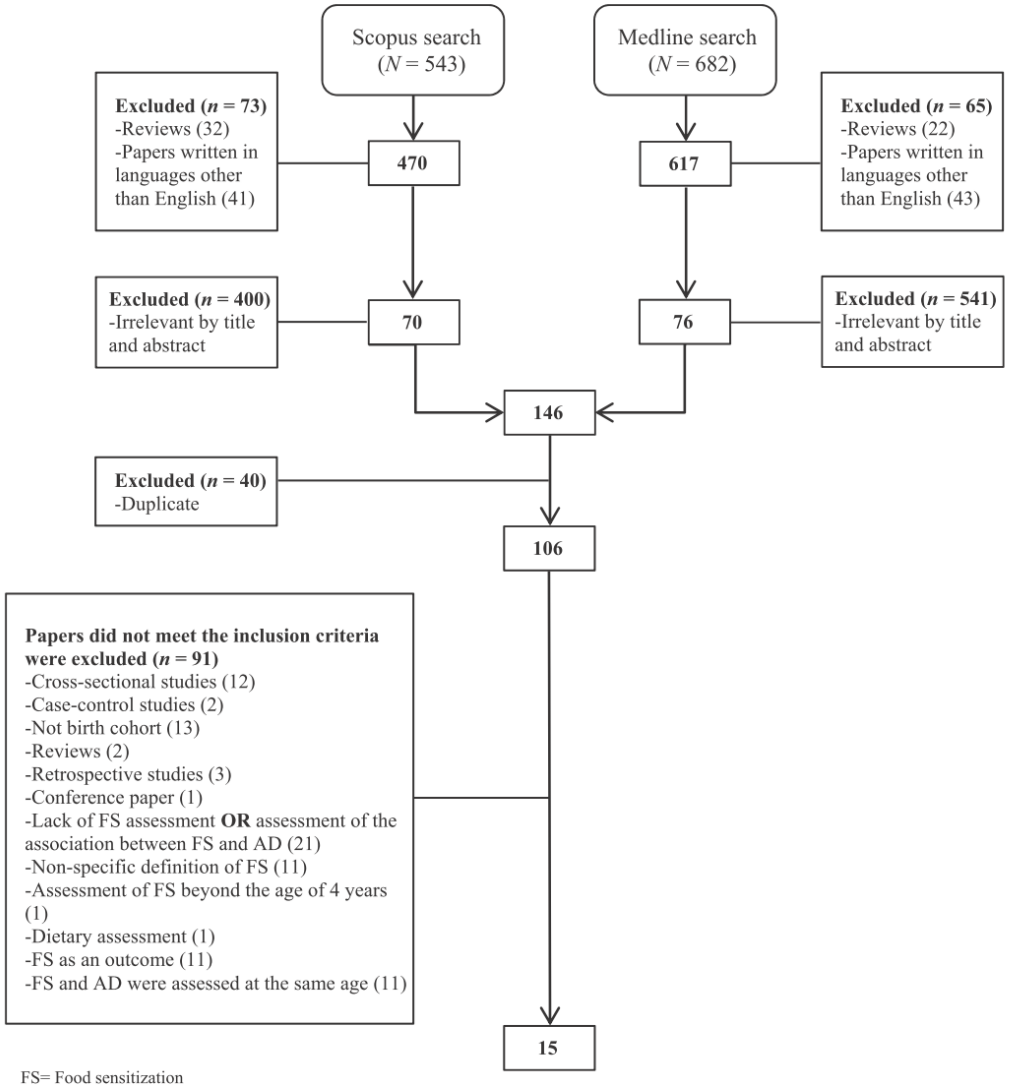

Figure 1 Flow chart for systematic searching process.

two in United States (30, 31), two in Australia (13, 32) and two in Asia (33, 34). Of the 13 birth cohorts, five were population based (21, 22, 30, 33, 34) and eight were defined as high-risk (i.e. infants who had first degree relatives with history of asthma, eczema, allergic rhinitis or food allergy, children of mothers with allergies, parents or siblings with current asthma or wheezing, parent positive SPT) (13, 23–27, 31, 32) (Table S2). The original number of enrolled participants in each cohort ranged from 100 (27) to 2252 (23) (Table 1).

Exposure assessment

Food sensitization was assessed by SPT in five cohorts (25, 27, 31, 32, 35), s-IgE in seven cohorts (23, 24, 26, 29, 30, 33, 34) or both SPT and s-IgE in one cohort (22). The earliest age for assessment of food sensitization was 3 months (22). Hen's egg and cow's milk sensitization were investigated in all included cohorts, peanut in five (13, 26, 30–32), soya in four (21, 23, 26, 33), wheat in three (21, 33, 34), codfish in two (26, 31) and shrimp and walnut in one (31).

Among cohorts that assessed sensitization using s-IgE, the definition of a positive test (i.e. the level of specific IgE ≥ 0.35kU/l) was consistent across all cohorts except one. The Swedish cohort (26) defined sensitization as low (i.e. s-IgE between 0.1 and 0.7 kU_A/l) and very low (i.e. s-IgE < 0.1 kU_A/l). For the purpose of this review, we define low s-IgE as <0.1 kU_A/l. Additionally, all studies that assessed sensitization by SPT defined a positive test as the mean wheal diameter of ≥3 mm, with the exception of the Poole birth cohort (27) where a positive test was defined as a mean wheal diameter ≥2 mm in subjects <2 years and as ≥3 mm for subjects older than 2 years.

Outcome assessment

The age at which allergic outcomes were reported varied between articles, ranging from 14 months to 22 years.

Among the 13 articles that investigated wheeze/asthma as an outcome, two were from the same cohort with outcomes presented at both 5 years (21) and 7 years (29). The definition of wheeze/asthma varied between articles, and the majority defined it using data from parent questionnaires. Only three articles defined wheeze/asthma using information from a paediatric allergist or bronchial hyper-responsiveness (BHR) (27, 31, 33). Seven articles defined wheeze/asthma as current (21–24, 27, 29, 32) and six as ever (25, 26, 30, 31, 33, 34). (Table S3).

Eczema as an outcome was assessed in nine articles from different cohorts. Eczema was defined according to Hanifin and Rajka's criteria (22, 26, 33, 34), presence of eczematous rash during physical assessment (31, 32) or parental report of child's eczema or use of any topical treatment (13, 23, 25, 32).

The development of allergic rhinitis was investigated in six articles, representing five cohorts. Two articles were from one cohort, with outcomes presented at age 5 (21) and 7 years (28). Allergic rhinitis was defined as parental report of child's nasal symptoms or doctor diagnosis of allergic rhinitis in all studies (21–23, 28, 32).

Quality assessment of included articles

Study quality was assessed using the NOS for cohort studies. The majority of articles reported satisfactory selection of participants, in which exposed individuals were truly representative of the intended study population and nonexposed individuals were from the same community. The ascertainment of exposure in all included articles was from laboratory or medical records. Only one Danish population-based cohort (22) and two, Australian and German, high-risk cohorts considered all key confounders listed in the methods section of this review (13, 23). Five articles presented several statistical models with different levels of adjustment for other potential confounders (24, 28, 31–33). The remaining articles did not adjust for any confounders. The majority of included articles assessed asthma, eczema and allergic rhinitis based on reported data. Loss to follow-up was a potential source of bias given that the included studies were longitudinal in design. However, as we assessed the outcome at different age, the follow-up rate at the age of outcome was determined to be satisfactory in the majority of articles, with adequate comparability between included and nonincluded participants. Only two studies did not provide comparisons between included and nonincluded participants (24, 34). Overall, according to the suggested criteria for selection, comparability and outcome in NOS, the articles included in this review were of acceptable quality (Table S4).

Studies included in the meta-analyses

Due to insufficient number of studies and methodological heterogeneity, we meta-analysed only studies that reported the association between food without inhalant allergens sensitization and allergic diseases from 2 to 12 years.

One of the included studies reported results for both egg and milk sensitization separately (24). In the meta-analysis model, we included the results for egg and milk separately and observed no significant differences between the two models. Thus, egg sensitization results were plotted in the final models.

Two of the included studies investigated food sensitization at age 6, 12 and 24 months (26, 34). When conducting the meta-analysis, we computed separate models for each of the different time points and found no significant differences in the pooled estimates. Therefore, 6-month SPT results were included in the final meta-analyses models.

The association between food sensitization and wheeze/asthma (Table 2)

In infancy (1–2 years)

One population-based (33) and one high-risk cohorts (26) assessed the association between food sensitization and wheeze in the first 2 years of life (Table 2). The high-risk cohort showed an increase in the risk of ever wheezing at 2 years in children with an increased serum level of the sum of egg and milk s-IgE (26). The population-based cohort showed no statistical significant associations.

In childhood (>2–12 years)

Ten articles representing nine birth cohorts examined the association between food sensitization (with or without inhalant sensitization) and subsequent wheeze/asthma in childhood (Table 2). Of these, only five were from population-based cohorts (21, 22, 29, 30, 34).

Overall, positive associations were demonstrated in three articles from population-based (21, 22, 34) and four articles from high-risk cohorts (23–25, 31). The population-based MAS cohort found that food sensitization at both 1 and 2 years of age increased the risk of current wheeze/asthma at 5 (21), but not significantly at 7 years (29) (Table 2).

The results of seven articles that met the inclusion criteria for meta-analysis (22–25, 29, 30, 34) showed that food sensitization in the first 2 years was associated with an increased risk of asthma during childhood (OR = 2.8, 95% CI = 2.1–3.9) (Fig. 2). The pooled estimates remained almost the same when

Table 1 Birth cohort studies that assessed the association between food sensitization and subsequent development of allergic disease

Name of the cohort, Country	Years of enrolment (Original sample size)	Study population*	Tested food allergens (Methods for assessment)	Publication (s); First author (year), Ref.	Number of analysed participants (% from total cohort)	Allergic outcomes Assessed	Quality Score†
The German Multicenter Allergy Study (MAS), Germany	1990 (1314)	Population-based atopy enriched study (40%)	Egg, Milk, Wheat, Soya bean (s-IgE)	Kulig (1998), (21)	–	Asthma, Allergic rhinitis	6/9
				Kulig (2000), (28)	–	Seasonal allergic rhinitis	5/9
				Illi (2001), (29)	1062 (80.8%)	Asthma	6/9
The Wayne County Health, Environment, Allergy and Asthma Longitudinal Study (WHEALS) birth cohort, United States	2003–2007 (1258)	Population based	Egg, Milk, Peanut (s-IgE)	Havstad (2014), (30)	594 (47%)	Asthma	5/9
The Danish Allergy Research Centre cohort (DARC birth cohort), Denmark	1998–1999 (562)	Population based	Egg, Milk (SPT and s-IgE)	Kjaer (2009), (22)	S-IgE → 325 (57.8%) SPT → 382 (68%)	Asthma, Eczema, Allergic rhinitis	7/9
The Gifu Allergy and Immunology Cohort Study (GAICS), Japan	2004–2005 (314)	Population based	Egg, Milk, Wheat, Soya bean (s-IgE)	Kawamoto (2012), (33)	171 (54.5%)	Asthma, Eczema	5/9
The Prediction of Allergies in Taiwanese Children study (PATCH), Taiwan	2007–2010 (258)	Population based	Egg, Milk, Wheat (s-IgE)	Chiu (2014), (34)	182 (70.5%)	Asthma, Eczema, Allergic rhinitis	5/9
German Infant Nutritional Intervention (GINIplus) study, Germany	1995–1998 (2252)	High risk of atopy	Egg, Milk, Soya bean (s-IgE)	Brockow (2009), (23)	1290 (57.3%)	Asthma, Eczema, Allergic rhinitis	7/9
The Prevention and Incidence of Asthma and Mite Allergy (PIAMA) birth cohort, Netherlands	1996–1997 (855)	High risk of atopy	Egg, Milk (s-IgE)	Bekkers (2013), (24)	–	Asthma	6/9
The Melbourne Atopy Cohort Study (MACS), Australia	1990–1994 (620)	High risk of atopy	Egg, Milk, Peanut (SPT)	Lowe (2007), (13)	552 (89%)	Eczema	7/9
The Childhood Asthma Prevention Study (CAPS), Australia	1997–2000 (616)	High risk of asthma	Egg, Milk, Peanut (SPT)	Garden (2013), (32)	509 (83%)	Asthma, Eczema, Allergic rhinitis	6/9
No specific name was given, Referred to South Wales Birth Cohort, UK	– (497)	High risk of atopy	Egg, Milk (SPT)	Burr (1997), (25)	437 (88%)	Asthma, Eczema	6/9
No specific name was given, Referred to as Swedish Birth Cohort, Sweden	1997–2000 (268)	High risk of atopy	Egg, Milk, Peanut, Soya bean, Codfish (s-IgE)	Söderström (2011), (26)	–	Asthma, Eczema	6/9
No specific name was given, Referred to as Poole Birth Cohort, UK	1976–1977 (100)	High risk of atopy	Egg, Milk (SPT)	Rhodes (2001), (27)	63 (63%)	Asthma	6/9
No specific name was given, Referred to as San Diego Birth Cohort, USA	–	High risk of atopy	Egg, Milk, Peanut, Cod, Shrimp, Walnut (SPT)	Zeiger (1995), (31)	–	Asthma, Eczema, Allergic rhinitis	6/9

S-IgE = serum-specific IgE, SPT = skin prick testing.

*For definition of high- risk cohort refer to Table S2.

†For details of the quality assessment refer to Table S4.

Table 2 The association between food sensitization and subsequent development of wheeze/asthma in birth cohort studies stratified by the age of wheeze/asthma development

Name of the cohort (Ref.)	Age at assessment of Asthma/ wheezing*	Age at assessment of food sensitization	Main Findings OR (95% CI)	Assessed confounders
Late infancy				
GAICS (33)	14 months	6 months		The level of cord blood IgE and other immunological biomarkers.
		Egg white	1.8 (0.4–7.1)	
		Wheat	1.5 (0.1–13.9)	
Swedish Birth Cohort (26)	2 years	12 months		No adjustment
		Egg + Milk	2.6 (1.0–6.7)	
Childhood				
PIAMA (24)	Yearly from 3 to 8 years and then at 11 years	12 months		Sex, Maternal smoking during pregnancy, Breastfeeding, Presence of older siblings, Paternal allergy
		Hen's egg	Later asthma → 2.5 (1.2–5.1) At 6 years → 3.9 (1.6–9.4) At 7 years → 4.3 (1.8–10.5) At 8 years → 3.9 (1.6–9.5)	
		Cow's milk	Later asthma → 1.0 (0.6–1.6)	
WHEALS (30)	4 years	2 years		–
		Highly sensitized†	5.3 (1.6–17.4)	
		Egg + Milk	1.6 (0.8–3.0)	
		Peanut + any inhalant‡	1.8 (0.6–4.9)	
PATCH (34)	4 years	6, 12, 18 & 24 months		–
		Food only (6 months)	7.5 (1.7–33.0)	
		Food + inhalant (6 months)	2.8 (0.2–48.8)	
		Food only (12 months)	3.0 (0.8–10.4)	
		Food + inhalant (12 months)	4.8 (0.7–31.1)	
		Food only (18 months)	9.0 (1.0–78.1)	
		Food + inhalant (18 months)	57.0 (4.3–744.7)	
		Food only (24 months)	7.4 (0.8–68.1)	
		Food + inhalant (24 months)	38.4 (3.9–373.1)	
MAS (21)	5 years	1, 2 and 5 years		–
		Long lasting§ *vs* transient¶	5.5 (No 95% CI), $P < 0.0001$	
		Long lasting *vs* No sensitization	10.6 (No 95% CI)	
DARC (22)	6 years	Early food (3–18 months)	4.0 (1.1–12.5)	Early atopic dermatitis, Family history of allergy, Elevated cord blood IgE, Early wheeze, Male gender, Maternal history of allergy
		Early food + inhalant	3.8 (0.8–14.0)	
GINIplus (23)	6 years	12 month	3.9 (1.9–7.7) Adjusted; 3.9 (1.9–7.9)	Family history of atopic disease, Parental education, Siblings, Gender, Study region, and Type of milk feeding during the first 4 months of life
MAS (29)	7 years	1 and 2 years		
		Food only before 2 years.	2.2 (0.7–6.2)	–
		Food + inhalant before 2 years.	11.1 (4.7–26.1)	
South Wales Birth Cohort (25)	7 years	6 months		–
		Egg	2.6 (1.2–5.5)	
San Diego Birth Cohort (31)	7 years	12 months		Male gender, Maternal ethnicity, Parental atopic disease, Maternal smoking
		Egg	1.7 (No 95% CI)	
		Milk	Adjusted; 6 (1.3–28)	
		Peanut	Adjusted; 11.2 (1.2–107)	
CAPS (32)	8 years	18 months, 3, 5 and 8 years		Sex, The study intervention groups.
		Mixed food and inhalant**	16.4 (5.2–51.7) Adjusted; 14.3 (4.7–44.1)	

Table 2 (continued)

Name of the cohort (Ref.)	Age at assessment of Asthma/ wheezing*	Age at assessment of food sensitization	Main Findings OR (95% CI)	Assessed confounders
Adulthood				
Poole Birth Cohort (27)	22 years	3, 6 and 12 months Egg or milk before 1 year	12.3 (2.5–77.1)	–

*The definitions of outcome are listed in Table S3.
†Sensitization to at least 4 of the same allergens: milk, egg, peanut and timothy grass, as determined by latent class analysis.
‡Inhalant allergens were Dermatophagoides farinae, dog, cat, timothy grass, ragweed and Alternaria alternate.
§Long-lasting sensitization: sensitization at both 1 and 2 years.
¶Transient sensitization: sensitization at 1 or 2 years.
**Mixed food and inhalant (as determined by latent class analysis): Characterized by early and persistently sensitization to peanut, early, but decreasing sensitization to egg, and an increasing sensitization to HDM, Alternaria and grass.

studies that assessed wheeze/asthma before the age of 5 were excluded from the final model (OR = 3.2, 95% CI = 2.2–4.8).

The association between co-sensitization to food and inhalant allergens and subsequent wheeze/asthma was reported in five studies (22, 29, 30, 32, 34) (Table 2). All cohorts, except two populations based (22, 34), demonstrated a positive relationship.

Specific food allergen sensitization in relation to wheeze/asthma was assessed in three high-risk cohorts (24, 25, 31) (Table 2). Hen's egg sensitization in the first 2 years of life was found to be associated with increased risk of wheeze/asthma in early childhood in three high-risk cohorts (24, 25, 31). Conversely, the evidence for the association between cow's milk sensitization and wheeze/asthma was inconsistent. Cow's milk sensitization at 1 year was not related to wheeze/asthma from 3 to 11 years in Bekkers et al. (24) but significantly increased the risk of asthma at 7 years in Zeiger, et al. (31).

In adolescence (12–18 years)
None of the included articles investigated the association between food sensitization and subsequent asthma in this age group.

In adulthood (>18 years)
Only one birth cohort (27) assessed the association between early food sensitization and subsequent asthma in adulthood. In this high-risk cohort, sensitization to milk, egg or both before the age of 1 year was associated with an increased risk of asthma at 22 years (Table 2).

The association between food sensitization and eczema (Table 3)

In infancy (1–2 years)
One population-based (33) and one high-risk cohorts (26) assessed the association between food sensitization and eczema during late infancy. Although both cohorts showed a positive association, no adjustment for concurrent eczema was made at the time of sensitization assessment (Table 3). Egg white, milk or wheat sensitization at 6 months was found to be associated with eczema at 14 months in the population-based cohort (33). However, in the high-risk cohort that used the same eczema definition, it was found that milk but not egg sensitization at 6 months was significantly associated with eczema at 2 years (26).

In childhood (>2–12 years)
Eight articles representing eight cohorts investigated the association between food sensitization with or without inhalant sensitization and subsequent eczema at age 4–8 years (13, 22, 23, 25, 26, 31, 32, 34) (Table 3). Overall, two population-based (22, 34) and five of six high-risk cohorts reported positive associations (13, 23, 25, 31, 32). Conversely, a Swedish high-risk cohort (26) that assessed egg and milk sensitization at three time points (6, 12 and 24 months) found no significant association between s-IgE to egg or milk in the first year and the development of eczema at 5 years (Table 3).

Results from one population-based study (34) showed that only food sensitization at 6 but not at 12, 18 or 24 months was significantly related to increased risk of eczema at 4 years. Also, no confounders were considered in their analysis. Findings from a high-risk cohort observed a significant association between food sensitization at 12 months and eczema at 6 years (23), but this association was no longer present when children who had eczema in their first year of life were excluded from the analysis.

Five of the eight articles assessing early food sensitization and eczema during childhood met the meta-analyses inclusion criteria (22, 23, 25, 26, 34). Pooling of the findings showed that food sensitization in the first 2 years was associated with an increased risk of eczema during childhood (OR = 2.5, 95% CI = 1.8–3.6) (Fig. 2).

Co-sensitization to food and inhalant allergens and subsequent eczema during childhood was investigated in two population-based and one high-risk cohorts (22, 32, 34). Two of these studies found positive associations (22, 32) (Table 3), while the third study (34) showed significant positive associations when sensitization was assessed at 18 and 24 months, but not at 6 or 12 months.

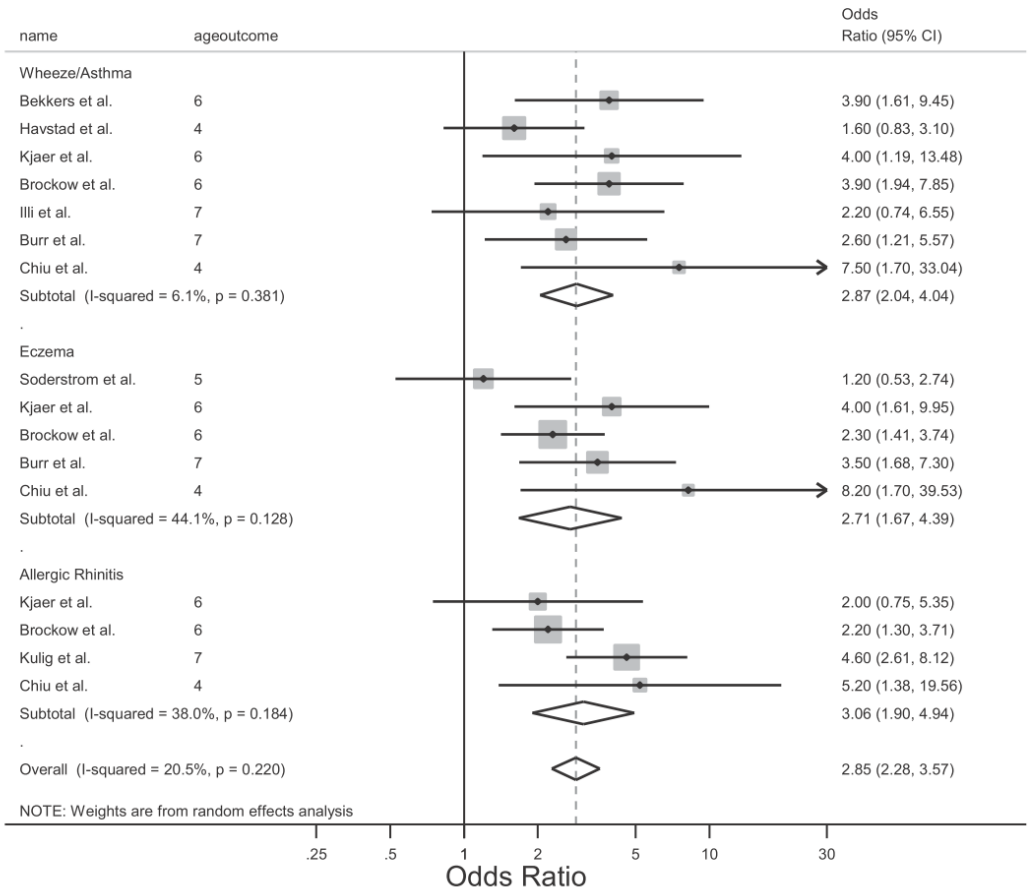

Figure 2 Association between early life food sensitization and asthma, eczema and allergic rhinitis during childhood (>2–12 years). Risk estimates and 95% confidence intervals estimated with random-effects modelling.

In adolescence and adulthood (>12 years)
None of the included articles examined the association between food sensitization and subsequent eczema in this age group.

The association between Food sensitization and allergic rhinitis (Table 4)

In infancy (1–2 years)
None of the included articles examined the association between food sensitization and subsequent allergic rhinitis during infancy.

In childhood (>2–12 years)
Three population-based (21, 22, 34) and two high-risk cohorts (23, 32) examined the association between food with or without inhalant sensitization and subsequent allergic rhinitis at age 4–8 years (Table 4). Overall, positive associations were observed in five (21, 23, 28, 32, 34) of six articles (21–23, 28, 32, 34). Two articles from the MAS cohort assessed allergic rhinitis at 5 years (21) and seasonal allergic rhinitis at 7 years (28). These articles showed that the risk of allergic rhinitis at 5 years and seasonal allergic rhinitis at 7 years in food sensitized compared to nonfood sensitized children was similar (OR = 4.7 and 4.6 at 5 and 7 years, respectively); however, 95% CI was not reported in the former.

Four of the six articles met the inclusion criteria to be included in meta-analysis of articles assessing early food sensitization and allergic rhinitis during childhood (22, 23, 28, 34). Our meta-analyses showed that food sensitization in the first 2 years of life was associated with an increased risk of allergic rhinitis during childhood (OR = 3.0, 95%

Table 3 The association between food sensitization and development of eczema in birth cohort studies, stratified by the age of eczema development

Name of the cohort (Ref.)	Age at assessment of eczema/atopic dermatitis*	Age at assessment of food sensitization	Main findings OR (95% CI)	Assessed confounders
Late infancy				
GAICS (33)	14 months	6 months		The level of cord blood IgE and other immunological biomarkers.
		Egg white	14.9 (4.1–53.2) Adjusted; 23.2 (2.8–192.9)	
		Cow's milk	8.1 (2.7–24.2)	
		Wheat	10.2 (2.3–44.5)	
Swedish Birth Cohort (26)	2 years	6 and 12 months		
		Egg (6 months)	1.7 (0.7–4.0)	No adjustments
		Egg (12 months)	3.3 (1.5–7.1)	
		Milk (6 months)	4.8 (1.8–12.8)	
		Milk (12 months)	3.5 (1.4–8.3)	
		Egg + /or Milk (6 months)	3.1 (1.4–6.5)	
		Egg + /or Milk (12 months)	3.3 (1.6–6.9)	
Childhood				
PATCH (34)	4 years	6, 12, 18 & 24 months		–
		Food only (6 months)	8.2 (1.7–39.5)	
		Food + inhalant (6 months)	8.2 (0.6–101.0)	
		Food only (12 months)	3.0 (0.7–11.6)	
		Food + inhalant (12 months)	6.0 (0.8–40.8)	
		Food only (18 months)	1.0 (0.2–4.0)	
		Food + inhalant (18 months)	7.6 (1.1–54.1)	
		Food only (24 months)	1.1 (0.2–5.1)	
		Food + inhalant (24 months)	5.6 (1.1–27.3)	
Swedish Birth Cohort (26)	5 years	6 and 12 months		No adjustments
		Egg (6 month)	1.0 (0.4–2.4)	
		Egg (12 month)	1.1 (0.5–2.6)	
		Egg (24 month)	1.5 (0.8–2.8)	
		Milk (6 month)	0.7 (0.2–1.9)	
		Milk (12 month)	0.8 (0.3–2.1)	
		Milk (24 month)	1.1 (0.6–2.0)	
		Egg + /or Milk (6 month)	1.1 (0.5–2.6)	
		Egg + /or Milk (12 month)	0.9 (0.4–2.1)	
		Egg + /or Milk (24 month)	1.2 (0.6–2.1)	
DARC (22)	6 years	Early food (3–18 month)	4.0 (1.6–9.9)	Early atopic dermatitis, Family history of allergy, Elevated cord blood IgE, Early wheeze, Male gender, Maternal history of allergy
		Early food + inhalant	6.2 (2.2–17.1)	
GINIplus (23)	6 years	12 month	2.3 (1.4–3.7) Adjusted; 2.1 (1.2–3.6) *Excluding children with a physician's diagnosis of eczema within the first year of life* → Adjusted; 0.8 (0.3–2.1)	Family history of atopic disease, Parental education, Siblings, Gender, Study region and Type of milk feeding during the first 4 months of life
MACS (13)	6 and 7 years	6 months		Maternal or paternal history of eczema, gender and presence of older siblings
		Any food	HR=1.6 (1.1–2.3)	
		Egg	HR=1.6 (1.1–2.5)	
		Milk	HR=2.1 (1.1–3.7)	
		Peanut	HR=1.7 (0.9–2.9)	
South Wales Birth Cohort (25)	7 years	6 months Egg	3.5 (1.7–7.4)	–

Table 3 (continued)

Name of the cohort (Ref.)	Age at assessment of eczema/atopic dermatitis*	Age at assessment of food sensitization	Main findings OR (95% CI)	Assessed confounders
San Diego Birth Cohort (31)	7 years	1 year Egg Peanut	 15 (No 95% CI) Adjusted; 34 (1.5–794)	Male gender, Maternal ethnicity parental atopic disease, maternal smoking
CAPS (30)	8 years	18 months, 3, 5 and 8 years Mixed food and inhalant†	 5.6 (1.8–17.2) Adjusted; 5.6 (1.9–16.8)	Sex, study intervention groups

HR, Hazard Ratio.

*The definitions of outcome are listed in Table S3.

†Mixed food and inhalant (as determined by latent class analysis): Characterized by early and persistently sensitization to peanut, early, but decreasing sensitization to egg, and an increasing sensitization to HDM, Alternaria and grass.

CI = 2.1–4.2) (Fig. 2). After excluding studies that assessed allergic rhinitis before the age of 5, there was no significant change in pooled estimates (OR = 2.9, 95% CI = 2.0–4.1).

Co-sensitization to food and inhalant allergens and subsequent allergic rhinitis during childhood was investigated in two population-based and one high-risk cohorts (22, 32, 34) (Table 4). Although a significant association was reported in the high-risk cohort (32), a nonsignificant association was found in one population-based cohort (22). The second population-based cohort showed significant positive associations when food and inhalant sensitization were assessed at 18 and 24 months but not at 6 or 12 months (34).

In adolescence and adulthood (>12 years)

None of the included articles assessed the association between food sensitization and subsequent allergic rhinitis in this age group.

Dose–response relationship between food sensitization and allergic diseases:

Only in MACS study (13) was the association between number and size of SPT reactions to food allergens and risk of developing eczema examined. The authors found that infants with the largest SPT wheal (≥6 mm) had the greatest risk of eczema (HR = 2.28, 95% CI = 1.34–3.87) and those who were sensitized to three food allergens had the greatest risk of developing eczema (HR = 4.73, 95% CI = 2.10–10.66) when compared to those sensitized to only one food allergen (HR = 1.53, 95% CI = 1.10–2.12).

