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ASTHMA AND IgE-REACTIVITY IN CHILDHOOD: RISK FACTORS AND CONSEQUENCES

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ASTHMA AND IgE-REACTIVITY IN CHILDHOOD: RISK FACTORS AND CONSEQUENCES

THESIS FOR DOCTORAL DEGREE (Ph.D.)

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If you fail, never give up because
F.A.I.L. means “First Attempt In Learning”
A.P.J. Abdul Karam

SUMMARY

Many children have asthma-like wheezing symptoms during their first years of life, yet only a minority develop asthma. Attempts to identify wheezing children at increased risk of asthma have been conducted, but the value for the clinician has been limited. The risk of developing asthma is influenced already *in utero* if the foetus is exposed to maternal tobacco smoke, but prior studies have not been able to differentiate pre- and postnatal smoke exposure effects. Conversely, the role of foetal or early postnatal smoke exposure for the risk of allergic sensitization in childhood is still under debate. It has been suggested that allergies may play a role in the pathogenesis of recurrent abdominal pain due to a mutual immunologic origin. Previous studies investigating potential associations between allergy-like symptoms, IgE-reactivity and abdominal pain have shown contradictory results.

The aim of the epidemiological studies in this thesis were to contribute to the knowledge on the role of pre- and early postnatal tobacco smoke exposure for the development of asthma and aeroallergen sensitization in children. We also explored which clinical risk factors and comorbidities to wheezing in infancy that were associated with having asthma at school age. Moreover, associations between allergy-related symptoms and IgE-reactivity during childhood and recurrent abdominal pain at age 12 years were investigated.

The results regarding risks of tobacco smoke exposure were based on data from eight European birth cohorts consisting of a total of 32,774 children. The studies on wheeze and school age asthma as well as allergy-related symptoms and abdominal pain were based on data from the population-based Swedish birth cohort BAMSE consisting of 4,089 children.

In study I we showed that among the children that wheezed at least once during their first two years of life, the risk of school age asthma was almost fourfold. Allergic heredity, increased severity of wheeze, infant eczema and recurrent abdominal pain were independent risk factors for having asthma at age eight years. In study II, maternal tobacco smoking during pregnancy was associated with an increased risk of wheeze or asthma in preschool children even among the children that had not been exposed late in pregnancy or after birth. We observed no convincing associations between early second hand tobacco smoke exposure and sensitization to pets, house dust mite, pollen or all aeroallergens combined in preschool or school age in study III. In study IV all allergy-related diseases at the age of 12 years were associated with recurrent abdominal pain at the same age. Moreover, food sensitization and food allergy at age four or eight years were also associated with recurrent abdominal pain at age 12 years.

The results of this thesis consolidate previous knowledge regarding early factors associated with childhood asthma development. The significant association between allergy-related diseases and recurrent abdominal pain supports the hypothesis of a mutual immunologic pathway. The knowledge that maternal smoking during pregnancy is associated with wheeze and asthma in preschool children, even if they were not exposed late in pregnancy or after birth, should be used to motivate women not to start smoking or to quit before conceiving to prevent asthma in their children.

LIST OF SCIENTIFIC PAPERS

I. Neuman Å, Bergström A, Gustafsson P, Thunqvist P, Andersson N, Nordvall L, Kull I, Wickman M. *Infant wheeze, comorbidities and school age asthma.* Pediatr Allergy Immunol 2014;134:428-434.

II. Neuman Å, Hohmann C, Orsini N, Pershagen G, Eller E, Fomsgaard Kjaer H, Gehring U, Granell R, Henderson J, Heinrich J, Lau S, Nieuwenhuijsen M, Sunyer J, Tischer C, Torrent M, Wahn U, Wijga AH, Wickman M, Keil T & Bergström A. *Maternal Smoking in Pregnancy and Asthma in Preschool Children: A Pooled Analysis of Eight Birth Cohorts.* Am J Respir Crit Care Med 2012;186:1037-1043.

III. Neuman Å, Hohmann C, Granell R, Henderson J, Torrent M, Bindslev-Jensen C, Eller E, Heinrich J, Smit HA, Nieuwenhuijsen M, Lau S, Thacher JD, Wickman M, Asarnoj A, Bergström A & Keil T. *Second Hand Tobacco Smoke Exposure and Aeroallergen Sensitization during Childhood: A Pooled Analysis.* Manuscript.

IV. Olén O, Neuman Å, Koopmann B, Ludvigsson JF, Ballardini N, Westman M, Melén E, Kull I, Simrén M & Bergström A. *Allergy-Related Diseases and Recurrent Abdominal Pain during Childhood – A Birth Cohort Study.* Aliment Pharmacol Ther 2014;40:1349-1358.

The studies will often be referred to by their Roman numbers in the text.

CONTENTS

LIST OF ABBREVIATIONS

ALSPAC	Avon Longitudinal Study of Parents and Children
AMICS	Asthma Multicenter Infant Cohort Study
aOR	Adjusted odds ratio
BAMSE	Barn, Allergi, Miljö, Stockholm, Epidemiologi
CI	Confidence interval
DARC	Danish Allergy Research Centre Cohort
ENRIECO	Environmental Health Risks in European Birth Cohorts
ETS	Environmental tobacco smoke
GER	Gastroesophageal reflux
GINI	German Infant Nutritional Intervention
IBS	Irritable bowel syndrome
IgE	Immunoglobulin E
ISAAC	International Study of Asthma and Allergies in Childhood
kU_A/l	Kilo Units (specified allergen IgE) per litre
LISA	Lifestyle-related Factors on the Development of the Immune System and Allergic Disease
MAS	Multizentrische Allergiestudie
OR	Odds ratio
PIAMA-NHS	The Prevention and Incidence of Asthma and Mite Allergy-Natural History Study
SHS	Second hand smoke
SPT	Skin prick test
Q0, 1,..,n	Q=Questionnaire, n=age of the child at follow-up

1 BACKGROUND

1.1 ALLERGIC DISEASES

1.1.1 Nomenclature

Allergic diseases such as asthma, eczema and allergic rhinitis are chronic inflammatory disorders caused by immune responses against common environmental substances, usually proteins[1]. Proteins that can cause allergy in susceptible individuals are called allergens. Allergy is defined as "a hypersensitivity reaction initiated by immunological mechanisms" whereas non-allergic hypersensitivity is used in the absence of proven immunological mechanisms[2-4]. The immunological mechanisms can be mediated by B-cells or T-cells. In most cases, the allergic reaction belongs to the IgE antibody isotype and the patient is said to have IgE mediated allergy[2]. The process when an individual acquires increased sensitivity to an allergen is called sensitization[2].