Three articles (21, 22, 29) investigated the association between specific patterns of food sensitization and subsequent allergic diseases. However, the patterns were different across the articles. The MAS (21) found that long-lasting food sensitization (i.e. food sensitization at age 1 and 2 years) was associated with increased risk of asthma and allergic rhinitis compared to transient (i.e. food sensitization at only at age 1 or 2 years) and never food sensitized (Tables 2 and 3). While articles from the MAS (29) and DARC study (22) have found that early food sensitization was associated with increased risk of subsequent asthma, the definition of early sensitization varied between the two studies. Early food sensitization was defined as sensitization before the age of 2 years in the MAS (29) and between 3 and 18 months in the DARC study (22).

Discussion

This is the first systematic review and meta-analyses that assessed the association between food sensitization and subsequent allergic diseases in birth cohort studies. We found that early life food sensitization was related to eczema in late infancy, wheeze/asthma, eczema or allergic rhinitis in childhood and asthma in young adults. Meta-analyses confirmed these relationships for childhood wheeze/asthma, eczema and allergic rhinitis. Association in relation to infant wheeze was inconsistent, and associations with adult eczema and allergic rhinitis have not been investigated.

Although a formal protocol was not registered, we developed an informal protocol prior to commencement of the search to ensure the review methodology was not altered based on the findings from individual studies. Two independent authors selected papers for inclusion by searching two large databases along with the reference lists of included papers. Two authors also independently performed data extraction and quality assessment. However, there is potential for publication bias affecting the pooled estimates. We chose not to include unpublished literature as we could not properly assess the quality of papers that have not been published in full.

Some of the included papers were from interventional studies (13, 23–25, 31, 32) where the intervention might influence the occurrence of food sensitization (such as dietary intervention) or allergic outcomes (such as HDM avoidance). The presented associations in these studies were from both control and intervention groups with only three studies (23, 24, 31) having considered the intervention in their statistical analyses. In a sensitivity analysis, excluding the studies that

Table 4 The association between food sensitization and subsequent development of allergic rhinitis in birth cohort studies stratified by the age of allergic rhinitis development

Name of the cohort (Ref.)	Age at assessment of allergic rhinitis*	Age at assessment of food sensitization	Main findings OR (95% CI)	Assessed confounders
Childhood				
PATCH (34)	4 years	6, 12, 18 & 24 months		–
		Food only (6 month)	5.2 (1.4–19.8)	
		Food + inhalant (6 month)	2.1 (0.1–24.3)	
		Food only (12 month)	1.1 (0.4–2.8)	
		Food + inhalant (12 month)	1.8 (0.3–8.5)	
		Food only (18 month)	1.8 (0.6–4.8)	
		Food + inhalant (18 month)	8.6 (1.5–46.8)	
		Food only (24 month)	2.4 (0.8–7.1)	
		Food + inhalant (24 month)	8.4 (2.3–30.6)	
MAS (21)	5 years	1, 2 and 5 years		–
		Long lasting† *vs* Transient‡	3.4 (No 95% CI), $P < 0.0001$	
		Long lasting *vs* No sensitization	4.7 (No 95% CI)	
DARC (22)	6 years	Early food (3–18 month)	2.0 (0.7–5.0)	Early atopic dermatitis, Family history of allergy, Elevated cord blood IgE, Early wheeze, Male gender, Maternal history of allergy
		Early food + inhalant	2.7 (0.9–7.5)	
GINIplus (23)	6 years	12 month	2.2 (1.3–3.7) Adjusted; 2.1 (1.2–3.7)	Family history of atopic disease, Parental education, Siblings, Gender, Study region, and Type of milk feeding during the first 4 months of life
MAS (28)	7 years	1 and 2 years	4.6 (2.6–8.1) Adjusted; 3.3 (1.7–6.5)	Parental educational level and Study centre
CAPS (32)	8 years	18 months, 3, 5 and 8 years		Sex, study intervention groups
		Mixed food and inhalant§	5.1 (1.8–14.8) Adjusted; 4.2 (1.5–11.7)	

*The definitions of outcome are listed in Table E3 at supplementary documents.
†Long-lasting sensitisation: sensitisation at both 1 and 2 years.
‡Transient sensitisation: sensitisation at 1 or 2 years.
§Mixed food and inhalant (as determined by latent class analysis): Characterized by early and persistently sensitisation to peanut, early, but decreasing sensitisation to egg, and an increasing sensitisation to HDM, Alternaria and grass.

had not considered the intervention from our meta-analyses did not appreciably change the pooled estimates.

The quality of included articles was acceptable as assessed by the NOS. However, in the majority of articles, there was no control for important confounding factors such as family history of allergy, concurrent eczema and wheezing. Most articles were from high-risk allergy cohorts, where it is not necessary to account for a family history of atopy. Family history of atopy was considered as a potential confounder in only two of five population-based cohorts. Eczema in the first 12 months is commonly associated with high levels of food-specific IgE (12) and has been associated with increased risk of asthma (35). Similarly, wheeze has been co-associated with food-specific IgE levels (or food allergy) (36). Thus, considering early life eczema and wheeze as potential confounders is essential for investigating the consequences of food sensitization. Only three articles, however, investigated this. One population-based cohort adjusted for early atopic dermatitis and early wheeze, and this showed a positive association even after these adjustments (22). Conversely, a high-risk cohort (23) reported a null association between food sensitization at 12 months and eczema at 6 years after excluding all children with eczema in the first year. However, this may have underestimated the true association as those who developed food sensitization before eczema may have been excluded. Another high-risk study (13) showed an increase in the incidence rate of eczema up to 7 years in children who had food sensitization at 6 months compared to nonsensitized children among those who did not have eczema in the first six months.

The majority of articles in our review were from high-risk cohorts (eight of fifteen), limiting the generalizability of findings. However, we observed positive associations in five articles from population-based cohorts (21, 22, 28, 33, 34) and the effect estimates from the high-risk *vs* general population studies were almost always similar. Another limitation is that the number of tested food allergens varied across studies. We cannot exclude the possibility of nondifferential misclassification

of the exposure, which may push the estimates towards null in cohorts with fewer tested food allergens. However, cow's milk and hen's egg, which are considered common food sensitizations in the first 2 years of life (37), were tested in all cohorts. A small number of articles defined outcomes as 'ever' (25, 26, 30, 31) instead of 'current' allergic disease. This may lead to imprecise estimation for the effect of early food sensitization as the outcome of interest may develop before, after or at the time of testing food sensitization status. If the disease is not current, the relevance of the observed association with long-term disease burden is also questionable.

The influence of early food sensitization on subsequent eczema and allergic rhinitis has not been investigated beyond childhood in any birth cohorts. Only one British birth cohort investigated the association with subsequent asthma in adulthood, finding an increased risk. However, small sample size and loss to follow-up (only 63 of 100 participants were included in analysis), limited number of food allergens (cow's milk and egg white) and being an atopy high-risk cohort might limit its generalizability (27). Given that the prevalence of asthma and allergic rhinitis increases with age (38), investigating the subsequent effect of early life food sensitization on allergic diseases in birth cohorts over a long period of follow-up is required.

In conclusion, our systematic review supports the hypothesis that food sensitization marches towards other allergies especially childhood wheeze/asthma, eczema and allergic rhinitis. Therefore, early life food sensitization can be used as an early indicator for identifying children at risk of subsequent allergic disease who may benefit from early life preventive strategies. However, due to lack of inclusion of key confounders in many of these studies, lack of follow-up data into adulthood and unclear data on the role of specific food allergens and specific pattern of sensitization, further research is needed to confirm these findings.

Author contributions

SA designed the systematic review and meta-analysis with input from SD, CL and AL and wrote the first draft of the article, BC conducted the parallel systematic search and data entry check. BE provided input to the meta-analysis methods, and CL, KA, BE, AL and SD revised and edited the manuscript and approved the final version.

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Conflict of interest

The authors declare that they have no conflicts of interest.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Search terms in Medline and Scopus databases.
Table S2. Definitions of high-risk cohort.
Table S3. Definitions of outcomes in included studies.
Table S4. NOS quality assessment for included studies.

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Table E1: Search terms in Medline and Scopus databases

Medline **Years**: 1950 - 2014	**Exposure Group: (MeSH heading)** Food or milk or egg or peanut or fish or wheat or soy ***and*** sensitisation or specific immunoglobulin E or skin prick test AND **Outcome Group: (MeSH heading)** Asthma or wheeze or eczema or "hay fever" or "allergic disease*" AND **Inclusion Group:** Cohort or prospective or longitudinal
Scopus **Years**: All years published	**Exposure Group: (Title, abstract and keywords)** Food or milk or egg or peanut or fish or wheat or soy ***and*** sensitisation or specific immunoglobulin E or skin prick test or SPT AND **Outcome Group: (Title, abstract and keywords)** Asthma or “asthma symptoms” or wheeze or eczema or “atopic dermatitis” or "hay fever" or “allergic rhinoconjunctivitis” or “allergic rhinitis” or "allergic disease*" AND **Inclusion Group:** Cohort or prospective or longitudinal

Table E 2: Definitions of high-risk cohort

Cohort	Definition
GINIplus (23)	Positive family history of atopic diseases
PIAMA (24)	Children of mothers with ever asthma, pet allergy, HDM allergy or nasal allergy
MACS (13)	At least one 1st degree relative with history of asthma, eczema, hay fever or food allergy
CAPS (32)	Parents or siblings with current asthma or wheezing
South Wales Birth Cohort (25)	Parental or siblings history of asthma, eczema or hay fever
Swedish Birth Cohort (26)	Positive SPT in parents
Poole Birth Cohort (27)	One or both parents having a history of asthma, hay fever or both
San Diego Birth Cohort (31)	Atopic parents

Table E 3: Definitions of outcomes in included studies.

Name of the cohort	Outcomes definition
MAS (21, 28, 29)	***Asthma at 5Y*** →The presence of one of the following criteria throughout the fifth year of life; 1. At least two reported episodes of wheezing or shortness of breath within the last 12 months, 2. Asthma diagnosed by the family practitioner or the study physician (considering that symptoms were not related to infections or were present especially in pollen season). ***Asthma at 7Y*** → Doctor's diagnosis of asthma and current wheeze at the age of 7 years ***Allergic rhinitis at 5Y*** → The presence of ≥1 throughout the 5th year of life: 1. Reported symptoms of blocked nose and rhinorrhoea not related to infections for 2 to 8 consecutive months and /or 2. Allergic rhinitis diagnosed by the family practitioner or the study physician (considering that symptoms, see above, were not related to infections or were present especially in pollen season) ***Seasonal Allergic rhinitis*** → Reported seasonal symptoms had to be associated with the respective seasonal sensitization to birch or grass pollen
WHEALS (30)	***Asthma*** → Parental report of child's doctor's diagnosis of asthma
DARC (22)	***Asthma*** → A clear subjective or objective response to treatment with b2-agonists and/or inhaled corticosteroids **AND** The presence of one of the following criteria;(i) recurrence of at least two of the three symptoms cough, wheeze and shortness of breath within the previous12 months (symptoms not triggered only by infections), (ii) doctor's diagnosis of asthma prior to follow-up visit (in combination with ongoing treatment), and (iii) symptoms suggestive of asthma within the previous 12 months in combination with a positive exercise or bronchodilator test. ***Eczema*** → According to Hanifin-Rajka criteria ***Allergic rhinitis*** → Two or more separate episodes of one or more typical symptoms from eyes and/or nose (not related to common colds)
GAICS (33)	***Asthma*** → Recurrent wheezing (RW) diagnosed by a pediatric allergist based on a history of more than 2 episodes of physician-diagnosed wheezing ***Eczema*** → diagnosed by a pediatric allergist according to Hanifin and Rajka's minor criteria
PATCH (34)	***Asthma*** → Ever having asthma with the occurrence of wheeze or current use of asthma medication. ***Eczema*** → Pruritic rash over the face and/or extensors with a chronic relapsing course as described by Hanifin and Rajka ***Allergic rhinitis*** → Ever having sneezing, nasal congestion, itching, rhinorrhea or current use of medication for these symptoms
GINIplus (23)	***Asthma*** → Parental report of a physician's diagnosis of asthma in the 12

	months ***Eczema*** → Parental report of a physician's diagnosis of eczema in the 12 months prior to their child turning 6 years ***Allergic rhinitis*** → Parental report of a physician's diagnosis of allergic rhinitis in the 12 months prior to their child turning 6 years)
PIAMA (24)	***Asthma*** → The presence of at least 2 of 3 parental-reported criteria: (1) a doctor's diagnosis of asthma ever, (2) wheezing during the last 12 months, and (3) a prescription of inhaled corticosteroids in the last 12 months.)
MACS (13)	***Eczema*** → Either a doctor diagnosis of eczema or any rash (excluding rash that only affected the nappy region or scalp) that was treated with topical steroid-based preparations
CAPS (32)	***Asthma*** → Wheeze in the last 12 months **AND** either ever-diagnosed asthma (reported at ages 18 months and 3, 5 or 8 years) or airway hyperresponsivenss (at 8 years) ***Eczema*** → Flexural rash at the time of physical assessment or a history of recurrent itchy rash and use of topical treatments in the last 12 months ***Allergic rhinitis*** → Positive response if the child had a problem with sneezing, or a runny, or blocked nose when he/she did not have a cold or the flu in the past12 months.
South Wales Birth Cohort (25)	***Asthma*** → Parental report of child's wheezing or asthma at 3, 6 and 12 months then annually up to the age of 7 years ***Eczema*** → Parental report of child's eczema at 3, 6 and 12 months then annually up to the age of 7 years)
Swedish Birth Cohort (26)	***Asthma*** → Any episode of wheezing or signs of hyper-reactivity reaction or wheezing after exposure to an allergen or respiratory symptoms treated with inhaled ***Eczema*** → According to Hanifin and Rajka criteria
Poole Birth Cohort (27)	***Asthma*** →Current wheeze (yes answer to the question, "Have you had wheezing (loud breathing with a whistling sound coming from the chest) at any time in the last 12 months) **AND** BHR (the maximum cumulative dose of 7.8 µ mol of histamine that caused a reduction in FEV1 by 20%)
San Diego Birth Cohort (31)	***Asthma*** → Physician-documented lower respiratory disorder with reversible bronchospasm, occurring at least twice and unassociated with other anatomic, congenital, or immunologic causes ***Eczema*** → An eczematous eruption associated with at least three of the following four criteria: (1) Pruritis, (2) typical appearance and distribution, (3) a tendency toward chronicity or recurrence, and (4) concurrent specific IgE

Table E 4: NOS quality assessment for included studies.

First author (Cohort)		S. Havstad (WHEALS)	C. Chiu (PATCH)	M. Bekkers (PIAMA)	F. Garden (CAPS)	N. Kawamoto (GAICS)	L. Söderström	I. Brockow (GINIplus)	H. Kjaer (DARC)
Selection	1) Representativeness of the exposed cohort								
	a) truly representative	★ Children born between 2003 and 2007 in Detroit and surrounding suburbs, USA	★ Children born between 2007 and 2010 in Taiwan	★ Children born between 1996 and 1997 in Netherland	★ Children born between 1997 and 2000 in Sydney, Australia	★ Children born between 2004 and 2005 in Gifu, Japan	★ Children born between 1997 and 2000 in Stockholm, Sweden	★ Children born between 1995 and 1998 in Germany	★ Children born between 1998 and 1999 in Germany
	b) somewhat representative	NA	NA	NA	NA	NA	NA	NA	NA
	c) selected group of users	NA	NA	NA	NA	NA	NA	NA	NA
	d) no description of the derivation of the cohort	NA	NA	NA	NA	NA	NA	NA	NA
	2) Selection of the non-exposed cohort								
	a) drawn from the same community as the exposed cohort	★ Exposed group from the same community	★ Exposed group from the same community	★ Exposed group from the same community	★ Exposed group from the same community	★ Exposed group from the same community	★ Exposed group from the same community	★ Exposed group from the same community	★ Exposed group from the same community
	b) drawn from a different source	NA	NA	NA	NA	NA	NA	NA	NA
	c) no description of the derivation of the non-exposed cohort	NA	NA	NA	NA	NA	NA	NA	NA
	3) Ascertainment of exposure								
	a) secure record (eg surgical records)	★ Laboratory records for	★Laboratory records for	★ Laboratory records for	★ Clinical records for	★ Laboratory records for	★ Laboratory records for	★ Laboratory records for	★ Laboratory and clinical

		blood samples	blood samples	blood samples	SPT	blood samples	blood samples	blood samples	records for blood samples
	b) structured interview	NA	NA	NA	NA	NA	NA	NA	NA
	c) written self-report	NA	NA	NA	NA	NA	NA	NA	NA
	d) no description	NA	NA	NA	NA	NA	NA	NA	NA
	4) Demonstration that outcome of interest was not present at start of study								
	a) yes	★ Birth cohort and outcomes appear later in life	★ Birth cohort and outcomes appear later in life	★ Birth cohort and outcomes appear later in life	★ Birth cohort and outcomes appear later in life	★ Birth cohort and outcomes appear later in life	★ Birth cohort and outcomes appear later in life	★ Birth cohort and outcomes appear later in life	★ Birth cohort and outcomes appear later in life
	b) no	NA	NA	NA	NA	NA	NA	NA	NA
Comparability	1) Comparability of cohorts on the basis of the design or analysis								
	a) Family history of atopy	Not adjusted for family history of atopy	Not adjusted for family history of atopy	★ Adjusted for parental allergy	★ Asthma high risk cohort	Not adjusted for family history of atopy	★ Atopy high risk cohort	★ Adjusted for family history of atopy	★ Adjusted for maternal history of allergy
	b) Early life eczema or wheezing	Not adjusted for early life wheezing or eczema	Not adjusted for early life wheezing or eczema	Not adjusted for early life wheezing or eczema	Not adjusted for early life wheezing or eczema	Not adjusted for early life wheezing or eczema	Not adjusted for early life wheezing or eczema	★ Early life eczema was considered	★ Early life eczema was considered
Outcome	1) Assessment of outcome								
	a) independent blind assessment	-	-	-	-	-	-	-	-

	b) record linkage	-	-	-	-	-	-	-	-
	c) self-report	Data on asthma extracted by parental-reported questionnaires	Data on allergic diseases extracted by questionnaires and pediatric pulmonologist	Data on asthma extracted by parental-reported questionnaires	Data on allergic diseases extracted by parental-reported questionnaires	Data on allergic diseases extracted by pediatric allergist	Data on allergic diseases extracted by pediatric allergist	Data on allergic diseases extracted by parental-reported questionnaires	Data on allergic diseases extracted by parental-reported questionnaires
	d) no description	NA	NA	NA	NA	NA	NA	NA	NA
	2) Was follow-up long enough for outcomes to occur								
	a) yes	★ 4 years follow up	★ 4 years follow up	★ 11 years follow up	★ 8 years follow up	★ 14 months follow up	★ 5 years follow up	★ 6 years follow up	★ 6 years follow up
	b) no	NA	NA	NA	NA	NA	NA	NA	NA
	3) Adequacy of follow up of cohorts								
	a) complete follow up	Loss to follow up	Loss to follow up	NA	Loss to follow up	Loss to follow up	Loss to follow up	Loss to follow up	Loss to follow up
	b) subjects lost to follow up unlikely to introduce bias	Both analyzed and non-analyzed children were comparable	NA	NA	Lost to follow-up had younger and less educated parents	Both included and non-included children were comparable	NA	Lost to follow-up were from less educated parents with more siblings	NA
	c) follow up rate	47% included in the analysis	NA	NA	83% at 8 years	54% included in the analysis	90% at 5 years	57% included in the analysis	68% included in the analysis
	d) no statement	NA	No statement	No statement	NA	NA	NA	NA	NA

First author (Cohort)		S. Illi (MAS)	H. Rhodes	M. BURR	R. Zeiger	M. Kulig (MAS)	M. Kulig (MAS)	A. Lowe (MACS)
Selection	1) Representativeness of the exposed cohort							
	a) truly representative	★ Children born during 1990 in 5 German cities.	★ Children born between 1976 and 1977 in Poole, UK	★ Children born in two areas of south Wales	★ Families from the Kaiser Foundation Health Plan of San Diego, USA	★ Children born during 1990 in 5 German cities.	★ Children born during 1990 in 5 German cities	★ Children born between 1990 and 1994 in Melbourne, Australia
	b) somewhat representative	NA	NA	NA	NA	NA	NA	NA
	c) selected group of users	NA	NA	NA	NA	NA	NA	NA
	d) no description of the derivation of the cohort	NA	NA	NA	NA	NA	NA	NA
	2) Selection of the non-exposed cohort							
	a) drawn from the same community as the exposed cohort	★ Exposed group from the same community	★ Exposed group from the same community	★ Exposed group from the same community	★ Exposed group from the same community	★ Exposed group from the same community	★ Exposed group from the same community	★ Exposed group from the same community
	b) drawn from a different source	NA	NA	NA	NA	NA	NA	NA
	c) no description of the derivation of the non-exposed cohort	NA	NA	NA	NA	NA	NA	NA
	3) Ascertainment of exposure							

	a) secure record (eg surgical records)	★ Laboratory records for blood samples	★ Clinical records for SPT	★ Clinical records for SPT	★ Clinical records for SPT	★ Laboratory records for blood samples	★ Laboratory records for blood samples	★ Clinical records for SPT
	b) structured interview	NA	NA	NA	NA	NA	NA	NA
	c) written self-report	NA	NA	NA	NA	NA	NA	NA
	d) no description	NA	NA	NA	NA	NA	NA	NA
	4) Demonstration that outcome of interest was not present at start of study							
	a) yes	★ Birth cohort and outcomes appear later in life	★ Birth cohort and outcomes appear later in life	★ Birth cohort and outcomes appear later in life	★ Birth cohort and outcomes appear later in life	★ Birth cohort and outcomes appear later in life	★ Birth cohort and outcomes appear later in life	★ Birth cohort and outcomes appear later in life
	b) no	NA	NA	NA	NA	NA	NA	NA
Comparability	1) Comparability of cohorts on the basis of the design or analysis							
	a) Family history of atopy	★ Stratified the analysis by family history of atopy or asthma	★ Atopy high risk cohort	★ Atopy high risk cohort	★ Adjusted for history of parental atopy	★ Stratified the analysis by family history of atopy or asthma	Not adjusted for family history of atopy	★ Adjusted for parental history of eczema
	b) Early life eczema or wheezing	Not adjusted for early life wheezing or eczema	Effect of early life wheezing and eczema was assessed separately from exposure	Not adjusted for early life wheezing or eczema	Not adjusted for early life wheezing or eczema	Not adjusted for early life wheezing or eczema	Effect of early life wheezing and eczema was assessed separately from exposure	★ Early life eczema was considered

Outcome	1) Assessment of outcome							
	a) independent blind assessment	-	-	-	-	-	-	-
	b) record linkage	-	-	-	-	-	-	-
	c) self-report	Data on asthma extracted by parental-reported questionnaires	Data on asthma extracted by parental-reported questionnaires	Data on allergic diseases extracted by parental-reported questionnaires	Data on allergic diseases extracted by parental-reported questionnaires	Data on allergic diseases extracted by pediatric allergist	Data on allergic rhinitis extracted by parental-reported questionnaires	Data on eczema extracted by parental-reported questionnaires
	d) no description	NA	NA	NA	NA	NA	NA	NA
	2) Was follow-up long enough for outcomes to occur							
	a) yes	★ 7 years follow up	★ 22 years follow up	★ 7 years follow up	★ 7 years follow up	★ 5 years follow up	★ 7 years follow up	★ 7 years follow up
	b) no	NA	NA	NA	NA	NA	NA	NA
	3) Adequacy of follow up of cohorts							
	a) complete follow up	Loss to follow up	Loss to follow up	Loss to follow up	Loss to follow up	Loss to follow up	Loss to follow up	Loss to follow up
	b) subjects lost to follow up unlikely to introduce bias	Lost to follow-up were from less educated and smoker parents and without history of atopy.	Lost to follow-up had parental history of asthma	NA	Both included and non-included children were comparable	Both analyzed and non-analyzed children were comparable	Both analyzed and non-analyzed children were comparable	NA

	c) follow up rate	81% included in the analysis	63% included in the analysis	88% at 7 years	NA	NA	71% included in the analysis	89% included in the analysis
	d) no statement	NA	NA	NA	NA	NA	NA	NA

CHAPTER 6 - Association between food sensitisation and subsequent asthma and allergic rhinitis during late childhood and adolescence

6.1. Chapter introduction

This chapter includes an accepted paper for publication in the *Pediatric Allergy and Immunology Journal* entitled "Is there a march from early food sensitisation to later childhood allergic airway disease? Results from two prospective birth cohort studies" (with data supplement) as well as the unpublished original analysis from the MACS study investigating the association between early life food sensitisation and asthma and allergic rhinitis at 18 years.

The accepted paper examines the association between food with or without aeroallergen sensitisation in the first two years of life and subsequent asthma and allergic rhinitis at 10-12 years, in both the allergy high-risk cohort (MACS) and the population-based cohort (LISAplus).

Findings from this chapter provide important information for predicting the risk of asthma and allergic rhinitis during late childhood and adolescence in individuals who had early life food sensitisation. Additionally, they provide evidence that supports the role of early life food sensitisation in the atopic march.

6.2. Association between early life food sensitisation and asthma and allergic rhinitis at 10-12 years: Results from the MACS and LISAplus cohorts

6.2.1. Main document and data supplement

Alduraywish SA, Standl M, Lodge CJ, Abramson MJ, Allen KJ, Erbas B, Berg Av, Heinrich J, Lowe AJ, Dharmage SC. Is there a march from early food sensitization to later childhood allergic airway disease? Results from two prospective birth cohort studies. *Pediatric Allergy and Immunology*. *Accepted date 01 Sep 2016.*

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Title page

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Abstract page

Alduraywish S, Standl M, Lodge C, Abramson M, Allen K, Erbas B, Berg A, Heinrich A, Lowe A, Dharmage S C

Title: Is there a march from early food sensitization to later childhood allergic airway disease? Results from two prospective birth cohort studies

Title of Journal: Pediatr Allergy Immunol

Abstract:

Background: The march from early aeroallergen sensitization to subsequent respiratory allergy is well established, but it is unclear if early life food sensitization precedes and further increases risk of allergic airway disease.

Objective: To assess the association between food sensitization in the first 2 years of life and subsequent asthma and allergic rhinitis by age 10-12 years.

Methods: We used data from two independent cohorts: the high-risk MACS (n=620) and the population-based LISAplus (n= 3094). Food sensitization was assessed at 6, 12 and 24 months in MACS and 24 months in LISAplus. Multiple logistic regressions were used to estimate associations between sensitization to food only, aeroallergen only or both and allergic airway disease.

Results: When compared to non-sensitized children, sensitization to food only at 12 months in MACS and 24 months in LISAplus was associated with increased risk of current asthma (aOR=2.2; 95%CI 1.1, 4.6 in MACS and aOR=4.9; 2.4,10.1 in LISAplus). Similar results were seen for allergic rhinitis. Additionally, co-sensitization to food and aeroallergen in both cohorts at any tested point was a stronger predictor of asthma (at 24 months, aOR=8.3; 3.7, 18.8 in MACS and aOR=14.4; 5.0, 41.6 in LISAplus) and allergic rhinitis (at 24 months, aOR=3.9;1.9,8.1 in MACS and aOR=7.6;3.0,19.6 in LISAplus).

Conclusions: In both cohorts, food sensitization (with or without aeroallergen sensitization) in the first two years of life increased the risk of subsequent asthma and allergic rhinitis. These findings support the role of early life food sensitization in the atopic march and suggest trials to prevent early onset have the potential to reduce the development of allergic airways disease.

Key words: Allergic rhinitis, Asthma, Atopy, Food Sensitization

Abbreviation:

aOR: Adjusted odd ratio

LISAplus: Influence of Life-style related factors on the development of the Immune System and Allergies in East and West Germany study

MACS: Melbourne Atopic Cohort Study

S-IgE: serum specific immunoglobulin E

SPT: Skin Prick Testing

Introduction

Over the past 50 years there has been a global epidemic of asthma, eczema and allergic rhinitis, especially in developed countries (1). Over the last 20 years, evidence suggests a second wave of the allergy epidemic with an increase in the prevalence of food allergies (2, 3). These allergic disorders pose a substantial health burden on affected individuals, their families and healthcare resources (4-6). Given the overlap between allergic disorders, the link between them has been a major research focus.

The “atopic march” refers to progression of allergic phenotype from early life eczema into later asthma and allergic rhinitis, which has been supported by many longitudinal and cross-sectional studies (7-9). There is increasing interest in these longitudinal relationships because the information could contribute in identifying early interventions and reduce burden of these disorders. Eczema is commonly associated with atopic sensitization, as assessed by either skin prick test (SPT) or serum specific IgE in vitro (s-IgE) (10). Previous studies suggest that eczema along with atopy is considered as a major risk factor for progression in the atopic march (11). Data from Melbourne Atopic Cohort Study (MACS) showed that children with atopic eczema in the first two years of life had a greater risk of asthma and allergic rhinitis at 6 and 7 years when compared with children with non-atopic eczema (9).

Although several epidemiological studies have shown that early aeroallergen sensitization is related to increased risk of allergic diseases in children (12-15) and adults (16), the role of food sensitization is less clear. Considering that food sensitization tends to develop earlier than aeroallergen sensitization, measuring food sensitization in the early years of life may allow earlier prediction of childhood and adolescent-onset allergic airway disease and potentially target intervention strategies in early life.

Food sensitization has been hypothesised to be related to development of other allergic diseases including asthma, allergic rhinitis (8, 17, 18) and food allergy (19). Although a number of epidemiological studies have assessed the association between food sensitization and subsequent asthma and/or allergic rhinitis up to seven years (12, 20, 21), only a few cohorts have assessed these associations beyond this age (22-24). However, concomitant early life eczema and/or wheeze have not been considered in most studies.

We conducted prospective analyses of two independent cohorts: the high-risk Australian based MACS cohort and the population based Influence of Life-style related factors on the development of the Immune System and Allergies in East and West Germany plus the influence of traffic emissions and genetics (LISAplus) cohort. We investigated the association between food sensitization, with or

without aeroallergen sensitization, at different time points in the first two years of life and risk of allergic airway disease by age 10-12 years, whilst taking into account various confounding factors.

Methods

Study populations

MACS began as a randomized controlled trial investigating the effect of three different infant formulas (cow's milk, partially hydrolysed whey and standard soy formulas) introduced at the time of weaning on the occurrence of allergic diseases. A total of 620 infants born between 1990 and 1994 were recruited from antenatal clinics at the Mercy Maternity Hospital in Melbourne, Australia. Children were eligible if they had a first degree relative with asthma, eczema, hay fever or food allergy. Baseline information was collected during pregnancy.