Atopy is a term for the personal and/or familial tendency, usually in childhood or adolescence, to become sensitized and produce IgE antibodies to "innocuous" proteins[4]. As a consequence, typical allergic symptoms can develop although the term atopy does not include clinical symptoms[4].

1.1.2 The sensitization process

The sensitization process starts when an allergen enters through the skin or through the mucosal membranes in the airways or gut and is taken up by an antigen presenting cell (APC) and transported to a regional lymph node where Th0 (naïve) CD4+ cells are stimulated to develop into Th2 cells (Th=T-helper cell)[5]. Cytokines (cell signalling proteins) such as IL-4 and IL-13 produced by the Th2 cells stimulate B-cells to form allergen-specific IgE-antibodies. These IgE-antibodies adhere to cell surfaces of mast cells and basophils which migrate to the mucous membranes and around blood vessels awaiting new contact with the allergen. On the other hand, if Th1, Th17 or T-regulatory cells are formed from naïve Th0 cells instead of Th2 cells when encountering the APC, the sensitization process is suppressed or not initiated. This description of the sensitization process is a simplified version. In reality it is much more complex, depending on a balance between Th1 and Th2 cell numbers and activity, specific local cytokine environment and type of encountered allergen[5].

When the sensitized individual is reexposed to the specific allergen, this is recognized by the previously formed IgE-antibodies on the mast cell or basophil surfaces. Cross-linking of two IgE-antibodies on the cell surface activates signalling pathways into the cell inducing degranulation with release of preformed inflammatory mediators such as histamine, leukotriens, heparine, proteases and tryptase[5] which give rise to the allergic inflammation.

1.1.3 Allergic inflammation

The allergic inflammation that arises upon mast cell or basophil activation causes different symptoms depending on affected organ but the underlying inflammation is similar, with increased capillary permeability and vasodilatation resulting in swelling/secretion and redness, and nerve stimulation causing itch, sneeze or constriction of smooth muscle[5]. For example, typical symptoms localized to the skin are itching rashes in eczema or red, raised itchy wheals in allergic urticaria. A runny, blocked, itchy nose with frequent sneezing is typical of allergic rhinitis. Usually symptoms develop within minutes after exposure.

Selected cytokines (such as IL-4 and IL-5) and chemokines produced by mast cells and Th2 cells also promote growth and development of inflammatory cells that migrate to the site. These cells, for example eosinophils, further enhance inflammation which can result in prolonged or biphasic allergic reactions[6]. These delayed reactions as well as chronic allergen exposure may cause tissue damage such as airway remodelling in asthmatics.

1.1.4 Disease prevalence

Allergic diseases such as asthma, eczema and allergic rhinitis have become more frequent during the latter half of the 20th century, particularly among children[7]. Recent worldwide population estimates show wide geographical variations and mixed time trend patterns[8]. Overall, allergic diseases are still on the rise, especially in countries with a historically lower prevalence of allergy[8], although asthma seems to have reached a plateau or even decreased slightly in the last decades in countries with an already high prevalence[8]. Today, allergic diseases affect up to every third child or adolescent in Europe[9, 10] and asthma is the most common chronic childhood disease in nearly all industrialized countries, giving rise to substantial morbidity[7, 11].

According to the International Study of Asthma and Allergies in Childhood (ISAAC), the prevalence of asthma symptoms in Sweden is around 10% in children aged six to seven years and 13 to 14 years, which is comparable with most of Western Europe[8]. The corresponding prevalence for allergic rhinitis is around 7% and 10%, and for eczema 22% and 13% at the two respective ages[8]. Apart from the negative impact on the quality of life of affected children and families, the high prevalence makes allergic diseases a serious public health issue.

1.1.5 Disease aetiology and risk factors

The aetiology of allergic diseases is multifactorial and complex. A genetic disposition as well as appropriate timing of environmental exposures to certain stimuli is required for atopy and allergic diseases to develop[1, 12, 13]. In addition, sex and allergic heredity may modify effects of exposures[14-16], and environmental exposures may influence the expression of important genes[17].

Changes in environment and lifestyle in the last decades are considered dominating factors for the increment in prevalence of atopy and allergic disease since the pace of this increase has been much faster than the genetic constitution can possibly shift[1]. Moreover, variations in allergy prevalence exist even between genetically similar groups[7, 8, 18]. A crucial window for harmful impact of environmental and lifestyle factors appear to exist in prenatal and early postnatal life, as immune and lung development occur largely during these periods[19, 20].

In 1989, Strachan suggested that reduced exposure to infections in early childhood could enhance development of allergic diseases[21]. This hypothesis that has later been known as "the hygiene hypothesis", was based on observations from a cohort of 17,414 British children, where the prevalence of hay fever and infantile eczema at age 11 and 23 years were inversely related to the number of older siblings[21]. An alternative interpretation of this hypothesis is that altered bacterial colonization in infancy could be responsible for the increase in allergic diseases due to changes in exposure to various microbial agents[1]. This is supported by observational studies confirming an inverse relation between other indirect markers of microbial exposure, such as living close to farm animals[22-24] and the development of atopy and allergic diseases. Thus, it is currently believed that changes related to improved hygiene and healthcare in the developed world have altered the pattern of exposure to infectious and/or microbial agents in early life, predisposing the immune system towards an atopic response[1, 18, 21, 25]. However, which microbiota that are involved and the mechanisms are still unclear.

Furthermore, the steady increase of asthma prevalence shares the trajectory of increasing air pollution and population trends towards urban centres[26]. Current research suggests that air pollution from different sources such as traffic, heating and environmental tobacco smoke[27-29] likely play an important role in the pathogenesis of asthma[30].

Several birth cohorts have demonstrated a relationship between early sensitization to aeroallergens and subsequent asthma persisting into school age and beyond[26, 31-34]. Infants and children spend most of their time at home or at day care, making the indoor environment an important source of early life allergens. Aeroallergens accounting for the vast proportion of sensitization in this context are house dust mite and furred pets (cats)[35-37]. Indoor dampness/mould is also associated with increased asthma prevalence[38]. Moreover, viral respiratory infections in infancy are associated with asthma development[39, 40] although it is still unclear whether the virus itself causes immunological changes that lead to asthma or if affected infants already destined for asthma develop more severe symptoms[26]. Many other factors than the ones reported in this thesis have been associated with asthma or atopy, for example dietary factors and obesity[41, 42].

1.1.6 Disease progression during childhood

Symptoms of allergic disease often follow a typical sequence of progression during childhood, starting with eczema in the first months of life[43]. Food allergies, especially to cow's milk and egg are also common early in life and wheeze often debuts in the second half of the first year[44, 45]. In school-aged children allergic rhinitis and asthma become more prevalent, combined with increasing aeroallergen sensitization[43, 46]. However, although different allergic symptoms are more common at different ages as described above, the same symptom sequence is not always present at the individual level and comorbidity of symptoms are more of a rule than an exception[47]. Furthermore, allergic disease development is a dynamic process, with both new cases and remission being common throughout childhood[47].

1.1.7 Comorbid symptoms and consequences of disease

Besides anaphylaxis that is potentially life-threatening with an allergic reaction involving several organs, data indicate that local exposure to an allergen can mediate systemic disease manifestations expressed also beyond organs commonly viewed as part of an allergic disease due to common immunological mechanisms[5, 48, 49]. Already in the seventies, Peckham et al. observed that recurrent headaches, migraine and recurrent abdominal pain were more frequent among eleven-year-olds with "wheezy bronchitis" compared to children without symptoms[50]. Later studies have shown similar associations[48, 51-55]. For example, Tollefsen et al. found that allergic wheeze and involvement of increasing combinations of wheeze and other allergic expressions (allergic rhinitis and eczema), increased the reporting of other health problems such as headache, abdominal pain or muscle pain among Norwegian adolescents[48]. Moreover, several studies have reported that allergic conditions are found in excess among patients with functional gastrointestinal disorders and irritable bowel syndrome (IBS), suggesting that this might (at least partly) be due to coexisting low-grade inflammation[53, 54, 56-62]. With regard to associations between food hypersensitivity and abdominal pain, the results have been conflicting although patients with abdominal pain often report that specific foods induce or worsen gastrointestinal symptoms[62-67].

The concept of a common mucosal immune system suggests that, since activated lymphocytes can migrate from one mucosal site to another for example between the lung and gut, there exists a potential to cause an inflammatory response at both sites[6, 49, 68, 69]. This concept is supported by the fact that airway-like cell infiltration of the duodenal and intestinal mucosa in patients with asthma and allergic rhinitis has been described[6, 69]. Several studies have observed disturbances in the immune system in the intestinal mucosa and peripheral blood of irritable bowel syndrome (IBS) patients such as an increased number of eosinophils and mast cells[70-72]. These cells are central in the pathogenesis of allergic disease, but mast cells also play a role for chronic pain perception, particularly at the visceral level[73]. Barbara et al. have shown that mast cell degranulation in close proximity to nerves innervating the colonic mucosa is correlated with abdominal pain perception in patients with IBS[74]. Thus, allergies are suggested to play a role in the pathogenesis of chronic abdominal pain due to a mutual immunologic origin, at least in a subgroup of patients[62, 70, 75]. Several studies have explored the relationship between asthma[50, 55, 58, 60, 76-78], allergic rhinitis[55, 62, 79], food hypersensitivity[61, 65, 66, 77, 80, 81] and eczema[55, 62, 79] in relation to abdominal pain, with

contradictory results. Most studies have been based on small, selected study samples and the study designs have not often been prospective and few have included children[50, 66, 79]. More knowledge about the relationship between allergy-related symptoms, IgE-reactivity and recurrent abdominal pain in children is of importance since therapeutic options may differ between atopic and non-atopic children with recurrent abdominal pain.

Although not studied in this thesis, there are also other consequences of allergic disease in children such as reduced health-related quality of life[82] and increased susceptibility to various infections[83, 84].

1.2 ASTHMA

1.2.1 Definition and phenotypes

According to the latest version of the international guidelines for asthma treatment, GINA 2015, asthma is "a heterogeneous disease, usually characterized by chronic airway inflammation. It is defined by the history of respiratory symptoms such as wheeze, shortness of breath, chest tightness and cough that vary over time and in intensity, together with variable expiratory airflow limitation"[85]. Chronic and/or recurrent airway inflammation, mucous hypersecretion, and airway smooth muscle mediated bronchoconstriction cooperate to create the airflow limitation, symptoms and signs of asthma[12, 26].

Many different phenotypes of asthma have been identified, but more research is needed to prove their clinical usefulness. The dominating phenotype in school children and adolescents is allergic asthma[13, 26]. Airway inflammation with eosinophilia and/or neutrophilia is often present, with good therapeutic response to inhalant steroid medication. This phenotype is often associated with other allergic manifestations and the vast majority of teenagers with asthma are sensitized to aeroallergens. A less common asthma phenotype in childhood, non-allergic asthma, comprises variable expiratory airflow limitation and typical respiratory symptoms without inflammatory markers.