Telephone-based interviews were conducted every 4 weeks until 15 months, at 18 months, 2 years then annually up to the age of 7 years, then at 12 and 18 years. At the 12 year follow-up, the mean (±SD) age of participants was 11.5±2 years. The study was approved by the Human Research Ethics committees of the Mercy Maternity Hospital and University of Melbourne. Written informed consent was obtained from all mothers and/or participants.

The baseline demographics of MACS participants have been published previously (9). Briefly, parents of MACS children were mainly Australian born (83% of fathers and 87% of mothers) and well educated (61% of fathers and 59% of mother had higher education).

Using data from randomized controlled trials to test additional hypotheses about the association between non-randomized exposures and outcomes determined during long-term follow-up is a well-established method. It is based on the testable assumption that the randomized intervention does not influence the associations of interest (25). A previous MACS publication showed that the randomization status (infant formula allocation) was not associated with the outcome of interest (26),

therefore MACS continues as an observational study. Despite this, the effect of an intervention formula (by intention to treat at baseline) was considered as a potential confounder in all analyses.

LISAplus is a German population based birth cohort study that recruited 3094 neonates between 1997 and 1999 from the cities of Munich, Leipzig, Wesel and Bad Honnef. Questionnaires were completed by parents at birth, 6 months, 1, 1.5, 2, 4, 6 and 10 years of age. Details of the study design have been described elsewhere (27). The study was approval by the local Ethics Committees (Bavarian Board of Physicians, University of Leipzig, and Board of Physicians of North-Rhine-Westphalia) and written parental consent was obtained.

The demographic characteristics for the initial LISAplus cohort have been described in previous publications (27).

Sensitization assessment

In MACS, Skin Prick Tests (SPT) were performed at 6, 12 and 24 months, according to a standard technique (28). Allergens tested included egg white, cow's milk, peanut, house dust mite *(Dermatophagoides pteronyssinus)*, rye grass (*Lolium perenne*) and cat dander (Bayer, Spokane, WA, USA). A positive histamine control (1 mg/mL) was used. SPTs were read at 15-20 minutes. Wheal size was measured by calculating the mean length of the longest and perpendicular wheal diameters (15). Sensitization was defined as wheal size of ≥2mm (29).

In LISAplus, blood samples were collected at the age of 2 years. Serum-specific IgE antibodies (s-IgE) were measured using a mix of common food allergens (FX5: hen's egg, cow's milk, peanut, wheat flour, soybean, and codfish). If the specific IgE exceeded 0.35 kU_A/L, egg white, milk protein and peanuts protein were tested separately. The inhalant allergens HX2 (mite), E1 (cat), MX1 (mold),

RX1 (pollen) were tested separately. Standardised methods with the CAP System FEIA (Pharmacia Diagnostics, Freiburg, Germany) were used. Sensitization was defined as an IgE antibody level ≥0.35 kU_A/L.

Outcome definitions

Allergic outcomes were defined by questionnaire responses at age 10 year follow-up in LISAplus and at 12 year follow-up in MACS.

Current asthma

MACS defined current asthma as one or more episodes of asthma and/or the use of any asthma medications in the last 12 months. LISAplus defined current asthma as doctor diagnosis of asthma at the age of 10 years or intake of asthma medication during the past 12 months.

Current Allergic rhinitis

MACS defined current allergic rhinitis as one or more episodes of allergic rhinitis and/or the use of any treatment for allergic rhinitis in the last 12 months. LISAplus defined allergic rhinitis as doctor diagnosis of seasonal and/or perennial rhinitis at the age of 10 years.

Confounder definitions

Eczema by the age at which the sensitization was assessed:

Defined as doctor diagnosis or treatment of rash with topical steroid (excluding nappy or scalp areas) by the age of SPT in MACS and as doctor diagnosis of eczema in the past 6 months and/or rash in the past 6 months asked at the age of 2 years in LISAplus.

Wheeze by the age at which the sensitization was assessed:

Defined if the response to the following question was >5 days "How many days of cough and/or chest rattle and/or wheeze has your child had in the past 4 weeks?" in MACS and according to the response to the following question "In the past 6 months, has your child had whistling or wheezy sound of breathing in the chest?" asked during the follow-up at age 2 years in LISAplus.

Statistical Analysis

Logistic regression models were constructed with asthma or allergic rhinitis as dependent variables and food and/or aeroallergen sensitization at 6, 12 or 24 months (in MACS) or at 24 months (in LISAplus) as independent variables. The predictive evaluation of sensitization was based on four groups: (1) no sensitization, (2) food sensitization only, (3) aeroallergen sensitization only and (4) sensitization to both food and aeroallergen. In MACS, the associations were evaluated at each time point separately, irrespective of previous status of sensitization. All models were adjusted for sex, maternal smoking during pregnancy, parental level of education, exclusive breastfeeding for at least 4 months (30) and eczema by the age of sensitization. The association between atopic sensitization and asthma was further adjusted for wheeze by the age at the assessment of sensitization.

Additional adjustment for formula allocation was performed in MACS analyses and for study center and family history of allergy (whether mother, father or a sibling ever had asthma, eczema or hay fever; asked at birth) in LISAplus. An interaction between allergic sensitization and family history of allergy was tested in LISAplus.

Results are presented as crude and adjusted Odd Ratios (OR) and 95% confidence intervals. All statistical tests were two sided with a p value of <0.05 considered as statistically significant. STATA 13 (StataCorp, College Station TX) was used in all analyses in MACS and R version 3.1.0 was used for all analyses in LISAplus (31).

Results

Characteristics of the study populations

The characteristics for analyzed MACS participants are presented in **Table 1**. At all time points tested in MACS, egg white was the commonest food allergen (13%, 18% and 15% at 6, 12 and 24 months, respectively), while house dust mite was the most prevalent aeroallergen (5%, 12% and 23% at 6, 12 and 24 months, respectively. With the exception of maternal education and paternal smoking, MACS participants who did not attend the 12 year follow-up were similar on all demographic characteristics and early life sensitization compared to those who did attend **(see Table E1 in the Online Repository)**.

The characteristics for participants analyzed from LISAplus (630, 292, 138 and 120 participants from Munich, Leipzig, Bad Honnef and Wesel, respectively) are presented in **Table 1.** At 24 months in LISAplus, the major food allergens were egg white and milk protein (5% each) and the major aeroallergen was house dust mite (3%). Apart from parental education, maternal smoking, number of older siblings, a sibling ever having asthma and parental history of food allergy or hay fever, LISAplus participants who did not attend the 10 year follow-up were similar on other demographic characteristics and sensitization at 24 months compared to those who did attend **(see Table E1 in the Online Repository).**

Atopic sensitization and allergic airway diseases

Food sensitization and asthma and allergic rhinitis

In MACS, infants who were sensitized to food allergens without concurrent aeroallergen sensitization at 12 months had an increased risk of current asthma and allergic rhinitis compared to non-sensitized infants. While there were similar trends at 6 and 24 months, these were not significant (**Tables 2&3)**. Additionally, children who had co-sensitization to both food and aeroallergen at 6, 12 or 24 months

had increased risk of current asthma and allergic rhinitis. These associations, at all three time points, appeared stronger than sensitization to food without sensitization to aeroallergen (**Tables 2&3)**.

In LISAplus, children who were sensitized to food without concurrent aeroallergen at 24 months had an increased risk of current asthma and allergic rhinitis compared to non-sensitized children (**Tables 2&3)**. Similar to MACS, the strongest effect on asthma and allergic rhinitis risk was observed in children who had co-sensitization to food and aeroallergen at 24 months (**Tables 2&3)**.

Aeroallergen only sensitization and asthma and allergic rhinitis

In MACS, sensitization to aeroallergen without food at 12 months, but not at 6 months was associated with increased risk of current asthma and allergic rhinitis (**Tables 2&3)**. Moreover, children who had aeroallergen without food sensitization at 24 months in both cohorts had increased risks of current asthma and allergic rhinitis and these associations were weaker than sensitization to both food and aeroallergen (**Tables 2&3)**.

None of the above associations were modified by family history of allergy in LISAplus study (i.e. *p* value of interaction was >0.1).

Discussion

Our study has shown that food sensitization in the first two years, independent of early life eczema and wheeze, predicts asthma and allergic rhinitis in later childhood. Additionally, co-sensitization to both food and aeroallergen was the strongest predictor at any time point tested. Our findings were mostly consistent across two cohorts, where data were available, with different populations in relation to co-sensitization to food and aeroallergen and sensitization to aeroallergen only. The MACS is an Australian cohort of children with a family history of allergic diseases while LISAplus is a German

population-based cohort. Interestingly, our findings were similar among those with and without a family history within the LISAplus study.

Earlier studies have established the role of aeroallergen sensitization on development of allergic airway diseases (13-16). We established that food sensitization itself was related to subsequent increased risk of asthma and allergic rhinitis. The current analysis compared the effect of different mutually exclusive groups of atopic sensitization on asthma and allergic rhinitis in later childhood allowing us to draw stronger conclusions on the different patterns of sensitization. Few studies have investigated the role of early life food sensitization on development of asthma and allergic rhinitis beyond the age of 7 years (22-24). Bekkers and colleagues (24) showed that egg, but not cow's milk sensitization at 12 months was associated with increased risk of asthma up to the age of 10 years. However, whether the observed effect was due to sensitization to food alone without concurrent aeroallergen was not assessed. Additionally, potential confounding by early life eczema and/or wheeze was not considered in this analysis.

Our study showed that food without aeroallergen sensitization at 24 months was associated with increased risks of asthma and allergic rhinitis in the LISAplus study but not in MACS. This appears to be due, at least in part, to only a small number of MACS participants only having food sensitization at 24 months compared to the LISAplus study. In contrast, findings related to aeroallergen without food sensitization and co-sensitization to foods and aeroallergen were consistent across both cohorts.

We found also that children co-sensitized to common foods and aeroallergen at any time point tested had a markedly higher risk of developing respiratory allergic diseases than sensitization to food or aeroallergen alone when compared to non-sensitized children. Few longitudinal studies have assessed the association between co-sensitization to food and aeroallergen and development of atopic diseases in childhood. A German study by Illi *et al.* (32) showed that concurrent sensitization to food and aeroallergen was the strongest predictor for asthma up to school age. In an Australian study, Garden *et al.* (23) found that mixed food and inhalant sensitization phenotype had the strongest associations

with allergic disease at the age of 8 years, and particularly with asthma. However, co-manifestation of early life wheeze and/or eczema was not been taken into account in the analysis and there was a shorter period of follow-up.

Eczema in early life is commonly associated with high levels of food specific IgE (33), and has been associated with increased risk of later asthma and allergic rhinitis (9). Similarly, wheeze has been related to food specific IgE (34). When early life eczema and/or wheezing, reported at the same age of testing for atopic sensitization, were considered in our analysis, the associations between atopic sensitization and allergic outcomes did not change significantly. This suggests that the role of early life sensitization on later childhood allergic airway diseases is independent of eczema and/or wheezing.

The strengths of this study are that we have analyzed longitudinal data from two independent cohorts with long periods of follow-up (extending from infancy to later childhood), the relatively large sample sizes and early objective assessment of sensitization to common allergens. These cohorts were from two different regions of the world, but both were high income countries with high prevalences of food sensitization (35) and allergic diseases (36-38). It is often assumed that results from a high-risk cohort may not be applicable to the general population, but interestingly our results were similar across the two cohorts. Also, family history of allergic diseases did not modify our associations in the population-based cohort suggesting that family history may not be a major modifier of risk and that other factors should be considered, for example environmental exposures.

This study has a number of limitations. Our analyses were based on longitudinal data and loss to follow-up needs to be considered when interpreting the results as it could be a potential source of bias. However, these studies achieved a 57% attendance at the 10 year follow-up in LISAplus and 59% attendance at the 12 year follow-up in MACS. In addition, apart from parental education and paternal smoking, there were no significant demographic and/or early sensitization differences between children who did and did not attend in either cohort. Although the reported paternal history of food allergy and hay fever was higher in those who attended the 10 years follow-up in LISAplus, this was

unlikely to bias findings. Another possible limitation is that the definitions of asthma and allergic rhinitis were based on questionnaire data. However, these definitions have been commonly used in epidemiological studies (20, 39). The findings of this study could have been strengthened if participants were examined for objective evidence of asthma and allergic rhinitis. Moreover, we were unable to establish the associations between specific allergen sensitization and subsequent development of asthma and allergic rhinitis due to limited statistical power.

Previous studies have observed that positive SPT reactions are likely to be smaller in infants and children younger than two years (40) presumably because of a relative lack of allergen-specific IgE and skin reactivity (41). Therefore, in the MACS cohort, a 2 mm cut-off point was used to define positive skin prick test reactions at 6, 12 and 24 months (42).

We acknowledge that food sensitization was assessed by SPT in MACS and by serum IgE in LISAplus. These two methods are commonly used to evaluate sensitization in epidemiological studies (43). Many studies have assessed sensitization as a predictor of allergic diseases, either by SPT (12, 22, 23) or s-IgE (12, 20, 24, 44). A recent study by Ro *et al.* (45) showed that the predictive value of SPT and s-IgE performed at 2 years of age was generally comparable in predicting allergic diseases in later childhood.

In conclusion, assessment of food sensitization in infants provides valuable information on the risk of later childhood asthma and allergic rhinitis. Additionally, we provide evidence for the role of early life food sensitization with or without co-sensitization to aeroallergen, independent of early life eczema, on the atopic march. Developing interventions that prevent early life food sensitization, such as food allergen avoidance or dietary modification, may reduce the likelihood of atopic march to asthma and allergic rhinitis occurring.

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The LISAplus Study Group consists of the following: Helmholtz Zentrum München, German Research Center for Environmental Health, Institute of Epidemiology, Munich (Heinrich J, Wichmann HE, Sausenthaler S, Chen CM, Schnappinger M); Department of Pediatrics, Municipal Hospital "St.Georg", Leipzig (Borte M, Diez U), Marien-Hospital Wesel, Department of Pediatrics, Wesel (von Berg A, Beckmann C, Groß I); Pediatric Practice, Bad Honnef (Schaaf B); Helmholtz Centre for Environmental Research – UFZ, Department of Environmental Immunology/Core Facility Studies, Leipzig (Lehmann I, Bauer M, Gräbsch C, Röder S, Schilde M); University of Leipzig, Institute of Hygiene and Environmental Medicine, Leipzig (Herbarth O, Dick C, Magnus J); IUF – Leibniz Research Institute for Environmental Medicine, Düsseldorf (Krämer U, Link E, Cramer C); Technical University Munich, Department of Pediatrics, Munich (Bauer CP, Hoffmann U); ZAUM - Center for Allergy and Environment, Technical University, Munich (Behrendt H, Grosch J, Martin F).

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Conflict of interest

No conflict of interest to declare.

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Table 1: Characteristics of participants analyzed from the MACS and LISAplus studies

	MACS			LISAplus
	6 Months	**12 Months**	**24 Months**	**24 Months**
	N*=335	N*=343	N*=307	N= 1180
	n (%)	n (%)	n (%)	n (%)
Sex				
Male	180 (54)	180 (52)	163 (53)	617 (52)
Female	155 (46)	163 (48)	144 (47)	563 (48)
Family history of allergy				
Yes	(100)	(100)	(100)	734 (62)
Maternal smoking during pregnancy				
Yes	18 (5)	20 (6)	19 (6)	148 (13)
Parental education level				
high	247 (74)	246 (72)	227 (74)	875 (74)
Exclusive breastfeeding ≥ 4m				
No	187 (56)	192 (56)	170 (55)	459 (39)
Yes	147 (44)	151 (44)	137 (45)	721 (61)
Current allergic disease**				
Asthma				
No	255 (76)	261 (76)	235 (76)	1118 (95)
Yes	80 (24)	82 (24)	72 (24)	62 (5)

Allergic rhinitis				
No	212 (63)	214 (62)	193 (63)	1057 (90)
Yes	123 (37)	129 (38)	114 (37)	123 (10)

* N represents the number of participants who had available data on both sensitization and allergic diseases (asthma and allergic rhinitis).

**At 10Y in LISAplus and 12Y in MACS.

Table 2: The association between food only, aeroallergen only and both food and aeroallergen sensitization and asthma in MACS and LISAplus.

Cohort	Atopic sensitization	n (%)	Asthma‡				
			Prevalence* (%)	Crude OR (95%CI)	*p*	Adjusted** OR (95% CI)	*p*
MACS	**6 months**						
	Non-sensitized	251 (75)	18	-		-	
	Food only	49 (15)	31	1.9 (1.0, 3.9)	0.05	1.8 (0.9, 3.8)	0.08
	Aero only	14 (4)	36	2.5 (0.8, 7.7)	0.11	2.2 (0.7, 7.4)	0.18
	Food and aero	21 (6)	67	8.9 (3.4, 23.3)	**<0.01**	6.1 (2.3, 16.7)	**<0.01**
	12months						
	Non-sensitized	233 (68)	15	-		-	
	Food only	48 (14)	31	2.6 (1.3, 5.2)	**<0.01**	2.2 (1.1, 4.6)	**0.03**
	Aero only	25 (7)	48	5.2 (2.2, 12.4)	**<0.01**	5.1 (2.1, 12.3)	**<0.01**
	Food and aero	36 (11)	53	6.3 (2.9, 13.3)	**<0.01**	5.6 (2.5,12.3)	**<0.01**
	24 months						
	Non-sensitized	192 (63)	13	-		-	
	Food only	22 (7)	23	2.1 (0.7, 6.1)	0.19	1.7 (0.6, 5.4)	0.34
	Aero only	53 (17)	40	4.6 (2.3, 9.2)	**<0.01**	4.9 (2.4, 10.2)	**<0.01**
	Food and aero	39 (13)	54	8.2 (3.8, 17.4)	**<0.01**	8.3 (3.7,18.8)	**<0.01**

LISAplus	**24 months**						
	Non-sensitized	1041 (88)	3	-		-	
	Food only	94 (8)	14	4.6 (2.3,9.1)	**<0.01**	4.9 (2.4,10.1)	**<0.01**
	Aero only	25 (2)	24	9.1 (3.4,24.1)	**<0.01**	10.2 (3.6,28.5)	**<0.01**
	Food and aero	20 (2)	40	19.2 (7.4,49.8)	**<0.01**	14.4 (5,41.6)	**<0.01**

‡At age 10 year follow-up in the LISAplus study and at 12 year follow-up in the MACS study.

*Prevalence refers to the proportion of individuals who developed asthma in each sensitization group.

** Adjusted for maternal smoking during pregnancy, parental level of education, sex, exclusive breast feeding for at least 4 months, eczema and wheeze by the age of sensitization assessment and formula allocation (in MACS only) and study center and family history of allergy (in LISAplus only).

Table 3: The association between food only, aeroallergen only and both food and aeroallergen sensitization and allergic rhinitis in MACS and LISAplus

Cohort	**Atopic sensitization**	n (%)	**Allergic Rhinitis‡**				
			Prevalence* (%)	Crude OR (95%CI)	*p*	Adjusted OR (95% CI)	*p*
MACS	**6 months**						
	Non-sensitized	251 (75)	34	-		-	
	Food only	49 (15)	38	1.2 (0.6, 2.2)	0.61	1.1 (0.5, 1.9)	0.93
	Aero only	14 (4)	43	1.4 (0.5, 4.2)	0.51	1.3 (0.4, 4.0)	0.67
	Food and aero	21 (6)	62	3.1 (1.2, 7.8)	**0.01**	2.8 (1.0, 7.7)	**0.04**
	12months						
	Non-sensitized	233 (68)	30	-		-	
	Food only	48 (14)	48	2.1 (1.1, 3.9)	**0.01**	2.1 (1.1, 3.9)	**0.02**
	Aero only	25 (7)	52	2.5 (1.1, 5.8)	**0.02**	2.4 (1.0, 5.6)	**0.03**
	Food and aero	36 (11)	62	3.8 (1.8, 7.8)	**< 0.01**	3.6 (1.7, 7.7)	**< 0.01**
	24 months						
	Non-sensitized	192 (63)	30	-		-	

	Food only	22 (7)	36	1.4 (0.5, 3.4)	0.52	1.3 (0.5, 3.3)	0.60
	Aero only	53 (17)	45	1.9 (1.1, 3.6)	**0.03**	1.9 (0.9, 3.5)	**0.05**
	Food and aero	39 (13)	63	3.9 (1.9, 8.0)	**< 0.01**	3.9 (1.9, 8.1)	**< 0.01**
LISAplus	**24 months**						
	Non-sensitized	1041 (88)	8	-		-	
	Food only	94 (8)	20	2.7 (1.6,4.7)	**< 0.01**	2.8 (1.6,4.8)	**< 0.01**
	Aero only	25 (2)	24	3.4 (1.3,8.8)	**0.01**	3.0 (1.1, 7.9)	**0.03**
	Food and aero	20 (2)	50	10.8 (4.4, 26.7)	**< 0.01**	7.6 (3.0, 19.6)	**< 0.01**

‡At age 10 year follow-up in the LISAplus study and at 12 year follow-up in the MACS study.

*Prevalence refers to the proportion of individuals who developed allergic rhinitis in each sensitization group.

** Adjusted for maternal smoking during pregnancy, parental level of education, sex, exclusive breast feeding for at least 4 months, eczema by the age of sensitization assessment and formula allocation (in MACS only) and study center and family history of allergy (in LISAplus only)

Table E1: Baseline characteristics of attendees and non-attendees to the LISAplus study at age 10 follow-up and to the MACS study at age 12 follow-up

	MACS			LISAplus		
	Attendees N=366 (59%) n (%)	Non-attendees N=254 (41%) n (%)	*p* value*	Attendees N=1761 (57%) n (%)	Non-attendees N= 1333 (43%) n (%)	*p* value*
Sex						
Male	194 (53)	123 (48)	0.26	910 (52)	674 (51)	0.56
Female	172 (47)	131 (52)		851 (48)	659 (49)	
Parental education						
Maternal higher education	228 (62)	137 (54)	**0.03**	989 (57)	562 (43)	**<0.01**
Paternal higher education	225 (62)	153 (60)	0.70	1068 (63)	555 (46)	**<0.01**
Parental smoking						
Maternal	20 (6)	15 (6)	0.81	238 (14%)	298 (23%)	**<0.01**
Paternal	55 (15)	55 (22)	**0.03**	NA	NA	NA
Number of older siblings						
No siblings	139 (38)	110 (43)	0.26	958 (54)	781 (59)	**<0.01**
1	118 (32)	82 (32)		637 (36)	392 (29)	
≥2	109 (30)	62 (24)		163 (9)	156 (12)	
History of atopic diseases in siblings						
Food allergy	140 (38)	92 (36)	0.60	55 (3)	39 (3)	0.80
Asthma	135 (37)	84 (33)	0.32	10 (1)	18 (1)	0.04
Eczema	144 (39)	94 (37)	0.55	142 (8)	102 (8)	0.67
Hay fever	84 (23)	51 (20)	0.39	39 (2)	25 (2)	0.57
History of maternal atopic diseases						
Food allergy	137 (37)	102 (40)	0.49	159 (9)	100 (8)	0.14
Asthma	159 (43)	109 (43)	0.89	120 (7)	75 (6)	0.20
Eczema	150 (41)	91 (36)	0.19	180 (10)	124 (9)	0.40
Hay fever	212 (58)	163 (64)	0.11	511 (30)	379 (29)	0.71

History of paternal atopic diseases						
Food allergy	79 (22)	52 (21)	0.73	120 (7)	54 (4)	**<0.01**
Asthma	99 (27)	59 (23)	0.28	96 (6)	67 (5)	0.85
Eczema	72 (20)	54 (21)	0.61	77 (5)	75 (6)	0.08
Hay fever	172 (47)	112 (44)	0.47	486 (30)	298 (25)	**<0.01**
Early food and aeroallergens sensitization						
Food_6M	71 (21)	53 (24)	0.32	NA	NA	NA
Food_12M	84 (24)	61 (30)	0.11	NA	NA	NA
Food_24M	61 (20)	30 (21)	0.79	137 (9)	67 (10)	0.91
Aero_6M	37 (11)	23 (11)	0.92	NA	NA	NA
Aero_12M	64 (18)	38 (19)	0.89	NA	NA	NA
Aero_24M	93 (30)	38 (26)	0.41	62 (4)	41 (6)	0.11

*From Pearson’s Chi-squared test..

6.3. Association between early life food sensitisation and asthma and allergic rhinitis at 18 years: Results from the MACS cohort

6.3.1. Introduction

While the prevalence and risk factors for asthma and allergic rhinitis in childhood have been studied extensively (Asher *et al.*, 2006), the data for late adolescence are less abundant. Understanding potential risk factors for the development of asthma and allergic rhinitis during adolescence is important, as this stage of life represents the transitional period between childhood and adulthood and is characterised by intense developmental, psychological and social change (de Benedictis and Bush, 2007). It has been shown that the prevalence of asthma peaks during childhood and then decreased afterward (ACAM, 2011). Until age 13–14 years, the incidence and prevalence of asthma is greater among boys than girls and during adolescence and adulthood, the prevalence switches to a female predominance (Osman *et al.*, 2007). Most authors have attributed these changes in prevalence to events of puberty (Subbarao *et al.*, 2009), although the exact mechanisms for differences between the sexes have not been established. On the other hand, the lifetime prevalence of allergic rhinitis peaks in the 15–19 years age group. Similar to asthma, the prevalence of allergic rhinitis is greater in males than in females before the adolescent prevalence peak; female prevalence exceeds that of males in the following years (Ghouri *et al.*, 2008; AIHW, 2011).

Food sensitisation in early life could be a potential risk factor for development of asthma and allergic rhinitis during adolescence. However, the association between early life food sensitisation and asthma during adolescence or early adulthood has been assessed in only one birth cohort, which found a positive association (Rhodes *et al.*, 2001). This study was limited by a small sample size (N=100). Moreover, there were no studies identified in my systematic review (**Chapter 5**) that assessed the association between food sensitisation and allergic rhinitis beyond the age of 8 years. Therefore, in this section, I examine these associations within the MACS cohort, in which participants were followed up to 18 years of age. I could not investigate these associations within LISAplus (15 year follow-up) as the data collection, entry and cleaning for this study was not completed at the time of analysis.

6.3.2. Methods

The MACS methods related to the current analysis are described in detail in the attached PDF file on the association between food sensitisation in the first two years and asthma and allergic rhinitis during late childhood (*Section 6.2.1*).

6.3.3. Results

6.3.3.1. Associations between food and/or aeroallergen sensitisation and asthma at 18 years

At 18 years, 25.4% of MACS participants had asthma. The associations between food only, aero only and food and aeroallergen sensitisation at 6, 12 and 24 months and asthma at 18 years are presented in **Table 6.1**.

Food with or without aeroallergen sensitisation and asthma

My results showed that sensitisation to food allergens without concurrent aeroallergen sensitisation at all tested time points was not associated with increased risk of asthma at 18 years. On the other hand, co-sensitisation to both food and aeroallergen at 12 or 24 months was related to increased risk of current asthma (aOR= 2.1, 95% CI= 1.0, 4.2 and aOR= 4.9, 95% CI= 2.3, 10.2, respectively) (**Table 6.1**).

Aeroallergen only sensitisation and asthma

Sensitisation to aeroallergen only at 24 months was associated with increased risk of asthma (aOR= 2.9, 95% CI= 1.5, 5.7). Although a similar trend was observed for sensitisation to aeroallergen at 12 months, this was not statistically significant (**Table 6.1**).

Table 6.1- Associations between food only, aeroallergens only and both food and aeroallergen sensitisation and asthma at 18 years in MACS.

Atopic sensitisation	Asthma at 18 years					
	n (%)	Prevalence* (%)	Crude OR (95%CI)	*P*	Adjusted** OR (95% CI)	*p*
6 months						
Non-sensitised	287 (74)	25	-		-	
Food only	62 (16)	24	0.9 (0.5, 1.8)	0.88	0.9 (0.5, 0.8)	0.82
Aero only	15 (4)	27	1.1 (0.3, 3.5)	0.89	1.0 (0.3, 3.4)	0.94
Food and aero	22 (6)	41	2.1 (0.8, 5.0)	0.11	1.8 (0.7, 4.5)	0.21
12months						
Non-sensitised	263 (67)	22	-		-	
Food only	59 (15)	27	1.3 (0.7, 2.4)	0.44	1.3 (0.7, 2.4)	0.48
Aero only	27 (7)	37	2.0 (0.9, 4.7)	0.09	1.9 (0.8, 4.5)	0.12
Food and aero	44 (11)	36	1.9 (1.0, 3.8)	**0.04**	2.1 (1.0, 4.2)	**0.04**
24 months						
Non-sensitised	204 (61)	17	-		-	
Food only	31 (9)	29	1.9 (0.8, 4.6)	0.11	1.8 (0.8, 4.4)	0.18
Aero only	57 (17)	39	3.0 (1.6, 5.8)	**<0.01**	2.9 (1.5, 5.7)	**0.001**
Food and aero	42 (13)	50	4.8 (2.4, 9.8)	**<0.01**	4.9 (2.3, 10.2)	**<0.01**

***Prevalence refers to the proportion of individuals who developed asthma in each sensitisation group.**

**** Adjusted for maternal smoking during pregnancy, parental level of education, sex, exclusive breast feeding for at least 4 months, eczema and wheeze by the age of sensitisation assessment and formula allocation.**

6.3.3.2. Associations between food and/or aeroallergen sensitisation and allergic rhinitis at 18 years

At 18 years, 36% of the MACS participants had allergic rhinitis. The associations between food only, aero only and food and aeroallergen sensitisation at 6, 12 and 24 months and allergic rhinitis at 18 years are presented in **Table 6.2**.

Food with or without aeroallergen sensitisation and allergic rhinitis

My findings showed that sensitisation to food allergens without concurrent aeroallergen sensitisation at 12 months was associated with increased risk of allergic rhinitis at 18 years (aOR= 2.1, 95% CI= 1.1, 3.7). Moreover, co-sensitisation to both food and aeroallergens at 24 months was related to increased risk of current allergic rhinitis (aOR= 3.1, 95% CI= 1.6, 6.4) **(Table 6.2)**.

Aeroallergen only sensitisation and allergic rhinitis

Sensitisation to aeroallergen only at 24 months was associated with increased risk of allergic rhinitis (aOR= 3.8, 95% CI= 2.1, 7.2). Although similar trend was observed for sensitisation to aeroallergen at 12 months, this was not statistically significant (**Table 6.2**).

Table 6.2 - Associations between food only, aeroallergens only and both food and aeroallergen sensitisation and allergic rhinitis at 18 years in MACS.