1.2.2 Diagnostic criteria, clinical features and trigger factors

The diagnosis of asthma in infants and preschoolers is based on patient's history and typical symptoms. The main symptoms are wheezing or whistling breathing sounds, forced or heavy breathing, nocturnal cough, cough during activity, prolonged cough after common colds and reduced energy/physical performance. The most common trigger factors for exacerbations and worsening of asthma symptoms are viral respiratory tract infections and exercise but also allergens, cold air, tobacco smoke and strong scents. In some cases, simple exercise tests are performed and in less certain cases, treatment with inhalant steroids can be tested to see if improvement occurs. Allergy testing is most often conducted. However, it is important to keep in mind that all that wheezes is not asthma, and differential diagnoses must be considered, especially in wheezy infants below age six months. In children from age five to

six years, objective measurements of lung function such as peak expiratory flow curves, spirometry and fractional exhaled Nitrous Oxide can be used to verify the diagnosis and for follow-up of symptoms and treatment effects.

1.2.3 Prediction of disease

In a large proportion of school age children with asthma, asthma-like symptoms were present already during the first years of life. On the other hand, only approximately 30% of preschool wheezing children have asthma at school age[31]. It is currently believed that infants and preschoolers with chronic wheezing symptoms that are triggered by multiple stimuli are at higher risk of developing asthma due to underlying structural or functional airway changes aggravated by airway inflammation, compared to children with mild episodic wheeze[86].

Since data suggest that early asthma symptoms are associated with deterioration of lung function and persistence of symptoms into adulthood, identifying these children early is of importance for developing tailored treatment strategies and improve follow-ups to decrease disease burden[31, 87, 88]. Detecting children at low risk of asthma is also important in order to reduce unnecessary treatment with potential side-effects. One obstacle is that no objective measurements for asthma can be used in this young age group[31].

Several attempts have been done over the years to develop predictive risk indices that can be used in clinical practice for identifying children with early respiratory symptoms at higher risk of developing asthma, such as the asthma predictive index[31] the PIAMA risk score[87] and the recent clinical asthma prediction score[88]. Factors suggested useful for identification of early-onset asthma in wheezers include a family history of asthma or atopy[31, 87, 88], eczema[87], allergic rhinitis[31], low socio-economy[87], respiratory tract infections[87, 89] and sex[87, 90]. Some prediction rules include results from IgE or eosinophil screening tests[31, 88, 89], and it may be argued that this is not simple to incorporate in general clinical practice[91]. Subdivision of wheeze into categories with regard to timing and persistence of symptoms such as early-onset, persistent, late, transient and intermediate wheezers up to early school age have been done in several studies[32, 86, 89]. These categories are relevant in epidemiological studies but less useful for clinicians since asthma prediction becomes important as soon as symptoms appear. Caregivers seeking health care for their wheezing children want to know the prognosis at the time being, not later on.

In conclusion, the tools for identifying wheezers that will develop asthma are not yet applicable in clinical practice[92]. Thus, the challenges remain for clinicians to decide on which treatment that can be effective and which information to give the parents.

1.3 SECOND HAND TOBACCO SMOKE

1.3.1 Second hand smoke exposure prevalence in children

Exposure to second hand smoke (SHS), also known as passive smoking or environmental tobacco smoke, is the involuntary inhalation of other people's cigarette smoke[93]. Globally, around 40% of children are exposed to SHS[94] and the main source of smoke exposure during childhood is from tobacco smoking by the caregivers in the home environment[93, 95]. The greatest contributor of SHS exposure in children is a smoking mother[96]. Newborns and infants spend most of their time in the home environment, thereafter the prevalence of SHS exposure declines with age due to more time being spent outside the home[97]. The smoke-free legislations banning smoking in public places that have been introduced in many countries in the last decade do not include private households[93]. According to information from the World Health Organization, children accounted for 28% of the deaths attributable to SHS in 2004[98].

Results from the Children's Environmental Health Survey, a comprehensive survey including 36,000 Swedish children[99], showed that 5% of women reported that they smoked at some point during pregnancy in 2011. This is a decrease compared to results from the same study conducted in 2003, when 9.5% of the women reported smoking during pregnancy. Moreover, the proportion of children with at least one parent being a daily smoker almost halved between 2003 and 2011. In 2011, about 11% of 12-year-olds, 9.6% of four-year-olds, and 7.5% of eight-month-old Swedish children had at least one parent who smoked. However, there was a clear difference between children's environments with regard to parental education. No reduction of smoking during pregnancy was seen in women with less than high school education and the decrement in tobacco smoking was seen in the group of parents that had completed high school, but among less educated parents the prevalence remained unchanged at almost 40%.

1.3.2 Composition of second hand tobacco smoke

Cigarette smoke is an aerosol that contains extremely fine droplets suspended in a complex gaseous system. The two kinds of SHS that are produced while smoking are side stream and mainstream smoke. Side stream smoke is released from the lit end of the cigarette and mainstream smoke is the smoke exhaled by the smoker. When burned, cigarettes emit more than 7,000 chemicals including 70 mutagens according to the American Cancer Society[100]. Although SHS becomes diluted in ambient air it contains significant levels of nicotine. Nicotine has been the focus of most animal model studies and human epidemiological studies on the effects of smoking[101] and despite the large number of chemicals present in tobacco smoke it has been suggested that many of the adverse effects on the foetus and neonate are due to the nicotine[102].

1.3.3 Foetal and early second hand smoke exposure and childhood respiratory morbidity

Children are especially susceptible to SHS due to their growing and differentiating organs and tissues[94, 96, 103]. Infants under two years of age may be particularly susceptible to the adverse effects of SHS exposure as they have a high respiration rate[103, 104] and immature lungs[95]. After delivery, infants are exposed to tobacco constituents through the breast during lactation besides through respiration, and the nicotine concentration in mother's milk is two to three times higher than in the mother's plasma[102].

Unborn children are also passive smokers, and the levels of tobacco constituents from maternal smoking during pregnancy have been calculated to be at least 30 times higher than exposure levels achieved by environmental tobacco smoke alone[105]. Circulating nicotine in the mother's blood reaches the foetus through the placenta together with harmful particulate matter, resulting in decreased amounts of oxygen and nutrients to the foetus which impairs foetal cell growth and development[96].

The foetal lungs have a very high affinity for nicotine due to nicotinic acetylcholine receptors present in the foetal lung[102]. There is growing evidence that nicotine may be the key constituent of cigarette smoke that alters lung development in the offspring, leading to impaired lung function and an increased risk of respiratory illness[102]. According to a recent review by Maritz et al, several nicotine induced changes have been demonstrated in the lungs of animal models after foetal SHS exposure, for example thickening of airway walls, collagen accumulation, airway narrowing, impaired cell proliferation in the bronchial epithelium, reduced surface complexity in the lung parenchyma and reduced internal surface area[102].

Epidemiological studies indicate that lung function and susceptibility to respiratory diseases throughout life can be programmed by environmental factors, such as SHS, operating during foetal and early postnatal life[102]. Associations have been reported between smoking during pregnancy or infancy and impaired lung function[106] as well as lower airway obstruction with symptoms of wheeze[107-113] and asthma[108, 114-117] in children.

Despite extensive research, the potential independent role of *in utero* tobacco smoke exposure on childhood wheeze and asthma has been unclear due to the challenge of disentangling prenatal and postnatal associations since most women continue smoking after delivery[118, 119]. Since different biological mechanisms may influence respiratory disease development before and after birth[116], assessing associations between pre- and postnatal SHS exposure and childhood wheeze or asthma separately could provide new insights. Moreover, the impact of smoke exposure on respiratory disease development may differ early and late in pregnancy and in previous epidemiological studies the timing of SHS exposure in pregnancy in relation to asthma in the offspring has not been possible to assess.

1.3.4 Foetal and early life second hand smoke exposure and aeroallergen sensitization development during childhood

The evidence for a causal relationship between pre- and postnatal SHS exposure and asthmatic symptoms and reduced lung function in children is quite strong. In contrast, the evidence related to the development of aeroallergen sensitization is much weaker[95]. In theory and with support from some studies based on animal models[120-122], a number of potential mechanisms exist by which air pollutants such as SHS may enhance aeroallergen sensitization. For example, SHS may act as an independent aggravator of the airway epithelium, or aeroallergens may adhere to SHS particulate matter making this an easy conduit for allergen-induced airway inflammation. SHS may also have an adjuvant effect on local allergens[26, 30]. Furthermore, SHS may influence the maturation of the immune system and modulate immune responses[123, 124].

In 1998, Strachan and Cook concluded that pre- or early postnatal parental smoking is unlikely to increase the risk of allergic sensitization in children after having reviewed existing epidemiological studies[125]. Later studies have shown conflicting results[126] reporting increased[29, 110, 127], reduced[128] as well as no[111, 129, 130] associations between early SHS exposure and aeroallergen sensitization. However, some of these studies observed significant associations only in children with or without allergic heredity, thus the role of heredity as an effect modifier is also unclear. A recent review by Feleszko et al based on nineteen population-based studies concluded that household SHS increases the sensitivity to allergens in children by influencing postnatal immunoregulation[28].

In conclusion, both reviews and studies report diverging results regarding the association between SHS and aeroallergen sensitization although some evidence exists regarding altered immune responses and tobacco smoke induced sensitization in animal models.

1.4 AIMS

The overall aim of this thesis was to assess potential consequences of early life tobacco smoke exposure and allergic comorbidity for the development of asthma, IgE-reactivity or recurrent abdominal pain during childhood in the general paediatric population.

The study-specific aims were:

- To identify clinical risk factors for asthma in eight-year-old children that wheezed during infancy.

- To assess associations between exposure to maternal smoking during pregnancy and wheeze or asthma in preschool children.

- To investigate associations between exposure to maternal smoking during pregnancy or early infancy and aeroallergen sensitization to pets, house dust mite and pollen in preschool and school age children.

- To examine if allergy-related diseases or IgE-reactivity during childhood are associated with increased risks of recurrent abdominal pain at age 12 years.

2 MATERIALS AND METHODS

2.1 STUDY SUBJECTS

Study I and IV are based on data from the BAMSE birth cohort. Study II and III are pooled analyses containing individual participant data from eight European cohorts including BAMSE.

2.1.1 The BAMSE birth cohort

BAMSE is a Swedish acronym standing for Barn (Children), Allergi (Allergy), Miljö (Environment), Stockholm, Epidemiologi (Epidemiology). This longitudinal, population-based birth cohort consists of children born in four predefined areas in the central and north-western parts of Stockholm (Järfälla, Solna, Sundbyberg and parts of Stockholm inner city). These areas cover both urban and suburban environments with varying socio-economy and living conditions[131].

The primary aim of the BAMSE study is to assess risk factors for developing allergy-related diseases in childhood[131]. The study is ongoing, and the originally recruited "BAMSE-children" are currently in their early twenties.

2.1.1.1 Recruitment

Enrolment took place between February 1994 and November 1996. Information on newborns in the areas of interest was retrieved from the community population register. Parents were asked about participation at the first scheduled postnatal visit at the child health care centre. During time of enrolment, 7,221 children were born in the study areas. Out of these children, 477 could not be reached and another 1,256 were actively excluded leaving 5,488 eligible children for the study. The flow chart in Figure 1 describes the included and excluded children at recruitment.