Atopic sensitisation	Allergic rhinitis at 18 years					
	n (%)	Prevalence* (%)	Crude OR (95%CI)	*P*	Adjusted** OR (95% CI)	*p*
6 months						
Non-sensitised	287 (74)	32	-		-	
Food only	62 (16)	40	1.4 (0.8, 2.5)	0.21	1.4 (0.8, 2.5)	0.25
Aero only	15 (4)	27	0.8 (0.2, 2.5)	0.66	0.7 (0.2, 2.4)	0.60
Food and aero	22 (6)	50	2.1 (0.9, 5.1)	0.09	2.0 (0.8, 5.1)	0.13
12months						
Non-sensitised	263 (67)	30	-		-	
Food only	59 (15)	47	2.1 (1.2, 3.7)	**0.01**	2.1 (1.1, 3.7)	**0.01**
Aero only	27 (7)	48	2.2 (0.9, 4.8)	**0.05**	2.2 (0.9, 4.9)	0.06
Food and aero	44 (11)	41	1.6 (0.8, 3.1)	0.15	1.5 (0.7, 2.9)	0.27
24 months						
Non-sensitised	204 (61)	26	-		-	
Food only	31 (9)	35	1.5 (0.7, 3.4)	0.29	1.7 (0.7, 3.8)	0.21
Aero only	57 (17)	58	3.8 (2.1, 7.0)	**<0.01**	3.8 (2.1, 7.2)	**<0.01**
Food and aero	42 (13)	52	3.1 (1.5, 6.0)	**<0.01**	3.1 (1.6, 6.4)	**<0.01**

***Prevalence refers to the proportion of individuals who developed allergic rhinitis in each sensitisation group.**

**** Adjusted for maternal smoking during pregnancy, parental level of education, sex, exclusive breast feeding for at least 4 months, eczema by the age of sensitisation assessment and formula allocation.**

6.3.4. Discussion

The relationship between early life food sensitisation and later development of allergic airway diseases has been discussed extensively in the accepted paper (presented in *Section 6.2.1*). In this section, I discuss my results related to food with or without aeroallergen sensitisation in the first two years of life as a predictor for having asthma and allergic rhinitis at 18 years. Then I compare the observed associations at 12 and 18 years using outcomes from the MACS cohort.

6.3.4.1. Association between early life food sensitisation and asthma and allergic rhinitis at 18 years

Early identification of children at greater risk for the development of asthma and allergic rhinitis in later life is important for the implementation of prevention strategies. Although several studies have examined the association between early life food sensitisation and subsequent development of asthma during childhood (Michael Kulig *et al.*, 1998; Brockow *et al.*, 2009; Kjaer *et al.*, 2009; Bekkers *et al.*, 2013), only one birth cohort has assessed these associations in early adulthood (Rhodes *et al.*, 2001). Findings from this high-risk cohort showed that sensitisation to milk, egg or both before the age of 1 year was associated with an increased risk of asthma (as defined based on reported history of current wheeze and BHR) at 22 years. On the other hand, my analysis from MACS, an allergy high-risk cohort, found that sensitisation to food (cow's milk, egg white and/or peanut) without aeroallergen in the first year was not associated with increased risk of asthma at 18 years. However, co-sensitisation to both food and aeroallergen at 12 months was related to increased risk of asthma at 18 years.

The association between early life food sensitisation and subsequent development of allergic rhinitis has not been assessed beyond the age of 8 years (Garden *et al.*, 2013). My analysis was the first to examine these associations at the age of 18 years.

6.3.4.2. Comparing the observed associations between food sensitisation and subsequent development of asthma and allergic rhinitis at 12 and 18 years in the MACS cohort

Unlike asthma and allergic rhinitis development during later childhood, I found that food without aeroallergen sensitisation in the first two years of life was not a predictor for asthma but was a predictive marker for allergic rhinitis during adolescence (i.e. at 18 years). This suggests that the asthma phenotype of later childhood may be different from that of adolescence. Several other factors have been documented to operate the airway inflammatory response and contribute to asthma development. Likely candidates for the role of co-factors include respiratory infection, personal smoking, exposure to environmental tobacco smoke and allergens and occupational exposures in later life.

CHAPTER 7 - Association between food sensitisation and lung function in adolescence

7.1. Introduction

Lung development commences during the gestational period and continues after birth, with almost 85% of alveoli developing postnatally (Merkus *et al.*, 1996). Thus, factors that interfere with this process may affect lung function in later life and increase the risk of respiratory disease. It is therefore important to understand potential risk factors during this critical period of lung development.

It has been shown that sensitisation to aeroallergens in early life is associated with loss of lung function during childhood (Lowe *et al.*, 2004; Illi *et al.*, 2006). The mechanisms underlying the relationship between allergen sensitisation and lung function remain unclear. It has been suggested that persistent environmental exposures could induce inflammatory processes that lead to airflow limitation (Gottlieb *et al.*, 1996).

Although few studies have examined the relationship between aeroallergen sensitisation and lung function (Sunyer *et al.*, 2000; Langley *et al.*, 2003; Lowe *et al.*, 2004; Illi *et al.*, 2006), the association between food sensitisation and subsequent lung function has not been evaluated. A recent population-based study has shown that early atopic sensitisation (defined as a positive skin prick test to at least one tested food or aeroallergen in the first year of life) was associated with persistent reduction in lung function from infancy up to adulthood (Owens *et al.*, 2016). However, they did not assess the role of food sensitisation separately. This is an important research question in the context of the recently observed rise in food allergies as a potential "second wave of allergy epidemic" (Prescott and Allen, 2011). Additionally, it is well known that sensitisation to food allergens is associated with asthma, which itself can affect lung function (Nickel *et al.*, 1997; Michael Kulig *et al.*, 1998; Brockow *et al.*, 2009). Therefore an analysis that looks at the association of early life food sensitisation on subsequent lung function needs to take into account the potential mediation role that concurrent asthma may play in altered lung function.

7.2. Rationale and aims

Several epidemiological studies have shown that aeroallergen sensitisation is associated with subsequent poor lung function, but it is unknown whether early life food sensitisation is similarly associated with subsequent lung function deficit. Therefore, I aimed

to assess the association between food sensitisation in the first two years of life and adolescent lung function measures and to examine if these associations were mediated by early life aeroallergen sensitisation or asthma.

7.3. Methods

7.3.1. Study populations and data collection

This research question was investigated within the MACS, allergy high-risk cohort, and the findings related to 24 months sensitisation were replicated in the LISAplus, a population-based study. Details description of participants' recruitment, the study population and data collection for both cohorts was provided in **Chapter 3**.

7.3.2. Sensitisation assessment

In the MACS, SPTs were conducted at 6, 12 and 24 months, according to a standard technique (Heinzerling *et al.*, 2013) as described in *Section 3.2.4.2*. Tested allergens include egg white, cow's milk, peanut, *Dermatophagoides pteronyssinus* (dust mite), *Lolium perenne* (rye grass) and cat dander *(*Bayer, Spokane, WA, USA*).*

In the LISAplus, sensitisation was assessed at the age of 24 months using the CAP System FEIA (Pharmacia Diagnostics, Freiburg, Germany) according to the manufacturer's instructions, as described in *Section 3.3.2.2*. Food sensitisation was measured using a mix of six common food allergens (fx5: hen's egg, cow's milk, peanut, wheat flour, soybean, and codfish). Serum-specific Immunoglobulin E antibodies (s-IgE) to aeroallergens of mould (MX1), cat (E1), a mixture of mites and cockroach (HX2), and of pollen (RX1) were all tested separately.

7.3.3. Lung function assessment

In the MACS, spirometry was conducted at 12 and 18 years following standardized techniques (American Thoracic Society (ATS) Guidelines (Crapo *et al.*, 1995) and ATS / European Respiratory Society Guidelines (ERS) (Miller *et al.*, 2005)). Lung function assessment (pre-bronchodilator) was performed at 12 years by trained research nurses using the Spirocard system (SpiroCard **™** PC Spirometer, QRS Diagnostic, Plymouth, MN, USA).

At 18 years, pre- and post-bronchodilator (salbutamol) spirometry was conducted by trained respiratory scientists using the EasyOne^{TM} Spirometer (NDD Medical Technologies Inc, Andover MA), as described in details in *Section 3.2.4.2.*

In the LISAplus, spirometry was performed at the 15-years follow-up pre- and post-bronchodilation (200µg salbutamol). The procedure, all measurements as well as the evaluation of the results were in accordance with ATS/ERS recommendations (Miller *et al.*, 2005). Flow-volume curves were obtained using a pneumotachograph-type (EasyOne Worldspirometer, ndd, Zurich, Switzerland), as described in details in *Section 3.3.2.2.*

7.3.4. Definitions used within this chapter

The following definitions have been used within this chapter:

Sensitisation

It was defined as a wheal size of ≥2 mm in MACS (Rhodes *et al.*, 2001), and as a s-IgE antibody level ≥0.35 kUA/L in LISAplus.

Current asthma

It was defined as one or more episodes of asthma and/or the use of any asthma medications in the last 12 months in the MACS. In LISAplus, it was defined based on the Global Allergy and Asthma European Network (GA2LEN) definition (Carlsen *et al.*, 2006); subjects providing a positive response to at least two of the following three questions were considered as currently having asthma: (1) “Has a doctor-diagnosed asthma in your child at age 3 to 15 years?” (2) “Has your child taken asthma medication during the last 12 months?” (3) “Has your child had wheezing or whistling in the chest in the last 12 months?”

Wheeze by the age at which the sensitisation was assessed

In MACS, defined if the response to the following question was >5 days “How many days of cough and/or chest rattle and/or wheeze has your child had in the past 4 weeks?”, and in LISAplus according to the response to the following question “In the past 6 months, has your child had whistling or wheezy sound of breathing in the chest?” asked during the follow-up at age 2 years.

Family history of atopy (in LISAplus only)

Defined if the participant had at least one first degree relative with asthma, eczema or hay fever; asked at birth.

7.3.5. Statistical analysis

7.3.5.1. Examining the association between atopic sensitisation and lung function

Linear regression models were used to assess the association between food with or without aeroallergen sensitisation at 6, 12 or 24 months in MACS or at 24 months in LISAplus, and lung function outcomes including FEV_1, FVC and FEV_1/FVC ratio at 12 and 18 years (in MACS) and at 15 years (in LISAplus). I modelled sensitisation as: (1) no sensitisation; (2) food sensitisation only; (3) aeroallergen sensitisation only; and (4) sensitisation to both food and aeroallergen, using those who were not sensitised as the reference group. In MACS, the associations were evaluated at each time point separately, regardless of previous sensitisation status. All results were presented as regression coefficients β with corresponding 95% confidence interval (CI).

All models were adjusted for sex, age, height and formula allocation in MACS and study centre in LISAplus. All models were then adjusted for other potential confounders including maternal smoking during pregnancy, parental level of education, wheezing by the age of sensitisation testing and exclusive breastfeeding for at least 4 months (Fleischer *et al.*, 2013). Interactions of atopic sensitisation with concurrent asthma (i.e. at the time of lung function assessment), personal smoking (only at 15 or 18 years) and family history of allergic diseases in LISAplus study and formula allocation in MACS study were assessed. Interaction terms were not included in final models if *p* values were > 0.10.

STATA 13 (StataCorp, College Station TX) was used in all analyses in MACS and *R* version 3.2.0 was used for all analyses in LISAplus.

7.3.5.2. Mediation analysis

To assess whether aeroallergen sensitisation at 12 or 24 months and asthma at 6, 12 or 18 years acted as mediators of the relationship between food sensitisation and lung function

outcomes in MACS study, I used the “medeff” command in STATA version 13 to estimate the magnitude of the natural direct effect and natural indirect effect (called the “average causal mediation effect” or ACME in the output from the “medeff” command) as the two additive components of the total causal effect (Imai *et al.*, 2010; Hicks and Tingley, 2011).

7.4. Results

7.4.1. Characteristics of Participants

In the current analysis, 364 of the MACS participants (59% of original cohort) at 12 years and 399 participants (64% of original cohort), who had data on both sensitisation and lung function testing, were included. The characteristics of those participants are presented in **Table 7.1**. In LISAplus, 796 participants from four study centres (358, 250, 100 and 88 from Munich, Leipzig, Bad Honnef and Wesel, respectively) were included in the current analysis. The characteristics of analysed participants are summarised in **Table 7.1**.

Table 7.1 - Characteristics of participants with both sensitisation and lung function data in MACS and LISAplus cohorts.

	MACS cohort		LISAplus cohort
	At 12 years	At 18 years	At 15 years
N	364	399	796
Sex, n (%)			
Male	189 (52)	201 (51)	425 (53)
Female	175 (48)	198 (49)	371 (47)
Age (years), mean (SD)	11.5 (1.9)	17.9 (1.3)	15.1 (0.3)
Height (cm), mean (SD)	149.5 (13)	172.4 (9.4)	171.3 (8.5)
Family history of atopy, n (%)	364 (100)	399 (100)	461 (62)
Current asthma, n (%)	82 (24)	104 (27)	43 (5)
Sensitisation, n/N (%)			
Food allergens only			
6 months	51/342 (15)	59/373 (16)	-
12 months	47/349 (13)	55/381 (14)	-
24 months	24/312 (8)	28/326 (9)	62/796 (8)
Aeroallergens only			
6 months	17/342 (5)	15/373 (4)	-
12 months	26/349 (7)	27/381 (7)	-
24 months	53/312 (17)	56/326 (17)	19/796 (2)
Food and aeroallergens			
6 months	20/342 (6)	23/373 (6)	-
12 months	40/349 (11)	44/381 (12)	-
24 months	40/312 (13)	44/326 (14)	19/796 (2)
Lung Function parameter, adjusted mean (SD)*			
Pre-BD FEV1 (ml)	2366 (651)	3832 (791)	3514 (480)
Post-BD FEV1 (ml)	-	4020 (804)	3619 (501)
Pre-BD FVC (ml)	2582 (678)	4543 (975)	4085 (617)
Post-BD FVC (ml)	-	4578 (975)	4075 (611)
Pre-BD FEV1/FVC ratio (%)	92 (7)	85 (8)	86 (2)
Post-BD FEV1/FVC ratio (%)	-	88 (6)	89 (1)

***Adjusted for age, sex and height.**

7.4.2. Loss to follow-up

In MACS, participants who did not attend follow-ups at 12 and 18 years had less educated parents (**Table 7.2**). In LISAplus, the participants who attended lung function measurement at the 15-year follow-up were similar in all demographic characteristics to those from the initial cohort who did not attend, with the exception of a higher proportion of highly educated parents and a lower number of older siblings (**Table 7.2**).

Table 7.2 - Comparison of baseline characteristics between attendees and non-attendees at 12-year and at 18-year follow-up in MACS and at 15-year follow-up in LISAplus.

	MACS						LISAplus		
	At 12 years			At 18 years			At 15 years		
	Attendees *N* = 366	Non-attendees *N* = 254	*P** value	Attendees *N* = 404	Non-attendees *N* = 216	*P** value	Attendees *N* = 991	Non-attendees *N* = 2103	*P** value
Sex									
Male	194 (53)	123 (48)	0.26	206 (51)	111 (51)	0.91	512 (52)	1072 (51)	0.75
Female	172 (47)	131 (52)		198 (49)	105 (49)		479 (48)	1031 (49)	
Parental education									
Maternal higher education	228 (62)	137 (54)	**0.03**	260 (64)	105 (49)	**<0.01**	536 (54)	1015 (49)	**<0.01**
Paternal higher education	225 (62)	153 (60)	0.70	265 (66)	113 (52)	**<0.01**	576 (61)	1047 (54)	**<0.01**
Number of older siblings									
No siblings	139 (38)	110 (43)	0.18	163 (40)	86 (40)	0.89	532 (54)	1207 (58)	**0.01**
1	118 (32)	82 (32)		129 (32)	71 (33)		365(37)	664 (32)	
≥2	109 (30)	62 (24)		112 (28)	59 (27)		93 (9)	226 (11)	
History of atopic diseases in siblings									
Food allergy	140 (38)	92 (36)	0.6	155 (38)	77 (36)	0.50	25 (3)	69 (3)	0.29
Asthma	135 (37)	84 (33)	0.32	141 (35)	78 (36)	0.76	5 (1)	23 (1)	0.15
Eczema	144 (39)	94 (37)	0.55	155 (38)	83 (38)	0.98	78 (8)	166 (8)	1.00
Hay fever	84 (23)	51 (20)	0.39	90 (22)	45 (21)	0.67	17 (2)	47 (2)	0.41

History of maternal atopic diseases									
Food allergy	137 (37)	102 (40)	0.49	155 (38)	84 (39)	0.89	86 (9)	173 (8)	0.73
Asthma	159 (43)	109 (43)	0.89	169 (42)	99 (46)	0.33	64 (7)	131 (6)	0.88
Eczema	150 (41)	91 (36)	0.19	158 (39)	83 (38)	0.86	87 (9)	217 (10)	0.19
Hay fever	212 (58)	163 (64)	0.11	242 (60)	133 (62)	0.60	279 (29)	611 (30)	0.50
History of paternal atopic diseases									
Food allergy	79 (22)	52 (21)	0.73	78 (19)	53 (25)	0.12	63 (7)	111 (6)	0.40
Asthma	99 (27)	59 (23)	0.28	110 (28)	48 (22)	0.17	48 (5)	115 (6)	0.40
Eczema	72 (20)	54 (21)	0.61	85 (21)	41 (19)	0.54	46 (5)	106 (6)	0.50
Hay fever	172 (47)	112 (44)	0.47	188 (47)	96 (44)	0.61	260 (28)	524 (28)	0.97
Early food and aeroallergens sensitisation									
Food sensitisation at 6 months	71 (21)	53 (24)	0.32	79 (22)	45 (23)	0.66	NA	NA	
Food sensitisation at 12 months	84 (24)	61 (30)	0.11	97 (26)	48 (27)	0.76	NA	NA	
Food sensitisation at 24 months	61 (20)	30 (21)	0.79	71 (22)	20 (15)	0.06	87 (10)	117 (9)	0.47
Aero sensitisation at 6 months	37 (11)	23 (11)	0.92	37 (10)	23 (12)	0.52	NA	NA	
Aero sensitisation at 12 months	64 (18)	38 (19)	0.89	69 (18)	33 (19)	0.95	NA	NA	
Aero sensitisation at 24 months	93 (30)	38 (26)	0.41	96 (30)	35 (26)	0.35	40 (5)	63 (5)	0.89

***From Pearson's Chi-squared test.**

7.4.3. Atopic sensitisation in the first two years and lung function at 12, 15 and 18 years

7.4.3.1. Sensitisation and pre-BD FEV_1

Sensitisation to food only (i.e. without aeroallergen sensitisation) at 6 months was associated with a reduction in pre-BD FEV_1 at both 12 (-149 ml; 95% CI -251 ml to -47 ml; *p* <0.01) and 18 years (-208 ml; 95% CI -349ml to -66 ml; p <0.01). Similar findings were observed for food only sensitisation at 12 months (-135 ml; 95% CI -247 ml to -24 ml; *p* 0.01 and -149 ml; 95% CI -297ml to -1 ml; *p*=0.04 at 12 and 18 years, respectively)**.** Additionally, sensitisation to aeroallergens without co-existing food sensitisation at 24 months was associated with a reduction in pre-BD FEV_1 at 15 (-208 ml; 95% CI -410 ml to -5 ml; *p*=0.04) and at 18 years (-158 ml; 95% CI -310ml to -6ml; *p*=0.04) (**Figure 7.1**).

Co-sensitisation to food and aeroallergens at all three time points was not associated with any deficit in pre-BD FEV1 at 12, 15 or 18 years (**Figure 7.1**).

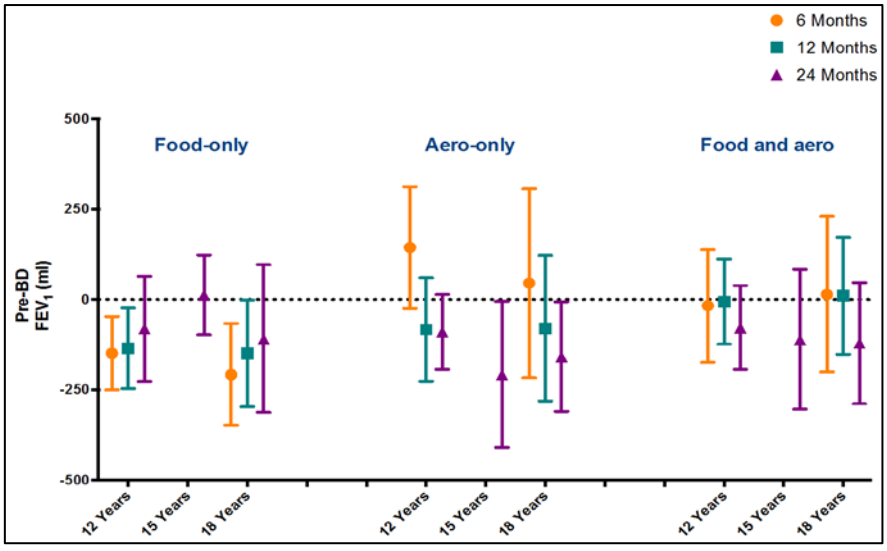

Figure 7.1 - The adjusted‡ association between atopic sensitisation (food only, aero only and both food and aero sensitisation) in the first 2 years of life and pre-BD FEV1 at 12, 15 and 18 years. * *P* value <0.05.

‡Adjusted for sex, age, height, wheezing by the age of testing food sensitisation, maternal smoking during pregnancy, parental level of education and exclusive breast feeding for at least 4 months (formula allocation in MACS and study center in LISAplus).
NB: 12 and 18 year data from MACS, 15 year data from LISAplus.

7.4.3.2. Sensitisation and post-BD FEV_1

Sensitisation to food without co-existing aeroallergen sensitisation at 6 months was associated with a reduction in post-BD FEV_1 at 18 years (-182 ml; 95% CI -313 ml to -52 ml; p <0.01). However, no other combination of food and/or aeroallergen sensitisation at any time point was related to post-BD FEV_1 at 15 or 18 years (**Figure 7.2**).

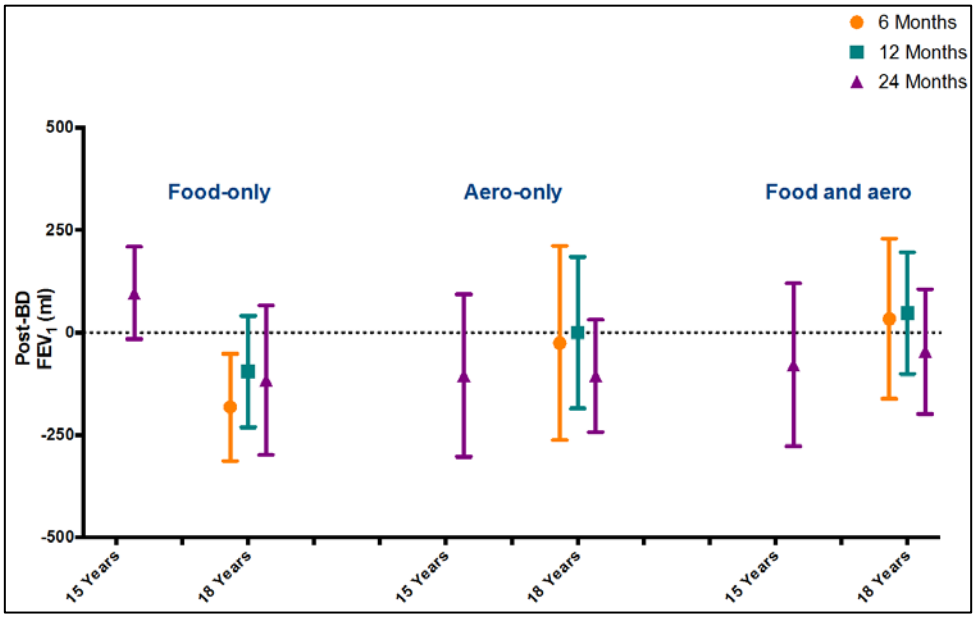

Figure 7.2 - The adjusted‡ association between atopic sensitisation (food only, aero only and both food and aero sensitisation) in the first 2 years of life and post-BD FEV1 at 15 and 18 years. * *P* value <0.05.

‡Adjusted for sex, age, height, wheezing by the age of testing food sensitisation, maternal smoking during pregnancy, parental level of education and exclusive breast feeding for at least 4 months (formula allocation in MACS and study center in LISAplus).
NB: 18 year data from MACS, 15 year data from LISAplus, post-BD FEV_1 was not assessed at 12 years.

7.4.3.3. Sensitisation and pre-BD FEV₁/FVC ratio

Sensitisation to food only without aeroallergen sensitisation at 6 months was associated with a reduction in pre-BD FEV_1/FVC ratio at 12 years (-2.2%; 95% CI -4.2% to -0.2%; *p*=0.03). Furthermore, sensitisation to aeroallergens without concurrent food sensitisation at 24 months was associated with a reduction in pre-BD FEV_1/FVC ratio at both 15 (-3.3%; 95% CI -6.1% to -0.4%; *p*=0.03) and 18 (-3.0%; 95% CI -5.3% to -0.8%; *p* <0.01) years. Surprisingly, sensitisation to aeroallergens only at 6 months was associated with an increase of pre-BD FEV_1/FVC ratio at 12 years (3.8%; 95% CI 0.5% to 7.1%; *p*=0.02). Co-sensitisation to food and aeroallergens at all three time points was not associated with any deficit in pre-BD FEV_1/FVC ratio at 12, 15 and 18 years (**Figure 7.3**).

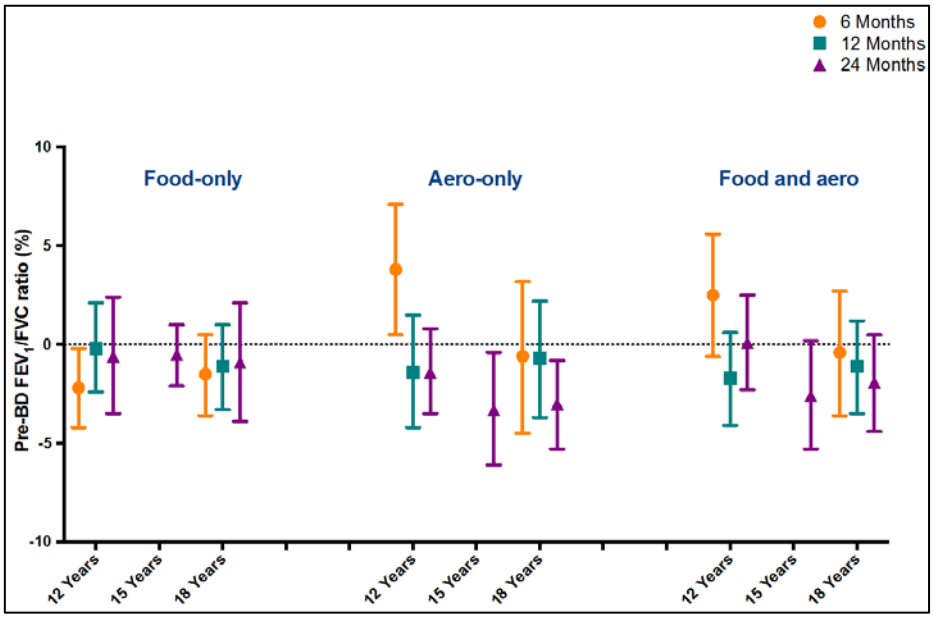

Figure 7.3 -The adjusted‡ association between atopic sensitisation (food only, aero only and both food and aero sensitisation) in the first 2 years of life and pre-BD FEV1 /FVC ratio at 12, 15 and 18 years. * *P* value <0.05.

‡Adjusted for sex, age, height, wheezing by the age of testing food sensitisation, maternal smoking during pregnancy, parental level of education and exclusive breast feeding for at least 4 months (formula allocation in MACS and study center in LISAplus).
NB: 12 and 18 year data from MACS, 15 year data from LISAplus.

7.4.3.4. Sensitisation and post-BD FEV_1/FVC ratio

Sensitisation to food only without concurrent aeroallergen sensitisation at 6 months was associated with a reduction in post-BD FEV_1/FVC ratio at 18 years (-2.3%; 95% CI -4.0% to -0.5%; *p*=0.01). Also, co-sensitisation to food and aeroallergen at 12 months was related to reduced post-BD FEV_1/FVC ratio at 18 years (-2.1%; 95% CI -4.1% to -0.1%; *p*=0,03) and co-sensitisation to food and aeroallergen at 24 months was related to reduced post-BD FEV_1/FVC ratio at 15 years (-2.8%; 95% CI -5.3% to -0.4%; *p*=0.02). In contrast, sensitisation to aeroallergen only at any time point was not associated with post-BD FEV_1/FVC ratio at 15 or 18 years (**Figure 7.4**).

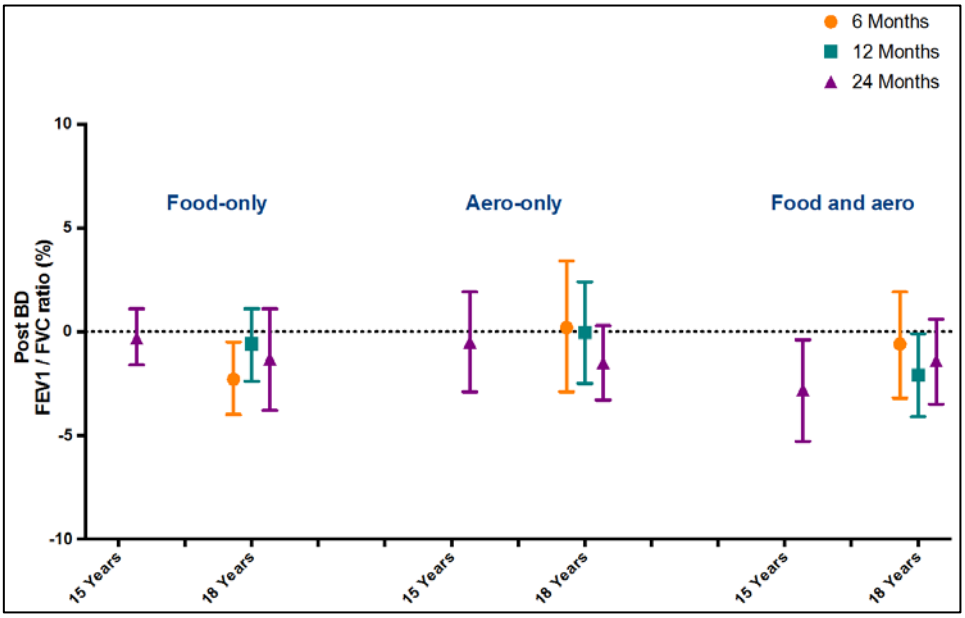

Figure 7.4 - The adjusted‡ association between atopic sensitisation (food only, aero only and both food and aero sensitisation) in the first 2 years of life and post-BD FEV1 /FVC ratio at 15 and 18 years. * *P* value <0.05.

‡Adjusted for sex, age, height, wheezing by the age of testing food sensitisation, maternal smoking during pregnancy, parental level of education and exclusive breast feeding for at least 4 months (formula allocation in MACS and study center in LISAplus).
NB: 18 year data from MACS, 15 year data from LISAplus, post-BD FEV_1/FVC ratio was not assessed at 12 years.