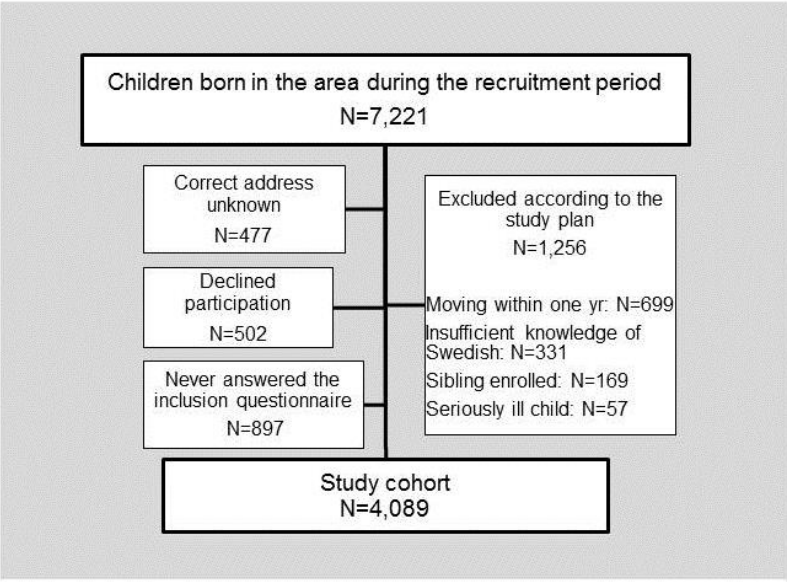


Figure 1 Flow chart of included and excluded children at recruitment to BAMSE

In 1996, the families who declined participation or were actively excluded were sent a short questionnaire on background characteristics to enable evaluation of potential selection into the study. The response rates for families declining to participate was 83%, and for the actively excluded families 58%. Smoking was more common among these parents, but no significant differences were seen with regard to pet ownership or allergy-related disease[131].

The final number of included children was 4,089 which was about 75% of all eligible newborns. When the infants were two months old (median age), the parents answered postal questionnaires regarding parental allergy-related diseases, lifestyle factors, socio-economy and residential characteristics.

2.1.1.2 Follow-ups

When the children were one, two, four, eight, and 12 years, questionnaires focusing on key exposures and allergy-related symptoms in the children were answered by the parents. The response rates at the respective ages were 96%, 94%, 91%, 84% and 82%. At the age of 12 years, apart from the parents, questionnaires were also answered by the children themselves. The 12-year questionnaires were sent out at a single time point in springtime 2008 when the children were at a mean age of 12.9 years, (eleven to 14 years).

All children whose parents answered the questionnaires at four and eight years were offered clinical examination including blood sampling regarding common food and airborne allergens. Serum samples were drawn from 2,614 (64%) and 2,461 children (60%) at the respective ages. Figure 2 describes the periods of data collection up to 12 years.

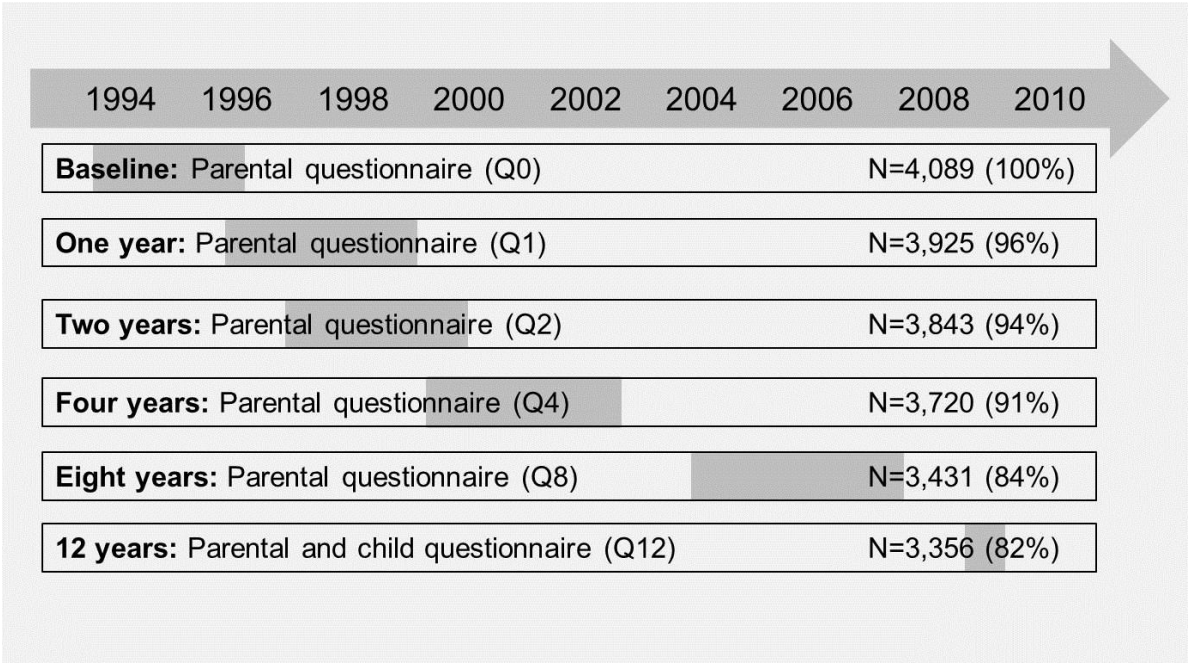


Figure 2 Periods of data collection and response rates up to 12 years in the BAMSE birth cohort

2.1.2 The ENRIECO collaboration

The ENRIECO (Environmental health risks in European birth cohorts) project started in 2009 with the objective to advance knowledge on relationships between environmental contaminants and child health[132]. The project received financial support from the European Commission for this purpose. European pregnancy and birth cohorts with ethical approval from local review boards were allowed to participate.

2.1.2.1 Overview of the birth cohorts

Researchers from BAMSE participated in the identification and evaluation of the cohorts with data on tobacco smoke and allergy-related symptoms in ENRIECO[133]. The cohorts with sufficient data (including BAMSE) were included in the pooled analyses in study II and III. An overview of these birth cohorts is provided in Table 1.

Table 1 Overview of the European cohorts: cohort acronym and country, years of recruitment, the number of children enrolled in each cohort at baseline and proportions included in study II and III

Acronym, country	Recruitment	N, Baseline	N, Study II	N, Study III
ALSPAC, U.K.	1991-1992	14,057	7,608 (54%)	5,655 (40%)
AMICS-Menorca, Spain	1997-1998	482	441 (91%)	349 (72%)
BAMSE, Sweden	1994-1996	4,089	3,673 (90%)	3,041 (74%)
DARC, Denmark	1998-1999	562	495 (88%)	396 (70%)
GINIplus, Germany	1995-1998	5,991	3,794 (63%)	1,931 (32%)
LISAplus, Germany	1997-1999	3,097	1,760 (57%)	1,054 (33%)
MAS, Germany	1990	1,314	894 (68%)	793 (60%)
PIAMA-NHS, The Netherlands	1996-1997	3,182	2,935 (92%)	1,394 (44%)
Total		32,774	21,600 (66%)	14,613 (45%)

Three additional cohorts provided data but were excluded before the analysis stage due to insufficient exposure or outcome information. These were NINFEA, Co.N.ER (Italy) and KOALA (The Netherlands).

2.2 STUDY POPULATIONS

In study I we included 3,251 children from the BAMSE birth cohort who participated at one, two, and eight years with available information on symptoms related to wheeze, eczema and rhinorrhoea at one and two years and on asthma at age eight years. There were 823 children that wheezed before age two years in the study population (infant wheezers).

In study II we required complete data on maternal smoking from at least one time point during pregnancy and from the first year after birth as well as information regarding wheeze symptoms or asthma between ages four to six years for cohorts from the ENRIECO collaboration. In total, 21,600 children could be divided into four exclusive pre- and postnatal smoke exposure groups.

Study III is based on 14,613 children from the ENRIECO collaboration with available data on maternal smoking from any time point during pregnancy and maternal smoke exposure assessment in the first year after delivery. We also required data on IgE antibody concentrations to at least one specific allergen based on serum or SPT tests from at least one of ages three or four years or six, seven or eight years from all cohorts.

Study IV comprised 2,610 children from the BAMSE birth cohort that participated in the one, two, four, eight and 12 year follow-ups without diagnosed coeliac disease or irritable bowel disease. Available answers on questions on abdominal pain and coeliac/inflammatory disease were required. Subgroup analyses were performed in 2,289 children with available serum IgE samples age four and/or eight years.

2.3 DEFINITIONS OF EXPOSURES AND OUTCOMES

2.3.1 Definitions of background characteristics (I, II, III, IV)

The study populations were compared to the original cohorts regarding certain characteristics in order to assess potential selection bias and generalizability of the results. These characteristics are defined in Table 2. Recruitment and collection of background information took place during pregnancy or in the first months postpartum in the European cohorts.

Although data were harmonized before the analyses in study II and III, some variation in the definitions was unavoidable due to differences between the wordings in the original questionnaires. For example, exclusive and partial breast feeding was rarely reported separately and duration was recorded no longer than six months in half of the cohorts. Moreover, questions regarding home dampness and mould were particularly heterogeneous.

Table 2 Definitions of background characteristics

Variable	Definition	Study
Socioeconomic status	Socioeconomic status for the household according to dominance, dichotomized into blue collar worker (low) and white collar worker (high).	I, IV
Maternal age	Mother's age 25 years or less at delivery.	I, IV
Parental education	Divided into high, middle and low depending on completed educational stage or years of education at birth of the child. The parent with the highest educational level was counted.	II, III
Immigrant parent	At least one parent born outside the Nordic countries (Sweden, Finland, Norway or Denmark).	IV
Allergic heredity/ Parental allergic disease	Mother and/or father with a doctor's diagnosis of asthma and asthma medication and/or a doctor's diagnosis of hay fever in combination with furred pets- and/or pollen allergy.	I, IV
Parental allergy	Mother or father has or have had asthma, rhinitis or eczema.	III
Parental asthma	Mother or father has a history of asthma or current asthma.	II
Older siblings	Older siblings at birth.	II, III
Home dampness or mould	Current or past smell of mould and visible mould in the home in the last year and/or known dampness damage in the home and building construction or visible condensation on the inner surface of the windows combined with known dampness damage in the building construction. BAMSE.	I
Home dampness or mould	Dampness, moisture or mould stains somewhere in the house during the child's first year. ENRIECO.	II, III
Early day care attendance	Child attending day care between ages one to two years.	III
Pet ownership	Furry or feathery pets in the home at birth.	III
Tobacco smoke exposure	Mother smoked at least one cigarette daily at two months of age of the child and/or at any point of time during pregnancy.	I
Caesarean section	Delivery by caesarean section (planned or emergency section).	III
Birth weight	Continuous variable reported in grams.	II
Birth weight	Low birth weight defined as 2600 grams or below.	IV
Gestational age	Reported as weeks of gestation at delivery.	II
Preterm delivery	Delivery before gestational week 37.	I
Breast feeding duration	Exclusive breast feeding for at least four months (BAMSE). Exclusive or partial breast feeding for at least four months (ENRIECO).	I, II, III, IV

2.3.2 Definitions of clinical characteristics and factors related to recurrent abdominal pain in infancy (I)

Exposures describing clinical characteristics and factors related to recurrent abdominal pain are defined in Table 3. All exposure information was based on parental reports, except for measurements of IgE-reactivity.