7.4.3.5. Sensitisation and pre- and post-BD FVC

Sensitisation to food and/or concurrent aeroallergen at any tested time point was not related to FVC in adolescence, with exception of food without aeroallergen sensitisation at 12 months and pre-BD FVC at 12 years (-144ml; 95% CI -271 ml to -16 ml; p=0.02).

7.4.4. Interaction with concurrent asthma, personal smoking, family history of allergic diseases (in LISAplus) and formula allocation (in MACS)

In MACS, there were no significant interactions between food sensitisation and concurrent asthma, personal smoking and formula allocation. All p values for interactions were >0.1. In LISAplus, there was an interaction between food sensitisation status and family history of allergic diseases only for post-BD FEV_1/FVC ratio (p=0.08). After stratification, co-sensitisation to food and aeroallergens was not significantly associated with post-BD FEV_1/FVC ratio in the group without family history of atopy, but the association remained the same in those with family history of atopy. However, there were few individuals who had co-sensitisation without family history of allergy (N=3). There were no interactions between food sensitisation and concurrent asthma and personal smoking in LISAplus.

7.4.5. Assessment of mediators in MACS study

Given the consistent association between food sensitisation in the first year of life and lower pre-BD FEV_1 at both 12 and 18 years, I investigated the potential for asthma and aeroallergen sensitisation to mediate this pathway in the MACS. Results from the causal mediation analysis showed evidence of a direct effect of food only sensitisation at 6 or 12 months on pre-BD FEV_1 at 12 and 18 years, and an indirect effect mediated through asthma at 6 years.

The proportion of the total causal effect of food sensitisation at 12 months mediated by asthma at 6 years was 15.3% (95%CI 7.9 to 71.4%) for pre-BD FEV1 at 12 years (direct effect -114ml, 95%CI= -230ml, -2ml; indirect effect -20ml, 95%CI= -48ml, -2ml). A similar proportion mediated by asthma at 6 years was observed for food sensitisation at 6 months and

pre-BD FEV_1 at 18 years 15% (95%CI 9 to 46%) (direct effect -184ml, 95%CI= -334ml, -39ml; indirect effect -32ml, 95%CI= -77ml, -4ml).

7.5. Discussion

7.5.1. Summary of the findings

This is the first study to evaluate the association between early life food and/or aeroallergen sensitisation and lung function in adolescence. I observed that early life sensitisation to food allergens, up to 12 months, was associated with a number of lower spirometric indices during adolescence. These effects were consistent for pre-BD FEV_1 at 12 years and 18 years in relation to both 6 months and 12 months food sensitisation. The observed associations between food sensitisation and pre-BD FEV_1 were partially mediated by asthma at six years. Sensitisation to aeroallergens only at 24 months was also associated with a reduction in pre-BD FEV_1 and FEV_1/FVC ratio in adolescence. Findings were largely consistent between the two cohorts in relation to 24 months sensitisation.

7.5.2. Association between atopic sensitisation and lung function

In individuals sensitised to food and/or aeroallergen, a series of cellular and molecular interactions involving B cells, T cells and antigen presenting cells (APCs) result in the release of several pro-inflammatory mediators and cytokines (Galli *et al.*, 2008). The release of these mediators contributes to the acute symptoms and signs related to allergic reactions, including vasodilatation, increased vascular permeability and bronchial smooth muscle contraction (Hamelmann *et al.*, 1999; Galli *et al.*, 2008). After repeated exposure to allergens, persistent inflammation occurs that may lead to long term structural and functional changes of affected tissues (Galli *et al.*, 2008).

Although these inflammatory mechanisms are associated with atopy and also with loss of lung function, it is not clear whether atopy in early life causes lung function deficits or whether both atopy and reduced lung function are manifestations of a common underlying condition, with early life atopy being a measurable marker. It has been reported that infant-onset atopy (defined as positive SPT to any tested allergen on at least one occasion during

infancy) was associated with a reduction in FEV1 at 11 years of age (Turner *et al.*, 2007). Likewise, our results have shown that food sensitisation in the first year of life was related to decrease FEV_1 and FEV_1/FVC ratio.

7.5.3. The role of asthma and parental/personal smoking in the association between atopic sensitisation and lung function

Asthma in early childhood has been shown to be associated with airway remodelling which may result in fixed airflow obstruction (Ulrik and Backer, 1999) and subsequently a decrease in lung function mainly in individuals with moderate to severe asthma (James *et al.*, 2005). Nevertheless, to what extent asthma influences this decrease, in sensitised individuals, is less obvious. My results showed that the associations between food sensitisation and decreased FEV_1 were partially mediated by asthma at 6 years, suggesting that food sensitisation per se may lead to subsequent airway changes and reduced lung function.

Asthma and parental or personal smoking are known to be associated with a decline in lung function (James *et al.*, 2005). A previous study has shown that infants born to smoking mothers have reduced forced expiratory flows compared to those born to non-smoking mothers (Hofhuis *et al.*, 2003). I adjusted for maternal smoking during pregnancy in all analyses. Interestingly, personal history of smoking did not confound or modify the associations observed. However, my analysis was based on a young age group in which the prevalence of smoking was low (<7%).

7.5.4. Strengths and limitations

The current study investigated the association between early life sensitisation and adolescent lung function both in a high allergy risk and an unselected population-based cohort. These cohorts were from different regions of the world, but both were westernised countries with a high prevalence of food sensitisation (Burney *et al.*, 2010). It is often assumed that results from a high-risk cohort may not be applicable to the general population, but my results were mostly consistent across the two cohorts where sensitisation data were available, despite the geographical differences between cohorts as well as some differences in the methods of sensitisation assessment.

The strengths of this study are that it includes longitudinal data from two independent cohorts with long period of follow-up that extended from infancy to adolescence. A large number of participants have performed lung function testing according to internationally recognised standards during the adolescent period. Additionally, I was able to examine the relationship between early life food and/or aeroallergen sensitisation and adolescent lung function. This is a significant period for growth that is associated with changes in the rate of growth of lung volumes, flow and body dimensions. In MACS, frequent follow-ups, particularly in the first 7 years of life, and early ages for sensitisation assessment to a standard battery of allergens allowed exploring the potential pathways for the association between sensitisation to food allergens only and adolescent lung function through aeroallergen sensitisation at 1 and 2 years and asthma at 6 years.

However this study also has a number of limitations. The longitudinal nature of the studies means that attrition of the cohort has occurred over time, and is a potential source of bias. However, it is somewhat reassuring that with the exception of parental education and the number of older siblings, there were no significant demographic and/or early sensitisation differences between attending and non-attending children in either cohort. The second limitation is that I was unable to establish the effect of food sensitisation at 6 and 12 months in the population-based LISAplus study, as sensitisation was not assessed in the first year of life. Since the significant deficit in FEV_1 and FEV_1/FVC ratio in MACS was demonstrated in those who had early life food sensitisation, I was unable to confirm if these findings in addition to mediation analysis were limited to individuals at high-risk of atopy.

7.6. Conclusion

In conclusion, food sensitisation without co-existent aeroallergen sensitisation in the first year of life was associated with declines in FEV_1 and FEV_1/FVC ratio during adolescence in the high allergy risk cohort. Early childhood asthma has partially mediated the associations between food only sensitisation at 6 and 12 months and pre-BD FEV_1 at 12 and 18 years. As such, there appears to be a direct pathway from food sensitisation in infancy to decreased FEV_1. Food sensitisation in infancy could be a potential risk factor, or early immunological marker, for adolescent lung function impairment. Further research is required to confirm these findings in other settings. It remains to be determined if more appropriate management of asthma during early childhood can improve lung function in adolescence.

CHAPTER 8 - Overall Discussion

8.1. Thesis summary

The prevalence of asthma, eczema and allergic rhinitis has increased over the past 50 years, particularly in industrialised countries (Mallol *et al.*, 2013). More recently, evidence suggests a second wave of the "allergy epidemic", with an increase in the prevalence of food allergies (Prescott *et al.*, 2013). Australia has one of the highest prevalences of childhood asthma, eczema and allergic rhinitis in the world (Asher *et al.*, 2006). The coincident increase in the prevalence of allergic conditions along with atopic sensitisation has raised questions concerning the role of sensitisation in the pathogenesis of these conditions (J. Heinrich *et al.*, 2002). Nevertheless, the evidence concerning the association between atopy and allergic diseases is controversial, possibly due to differences in the study design, number and type of tested allergens and the age at which atopy and allergic diseases was assessed. More importantly, there is a scarcity of data on the long term consequences of food sensitisation in the context of allergic diseases, as the major focus of previous work on atopic sensitisation has been on aeroallergen sensitisation. Longitudinal studies, where the same individuals are followed over time, are the appropriate design to address questions related to the natural history and consequences of food sensitisation.

My doctoral work has critically examined the natural history of food sensitisation from infancy up to adolescence. Additionally, I investigated whether the natural history of egg, milk and peanut sensitisation was modified by early life eczema and/or aeroallergen sensitisation. Moreover, I examined the relationships between early life food sensitisation and subsequent probable food allergy, asthma, allergic rhinitis and lung function in later childhood and adolescence.

I used data from the Australian MACS and German LISAplus birth cohorts to address these aims. The results generated from MACS, an allergy high-risk cohort, may not be directly applicable to the general population. The consequences of food sensitisation were therefore examined also, where possible, within the population-based (LISAplus) cohort. These two cohorts were from two different regions of the world, but both were high income countries with high prevalences of food sensitisation (Burney *et al.*, 2010) and allergic diseases (Pearce *et al.*; Maziak *et al.*, 2003; Stock *et al.*, 2005).

While I have confirmed that the prevalence of food sensitisation was highest during infancy and declined after that, I found that boys with early life eczema had the highest prevalence of

egg and milk sensitisation. During my doctoral work, I also found that food sensitisation at 12 months can predict children at greater risk of adolescent sensitisation and probable food allergy. Moreover, I observed an association between food sensitisation in the first two years and asthma and allergic rhinitis during later childhood (i.e. 10-12 years of age) in both the MACS and LISAplus cohorts. In addition, I also demonstrated in the MACS results that sensitisation to food allergens only at 6 or 12 months was associated with reduced FEV_1 in adolescence. The majority of this effect was a direct association, although early life asthma (but not aeroallergen sensitisation) partially mediated these associations.

I discuss these findings in more details in the individual results chapters (**Chapters 4, 5, 6 and 7**), and summarise them in the concluding chapter (**Chapter 9**). In this discussion chapter, I explain the potential for the observed findings to advance the knowledge in the field of allergy and epidemiology and provide an assessment with regard to causality. I then summarise the study strengths and limitations. Moreover, I review general methodological and analytical considerations that may influence the interpretation of my results chapters.

8.2. Contribution of my findings to the existing literature

8.2.1. Natural history of food sensitisation

While a number of prospective studies have examined the natural history of food sensitisation during infancy and early childhood, only a few studies have assessed the course of food sensitisation up to adolescence and young adulthood. Additionally, none of these studies have investigated potential modifiers for the rates of food sensitisation. Therefore, I examined the natural history of food sensitisation from infancy up to adolescence in MACS, an allergy high-risk cohort. In my analysis (**Chapter 4**), I confirmed the findings from previous studies that sensitisation to food allergens was predominant during infancy and early childhood, whereas sensitisation to aeroallergens was low during this period, and steadily increased at later ages. Given that the tested food allergens were different between studies, the prevalences of food sensitisation were not consistent among previous studies. Therefore, the results of these studies cannot be compared with my results.

Additionally, while sensitisation to cow's milk is known as the most common food allergen during infancy (Dubakiene *et al.*, 2012), I found that egg white and peanut were the most

common sensitised foods in infancy. This finding suggests that the tested food allergens in epidemiological and clinical studies should be related to the most prevalent food allergens in that region.

My analysis was the first to assess possible modifiers of the natural course of egg, cow's milk and peanut sensitisation. I found that sex, early life eczema and/or aeroallergen sensitisation modified the natural history of milk and egg, but not peanut sensitisation. Boys with early eczema had the highest prevalence of milk and egg sensitisation throughout follow-ups, while boys with aeroallergen sensitisation had a non-linear variation in the prevalence of milk sensitisation. These findings highlighted the importance of early life eczema and/or aeroallergen sensitisation in determining the natural history of milk and egg sensitisation. Thus, this suggests that the environment may play a significant role in changing the sensitisation pattern over time. Further studies are needed to confirm these findings in a population-based setting, and to explore the natural history of other food allergens such as wheat, sesame, soy and fish.

8.2.2. Consequences of early life food sensitisation

In this thesis, I investigated the associations between food sensitisation in early life and subsequent allergic disease outcomes and lung function measures, by undertaking a systematic review and performing original data analyses (**Chapters 4, 5, 6 and 7**). I found that food sensitisation in infancy may predict persistence of sensitisation and/or probable food allergy during adolescence. Individuals with sensitisation to more than one food allergen carry a greater risk of later food allergy. Results from my systematic review and meta-analysis (**Chapter 5**) showed that sensitisation to food allergens in early life was associated with an increased risk of asthma/wheeze, eczema and allergic rhinitis from 4 to 8 years of age. I found inconsistent findings in relation to the association between food sensitisation and asthma/wheeze in infancy, and only one high-risk cohort (Rhodes *et al.*, 2001) had assessed asthma as an outcome during adulthood (at 22 years), demonstrating a positive association. I did not identify any studies in my literature review that assessed the association between food sensitisation and allergic rhinitis beyond the age of 8 years. However, in most included studies, concomitant early life eczema and/or wheeze had not been considered. This is an important issue as eczema in early life is commonly associated with high levels of food specific IgE (Hill *et al.*, 2000) and has been associated with increased risk of later asthma and

allergic rhinitis (A. Lowe *et al.*, 2007). Similarly, wheeze has been related to food specific IgE (Wang *et al.*, 2005).

In my original analysis, I assessed the association between mutually exclusive groups of food sensitisation (no sensitisation, food only, aero only, both food and aero) and subsequent asthma and allergic rhinitis at ages 10-12 years (in MACS and LISAplus cohorts) and 18 years (in MACS only). I found that food without aeroallergen sensitisation in the first two years, independent of early life eczema and wheeze, predicts asthma and allergic rhinitis in later childhood (10-12 years). Moreover, I observed that co-sensitisation to both food and aeroallergen was the strongest predictor at any time point tested. In contrast, sensitisation to food without aeroallergen in the first two years was not significantly associated with asthma at 18 years in MACS, while significant associations were observed for co-sensitisation to both food and aeroallergens at 1 or 2 years of age. The associations I observed at 18 years were not as strong as those observed at 12 years. To date, none of the other cohorts have published associations between early life food sensitisation and asthma and allergic rhinitis at 12 or 18 years. My analysis was the first to explore these associations using well defined food sensitisation groups.

Additionally, I examined the relationships between mutually exclusive groups of food sensitisation and lung function (FEV_1, FVC and FEV_1/FVC ratio) during adolescence (at 12 and 18 years in MACS and at 15 years LISAplus). None of the available literature examined these associations with food sensitisation separately. Interestingly, I found that sensitisation to food without aeroallergen at 6 or 12 months was associated with reduced pre-bronchodilator (pre-BD) FEV_1 at both 12 and 18 years. I further investigated whether the observed association between food sensitisation and FEV_1 was mediated by aeroallergen sensitisation at 12 or 24 months, or asthma at 6, 12 or 18 years. I found that asthma at 6 years, but not early life aeroallergen sensitisation partially mediated this association. Furthermore, I found that aeroallergen without food sensitisation at 2 years was associated with a reduction in pre-BD FEV_1 and FEV_1/FVC ratio in adolescence. This is in line with findings from previous research that assessed the lung function measures during childhood (Lowe *et al.*, 2004; Illi *et al.*, 2006). However, I was unable to establish the effect of food sensitisation at 6 and 12 months in the population-based LISAplus study, as sensitisation was not assessed in the first year of life. Since the significant deficit in FEV_1 in MACS was demonstrated in those who had food sensitisation in infancy, I was unable to confirm this finding in LISAplus

as sensitisation was not assessed at 6 and 12 months. Food sensitisation in infancy could be a potential risk factor, or an early immunological marker, for adolescent lung function impairment. However, additional research is required to confirm these associations in population-based settings.

Finally, I was unable to explore the consequences of specific food allergen sensitisation due to small numbers in each group. Further research is required to investigate the role of specific food allergens which may have implications for implementation of preventive strategies.

8.3. Evaluation of the evidence for causality versus association

The observed statistical associations throughout my thesis do not necessarily indicate causal associations. Criteria first suggested by Bradford Hill provide evidence that associations may be causal in nature (Höfler, 2005). The main Bradford Hill criteria include: temporal sequence, strength and consistency of an association and biological plausibility.

In order to assess whether a causal relationship exists between food sensitisation in early life and the development of probable food allergy, asthma, allergic rhinitis and reduced lung function in later childhood and adolescence, the guidelines by Bradford Hill were used. The only essential, but not sufficient, criterion is temporality: the cause must antedate the effect. Birth cohort studies with prospective collection of data and frequent assessment of exposures and early signs of allergic diseases are the ideal design to evaluate a potential temporal relationship. In this thesis, all investigated associations originated from longitudinal birth cohort studies, MACS and LISAplus, both of which have a temporal sequence between exposure (food sensitisation) and outcomes of interest (probable food allergy, asthma, allergic rhinitis and lung function).

Consistent observation of an association in different populations and with different study designs also provides support for a causal effect. Even though I found associations between food sensitisation in the first two years and development of asthma and allergic rhinitis in later childhood, not all previous studies showed statistically significant associations. This could be explained by the differences in the ages of outcomes assessment, the cut-off point that has been used for defining food sensitisation, or the way outcomes were defined.

Regarding lung function outcomes, I was unable to compare my findings with previous literature as, to my knowledge, this is the first study that has assessed the association between food sensitisation in early life and lung function during adolescence. Therefore, the Bradford Hill criterion of consistency has not been met.

Strong associations are more likely to be causal than modest associations, however, there is no recognised definition of a "strong association". Overall, the observed associations in my results varied in strength according to the presence or absence of co-existing aeroallergen sensitisation. I observed weak associations between sensitisation to food allergens only and asthma and allergic rhinitis in late childhood (ORs ranged from 1.1 to 2.2). On the other hand, stronger associations were observed between sensitisation to both food and aeroallergens and subsequent asthma and allergic rhinitis (ORs ranged from 2.8 to 8.3). However, weak associations in observational studies can easily be due to bias such as misclassification of the exposure (as discussed below in *Section 8.5.1*) or unmeasured confounding.

Although several cohort studies have shown associations between food sensitisation and asthma and allergic rhinitis during early childhood, the biologically plausible pathways are not fully understood. It has been suggested that in individuals sensitised to allergens, a series of cellular and molecular interactions involving B cells, T cells and antigen presenting cells (APCs) result in the release of several pro-inflammatory mediators and cytokines (Galli *et al.*, 2008). The release of these mediators contributes to the acute symptoms and signs related to allergic reactions, including vasodilatation, increased vascular permeability and bronchial smooth muscle contraction (Hamelmann *et al.*, 1999; Galli *et al.*, 2008). After repeated exposure to allergens, persistent inflammation occurs that may lead to long term structural and functional changes of affected tissues (Galli *et al.*, 2008).

While it has been shown that sensitisation to aeroallergens in early life is associated with loss of lung function during childhood (Lowe *et al.*, 2004; Illi *et al.*, 2006), the mechanisms underlying the relationship between allergen sensitisation and lung function remain unclear. It has been suggested that persistent environmental exposures could induce inflammatory processes that lead to airflow limitation (Gottlieb *et al.*, 1996). However, the role of early life food sensitisation on lung function has not been assessed separately in previous studies. Therefore, further research in this area may reveal the potential pathways for these relationships.

In summary, according to the main Bradford Hill criteria that have been assessed above, the associations between early life food sensitisation and subsequent development of probable food allergy, asthma, allergic rhinitis and reduced lung function in later childhood and adolescence have shown clear temporal relationships, inconsistent findings between studies, variable strength of associations as well as unclear biological plausibility. Therefore, to date, it is not known whether these associations are causal or as a result of shared environment and/or shared genetics factors.

8.4. Methodological strengths and limitations

8.4.1. Study strengths

In my thesis, I analysed data from the MACS birth cohort to examine the natural history of food sensitisation and presented this work in **Chapter 4**. Moreover, I further analysed data from two birth cohort studies, the Australian MACS and the German LISAplus, and presented the findings in **Chapters 6** and **7**. Birth cohort studies are the best design for exposures that cannot be randomised, such as food sensitisation, and for documenting the natural history. One of the major strengths of this work is the length of follow-up of both cohorts, which extends up to 18 years in MACS and up to 15 years in LISAplus.

In my thesis, assessment of the exposure (food sensitisation) and some of the investigated outcomes (such as lung function) were based on objective measures. Sensitisation was assessed by clinical testing including SPTs in MACS and s-IgE in LISAplus, while lung function was assessed by spirometry following a standardised technique. Using objective measures to examine particular associations is important to reduce the possibility of misclassification and strengthen the observed findings. Furthermore, both cohorts assessed atopic sensitisation frequently throughout the follow-up period and from early life. In particular, sensitisation was evaluated at three time points in the first two years of life in MACS. Considering that food sensitisation is mainly an early life event, it has been suggested that exposure to the appropriate dose of food allergens during this critical period is important for proper immune response (or immune tolerance) to foods and for the development of sensitisation.

The two cohorts that have been analysed in my thesis have also collected extensive information on early life related factors along with careful documentation of allergic outcomes and related symptoms several times over the length of these studies. The frequency of data collection minimises the possibility of recall bias. Moreover, the temporal sequence of collected data adds a high level of evidence to any causal hypotheses explored through an understanding of the sequence involved with exposures and outcomes. This is also one of the major strengths of this work.

In **Chapters 6 and 7**, data were analysed from two independent cohorts from different regions of the world, but both in high income countries with high prevalences of food sensitisation (Burney *et al.*, 2010) and allergic diseases (Pearce *et al.*; Maziak *et al.*, 2003; Stock *et al.*, 2005). It is often assumed that results from a high-risk cohort may not be applicable to the general population, but interestingly I found almost similar results across the two cohorts whenever sensitisation data were available.

The sampling frame used in MACS is both a strength and limitation. MACS is an allergy high-risk cohort in which the recruitment of participants was based on the presence of family history of asthma, eczema, food allergy and/or allergic rhinitis. The main advantage of studying a high-risk group is that the allergic disease outcomes are more common, which increases the statistical power of the study. Additionally, high-risk infants are of particular interest as a potential target group for interventions to attempt to prevent the development of allergic diseases. On the other hand, by considering the population source of the MACS cohort, results obtained from this cohort (all results presented in **Chapter 4**; 6 and 12 months sensitisation results presented in **Chapters 6** and **7**) might not be directly extrapolated to the general population. Limiting the sample to children, who had familial high-risk of allergic disease, may mean that it is not possible to extend the findings to children who are not at high-risk of allergic disease. Although a high-risk cohort might alter the observed prevalence of sensitisation and allergic disease, it is less likely to alter the associations found. Additionally, MACS was carried out in a country with a high prevalence of allergic diseases. Therefore, these issues should be considered when interpreting my results and prior to generalising findings.

8.4.2. Study limitations

Loss to follow-up is a common issue in longitudinal studies and its rate increases with increasing duration of the study. Loss to follow-up is inevitable in most cohort studies and commonly leads to selection bias, particularly when it is non-random and linked to the study outcomes. Additionally, as a result of loss to follow-up, the statistical power of the study to address specific research questions may reduce, especially in relatively small studies like MACS. However, both MACS and LISAplus studies achieved good follow-up rates, with more than 50% attendance at the 12 and 18-year follow-ups in MACS and 10 and 15 year follow-ups in LISAplus. The characteristics of participants who were lost to follow-up, along with the impact on results, have been discussed in **Chapters 4**, **6** and **7**. Briefly, parental education and smoking status were the only baseline factors that influenced the participation rate in MACS. Although both lower educational level and smoking are proxy measures for lower socioeconomic class, the relationship between socioeconomic status and atopic sensitisation and allergic diseases is controversial. However, previous studies suggest a higher risk of atopic sensitisation, asthma and allergic rhinitis in individuals from lower socioeconomic classes (Almqvist *et al.*, 2005; Litonjua *et al.*, 2005). Therefore, the results related to the natural history of food sensitisation in MACS (**Chapter 4**) and the observed associations between sensitisation and asthma and allergic rhinitis (**Chapter 6**) may be an underestimate, due to the reduced number of children from lower socioeconomic groups included.

8.5. Methodological considerations within my thesis

The overall methodological issues and study strengths and limitations related to my results chapters will be discussed in this section. Issues that are related to each specific results chapter are discussed in that particular chapter.

8.5.1. Exposure variable

8.5.1.1. Skin prick test (SPT) versus measurement of serum specific IgE (s-IgE)

There are different confirmatory tests that can be conducted to detect allergen specific IgE antibodies in an individuals' skin or blood. The most commonly used tests in clinical practice and epidemiological studies are SPT and the measurement of s-IgE levels. Results of SPT and s-IgE are often used interchangeably in clinical practice and allergy research even though there is little evidence on how well these two diagnostic methods correlate with each other. However, the prevalence of sensitisation has been reported to be higher when measured by s-IgE compared to SPT (Kjaer *et al.*, 2009; Rø *et al.*, 2014). Agreement between s-IgE and SPT in the etiological diagnosis of allergic diseases is reported to be greater than 90% (Williams *et al.*, 1992). However, SPT and s-IgE measurement provide different information. One major difference is that SPT detects IgE attached to mast cells, while the in vitro test detects IgE in serum (Leto-Barone *et al.*, 2013).

Although the agreement between SPT and s-IgE is controversial, both tests have been shown to reliably predict the risk of allergic diseases. In **Chapter 5** (systematic review), I examined the current evidence for the association between early food sensitisation and subsequent asthma, eczema and allergic rhinitis. I concluded that food sensitisation, as assessed by either SPT or s-IgE, was associated with increased risk of later development of allergic conditions. In my thesis, sensitisation was assessed by SPT in the MACS cohort and by s-IgE in the LISAplus study. A recent study by Ro *et al.* (Rø *et al.*, 2014) showed that the predictive value of SPT and s-IgE performed at 2 years of age was generally comparable in predicting allergic diseases in later childhood. Another study by Kjaer *et al.* (Kjaer *et al.*, 2009) demonstrated that early food sensitisation with or without aeroallergen sensitisation, as assessed by either s-IgE or SPT, was a highly significant predictor for allergic disease at the age of 6 years.

8.5.1.2. Definition of positive SPT

A positive SPT reflects the existence of mast cell-bound IgE specific to the tested allergen (Plebani, 2003). While different cut-off points have been used for defining a positive SPT reaction, the most widely accepted cut-off is a wheal diameter of 3 mm or more (P. A. Eigenmann *et al.*, 2013); this represents the standard clinical definition of sensitisation

(Sampson, 2004). This cut-off point has been utilised in the majority of previous epidemiological longitudinal studies (Burr *et al.*, 1997; Kjaer *et al.*, 2009; Garden *et al.*, 2013). To my knowledge, only one birth cohort study has used another threshold for defining positive SPT responses. This British study defined the SPT reaction as positive if the mean diameter of the skin wheal at 15 minutes was 2 mm or greater in subjects less than 2 years of age and 3 mm or greater in subjects older than 2 years (Rhodes *et al.*, 2002).

It has been reported that positive SPT reactions are likely to be smaller in infants and children younger than 2 years of age (Bernstein *et al.*, 2008; Osborne *et al.*, 2011), presumably because of a lack of antigen-specific IgE and skin reactivity (Ménardo *et al.*, 1985). Therefore, throughout this thesis for the MACS cohort, I used a 2 mm cut-off point to define positive skin prick test reaction at 6, 12 and 24 months and 3 mm cut-off point in participants older than 2 years. This had the benefit of including more children at increased risk of allergy and perhaps increased the power in my analysis to investigate associations. On the other hand, using a lower threshold for defining positive SPT might increase the number of false positive cases and hence overestimate the actual prevalence rates of sensitisation when comparing the results with other studies.

8.5.1.3. The number of tested food allergens

In the MACS cohort, SPT was performed using three of the most common food allergens for the region (egg white, cow's milk and peanut) (Hill *et al.*, 1999), up to the 12-year follow-up. Additional food allergens, including cashew and shrimp, were added at the 18-year follow-up. For consistency in estimating the prevalences of food sensitisation from infancy up to young adulthood, I restricted my analysis in **Chapter 4** (natural history of food sensitisation) to the three food allergens that were tested in all follow-ups.

Even though the tested food allergens were the most prevalent allergens in Australia, I cannot exclude the possibility that participants were sensitised to other untested food allergens. Cross-reactivity between the tested allergens with untested allergens may have partially reduced the number of missed cases (De Leon *et al.*, 2003), but may overestimate the observed prevalence of food sensitisation. It has been reported that individuals with peanut allergy have IgE antibodies to more than one tree nut (Sicherer and Sicherer, 2001). Considering only three food allergens to evaluate food sensitisation may increase the probability of false negative results. Additionally, differential misclassification of children as

not being sensitised may underestimate the observed association between early life sensitisation and subsequent probable food allergy, asthma, allergic rhinitis and lung function because sensitised infants would have been analysed in the non-sensitised group.

8.5.1.4. Lack of normal saline for SPT

In MACS, a saline negative solution was not performed for SPT up to 18-year follow-up. Thus a positive SPT was defined as "a wheal of 2 mm diameter or greater (in children age 2 years or younger) and 3 mm diameter or greater (in children older than 2 years)". A negative control solution is required to evaluate unspecific reactions related to prick testing trauma. Using the original wheal of an allergen without reference to the saline control raises the possibility of false positive due to the presence of dermographism. However, this phenomenon is rare and almost never observed in infants, therefore it is unlikely to alter the results (Kontou-Fili *et al.*, 1997; Antunes *et al.*, 2009).

8.5.2. Outcome variables

8.5.2.1. Definitions of asthma, allergic rhinitis and food allergy

In my thesis, the definitions of asthma and allergic rhinitis relied on the participants' responses to distributed questionnaires. The details of these definitions have been given in the general methods (**Chapter 3**). Asthma and allergic rhinitis symptom questionnaires have been used widely in epidemiological research, particularly in longitudinal surveys or large surveys where it may not be feasible to perform physiological tests for all participants. The definition for food allergy at ages 12 and 18 was based on predictive values of SPT wheal sizes to specific food allergens (for a detailed description, see *Section 2.6.2.1*) and the reported history of allergic reaction when consuming that food. Although oral food challenge is the gold standard test to diagnose food allergy, relatively few epidemiological studies have utilised this measure for defining food allergy. Many epidemiological studies have depended on the detection of food specific IgE antibodies, either by SPT or serum s-IgE measurement, to diagnose food allergy (Osborne *et al.*, 2011; Peters *et al.*, 2013). Larger wheal sizes on skin prick testing have been shown to be associated with an increased likelihood that individuals are truly food allergic (Peters *et al.*, 2013).

Some non-differential misclassification of outcomes may have occurred due to lack of objective measures to define these disorders. The conclusions drawn from this thesis could have been strengthened if participants were examined for objective evidence of asthma, allergic rhinitis and food allergy.

8.5.3. Confounding Issues

The MACS and LISAplus datasets contain many exposure variables, potential confounders, effect modifiers and/or mediators that could be considered. A range of potential confounders for the relationship between the exposure (food sensitisation) and the outcomes of interest were included in the final model if they resulted in significant confounding (10% or greater change in the odds ratio for the primary predictor) or if there was evidence in the literature that they were associated with the outcomes. The results from my thesis are observational in nature. It is possible that some of the observed associations (between food sensitisation and subsequent allergic diseases or lung function) are due to confounding by factors that were not measured such as ethnicity.

Originally, the main aim of MACS was to determine whether the use of different feeding formulas attenuated the development of allergic symptoms in allergic high-risk cohort of infants. Using data from randomised controlled trials to test additional hypotheses about the association between non-randomised exposures and outcomes determined during long term follow-up is a well-established method. It is based on the testable assumption that the randomised intervention does not influence the associations of interest (Howard and Howard, 2012). A previous MACS publication showed that the randomisation status (infant formula allocation) was not associated with the outcomes of interest (Lowe *et al.*, 2011). Therefore, MACS continues as an observational study and I adjusted for the intervention group allocation in all analyses.

8.5.4. Subgroup analysis

Although the initial participants of the MACS birth cohort were 620 infants, the number of individuals who attended the 12 and 18-year follow-ups was 366 and 404, respectively. While this is still a relatively large sample, there was limited statistical power to detect associations. Some of my results did not reach statistical significance, however these associations might still be clinically relevant. I have considered the confidence intervals and

the p values in interpreting these findings. Wide confidence intervals indicate lack of precision in estimating relationships. I have deemed some of these associations to be clinically relevant whilst acknowledging the lack of statistical precision.

I also have limited statistical power to detect relationships within subgroups, such as the associations between specific food allergen sensitisation and asthma, allergic rhinitis or lung function. This has significant implications in advancing the field of allergy by identifying individuals at greater risk of allergy who could be targeted for preventive measures.

CHAPTER 9 - Conclusions and Recommendations

9.1. Introduction

Food allergy and other allergic diseases are a major global health problem. They are accountable for a high proportion of child and adult morbidity, substantial school absenteeism and loss of productivity in the workforce, significant psychological stress in affected individuals and their families, in addition to a high financial burden in terms of direct and indirect health costs.

A better understanding of potential risk factors that predict the development of allergic disease provides a starting point for public health measures aimed at preventing or delaying the progression of these conditions. Additionally, as most allergic disease arises in early childhood it is essential to evaluate these factors during early life. This is also the time of life when modifications to potential risk factors may provide the greatest benefit.

The overall aims of my thesis were to investigate the natural history of food sensitisation from infancy to adolescence and to examine the consequences of early life food sensitisation. By undertaking a systematic review of the literature and analysing data from two longitudinal birth cohorts with extended follow-up periods to adolescence, I have been able to achieve these aims. This chapter is a summary of the results relating to these objectives, along with their implications. Finally, I provide recommendations for further research to confirm and extend these findings where required.

9.2. Summaries and implications of the findings

9.2.1. Natural history of food sensitisation (Chapter 4)

9.2.1.1. Summary of the main findings

The key findings from this chapter were as follows:

- The prevalence of food sensitisation for the MACS, allergy high-risk cohort, was highest in infancy and declined after 12 months of age.

- Egg white was the most common food sensitisation in the first two years of life, followed by peanut and cow's milk. Sensitisation to peanut was most common at the age of 18 years.
- Sex, eczema in the first six months, and aeroallergen sensitisation at 6 months modified the natural history of milk sensitisation, while only sex and eczema modified the natural history of egg sensitisation. In contrast, none of these factors modified the natural history of peanut sensitisation.
- Boys with early life eczema had the highest prevalence of milk and egg sensitisation throughout follow-ups.
- A small proportion of individuals (7%) developed new onset food sensitisation after the age of 2 years. However, late onset food sensitisation was unlikely to be clinically relevant.
- At an individual level, food sensitisation status changed from positive to negative and from negative to positive throughout the follow-up period.
- Food sensitisation at 12 months was associated with increased risk of adolescent food sensitisation and probable food allergy, with sensitisation to more than one food allergen being the strongest predictor.

9.2.1.2. Implications of the findings

This is the first research to explore the natural course of SPT reactivity patterns for egg, milk and peanut from 6 months to 18 years in an allergy high-risk birth cohort. I confirmed that food sensitisation was more prevalent in the first two years of life. Although sensitisation to cow's milk is known as the most common food allergen during infancy, unlike other studies, I found a higher prevalence of egg and peanut sensitisation during this period. Although the type of intervention (as MACS was initiated as an RCT as described in *Section 3.2.3*) may influence the prevalence of milk sensitisation, I did not observe any significant interaction with the type of formula allocation. Differences in the prevalence of egg, milk and peanut sensitisation between the current analysis and previous studies could be due to variation in genetic background, geographical and environmental exposures, and infant feeding practices between different populations.

I found that sex, early life eczema and aeroallergen sensitisation at 6 months modified the natural history of milk sensitisation; sex and eczema modified the natural history of egg sensitisation; while the natural history of peanut sensitisation was not modified by any of these factors. The association between these factors and the development of food sensitisation is complex and was described in *Section 2.6.1.3*. This observation may highlight the differences in underlying pathophysiology between these three food allergens. Additionally, it appears likely they represent three different categories, each with different risk factors. Considering these potential risk factors in future clinical and epidemiological studies may contribute to the development of preventive strategies for each specific food allergen. Boys with eczema in the first six months had a higher prevalence of milk and of egg sensitisation throughout the first 18 years of life. Therefore, this group can be recognised as a higher risk population and can be monitored and targeted for allergy prevention measures (see *Section 9.3.3* for a more detailed description of potential allergy prevention strategies). Additionally, sex and early life eczema can be used as prognostic markers that could be used to inform parents of future risks for their children.

While food sensitisation is common in early life, I found that a number of individuals developed food sensitisation after the age of 2 years. However, when compared to those who developed early life food sensitisation, the wheal sizes of SPTs were smaller and most of them did not report a clinical reaction to the same food. This observation highlighted the importance of the timing of onset of food sensitisation. Although earlier assessment of food sensitisation may be a useful allergy bio-marker, late onset food sensitisation is more likely to be a transient phenomenon. Food sensitisation in infancy may predict persistence of sensitisation and/or probable food allergy during adolescence. Individuals with sensitisation to more than one food allergen carry a greater risk. These observations may benefit the clinician, allowing them to tailor the advice they give to parents on the clinical relevance of positive SPT.

In conclusion, the following factors should be considered when assessing and predicting the consequences of food sensitisation: sex, early life eczema status, early aeroallergen sensitisation, the timing of onset of food sensitisation, the number of food-specific positive SPTs and the type of food allergen.

9.2.2. Association between food sensitisation and subsequent allergic diseases: a systematic review and meta-analyses (Chapter 5)

9.2.2.1. Summary of the main findings

By searching two main databases, MEDLINE and SCOPUS, 15 original articles, from 13 birth cohorts, met my pre-defined inclusion criteria. Findings from these included articles showed that early life food sensitisation was associated with an increased risk of infantile eczema, childhood wheeze/asthma, eczema and allergic rhinitis and young adulthood asthma. Meta-analyses demonstrated that food sensitisation in the first two years of life was related to an increased risk of wheeze/asthma, eczema and allergic rhinitis from 4 to 8 years of age.

9.2.2.2. Implications of the findings

This was the first systematic review and meta-analyses that assessed the current evidence related to the association between early life food sensitisation and subsequent allergic diseases in birth cohort studies. My review supported the hypothesis that food sensitisation "marches" towards other allergies. Thus, early life food sensitisation can be used as an early marker for identifying individuals at greater risk of subsequent allergic disease who may benefit from early life preventive strategies. However, due to lack of follow-up data beyond childhood and a lack of inclusion of key confounders in the majority of studies, additional research is needed to confirm these findings.

9.2.3. Association between food sensitisation and subsequent asthma and allergic rhinitis during late childhood and adolescence (Chapter 6)

9.2.3.1. Summary of the main findings

The associations between food with or without aeroallergen sensitisation in the first two years and asthma and allergic rhinitis at **10-12 years** were examined within *both MACS and LISAplus* cohorts. The key findings from this analysis were as follows:

- In MACS, the allergy high-risk cohort, sensitisation to food allergens without concurrent aeroallergen sensitisation at **12 months** was associated with increased risk of current asthma and allergic rhinitis at 12 years, compared to no sensitisation. Similar trends were observed for sensitisation at 6 and 24 months, however these were not statistically significant.

- In LISAplus, the population-based cohort, sensitisation to food without concurrent aeroallergen at **24 months** was associated with increased risk of asthma and allergic rhinitis at 10 years, compared to no sensitisation.

- In both MACS and LISAplus, co-sensitisation to both food and aeroallergen at all tested time points (6, 12 and 24 months in MACS and 24 months in LISAplus) was associated with increased risk of asthma and allergic rhinitis at 10-12 years, compared to no sensitisation. These associations were stronger than sensitisation to food only.

The associations between food with or without aeroallergen sensitisation in the first two years and asthma and allergic rhinitis at **18 years** were examined within the *MACS study only*. The key findings from this analysis were as follows:

- Sensitisation to food only at all tested time points was not related to increased risk of asthma at 18 years.

- Co-sensitisation to both food and aeroallergen at **12 or 24 months** was associated with increased risk of asthma at 18 years. A stronger association was observed for sensitisation at 24 months.

- Sensitisation to food only at **12 months** was associated with increased risk of allergic rhinitis at 18 years.

- Co-sensitisation to food and aeroallergen at **24 months** was associated with increased risk of allergic rhinitis at 18 years.

9.2.3.2. Implications of the findings

The march from early aeroallergen sensitisation to subsequent asthma and allergic rhinitis is well established, but it is unclear if early life food sensitisation precedes and further increases the risk of these conditions. In this analysis, I provide evidence for the role of early life food sensitisation with or without co-sensitisation to aeroallergen, independent of early

life eczema, on the atopic march. Developing interventions that prevent early life food sensitisation may reduce the likelihood of the atopic march to asthma and allergic rhinitis occurring. Overall, my analyses showed that food sensitisation in the first two years, independent of early life eczema and/or wheeze, predicted asthma and allergic rhinitis in later childhood (i.e. 10-12 years). Although early life food sensitisation was associated with later allergic airway diseases, it is not known whether these associations are causal or arise as a result of shared environment and/or shared genetics factors (see *Section 8.3*). Nevertheless, assessment of food sensitisation in infants could provide valuable information on predicting the risk of later childhood asthma and allergic rhinitis.

On the other hand, I found that food without aeroallergen sensitisation in the first two years was not a predictor for asthma, but could be a predictor for allergic rhinitis, during adolescence (i.e. at 18 years) in the allergy high-risk cohort. This suggests that the asthma phenotype during later childhood may be different from asthma during adolescence. Therefore, the risk factors for asthma may vary between these two age groups. This factor should be considered when further assessment of the association between food sensitisation and subsequent asthma is undertaken.

Findings from both MACS and LISAplus demonstrated that co-sensitisation to food and aeroallergen was the strongest predictor for asthma and allergic rhinitis in later childhood and adolescence. This finding suggests that children with co-sensitisation carry a greater risk for these conditions compared to those who had early food only or aero only sensitisation. Children with early life co-sensitisation to both food and aeroallergen should be considered a high-risk group and targeted for early allergy preventive measures.

In summary, food sensitisation in the first two years of life may be considered an early biomarker for predicting which children are at greater risk for later asthma and allergic rhinitis. However, co-sensitisation to both food and aeroallergen is a stronger allergy predictor.

9.2.4. Association between food sensitisation and lung function in adolescence (Chapter 7)

9.2.4.1. Summary of the main findings

The associations between food with or without aeroallergen sensitisation in the first two years of life and lung function indices during adolescence (i.e. at 12, 15 and 18 years) were examined within *both the MACS and LISAplus* cohorts. The key findings from this chapter were as follows:

- In MACS, food without aeroallergen sensitisation at **6 or 12 months** was associated with a reduction in pre-BD FEV_1 at 12 and 18 years.
- While most of the effect of food sensitisation on pre-BD FEV_1 was direct, early life asthma, but not aeroallergen sensitisation, partially mediated these associations.
- In MACS, food without aeroallergen sensitisation at **6 months** was associated with a reduction in post-BD FEV_1 at 18 years.
- In MACS, food without aeroallergen sensitisation at **6 months** was associated with a reduction in pre-BD FEV_1/FVC ratio at 12 years and post-BD FEV_1/FVC ratio at 18 years.
- In both MACS and LISAplus, food with or without aeroallergen sensitisation at **24 months** was not associated with any of the assessed lung function measures.
- In both MACS and LISAplus, sensitisation to aeroallergens only at **24 months** was associated with a reduction in pre-BD FEV_1 and FEV_1/FVC ratio in adolescence.
- In both MACS and LISAplus, asthma and personal smoking at the age of lung function assessment did not modify any of the observed associations.

9.2.4.2. Implications of the findings

To my knowledge, this was the first analysis that examined the associations between early life food sensitisation and lung function measures during adolescence. I provide evidence, from the allergy high-risk cohort, for a relationship between early life foods without co-sensitisation to aeroallergen during infancy and reduced FEV_1 and FEV_1/FVC

ratio in adolescence. Therefore, food sensitisation in infancy could be a potential risk factor, or an early immunological marker, for adolescent lung function impairment. I also found that asthma in early childhood partially mediated the effect of food sensitisation on pre-BD FEV_1 in adolescence. Although the nature of the association between atopy, reduced lung function and asthma is complex, it remains to be determined if more appropriate management of asthma during early childhood can improve lung function in adolescence.

Given that the diagnosis and severity coding of obstructive airway diseases are based on lung function measures, factors associated with their reduction might be considered potential targets for disease prevention or treatment. I found that food sensitisation in infancy was associated with lung function impairment during adolescence. This suggests that the critical time for allergic sensitisation may be within the first year of life. The association between early onset food sensitisation and lung function reduction might suggest antenatal triggers or causes. Further studies are required to confirm these findings in a population-based setting.

In conclusion, food sensitisation without coexistent aeroallergen sensitisation in the first year of life, as well as aeroallergen sensitisation at 2 years of age could be regarded as early biomarkers for predicting lung function deficits during adolescence.

The findings within this thesis have a number of implications for researchers, clinicians and public health policy in this area. In the following section, I outline the substantial recommendation for researchers, clinicians and public health policy makers.

9.3. Recommendations

9.3.1. For researchers

I observed that sensitisation to egg white and peanut were the most common food sensitisations in the first year of life, which contrasts with the findings from previous literature. These results were consistent across the three arms of a formula intervention trial in children with a family history of allergic disease. Former studies have shown that cow's milk is the most prevalent food allergen during infancy. This result suggests that the prevalence of specific food allergens is different between regions according to their genetic predisposition as well as their exposure to food allergens. Thus, national studies are required to identify the local prevalent food allergens. Additionally, this finding could be restricted to

individuals with a family history of atopy. Therefore, further population-based studies are needed to explore the natural history of specific food allergen sensitisation.

Moreover, I found that factors such as sex, early life eczema and/or aeroallergen sensitisation modified the natural history of milk and egg white sensitisation from infancy up to adolescence. Therefore, these findings should be taken into account in studies that aim to assess the prevalence of food sensitisation or identify children at greater risk for allergy who could be targeted for allergy preventive strategies. Moreover, I found that food sensitisation after the age of 2 years was less likely to be clinically relevant. Thus, the timing of onset of food sensitisation should also be considered in epidemiological and clinical studies that propose to investigate the underlying pathophysiology, causes or consequences of food sensitisation. Further studies that explore the relationship between the process of maturation of the immune system in early life and the development of food sensitisation are required.

I also observed, in the allergy high-risk cohort, that food sensitisation in the first two years of life was a predictor for an increased risk of asthma in later childhood (12 years), but not in adolescence (18 years). This suggests that the potential risk factors for later childhood asthma and adolescent asthma could be different. Therefore, the age of current asthma should be considered whenever the associations between food sensitisation and subsequent asthma are assessed. On the other hand, co-sensitisation to both food and aeroallergen, in both the allergy high-risk cohort and the population-based cohort, was a stronger predictor for development of adolescent asthma and/or allergic rhinitis when compared to food only or aero only sensitisation.

Furthermore, I found that food sensitisation at 6 months in the allergy high-risk cohort was related to lung function deficits during adolescence. However, this observation could not be confirmed in the population-based study as atopic sensitisation was not assessed before the age of 2 years. Thus, further longitudinal population-based studies are needed to confirm these observed associations.

When the consequences of food sensitisation were assessed in my thesis, I used mutually exclusive groups to model the atopic sensitisation status (the exposure) which included: (1) No sensitisation; (2) Food sensitisation only; (3) Aeroallergen sensitisation only; and (4) Sensitisation to both food and aeroallergen, using those who were not sensitised as the reference group. I observed different associations between each group and subsequent

asthma, allergic rhinitis and lung function measures. This suggests that categorisation of individuals into a specific sensitisation group, according to the type of allergen sensitised to, is important in predicting the risk of subsequent allergies and identification of an allergy high-risk group.

9.3.2. For clinicians

The results within this thesis may have implications for clinicians in a number of areas.

- The finding that food sensitisation status changes over time, and that late onset food sensitisation was less likely to be clinically relevant informs the discussion between clinicians and parents regarding the results of subsequent SPTs and the prognosis of late onset food sensitisation. Full details of this finding are presented in **Chapter 4**.
- The findings may also aid clinicians in predicting which children with food sensitisation in the first two years have a high-risk of developing persistent sensitisation, probable food allergy, asthma, allergic rhinitis and lung function impairment in late childhood and adolescence. Performing SPT for food allergens at ages 6, 12 or 24 months in these children is a useful method for assessing the subsequent risk of these conditions. However, this should be done with caution so as not to imply that the foods, if tested positive, should be removed from the diet (positive test is not equivalent to allergy, as noted earlier in the thesis). Full details of this finding are presented in **Chapters 4, 6** and **7**. Clinicians could then use this information to develop specific plans and strategies for treatment and prevention of modifiable environmental triggers in high-risk children.

9.3.3. For public health policy makers

There is evidence from cross-sectional and prospective studies that the prevalence of allergic diseases is increasing. There is also intense interest in identifying modifiable risk factors for the prevention of allergic disease. The findings from my thesis showed that food sensitisation in early life is a predisposing factor for subsequent development of probable food allergy, asthma and allergic rhinitis. Thus, early life preventive measures aimed at reducing the occurrence of food sensitisation may consequently contribute to lessening the prevalence of these allergic disorders. The current evidence for allergy prevention includes the following: (1) Early introduction of solid food, starting from 4-6 months, while

continuing breastfeeding; (2) Introduction of allergenic solid food such as peanut, egg, diary and wheat products in the first year of life, particularly in infants at high-risk of allergy; and (3) Breastfeeding for at least 6 months (Du Toit *et al.*, 2015; ASCIA, 2016; Du Toit *et al.*, 2016).

There are three stages of allergy prevention: 1) Primary prevention blocks immunologic sensitisation to foods; 2) Secondary prevention suppresses disease expression after sensitisation; and 3) Tertiary prevention prevents symptoms after disease expression. Primary prevention of allergy would be ideal. Secondary prevention may be the option followed by families interested in allergy prevention only after atopy risk is documented in their offspring. However, secondary prevention is difficult because the mechanisms involved in the progression of sensitisation into allergic diseases are incompletely understood. Tertiary prevention is the stage in which clinicians treat patients to avoid recurrence of symptoms. A number of studies, in addition to the findings from my thesis, support the existence of a critical time early in infancy, possibly also prenatally, in which the genetically programmed atopic infant is at higher risk to become sensitised to ingested food allergens. Intervention efforts, therefore, must be introduced early (perhaps in the perinatal period) to have a chance of success.

The observation that food sensitisation in the first two years provides valuable information on predicting probable food allergy, asthma, allergic rhinitis and lung function impairment has implications for health policy makers. The routine assessment of food sensitisation on children may be warranted on this prognostic value alone. However, this test is not widely available at this time because there is currently no proven intervention available to prevent either the development of food sensitisation or the progression from food sensitisation in early life to later childhood and/or adolescent asthma, allergic rhinitis and impaired lung function. If it was recommended that all infants be investigated for food sensitisation, then availability of the test would need to be broadened. This requires additional health care resources and trained personnel. However, this would be costly and has adverse implications on infants and their families and healthcare providers. The prevention of sensitisation, and hence allergic diseases, requires collaboration between governments, communities, clinicians and other health care professionals, and patient organisations.

9.4. Future research

The findings within this thesis have raised further issues for future research including the following:

- I observed that food sensitisation in early life was associated with increased risk of probable food allergy, asthma, allergic rhinitis and lung function impairment. I was unable to assess the role of sensitisation to individual food allergens due to small sample sizes. Therefore, further studies with a larger sample size are required to examine the association between specific food allergens and these conditions. This could be useful in identifying specific subgroups of children at high-risk who should be targeted for preventive strategies.

- I found that early food sensitisation was associated with allergic disease outcomes in late childhood and adolescence. Additionally, co-sensitisation to both food and aeroallergen carries a greater risk for the development of allergic conditions. Therefore, further exploration of the role of early food and/or aeroallergen desensitisation as a potential therapy is needed.

- Determination of the dose response relationship between sensitisation (SPT wheal sizes or the level of s-IgE) and allergic outcomes and lung function could be an interesting area of research to predict a group of children at high-risk. This group could then be directed for close monitoring and prevention measures.

- I found that food sensitisation during infancy was associated with lung function deficits during adolescence. Future large scale population-based studies concerning the role of early life food sensitisation on subsequent lung function deficit are required, as my findings were restricted to children at high-risk of allergy. In the population-based cohort (LISAplus study), sensitisation was not assessed before the age of 2 years. On the other hand, the link between aeroallergen sensitisation at 24 months and subsequent lung function deficits were consistent across both studies. This highlights the importance of aeroallergen sensitisation as a predictor for lung function impairment in adolescence.

9.5. Conclusion

In conclusion, this thesis has addressed numerous issues in the epidemiology of food sensitisation in relation to allergic diseases and lung function during childhood and adolescence. It has answered some questions and illuminated the difficulties with others. I explored the natural history of food sensitisation from infancy up to adolescence (in an allergy high-risk cohort) (**Chapter 4**) and examined the relationship between food sensitisation in the first two years and probable food allergy, asthma, allergic rhinitis and lung function in later childhood and adolescence (Chapters **4, 5, 6** and **7**). I found that multiple factors, including sex, early life eczema and/or aeroallergen sensitisation, alter the natural course of egg white and cow's milk sensitisation. My findings concerning lung function impairment as a consequence of food sensitisation in infancy are novel. It is my hope that the results of this thesis will aid the current understanding of allergic conditions, identifying children at high-risk for asthma, allergic rhinitis and lung function deficits, help guide future research and, in the long term, help identify strategies to reduce the substantial burden of allergic disorders to the community.

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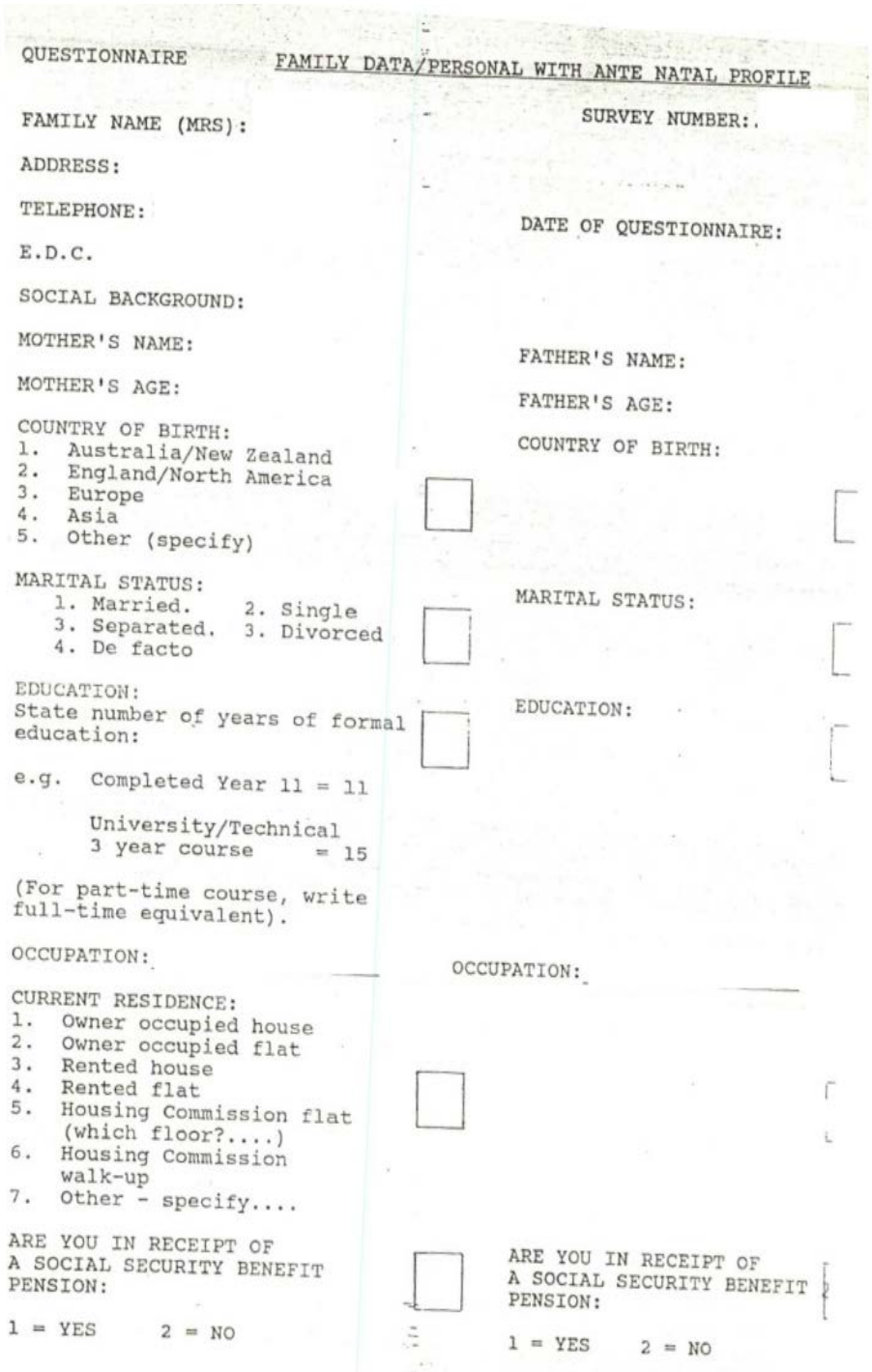
Appendix 1: Family Data/Personal with Ante Natal profile



QUESTIONNAIRE FAMILY DATA/PERSONAL WITH ANTE NATAL PROFILE

FAMILY NAME (MRS):

SURVEY NUMBER:.

ADDRESS:

TELEPHONE:

DATE OF QUESTIONNAIRE:

E.D.C.

SOCIAL BACKGROUND:

MOTHER'S NAME:

FATHER'S NAME:

MOTHER'S AGE:

FATHER'S AGE:

COUNTRY OF BIRTH:
1. Australia/New Zealand
2. England/North America
3. Europe
4. Asia
5. Other (specify)

COUNTRY OF BIRTH:

MARITAL STATUS:
1. Married. 2. Single
3. Separated. 3. Divorced
4. De facto

MARITAL STATUS:

EDUCATION:
State number of years of formal education:

e.g. Completed Year 11 = 11

University/Technical
3 year course = 15

(For part-time course, write full-time equivalent).

EDUCATION:

OCCUPATION:

OCCUPATION:

CURRENT RESIDENCE:
1. Owner occupied house
2. Owner occupied flat
3. Rented house
4. Rented flat
5. Housing Commission flat (which floor?....)
6. Housing Commission walk-up
7. Other - specify....

ARE YOU IN RECEIPT OF A SOCIAL SECURITY BENEFIT PENSION:

1 = YES 2 = NO

ARE YOU IN RECEIPT OF A SOCIAL SECURITY BENEFIT PENSION:

1 = YES 2 = NO

FAMILY HISTORY

Write in 1 = Yes 2 = No 9 = Don't know

SIBLINGS:- (NUMBERED FROM OLDEST (1) TO YOUNGEST)

1 = FEMALE 2 = MALE

1	2	3	4	5	6

1. Natural child to both parents.
2. Natural child to mother only.
3. Natural child to father only.
4. Adopted.
5. Other.

Suffers from now, or has in past had -

1.	MILK ALLERGY					
2.	EGG ALLERGY					
3.	OTHER FOOD ALLERGY (Specify... .)					
4.	ECZEMA					
5.	ASTHMA/WHEEZY BRONCHITIS					
6.	HAYFEVER					
7.	INFANTILE COLIC					
8.	DRUG ALLERGY (Specify.....)					
9.	OTHER ILLNESS (Specify.....)					

PARENTAL HISTORY:

Suffers from now, or has in past had -	MOTHER	FATHER
1. MILK ALLERGY	☐	☐
2. EGG ALLERGY	☐	☐
3. OTHER FOOD ALLERGY (Specify.....)	☐	☐
4. ECZEMA	☐	☐
5. ASTHMA	☐	☐
6. HAYFEVER	☐	☐
7. DRUG ALLERGY (Specify.	☐	☐
8. OTHER ILLNESS (Specify...	☐	☐

SMOKING HISTORY

1. is smoking now?
2. not smoking but has in past 3/12
3. not smoking now but has in past 6/12
4. has smoked > 6/12 ago
5. has never smoked.

INSERT CORRECT ANSWER ☐ ☐

Intensity of smoking:

i) Currently smokes:

1. no cigarettes
2. smokes 1 - 4 cigs./day
3. smokes 5 - 10 cigs./day
4. smokes 11 - 20 cigs./day
5. smokes > 21 cigs./day

☐ ☐

ii) At peak of smoking in last 6/12 smoked

1. no cigarettes
2. 1 - 4 cigs./day
3. 5 - 10 cigs./day
4. 11 - 20 cigs./day
5. > 21 cigs./day

INSERT CORRECT ANSWER ☐ ☐

Appendix 2: Initial Socio-Domestic Questionnaire

QUESTIONNAIRE INITIAL

SOCIO-DOMESTIC

FAMILY NAME (MRS):

ADDRESS:

TELEPHONE:

NAME OF CHILD:

SEX:

CHILD NUMBER IN STUDY:

DATE OF QUESTIONNAIRE =

QUESTIONNAIRE NUMBER =

AGE OF CHILD IN WEEKS =

Home factors

1. WHAT IS POSTCODE OF RESIDENCE?

2. IS CHILD'S BEDROOM CARPETED

ASSESSOR: 1 = Yes 2 = No 3 = Uncertain

3. IS MAIN LIVING AREA/CHILD'S PLAY AREA CARPETTED?

ASSESSOR: 1 = Yes 2 = No 3 = Uncertain

"ASSESSOR - Carpeted means wall-to-wall or mainly so.

4. WHICH OF THE FOLLOWING ANIMALS IS BEING IN REGULAR CONTACT WITH BY THE FAMILY:

CAT
DOG
HORSE
GUINEA PIG
BIRDS (Indoor)
OTHER (Specify .....)

ASSESSOR: WRITE 1. FOR YES 2. FOR NO 3. DON'T KNOW

5. THE CURRENT FAMILY COOKING FACILITY IS MAINLY

1. GAS
2. ELECTRIC
3. OIL
4. WOOD
5. OTHER (Specify.....)

ASSESSOR: WRITE CORRECT NUMBER IN BOX

6. THE CURRENT HEATING FACILITIES IN FAMILY RESIDENCE IS MAINLY:

1. GAS
2. ELECTRIC

3. OIL
4. WOOD
5. OTHER (Specify.....)
6. NO INTERNAL HEATING

ASSESSOR: MAIN HEATING MAY BE RADIATOR = LECTRIC, i.e. 2
OR DUCTED = GAS OR OIL, i.e. [illegible] OR 3

7. IN THE LAST 4 WEEKS WHAT IS AVERAGE MBER OF CIGARETTES SMOKED PER DAY BY

A) MOTHER
B) FATHER
C) OTHER HOUSEHOLD MEMBER

ASSESSOR: WRITE 0 IF NONE SMOKED.

Appendix 3: Food Reaction Questionnaire

FOODS REACTION QUESTIONNAIRE.

TO BE COMPLETED MONTHLY.

FAMILY NAME (MRS) :

NAME OF CHILD:

CHILD NUMBER IN STUDY:

DATE OF QUESTIONNAIRE

QUESTIONNAIRE NUMBER :

AGE OF CHILD IN WEEKS

	Age first given (weeks). If not given write 0.	Age last given (weeks). If not given write 0.	Average daily intake (in ml) write 0 if none given.	Other comment
BREAST MILK				
FORMULA HS050				
FORMULA HS064				
FORMULA HS012				
OTHER COW MILK FORMULA				
SOY MILK				
OTHER FORMULA (SPECIFY)				
COW MILK				

Appendix 4: Infant Illness Questionnaire

QUESTIONAIRE

INFANT ILLNESS, PHYSICAL EXAMINATION,

DIET CHART, AND SOCIO-DOMESTIC.

FAMILY NAME (MRS.)

ADDRESS.

TELEPHONE:

NAME OF CHILD

SEX:

CHILD NUMBER IN STUDY

QUESTIONNAIRE NUMBER

DATE OF QUESTIONAIRE:

AGE OF CHILD IN WEEKS

INFANT ILLNESS

1. TEMPERAMENT

In the last 3 nights between 7pm. and 12 midnight how many hours of irritable, colicky or distressed behavior has baby shown?

ASSESSOR: Write in average number of hours per night; i.e. 1,2,3, 6 etc.

2. GASTROINTESTINAL SYMPTOMS

a) In the last three days has there been any vomiting > 1 desertspoon i.e. 15 ml? How many times per day?

ASSESSOR : Write in number of episodes of vomiting.

b) In the last three days on average, how many bowel actions per day?

ASSESSOR: Write in number of bowel actions.

c) How many bouts of illness with vomit and/or diarrhoea have occured in the last 4 weeks?

ASSESSOR: Write in number of episodes.

3. SKIN SYMPTOMS

a) In the last 4 weeks has baby shown persistent skin rash for more than a week?

1 = Yes 2 = No 3 = Don't know.

ASSESSOR: Write in number.

If rash persistent > 1 week what is distibution?

1. Scalp
2. Face
3. Nappy Area
4. Trunk
5. Legs
6. Arms

ASSESSOR: 1 = Yes 2 = No
(If no rashes at all put 2 for each).

In the past 4 weeks how many days of Topical treatment were given?

i) Cortisone(steroid) application

ii) Non-cortisone applications

ASSESSOR: If no treatment = 0 , otherwise write in days of treatment required.

4. RESPIRATORY SYMPTOMS

In the last 4 weeks record the total number of days of:

a) blocked, snuffly or runny nose.

b) cough and/ or chest rattle and /or wheeze.

ASSESSOR : Write in days.

5. OTHER ILLNESSES IN THE LAST 4 WEEKS:

In the last 4 weeks record total number of days with, or episodes of:-

a) High temperature (fever) with illness.
Specify illness if known, e.g. Measles .....

b) Hives.
If cause known, specify.....

c) Otitis Media
i.e. Middle ear infection diagnosed by Doctor.

d) Hospital admission.
Specify illness if known.....
Operation .....

e) Episodes of allergic reaction not due to drugs.
Specify cause, if known .....

f) Episodes of allergic reaction to drugs.
Specify if known .....

ASSESSOR: Write in days of fever, or episodes of illness as indicated; 0 if no illness.

Has there been any visits to the Doctors, or any other allied Health Workers?

ASSESSOR: Write in who was visited and reason why. .
0 if no visits.

6. USE OF MEDICATIONS

In the last 4 weeks, specify the number of days baby has received:

i) Antibiotics .....
Specify type.

ii) Cough Mixtures

iii) Asthma medication
e.g. Ventolin, Nuelin, Intal, Steroids.
Specify .....

iv) Anti - allergy drugs.
i.e. Antihistamines

v) Nasal sprays or drops.

vi) Other
Specify .....

ASSESSOR : Write in days of treatment required with each.
Write in 0 if none given.

7. IMMUNIZATIONS

In the last 4 weeks specify any immunizations.

1 Triple Antigen
2. CDT
3. Sabin
4. MMR vacc.
5. Other

ASSESSOR: 1 = Yes 2 = None given 3 = Not sure

PHYSICAL EXAMINATION

1. Height = Percentile

2. Weight = Percentile

Is nose blocked, runny or snuffly?

1 = Yes 2 = No 3 = Don't know.

Is cough, chest rattle present.

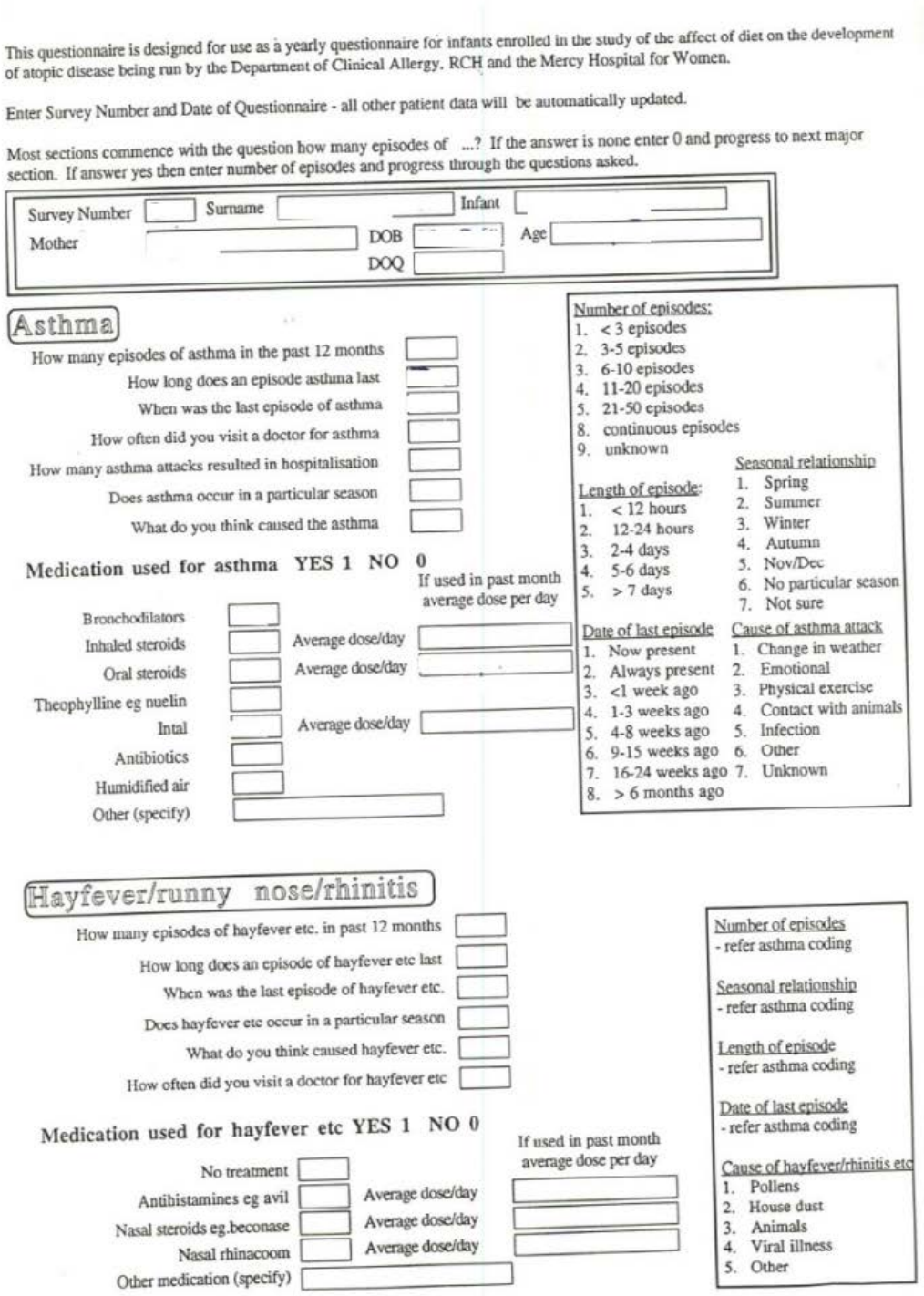
1 = Yes 2 = No 3 = Don't know

5. Is rash evident on:

1. Scalp
2. Nappy area
3. Face
4. Trunk
5. Arms/legs

ASSESSOR: 1 = Yes 2 = No 3 = Don't know

Appendix 5: MACS Follow-up Questionnaire



This questionnaire is designed for use as a yearly questionnaire for infants enrolled in the study of the affect of diet on the development of atopic disease being run by the Department of Clinical Allergy, RCH and the Mercy Hospital for Women.

Enter Survey Number and Date of Questionnaire - all other patient data will be automatically updated.

Most sections commence with the question how many episodes of ...? If the answer is none enter 0 and progress to next major section. If answer yes then enter number of episodes and progress through the questions asked.

Survey Number ____ Surname ____ Infant ____

Mother ____ DOB ____ Age ____

DOQ ____

Asthma

How many episodes of asthma in the past 12 months ____

How long does an episode asthma last ____

When was the last episode of asthma ____

How often did you visit a doctor for asthma ____

How many asthma attacks resulted in hospitalisation ____

Does asthma occur in a particular season ____

What do you think caused the asthma ____

Medication used for asthma YES 1 NO 0

			If used in past month average dose per day
Bronchodilators	____		
Inhaled steroids	____	Average dose/day	____
Oral steroids	____	Average dose/day	____
Theophylline eg nuelin	____		
Intal	____	Average dose/day	____
Antibiotics	____		
Humidified air	____		
Other (specify)	____		

Number of episodes:
1. < 3 episodes
2. 3-5 episodes
3. 6-10 episodes
4. 11-20 episodes
5. 21-50 episodes
8. continuous episodes
9. unknown

Length of episode:
1. < 12 hours
2. 12-24 hours
3. 2-4 days
4. 5-6 days
5. > 7 days

Seasonal relationship
1. Spring
2. Summer
3. Winter
4. Autumn
5. Nov/Dec
6. No particular season
7. Not sure

Date of last episode
1. Now present
2. Always present
3. <1 week ago
4. 1-3 weeks ago
5. 4-8 weeks ago
6. 9-15 weeks ago
7. 16-24 weeks ago
8. > 6 months ago

Cause of asthma attack
1. Change in weather
2. Emotional
3. Physical exercise
4. Contact with animals
5. Infection
6. Other
7. Unknown

Hayfever/runny nose/rhinitis

How many episodes of hayfever etc. in past 12 months ____

How long does an episode of hayfever etc last ____

When was the last episode of hayfever etc. ____

Does hayfever etc occur in a particular season ____

What do you think caused hayfever etc. ____

How often did you visit a doctor for hayfever etc ____

Medication used for hayfever etc YES 1 NO 0

			If used in past month average dose per day
No treatment	____		
Antihistamines eg avil	____	Average dose/day	____
Nasal steroids eg.beconase	____	Average dose/day	____
Nasal rhinacoom	____	Average dose/day	____
Other medication (specify)	____		

Number of episodes
- refer asthma coding

Seasonal relationship
- refer asthma coding

Length of episode
- refer asthma coding

Date of last episode
- refer asthma coding

Cause of hayfever/rhinitis etc
1. Pollens
2. House dust
3. Animals
4. Viral illness
5. Other

Eczema

How many episodes of eczema in past 12 months
How long since last episode of eczema
What areas of body are affected by eczema
Do you think there is a seasonal relationship with eczema
What do you think causes the eczema
If food specify which foods
How many visits to Dr for eczema in past 12 months

Medication used for eczema YES 1 NO 0

No treatment
Nonsteroid ointment
Steroid ointment
Oral steroid
Oral antihistamines
Other treatment for eczema - specify

Number of episodes(eczema)
1. 1 episode
2. 2 episodes
3. 3 episodes
4. 4 episodes
5. 5 episodes
6. 6 episodes
7. 7 episodes
8. 8 or more episodes
9. continuous

Areas of bodyaffected : In order of severity
1. Face and/or scalp
2. Chest
3. Upper back
4. Lower back
5. Stomach
6. Legs
7. Arms
8. Genitals
9. Trunk
10. All areas affected - enter 10 in first box & leave other boxes empty

Urticaria & hives

How many episodes of urticaria or hives in past 12 months
How long since last episode of urticaria
What areas of body are affected by urticaria
What do you think was the cause of the urticaria
If food specify which food
How long does the episode of urticaria last
Does urticaria/hives occur in a particular season
How often did you visit the doctor for urticaria/hives

Medication used for urtcaria/hives YES 1 NO 0

No treatment
Itch relief
Antihistamines
Diet change
Other (specify)

Number of episodes(urticaria)
1. 1-2 episodes
2. 3-5 episodes
3. 6-10 episodes
4. 11-20 episodes
5. 21-50 episodes
8. continuous
9. unknown

Length of episode
1. < 12 hours
2. 12-24 hours
3. 2-4 days
4. 5-6 days
6. > 7 days

Date of last episode
1. Now present
2. Always present
3. < 1 week ago
4. 1-3 weeks ago
5. 4-8 weeks ago
6. 9-15 weeks ago
7. 16-26 weeks ago
8. > 6 months ago
9. not sure

Seasonal relationship
1. Spring
2. Summer
3. Winter
4. Autumn
5. Nov/Dec
6. No particular season
7. Not sure

Cause of rash/urticaria or eczema
1. Inhalants
2. Food
3. Skin contact
4. Other
5. Unknown

Other rash

How many episodes of other rash in past year
How long since last episode of rash
Major areas of body affected by other rash
What is the major cause of the rash (specify)
Number of Doctor visits for rash

Medication used for other rash YES 1 NO 0

No treatment
Nonsteroid ointment or cream
Steroid ointment or cream
Other treatment for rash (specify)

URTI, colds or flu

How many episodes of colds flu or URTI in past 12 months
How long since last episode of colds etc
How many days did the cold flu URTI last (no. of days)
Are the colds more frequent at any particular time of the year
How many times did you visit the doctor for colds flu or URTI

Medication used for URTI etc YES 1 NO 0

No treatment
Symptomatic treatment
Antihistamines
Bronchodilators
Antibiotics
Other treatment (specify)

Number of episodes	Date of last episode
1. <3 episodes	1. Now present
2. 3-5 episodes	2. Always present
3. 6-10 episodes	3. < 1 week ago
4. 11-20 episodes	4. 1-3 weeks ago
5. 21-50 episodes	5. 4-8 weeks ago
8. continuous	6. 9-15 weeks ago
9. unknown	7. 16-26 weeks ago
	8. > 6 months ago
	9. not sure

Seasonal relationship
1. Spring
2. Summer
3. Winter
4. Autumn
5. Nov/Dec
6. No particular season
7. Not sure

Otitis Media (middle ear infection)

How many bouts of otitis media in the past 12 months
When was the last bout of OM
Did the ear discharge YES-1 NO-0
How long did the infection last (no. of days)
Did your child visit an ENT YES-1 NO-0
Did your child visit an Audiologist YES-1 NO-0
Have grommets been inserted YES-1 NO-0 If so when
How many visits to the doctor for OM

Medication used for otitis media YES 1 NO 0

No treatment
Symptomatic treatment
Antihistamines
Antibiotics
Nose drops
Ear drops
Other treatment (specify)

Number of episodes	Date of last episode & when grommets inserted
1. <3 episodes	1. Now present
2. 3-5 episodes	2. Always present
3. 6-10 episodes	3. < 1 week ago
4. 11-20 episodes	4. 1-3 weeks ago
5. 21-50 episodes	5. 4-8 weeks ago
8. continuous	6. 9-15 weeks ago
9. unknown	7. 16-26 weeks ago
	8. > 6 months ago
	9. not sure

LRTI (including bronchitis,bronchopneumonia, pneumonia & bronchioloitis

How many episodes of LRTI in past 12 months
How long does an episode of LRTI last
When was the last bout of LRTI
How many bouts of LRTI resulted in hospitalisation
How many Dr visits for LRTI

Medication used for LRTI YES 1 NO 0

No treatment
Symptomatic treatment
Antihistamines
Bronchodilators
Antibiotics
Other treatment (please specify)

Number of episodes	Date of last episode
1. <3 episodes	1. Now present
2. 3-5 episodes	2. Always present
3. 6-10 episodes	3. < 1 week ago
4. 11-20 episodes	4. 1-3 weeks ago
5. 21-50 episodes	5. 4-8 weeks ago
8. continuous	6. 9-15 weeks ago
9. unknown	7. 16-26 weeks ago
	8. > 6 months ago
	9. not sure

Croup

How many episodes of croup in the past year
How many days did croup last
When was the last bout of croup
How many times did your child visit the doctor for croup
How many bouts of croup resulted in hospitilisation

Number of episodes	Date of last episode
1. <3 episodes	1. Now present
2. 3-5 episodes	2. Always present
3. 6-10 episodes	3. < 1 week ago
4. 11-20 episodes	4. 1-3 weeks ago
5. 21-50 episodes	5. 4-8 weeks ago
8. continuous	6. 9-15 weeks ago
9. unknown	7. 16-26 weeks ago
	8. > 6 months ago
	9. not sure

Medication used for Croup YES 1 NO 0

No treatment
Steam
Steroids
Antihistamines
Antibiotics
Inhaled Ventolin/Adrenalin
Other treatment for croup (please specify)

Adverse reactions due to food

How many bouts of adverse reaction due to food in past year

What type of reaction was associated with the following food
1. Intolerance
2. Possible allergy
3. Definite allergy

Symptoms of reaction
0. None
1. Skin - S
2. Nasal - R
3. Respiratory - R
4. GI - v/d
5. S + R
6. S + GI
7. R + GI
8. S + R + GI
9. Unknown

Rice	Rice
Wheat	Wheat
Milk	Milk
Egg	Egg
Peanut	Peanut
Corn	Corn
Codfish	Codfish
Beef	Beef
Legume	Legume
Soy	Soy
Other (specify)	Other

This section replaced by previous question no longer necessary to complete - November 1994

What were the foods implicated (specify)

How did the reaction manifest itself YES - 1 NO - 0

Abdominal pains	Colic
Vomiting	Diarrhoea
Hives/urticaria	Eczema
Breathing difficulties	Choking
Asthma	Other reaction - specify

Medication used for adverse food reaction YES 1 NO 0

Treatment by nothing ☐
Treatment by change in diet ☐
Treatment by other (specify) ☐

Continuing allergic food reactions

Does your child still have adverse reactions to food YES - 1 NO - 0 ☐
Which food (specify) ☐
How do you know that your child is still reacting ☐
Which foods are still restricted ☐

How do you know still reacting
1. +ve SPT
2. Hospital challenge
3. Accidental challenge
4. Home trial challenge
5. Other

Drug allergy

How many episodes of drug allergy in the past year ☐
What was the type of reaction ☐ ☐ ☐
What do you think was the cause of reaction ☐
If antibiotic specify which one ☐

Number of episodes
1.<3 episodes
2. 3-5 episodes
3. 6-10 episodes
4. 11-20 episodes
5. 21-50 episodes
8. continuous
9. unknown

Reaction type
1. nausea and/or vomiting
2. diarrhoea
3. skin rash
4. breathing problems
9. other

Cause of reaction
1. Antibiotic
2. Other
3. Unknown

Allergic reactions not drugs or food

Have there been any other allergic reactions YES - 1 NO - 0 ☐
If so, to what (specify) ☐
Describe reaction (specify) ☐

Gastroenteritis (bacterial or viral)

How many bouts of diarrhoea and/or vomiting due to infection in past 12 months ☐
Was it accompanied by Abdominal pains YES - 1 NO - 0 ☐
Was child admitted to hospital YES - 1 NO - 0 ☐

Medication used for gastroenteritis YES 1 NO 0

No treatment ☐
Change in diet ☐
Symptomatic eg kaomagma ☐
Antibiotics ☐
Flagyl ☐
Other treatment for gastro (specify) ☐

Other infections

YES 1 NO 0

Has your child had mumps ☐
Has your child had measles ☐
Has your child had chicken pox ☐
Has your child had whooping cough ☐
Has your child had hand, foot and mouth disease ☐
Has your child had any other major infection (please specify) ☐
How many times(excluding birth) has your child been admitted to hospital ☐

Inoculations in past year

YES 1 NO 0

Hep B ☐ Sabin ☐
CDT ☐ HiB ☐

Weight and height

Weight ☐ gm ☐ percentile
Height ☐ cm ☐ percentile

Socialisation

Did your child attend any of the following in the past 12 months.

	If so for how many months?	Frequency of attendance
Creche	☐	☐
Family day care	☐	☐
Kindergarten	☐	☐
School	☐	☐
Other outside care	☐	☐

Frequency of attendance
1. occasional
2. 1 day per week
3. 2-3 days per week
4. 4-5 days per week

How many siblings are there in the family ☐
If any younger siblings, do they have any allergy YES -1 NO - 0 ☐
If so what allergy (specify) ☐
How many people live in your household ☐

Appendix 6: The ISAAC Questionnaire

INTERNATIONAL SURVEY OF BREATHING, NOSE AND SKIN PROBLEMS IN CHILDREN

QUESTIONNAIRE FOR PARENTS OF 6 – 7 YEAR OLDS

Office Use Only ☐1 ☐☐ ☐☐ ☐☐☐☐ ☐☐ ☐☐☐

INSTRUCTIONS FOR COMPLETING THE QUESTIONNAIRE

On this sheet are questions about your child's name, school and birthdate. Please write your answers to these questions in the spaces provided.

All other questions require you to tick your answer in a box. If you make a mistake, put a cross in the box and tick the correct answer. Tick only one option unless otherwise instructed.

Examples of how to mark the questionnaire:

To answer "yes": Yes ☑ No ☐

To answer "no": Yes ☐ No ☑

Today's Date: ____ / ____ / ____ (Day / Month / Year)

Child's Name: ____ (First) ____ (Last Name)

Child's School: ____

Child's Date of Birth: ____ / ____ / ____ (Day / Month / Year) Child's Age: ____ Years

(Tick all your answers for the rest of the questionnaire)

Is your child a: ☐ Male or ☐ Female

In which country was your child born? ☐ Australia ☐ Other → ____ Please Specify ☐ Office Use

In which country was the child's Mother born? ☐ Australia ☐ Other → ____ Please Specify ☐ Office Use

1. Has your child ever had wheezing or whistling in the chest at any time in the past?

☐ Yes ☐ No

IF YOU HAVE ANSWERED "NO" PLEASE GO TO QUESTION 6

2. Has your child ever had wheezing or whistling in the chest in the past 12 months?

☐ Yes ☐ No

IF YOU HAVE ANSWERED "NO" PLEASE GO TO QUESTION 6

3. How many attacks of wheezing has your child had in the past 12 months?

☐ None ☐ 1 to 3 ☐ 4 to 12 ☐ More than 12

4. In the past 12 months, how often, on average, has your child's sleep been disturbed due to wheezing?

☐ Never woken with wheezing ☐ Less than one night per week ☐ One or more nights per week

5. In the past 12 months, has wheezing ever been severe enough to limit your child's speech to only one or two words at a time between breaths?

☐ Yes ☐ No

6. Has your child ever had asthma?

☐ Yes ☐ No

7. In the past 12 months, has your child had a dry cough at night, apart from a cough associated with a cold or chest infection?

☐ Yes ☐ No

8. In the past 12 months, has your child's chest sounded wheezy during or after exercise?

☐ Yes ☐ No

IF YOU HAVE ANSWERED "NO" PLEASE GO TO QUESTION 13

9. In the past 12 months, has asthma limited the amount of sport or physical activity your child does?

☐ Yes ☐ No

10. Do you sometimes try and stop your child from taking part in sports or physical education because your child has asthma? ☐ Yes ☐ No

11. Do the teachers at your child's school sometimes exclude your child from taking part in physical education or sport because your child has asthma? ☐ Yes ☐ No ☐ Do Not Know

12. Before your child does any exercise that may make him/her wheezy, do you give him/her any medicine? ☐ Yes ☐ No

a) If you answered "yes", what medicine do you give your child before he/she exercises?

Name of Medication	Office Use

IF YOU ANSWERED "YES" TO QUESTION 2 (HAS HAD WHEEZE IN THE LAST 12 MONTHS) THEN PLEASE ANSWER QUESTIONS 13 TO 17, OTHERWISE GO TO QUESTION 18

13. In the past 12 months, has your child taken any medication (medicines/pills/puffers) for wheezing or asthma? ☐ Yes ☐ No

IF YOU ANSWERED YES, THEN PLEASE NAME THE MEDICATIONS:

Name of Medication	When medication is taken (please circle one or both)	Office Use
	When wheezy / regularly*	
	When wheezy / regularly*	
	When wheezy / regularly*	
	When wheezy / regularly*	

(* every day for at least 2 months in the year)

14. Do you have a written plan which tells you how to look after your child's asthma? ☐ Yes ☐ No

15. In the past 12 months, how many visits has your child made to the doctor (family doctor, general practitioner or specialist) for his/her wheezing or asthma?

a). For a wheezy episode? ☐ None ☐ 1 to 3 ☐ 4 to 12 ☐ More than 12

b). For a regular "check-up" for asthma? ☐ None ☐ 1 to 3 ☐ 4 to 12 ☐ More than 12

16. In the past 12 months, how many visits has your child made to a hospital Casualty or Emergency Department because of his/her wheezing or asthma? ☐ None ☐ 1 ☐ 2 ☐ 3 or More

17. In the past 12 months, how many times has your child been admitted to hospital because of his/her wheezing or asthma? ☐ None ☐ 1 ☐ 2 ☐ 3 or More

Appendix 7: MACS 18-year follow-up Questionnaires

The Royal Children's Hospital, Melbourne

THE UNIVERSITY OF MELBOURNE

SURVEY 1: TO BE COMPLETED BY ALL PARTICIPANTS WHO ARE 14 YEARS AND OLDER

Date survey completed

_____

Day/month/year

First name

Last name _____

ID _____

(Or affix sticker here) _____

Respiratory health

1. Have you ever had wheezing or whistling in the chest at any time in the past? (Wheezing means a whistling sound, however high or low pitched and however faint).

☐ No→ go to Q3

☐ Yes→ continue

1.1 At what age did you first have wheezing or whistling in the chest?

_____ Years

1.2 At what age did you last have wheezing or whistling in the chest?

_____ Years

2. Have you had wheezing or whistling in the chest in the last 12 months?

☐ No→ go to Q3

☐ Yes→ continue

2.1 How many attacks of wheezing have you had in the last 12 months?

_____ attacks

2.2 In the last 12 months, how often, on average, has your sleep been disturbed due to wheezing?

☐ Never woken with wheezing

☐ Less than one night per week

☐ One or more nights per week

2.3 In the last 12 months, has wheezing ever been severe enough to limit your speech to only one or two words between each breath?

☐ No

☐ Yes

3. Have you ever had asthma?

☐ No→ go to Q4

☐ Yes→ continue

3.1 Was this asthma confirmed by a doctor?

☐ No

☐ Yes

3.2 How many episodes of asthma have you had in the past 12 months?

_____ episodes or ☐ continuous

3.3 Do you have a written plan which tells you how to look after your asthma?

☐ No

☐ Yes

4. In the past 12 months, have you seen a doctor (family doctor, general practitioner or specialist), attended a hospital casualty or emergency department or been admitted to hospital for your **wheezing OR asthma**?

☐ No→ go to Q5

☐ Yes→ continue

4.1 In the past 12 months, how many visits have you made to the doctor (family doctor, general practitioner or specialist) for your wheezing or asthma?

a) for a wheezy episode: _____ visits

b) for a regular "check-up for asthma: _____ visits

4.2 In the past 12 months, how many visits have you made to hospital Casualty or Emergency Department because of wheezing or asthma (but not subsequently admitted to hospital)?

_____ visits

4.3 In the past 12 months, how many times have you been admitted to hospital because of wheezing or asthma?

_____ times

5. In the last 12 months, has your chest sounded wheezy during or after exercise?

☐ **No**

☐ **Yes**

6. Before you do any exercise that may make you wheezy, do you take any medications?

☐ **No**

☐ **Yes**

7. Have you taken any medicines including inhalers or tablets for asthma or wheezy breathing, in the last 12 months?

☐ **No**

☐ **Yes**

7.1 If yes, please list these medications

_____

_____

_____

8. Have you, had **cough with phlegm** on most days for at least three months and for two years in a row?

☐ **No**

☐ **Yes**

9. In the past 12 months, have you had any episodes of cough?

☐ **No**→ go to Q10

☐ **Yes**→ continue

9.1 In the past 12 months, how long was the longest episode of cough?

☐ less than one week

☐ one week

☐ two weeks

☐ three or four weeks

☐ more than four weeks

9.2 In the past 12 months, how many episodes of cough have you had?

_____ episodes or ☐ continuous

9.3 In the last 12 months, have you had a dry cough at night, apart from a cough associated with a cold or chest infection?

☐ **No**

☐ **Yes**

9.4 In the past 12 months, have you taken any treatments for cough?

☐ **No**

☐ **Yes**

9.5 If Yes, please list these medications.

_____

_____

_____

_____

Nasal symptoms

THE FOLLOWING QUESTIONS ARE ABOUT PROBLEMS WHICH OCCUR WHEN YOU **DO NOT** HAVE A COLD OR THE FLU

10. Have you ever had a problem with sneezing, or a runny, or a blocked nose when you **DID NOT** have a cold or the flu?

☐ **No**→ go to Q11

☐ **Yes**→ continue

10.1 At what age did you first have a problem with sneezing, or a runny, or a blocked nose?

_____ Years

10.2 At what age did you last have a problem with sneezing, or a runny, or a blocked nose?

_____ Years

10.3 In the past 12 months, have you had a problem with sneezing, or a runny, or a blocked nose when you **DID NOT** have a cold or the flu?

☐ **No**→ go to Q11

☐ **Yes**→ continue

10.4 In the past 12 months, has this nose problem been accompanied by itchy-watery eyes?

☐ **No**

☐ **Yes**

10.5 In which of the past 12 months did this nose problem occur? (please tick any which apply)

☐ No particular time of year

☐ January	☐ May	☐ September
☐ February	☐ June	☐ October
☐ March	☐ July	☐ November
☐ April	☐ August	☐ December

10.6 In the past 12 months, how much did this nose problem interfere with your daily activities?

- ☐ Not at all
- ☐ A little
- ☐ A moderate amount
- ☐ A lot

11. Have you ever had hay fever?

- ☐ **No**→ go to Q12
- ☐ **Yes**→ continue

11.1 How many episodes of hay fever have you had in the past 12 months?

_____ episodes or ☐ continuous

12. Have you taken any medicines including nasal inhalers or tablets for hay-fever or problem with sneezing, or a runny, or a blocked nose in the last 12 months?

- ☐ **No**
- ☐ **Yes**

12.1 If yes, please list these medications

_____

_____

_____

13. Do you ever get a runny or stuffy nose or start to sneeze when you are near:

	No	Yes
cats	☐	☐
dogs	☐	☐
horses	☐	☐
feathers (including pillows, quilts or duvets)	☐	☐
a dusty part of the house	☐	☐
pollen, grass, trees or flowers	☐	☐
other	☐	☐

please list _____

_____

Skin conditions

14. Have you ever had an itchy rash which was coming and going for at least 6 months?

- ☐ **No**→ go to Q15
- ☐ **Yes**→ continue

14.1 At what age did this itchy rash first occur?

_____ years

14.2 At what age did you last have this rash?

_____ years

14.3 Have you had this itchy rash at any time in the last 12 months?

- ☐ **No**→ go to Q15
- ☐ **Yes**→ continue

14.4 Has this itchy rash at any time affected any of the following places: (tick all that apply)

- ☐ The folds of the elbows
- ☐ Behind the knees
- ☐ In front of the ankles
- ☐ Under the buttocks
- ☐ Around the neck, ears or eyes
- ☐ Trunk (chest, stomach or back)
- ☐ Other. Please list: _____

14.5 Has this rash cleared completely at any time during the last 12 months?

- ☐ **No**
- ☐ **Yes**

14.6 In the past 12 months, how often, on average, have you been kept awake by this itchy rash?

- ☐ Never in the last 12 months
- ☐ Less than one night per week
- ☐ One or more nights per week

15. Have you ever had eczema?

- ☐ **No**→ go to Q18
- ☐ **Yes**→ continue

15.1 How many episodes of eczema have you had in the past 12 months?

_____ episodes or ☐ continuous

If no episodes of eczema, please go to question 18

Severity of eczema – only complete if you have had eczema IN THE LAST 12 MONTHS

16. Please circle the number under the column that describes the skin on each part of your body. **Please circle any and all that apply.**

	Clear	Dry	Scaly	Redness	Cracks/ openings	Oozing
Head (face, ears scalp)	0	1	2	3	4	5
Neck	0	1	2	3	4	5
Trunk (body, not including limbs)	0	1	2	3	4	5
Arms	0	1	2	3	4	5
Hands	0	1	2	3	4	5
Legs	0	1	2	3	4	5
Feet	0	1	2	3	4	5

17. How itchy has your skin been in the following places? **Please circle only one**

	None at all	A little bit	Somewhat bothered	Quite bothered	Very bothered	Extremely bothered/losing sleep
Head (face, ears scalp)	0	1	2	3	4	5
Neck	0	1	2	3	4	5
Trunk (body, not including limbs)	0	1	2	3	4	5
Arms	0	1	2	3	4	5
Hands	0	1	2	3	4	5
Legs	0	1	2	3	4	5
Feet	0	1	2	3	4	5

18. Have you used any medicines including creams and ointments for rash or eczema during the last 12 months?

☐ **No**
☐ **Yes**

18.1 If yes, please list these medications

_____

_____

_____

_____

Food and other allergies

19. Do you have an allergy to any of the following? (tick all that apply to you)

	No	Yes
Latex	☐	☐
Medicines please list	☐	☐
Other please list	☐	☐

_____

_____

_____

_____

_____

_____

20. Have you ever had any food allergies?

☐ Yes → please continue
☐ No → go to Q21
☐ Not sure → please continue

		Food 1 (eg egg) _____	Food 2 _____	Food 3 _____
20.1 Who diagnosed this food allergy? (tick all that apply)	Self-diagnosis	☐	☐	☐
	Doctor diagnosis	☐	☐	☐
	Other health care professional: Please list	☐ _____	☐ _____	☐ _____
20.2 Have you ever had a skin prick test (SPT) or a food challenge at an allergy clinic (challenge) to confirm this food allergy? (tick all that apply)		SPT ☐ challenge ☐	SPT ☐ challenge ☐	SPT ☐ challenge ☐
20.2.1 What were the results of the SPT for this food?		+ve ☐ -ve ☐ unsure ☐	+ve ☐ -ve ☐ unsure ☐	+ve ☐ -ve ☐ unsure ☐
20.2.2 What were the results of food challenge for this food?		+ve ☐ -ve ☐ unsure ☐	+ve ☐ -ve ☐ unsure ☐	+ve ☐ -ve ☐ unsure ☐
20.3 Have you been prescribed with an epipen for this food allergy?		☐ No ☐ Yes	☐ No ☐ Yes	☐ No ☐ Yes
20.4 At what age were you **first diagnosed** with this food allergy?		___ years	___ years	___ years
20.5 At what age did you **last consume this food**?		___ years or ☐ never	___ years or ☐ never	___ years or ☐ never
20.6 At what age did you **last react to this food?**		___ years or ☐ never	___ years or ☐ never	___ years or ☐ never
20.7 What symptoms have you experienced when you have reacted to this food? Write "never", if you have never reacted to this food. Common symptoms of food include: hives/urticaria/wheals, (small raised itchy areas likened to mosquito bites), itchy skin or rash, swelling of the lips, red swollen eyes, vomiting, diarrhoea, wheezing, coughing, collapse.		_____ _____ _____ _____	_____ _____ _____ _____	_____ _____ _____ _____

Eczema and rash

WE REALISE THE FOLLOWING QUESTIONS ARE SIMILAR TO THOSE YOU HAVE ALREADY ANSWERED, BUT THE WORDING IS SLIGHTLY DIFFERENT

21. IN THE LAST YEAR, have you had an ITCHY skin condition - by itchy we mean scratching or rubbing the skin?

☐ **No**→ go to Q22

☐ **Yes**→ continue

21.1 How old were you when this skin condition began? (please tick one box only)

☐ Under 2

☐ 2 to 5

☐ 5 to 10

☐ Over 10

21.2 Has this skin condition ever affected the skin creases in the past - by skin creases we mean fronts of elbows, behind the knees, fronts of ankles, around the neck, or around the eyes?

☐ **No**

☐ **Yes**

22. Have you ever suffered from asthma - by asthma we mean bouts of wheezing or whistling in the chest?

☐ **No**

☐ **Yes**

23. Have you ever suffered from hay fever - by hay fever we mean bouts of sneezing with a runny nose or itchy eyes in the summer?

☐ **No**

☐ **Yes**

24. In the last year, have you suffered from a dry skin in general?

☐ **No**

☐ **Yes**

General questions

25. What is your highest level of education?

☐ Primary School

☐ High School

☐ Trade / Apprenticeship (eg Hairdresser, electrician, plumber etc)

☐ Certificate or Diploma (eg. Child care, technician etc)

☐ University degree (eg. Bachelor)

☐ Postgraduate university degree (eg. Graduate Diploma, Masters, PhD)

26. If you are currently working, what is your job?

_____

26.1 On average, how many hours per day do you sit at a desk during working hours?

☐ none, or ____ hours per day.

26.2 What industry would you like to work in?

_____

27. Have you smoked at least 100 cigarettes or equal amounts of cigars, pipes or any other tobacco product?

☐ **No**→ go to Q28

☐ **Yes**→ continue

27.1 How old were you when you started and stopped smoking, and how much did you smoke during these times?

Many people start and stop smoking, and smoke different amounts during these times. Please use a separate line for each period that you have smoked.

Age started	Age stopped (current age if you still smoke)	Average cigarettes smoked
___ years	___ years	___ per day
___ years	___ years	___ per day
___ years	___ years	___ per day

Quality of life

28. In general, would you say your health is:

☐ Excellent

☐ Very good

☐ Good

☐ Fair

☐ Poor

29. The following questions are about activities you might do during a typical day. Does your health *now* limit you in these activities? If so, how much?

a) **Moderate activities,** such as moving a table, pushing a vacuum cleaner, bowling, or playing golf

☐ Yes, limited a lot

☐ Yes, limited a little

☐ No, not limited at all

b) Climbing **several** flights of stairs

☐ Yes, limited a lot

☐ Yes, limited a little

☐ No, not limited at all

30. During the *past 4 weeks*, have you had any of the following problems with your work or other regular daily activities as a result of your physical health?

a) **Accomplished less** than you would like

☐ **No**

☐ **Yes**

b) Were limited in the **kind** of work or other activities

☐ **No**

☐ **Yes**

31. During the *past 4 weeks*, have you had any of the following problems with your work or other regular daily activities *as a result of any emotional problems* (such as feeling depressed or anxious)?

a) **Accomplished less** than you would like

☐ **No**

☐ **Yes**

b) Didn't do work or other activities as **carefully** as usual

☐ **No**

☐ **Yes**

32. During the *past 4 weeks*, how much did pain interfere with your normal work (including both work outside the home and housework)?

☐ Not at all

☐ A little bit

☐ Moderately

☐ Quite a bit

☐ Extremely

These questions are about how you feel and how things have been with you during the past 4 weeks. For each question, please give the one answer that comes closest to the way you have been feeling.

		All of the time	Most of the time	A good bit of the time	Some of the time	A little of the time	None of the time
33. How much of the time during the *past 4 weeks*...							
33.1	Have you felt calm and peaceful?	☐	☐	☐	☐	☐	☐
33.2	Did you have a lot of energy?	☐	☐	☐	☐	☐	☐
33.3	Have you felt downhearted and blue?	☐	☐	☐	☐	☐	☐

	All of the time	Most of the time	Some of the time	A little of the time	None of the time
34. During the *past 4 weeks*, how much of the time has your physical health or emotional problems interfered with your social activities (like visiting friends, relatives, etc.)?	☐	☐	☐	☐	☐

35. If you have any current pain, disability, or illness what is the main cause for this?

_____

		All of the time	Most of the time	Some of the time	A little of the time	None of the time
36. The following questions are about your feelings **in the past 4 weeks:**						
About how often did you feel						
36.1	Nervous?	☐	☐	☐	☐	☐
36.2	Hopeless?	☐	☐	☐	☐	☐
36.3	Restless or fidgety?	☐	☐	☐	☐	☐
36.4	That everything was an effort?	☐	☐	☐	☐	☐
36.5	So sad that nothing could cheer you up?	☐	☐	☐	☐	☐
36.6	Worthless?	☐	☐	☐	☐	☐

General health and activity

37. Has a doctor ever told you that you have or have had this condition? (please tick all that apply).

	No	Yes
Diabetes Mellitus?	☐	☐
Heart Disease?	☐	☐
High Blood Pressure?	☐	☐
Chronic Bronchitis?	☐	☐
Emphysema?	☐	☐
Chronic obstructive lung disease or chronic obstructive airways disease (COPD, COAD)?	☐	☐
Other medical condition/s	☐	☐

Please list: _____

38. During a normal week, how many hours of each type of exercise do you perform **per week**?

38.1 **moderate exercise** (for example walking)

☐ none, or _____ hours per week

38.2 **strenuous** physical exertion (for example running or lifting weights)

☐ none, or _____ hours per week

39. During a normal week, how many hours of each type of activity do you perform **per day**?

39.1 watch television

☐ none, or _____ hours per day

39.2 use a computer or games console for non-work related reasons (such as games, research or study)

☐ none, or _____ hours per day

40. In the past 12 months, how often, on average, have you taken paracetamol (eg Panadol), ibuprofen (eg Nurofen), or aspirin? (please tick all that apply)

Medicine	Never	Once per year	Monthly	Weekly
Paracetamol	☐	☐	☐	☐
Ibuprofen	☐	☐	☐	☐
Aspirin	☐	☐	☐	☐

40.1 Did you use each of these medicines for treatment of **migraine**?

Medicine	Yes	No
Paracetamol	☐	☐
Ibuprofen	☐	☐
Aspirin	☐	☐

Supplemental questions for females

Please continue if you are a female. Please skip to question 46 if you are a male.

41. Have you ever had a menstrual period?

☐ **No** → Go to question 46

☐ **Yes** → Please continue

42. At what age did you first have your first menstrual period?

_____ (for example "12 years")

43. What was the date of the first day of your last menstrual period?
If you have reached menopause, please list the year of your last menstrual period

_____ (date)
(For example 14 July 2008)

The "pill" (oral contraceptive pill) and hormone replacement therapy (HRT) are used for a number of reasons, including treatment for period pain and "hot flushes", and for birth control.

44. Have you ever used the "pill" or HRT?

☐ **No** → Go to question 45

☐ **Yes** → Please continue

44.1 Between what ages did you use the "pill" or HRT, and what type?

Ages (eg 18 to 25)	"Pill"	HRT	Type OR (brand name)	can't remember
___ to ___	☐	☐	_____	☐
___ to ___	☐	☐	_____	☐
___ to ___	☐	☐	_____	☐

45. Do you generally have regular menstrual periods? Regular menstrual periods are between 21 to 35 days apart, and do not vary by more than 4 days between cycles.

- If you have reached menopause, were your menstrual periods generally regular?
- If you take oral contraception or HRT, were your menstrual periods generally regular when you **were not taking** these medicines?

☐ **No**

☐ **Yes**

Attitudes and beliefs about asthma

Questions 46 to 52 should only be completed by participants **who have had symptoms of asthma in the last 12 months.** If you do not have current asthma, please skip to question 53.

INSTRUCTIONS: Below there is a list of statements. At the end of each statement, please tick ONE of the boxes to show how much you agree/disagree with the statement	Strongly disagree	Tend to disagree	Tend to agree	Strongly agree
46. Think first about how it feels to be an asthmatic:				
a) I feel different from other people because I am an asthmatic	☐	☐	☐	☐
b) Being an asthmatic often makes me feel angry	☐	☐	☐	☐
c) Being an asthmatic often makes me feel depressed	☐	☐	☐	☐
d) I feel somehow to blame for being an asthmatic	☐	☐	☐	☐
e) I became an asthmatic because of emotional upset	☐	☐	☐	☐
47. Consider the effect on your relationship to others:				
a) I avoid letting other people know I am an asthmatic	☐	☐	☐	☐
b) The people who are closest to me seem overly protective of me because of my asthma	☐	☐	☐	☐
c) I am worried that my asthma may interfere with the lives of the people closest to me	☐	☐	☐	☐
48. Now think about the severity of your asthma:				
a) My asthma is severe	☐	☐	☐	☐
b) Looking toward the future, I feel certain that my asthma will get better	☐	☐	☐	☐
c) I worry about the long-term effects of asthma on my health	☐	☐	☐	☐
d) Asthma has made me physically less attractive	☐	☐	☐	☐
e) I worry that I might die from asthma	☐	☐	☐	☐
49. Consider the effect of asthma on your activities:				
a) I can't enjoy a full life because of my asthma	☐	☐	☐	☐
b) I do all the things I want to, regardless of their effect on my asthma	☐	☐	☐	☐
c) I know what things start my asthma attacks	☐	☐	☐	☐
d) I don't do anything about my asthma until it gets bad	☐	☐	☐	☐

INSTRUCTIONS: Below there is a list of statements. At the end of each statement, please tick ONE of the boxes to show how much you agree/disagree with the statement

	Strongly disagree	Tend to disagree	Tend to agree	Strongly agree
50. Think now about your asthma attacks:				
a) I have confidence in my ability to cope with an asthma attack	☐	☐	☐	☐
b) I know when an asthma attack is beginning	☐	☐	☐	☐
c) I can prevent asthma attacks	☐	☐	☐	☐
d) Even when I feel well, I worry about getting an attack of asthma	☐	☐	☐	☐
e) At the first sign of an asthma attack I feel panicky and frightened	☐	☐	☐	☐
51. Now consider your asthma medication:				
a) I worry that my asthma medication may have unwanted effects on my health	☐	☐	☐	☐
b) I find it easy to remember to take my asthma medication	☐	☐	☐	☐
c) It embarrasses me to use asthma inhalers or other asthma medication in public	☐	☐	☐	☐
d) It's a good idea to increase or decrease asthma medication without consulting the doctor	☐	☐	☐	☐
52. Think about the doctor who looks after your asthma:				
a) My doctor has helped to make my asthma better	☐	☐	☐	☐
b) I have confidence in my doctor's management of my asthma	☐	☐	☐	☐
c) I avoid troubling the doctor about my asthma	☐	☐	☐	☐
d) 1 wish that my doctor talked more to me about my asthma	☐	☐	☐	☐
e) My doctor tells me everything I want to know about my asthma	☐	☐	☐	☐

Home Conditions

THESE QUESTIONS ARE TO BE COMPLETED BY ONE PERSON PER HOUSEHOLD.

Please tick all that are appropriate.

All family members live together ☐ → **only the mother OR father answers these questions**
Mother and father live separately ☐ → **both the mother AND father answers these questions**
If you have moved away from family home ☐ → **you also have to answer these questions**

53. What is the address of your home?

_____

_____

_____

54. Of your immediate family, who lives with you and how many days per week do they normally live in this home?

Name	Days per week
_____	_____
_____	_____
_____	_____
_____	_____
_____	_____
_____	_____
_____	_____
_____	_____

55. How long have you lived in this home?

_____ years

56. In total how many people live in this house?

_____ people

57. Which types of heating and cooling do you use at home? ***(tick all that apply)***

- ☐ Gas ducted central heating
- ☐ Coal or wood fire
- ☐ Gas room heater
- ☐ Electric heater (eg. Radiator, fan or dimplex-type)
- ☐ Other central heating (eg Electric, hydronic, slab floor)
- ☐ Reverse cycle air-conditioning
- ☐ Evaporative cooler
- ☐ Other
- ☐ No Heating

58. What kinds of stove do you **mostly** use for **cooking**? ***(tick one only)***

- ☐ Gas
- ☐ Coal, coke or wood
- ☐ Electric
- ☐ Other

59. Do you have an exhaust fan over the stove?

- ☐ No → go to Q60
- ☐ Yes → continue

59.1 When cooking how often do you use the fan? ***(tick one only)***

- ☐ All the time
- ☐ Some of the time
- ☐ None of the time

59.2 Does the fan take the fumes out of the house?

- ☐ No
- ☐ Yes
- ☐ Don't Know

60. Has there **ever** been mould or mildew on any surfaces, other than food, in your home?

- ☐ No → go to Q61
- ☐ Yes → continue

60.1 Which rooms have been affected? ***(tick all that apply)***

- ☐ Bathrooms
- ☐ The child's bedroom
- ☐ Other bedrooms
- ☐ Living areas
- ☐ Kitchen
- ☐ Any other area/s

60.2 Has there been mould or mildew on any surface, other than food, in your home **in the last 12 months**?

- ☐ No
- ☐ Yes

61. Which rooms have wall to wall carpet as the floor covering ***(tick all that apply)***

	No	Yes
Bedrooms	☐	☐
Living areas	☐	☐
Kitchen	☐	☐
Any other area/s	☐	☐

62. Do you usually sleep with the window open?

- ☐ No
- ☐ Yes

63. How often is the bedroom aired? (ie windows open for more than two hours)

- ☐ Most days (5 or more times per week)
- ☐ 2-4 times a week
- ☐ Once a week
- ☐ Less often than once a week

64. How often do you open windows in your house?

- ☐ Daily
- ☐ 2-4 times per week
- ☐ Weekly or less often
- ☐ Never

65. Do the windows you open have fly screens?

- ☐ Yes
- ☐ No
- ☐ Not all

66. What type of backyard do you have?

- ☐ Grass
- ☐ Dirt
- ☐ Concrete
- ☐ Paving
- ☐ Grass and combination of the above
- ☐ No backyard

67. How many people in your household currently smoke regularly (most days of the week) outside the house?

_____ people

68. How many cigarettes are smoked on average per day outside your home?

_____ per day

69. How many people in your household currently smoke regularly (most days of the week) inside the house?

_____ people

70. How many cigarettes are smoked on average per day inside your home?

_____ per day

71. In the last 12 months, did you normally have any animals living with you?

- ☐ **No** → Thanks! That completes the survey.
- ☐ **Yes** → please continue

If yes, which type(s) of animal do you have?	How many of these animals live with you?	Do they live inside your home	
		Inside	Outside
☐ Dog	____ dog/s	☐	☐
☐ Cat	____ cat/s	☐	☐
☐ Guinea pig	____ guinea pig/s	☐	☐
☐ Rabbit	____ rabbit/s	☐	☐
☐ Birds	____ bird/s	☐	☐
☐ Other (please list below)	____ animal/s	☐	☐

_____

Appendix 8: MACS protocol for SPT at 18 years

SKIN PRICK TESTING

ITEMS REQUIRED FOR EACH PARTICIPANT:

Item	Amount
Bluey	2
Battery of allergens	13
Lancets	15
Cotton wool	2pkts (5 in each packet)
Water wipes	2
Tissues	1 box
Sharps container	1
Access to adrenaline*	Crash cart in CTU
Results recording sheet (Labelled)	1
Timer	2
Ruler	2
Marker	2

* The weight of the participant should be recorded prior to Skin Prick Testing to allow calculation of adrenaline dose.

PLEASE NOTE:

THE ALLERGENS SHOULD BE KEPT IN THE FRIDGE WHEN NOT IN USE AND STORED BETWEEN 2 & 8 DEGREES.

PLEASE DO NOT KEEP THEM IN THE FREEZER AS IT WILL DESTROY THE POTENCY OF THE ALLERGENS.

Prior to skin prick test

- Ask participant/participant's parent the pre-SPT questions on SPT data collection sheet.
- Fill in weight and calculate adrenaline dose (0.01mls/kg of Adrenaline 1:1000, IM)
- Explain the procedure and the time involved before com ncing. Encourage the patient to resist scratching during the test as th will invoke an inflammatory response.

Skin prick test

- Clean skin surface of volar forearm (dominant arm) gently with sterile water and pat dry.
- Mark position for template on arm with pen and small dots next to test site:

  - do not go within 5cm of wrist or 3cm of antecubital fossa
- Mark the lancet strip with numbers 1 through 13 with a permanent marker. Fix the lancet strip with sticky tape and peel off protective covering to expose the base of the lancets.
- Set times T1 and T2 on timer. Set T1 to 10 minutes and T2 to 15 minutes.
- Place a small drop of each allergen alongside the appropriate dot on one side of the template line (numbers 1-7). Do not touch the skin with the dropper. The box of allergens will be set up in the corresponding order.
- Take a sterile lancet and make a small prick through each drop. Use a new lancet for each allergen and dispose off used lancets in the plastic container. Count the lancets to ensure that all the drops have been pricked (there should be 7 lancets).
- Once a prick has been made through each drop, blot off the excess allergen with a tissue and taking care not to cross-contaminate allergens.
- Now place a small drop of the remaining allergens (numbers 8-13) along the other side of the template line.
- With the remaining lancets make a small prick through each drop. Use a new lancet for each allergen and dispose of used lancets in the plastic container.
- Count the lancets to ensure that all the drops have been pricked (there should be a total of 13 lancets). Empty the plastic container into the sharps container.
- Once a prick has been made through each drop, blot off the excess allergen with a tissue taking care not to cross-contaminate allergens.
- On the timer press T1 and press start. Then press T2 and press start.
- After 10 minutes, timer T1 will sound; at this time measure and record positive control (Histamine on position 8 – Top Right). Use the metric card marked in mm to measure the diameter in 2 dimensions. Measure the width of each wheal, first at the widest part then at 90 degrees from this line. Record this measurement in “mm” in the boxes on the recording sheet. Record “0” for no response.
- After 15 minutes, timer T2 will sound; measure and record all the other allergens.
- Clean the remaining traces of allergen and pen from the arm with sterile water.
- If the patient has had a significant local responses apply an ice-pack.
- Observe participants with history of anaphylaxis or significant local responses for 30mins following Skin Prick Test.

Appendix 9: MACS Spirometry Protocol

LUNG FUNCTION TESTS

- Perform Lung Function Testing questionnaire (including questions on contra-indications to spirometry)
- The order of testing will be 1. EBC (if proband), 2. exhaled NO, 3. pre-BD Spirometry, 4. post-BD spirometry,
- A nose clip should be used for the spirometry only.
- All tests should be done in the seated position.

Spirometry (pre and post – Bronchodilator)

All spirometry should be performed using the EasyOne spirometer supplied. Note the following:

- Both expiratory and inspiratory flow volume curves should be recorded.
- FEV_1, FVC, FER, $FEF_{25-75\%}$, PEF, $FEF_{50\%}$, $FIF_{50\%}$, flow volume loop.
- The American Thoracic Society recommendations will be followed.
- Acceptability Criteria: Obtain at least three technically acceptable manoeuvres, ideally with less than 0.15 litres variability for FEV_1 and FVC between the highest and second highest result. Quote t largest value (FEV_1 and FVC), even if from separate manoeuvres.

FVC:

- Minimum of 3 technically acceptable blows (then apply he reproducibility criteria below).
- A rapid start is essential: this is defined as non-hesitant start or a back-extrapolated volume (see below) of <5% of the FVC or 0.15 litres, whichever is greater.
- At least 6 second expiration (if possible).
- End of test criteria: The end of the FVC manoeuvre is indicated by no change in volume (<0.025L) for at least 1 second after exhalation time of 6 seconds in adults (3s for children) or stopped for clinical reasons.
- Reproducibility criteria: the FVC from highest two of the three acceptable blows should not vary by more than 0.15 litres. Repeat the test if a criterion is not met.
- Largest FVC is recorded.

FEV_1:

- As for FVC.
- Take largest FEV_1 even if not from the same curve as the best FVC.
- "Zero time" determined by back-extrapolation: The extrapolated volume should be <5% of the FVC or 0.15 litres, whichever is w).
- Smooth, rapid take-off with no: hesitation, cough, leak, tongue obstruction, glottic closure, valsalva or early termination.
- Reproducibility criteria: The FEV_1 of two of the three acceptable blows should not vary by more than 0.15 litres.

$FEF_{25-75\%}$ and Expiratory Flows:

- From the single spirogram with the largest sum of FEV_1 + FVC.

Back-Extrapolation: Current electronic spirometers (including the EasyOne supplied) perform this procedure automatically and report whether or not this criteria has been met. The back-extrapolation procedure minimises errors in the measurement of FEV_1 if the expiratory manoeuvre was not performed rapidly from the start. The back-extrapolation procedure is as follows. Zero time is calculated by fitting a line through the steepest portion of the volume-time curve and extending it back to the time axis. The point where the line crosses the volume axis is taken as zero time, and it is from this point that the one second interval is obtained for the calculation of FEV_1. To meet the ATS criteria the extrapolated volume should be less than 5% FVC or 0.15 litres, whichever is greater (see ATS document for more details).

Assessment of Bronchodilator Response: Salbutamol is the preferred bronchodilator and is administered by metered dose inhaler (MDI) via spacer as follows:

- Dose: Three 100ug puffs separated by 30-60 seconds.
- Shake the MDI vigorously for a 10-20 seconds prior to use.
- Insert the MDI into a Volumatic spacer.
- The patient places his/her lips around the spacer mout piece & exhales gently to below FRC. The MDI is discharged once and the patient inhales slowly & steadily through mouth to TLC & then breath-holds for 5-10 seconds.
- The second dose is administered about 30 seconds later.
- The third dose is administered about 30 seconds after the second dose.
- 10 minutes should be allowed before repeating spirometry (post-BD).

Quality Assurance (Spirometry):

- Daily Calibration Check: Prior to each testing session, a 3-litre calibration syringe is used to assess the accuracy of the spirometer. The volume measured should be 3.00 L ±3% (ATPS) in accordance with ATS recommendations. If the measured volume is outside this range, repeat the calibration check. A printout of the calibration shoul be obtained as a record.
- Biological Control Subject: A normal, well-trained control subject is used to assess the dynamic performance and overall functioning of the spirometer. This should be done prior to each testing session involving a MACS biological control subject. The results should be recorded and compared previous results. If the readings are outside those usually ob d for that subject a second calibration check should be performed and the subject tested again. If the results remain outside the expected range, then a cond control subject should be tested.

Infection Control (Spirometry):

The EasyOne spirometer uses a spirette, which incorporates a filter to minimise cross-infection between patients. An additional filter is not required. However, the external surface of the spirometer should be cleaned between patients.

The Breath-a-tek spacers used in the delivery of salbutamol will be cleaned in accordance with the infection control procedures at the RCH.

1. Explain the test

2. Fill out Spirometry Questionnaire

3. Instruct and demonstrate the test to the subject, to include:
   - ☐ Correct posture with head slightly elevated
   - ☐ Inhale rapidly and completely
   - ☐ Position of the mouthpiece
   - ☐ Exhale with maximal force and length
   - ☐ Inhale completely

4. Have subject assume the correct posture

5. Attach nose clip.

6. Insert mouthpiece in Easyone, place mouthpiece in mouth and close lips around it.

7. Inhale completely and rapidly with a pause of <1 s at TLC

8. Exhale rapidly and maximally until no more air can be expelled while maintaining an upright posture

9. Repeat instructions as necessary, coaching vigorously

10. Repeat for a minimum of three manoeuvres; no more than eight are usually required.
If unable to reach Quality A or B after 5 attempts – NOTIFY STUDY CO-ORDINATOR.

11. Check test repeatability and perform more manoeuvres as necessary.

Criteria for test completion summary:

- ☐ 3 technically acceptable blows
  End of Test criteria - Subject cannot breathe out any more or the volume-time curve has plateaued for >= 1sec (after 3 sec in <10yrs and 6 sec >10 years)
  Other factors rendering the test unacceptable - Cough, Valsalva, leak, obstruction, extra breath.
- ☐ The difference between the largest and next largest FVC should be <= 0.15L or 5%
- ☐ The difference between the largest and next largest FEV1 should be <= 0.15L or 5%

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