Table 3 Definitions of clinical characteristics and factors related to recurrent abdominal pain in infancy

Variable	Definition	Study
Infant wheeze	Having at least one episode of wheezing or whistling breathing sounds during the two first years of life. (Q1, Q2)	I
Wheeze persistency	Wheezing age zero to one years, age one to two years or during both years. (Q1, Q2)	I
Wheeze episodes	Wheeze less than three times or at least three times. (Q1, Q2)	I
Use of inhaled steroids	Prescribed corticosteroid inhalants due to symptoms of wheezing and whistling breathing sounds or forced or heavy breathing. (Q1, Q2)	I
Croup-like cough	Breathing difficulties combined with barking cough. (Q1, Q2)	I
Night cough	Troublesome cough at night. (Q1, Q2)	I
Cough during activity	Cough during play, laughter or while being outdoors. (Q1, Q2)	I
Infant rhinorrhoea	Runny or blocked nose with duration of at least two months during the last 12 months. (Q1, Q2)	I
Recurrent abdominal pain	Recurrent episodes of abdominal pain after the age of six months. (Q1, Q2)	I
Acute media otitis	Otitis or otosalpingitis symptoms treated with antibiotics. (Q1, Q2)	I
Diarrhoea	Recurrent or long-term episodes of diarrhoea. (Q1, Q2)	I
A doctor's diagnosis of food allergy	Food allergy diagnosed by a doctor. (Q1, Q2)	I
Symptoms at ingestion of hens' egg, cows' milk or wheat	Symptoms related to ingestion of hens' egg/cows' milk/wheat: asthma, itchy eyes and/or runny nose, facial oedema, urticaria, eczema or vomiting/diarrhoea. (Q1, Q2)	I
Pneumonia	Pneumonia diagnosed by a doctor. (Q1, Q2)	I
Use of antibiotics	Having received antibiotics for any reason. (Q1, Q2)	I

Q=questionnaire. The number after corresponds to the age of the child.

2.3.3 Definitions of smoking (II, III)

2.3.3.1 Prenatal smoke exposure

Prenatal smoke exposure was defined as maternal smoking of at least one cigarette daily during any trimester. Information on smoking habits was collected during pregnancy or in the first months after delivery.

The data used when constructing the prenatal smoke exposure variables, as well as for performing the dose-response analyses are described in Table 4. Since six out of eight cohorts had information on daily cigarette consumption from the first trimester, this trimester was used in the dose-response analyses in study II, except for DARC for which the amount for the whole pregnancy was used. The MAS cohort was excluded from dose-response assessments due to lack of data.

Table 4 Prenatal maternal smoke exposure information

Cohort	Available trimester data for dichotomous smoke exposure variable (study II and III)	Available trimester data with information on daily consumption for dose-response analysis (study II)
ALSPAC	1st, 2nd, 3rd trimester	1st and 3rd trimester
AMICS-Menorca	1st, 2nd, 3rd trimester	1st and 3rd trimester
BAMSE	1st, 2nd, 3rd trimester	1st, 2nd, 3rd trimester
DARC	No trimester specific information. "Has the mother smoked during this pregnancy?"	No trimester specific dose information. "Number of cigarettes, pipes, cigarillos or cigars smoked during the whole pregnancy".
GINIplus	1st, 2nd, 3rd trimester	1st, 2nd, 3rd trimester
LISAplus	1st, 2nd, 3rd trimester	1st, 2nd, 3rd trimester
MAS	1st, 2nd, 3rd trimester	No trimester specific dose information.
PIAMA-NHS	1st, 2nd, 3rd trimester	1st and 2nd trimester

2.3.3.2 Postnatal smoke exposure

Postnatal SHS exposure was defined as maternal smoking in the dwelling or near the child during the child's first year of life (study II) or in infancy (study III). Any tobacco smoke exposure was defined as mother, father, partner or other person smoking in the dwelling or near the child during the child's first year of life (study II) or in infancy (study III). In study II, information from the first months of life and one year was combined in the variable for smoke exposure in the first year of life, whereas in study III, smoke exposure information from the first months of life was used when constructing the variable for SHS exposure in infancy.

Despite harmonization of data before analyses, some differences regarding smoke exposure remained. The information after harmonization is described in Table 5.

Table 5 Description of the available postnatal smoke exposure information used for constructing the exposure variables in study II and III

Variable for maternal smoking			
Cohort	**First months**	**One year**	**Four to six years**
ALSPAC	Actively smoking, not limited to smoking in dwelling	Actively smoking, not limited to smoking in dwelling	Actively smoking, not limited to smoking in dwelling
AMICS-Menorca	Smoking, location not specified	Currently smoking at home	Currently smoking at home
BAMSE	Smoking at home	Smoking, location not specified	Smoking, location not specified
DARC	Smoking indoors	Smoking indoors	Smoking indoors
GINIplus	Smoking, not specified where	-	Smoking, not limited to smoking in dwelling
LISAplus	Smoking at home	Smoking at home	Smoking at home
MAS	Smoking at home	Smoking at home	Smoking at home
PIAMA-NHS	Cigarettes, pipes, cigars smoked in the house	Cigarettes, pipes, cigars smoked in the house	Cigarettes, pipes, cigars smoked in the house
Variable for smoke exposure by anyone			
Cohort	**First months**	**One year**	**Four to six years**
ALSPAC	Mother actively smoking, not limited to smoking in the dwelling and "child in smoky room"	No available information	No available information
AMICS-Menorca	Maternal smoking, location not specified and/or father and/or other persons currently smoking in the dwelling	Mother, father and/or other persons currently smoking in the dwelling	Mother, father and/or other persons currently smoking in the dwelling
BAMSE	Mother, father, siblings or others smoking in the dwelling	Mother, father, siblings or others smoking in the dwelling	Mother, father, siblings or others smoking in the dwelling
DARC	Mother, father, siblings or others smoking indoors	Mother, father, siblings or others smoking indoors	Mother, father, siblings or others smoking indoors
GINIplus	Mother smoking, not specified where, and someone smoking (mother and/or other) in the dwelling	Someone smoking (mother and/or other) in the dwelling	Someone smoking (mother and/or other) in the dwelling
LISAplus	Mother, father and/or others smoking in the dwelling	Mother, father and/or others smoking in the dwelling	Mother, father and/or others smoking in the dwelling
MAS	Mother, father and/or others smoking at home	Mother, father and/or others smoking at home	Mother, father and/or others smoking at home
PIAMA-NHS	Cigarettes, pipes, cigars smoked in the house by mother, father and/or other household members	Cigarettes, pipes, cigars smoked in the house by mother, father and/or other household members	Cigarettes, pipes, cigars smoked in the house by mother, father and/or other household member

2.3.4 Definitions of allergy related diseases and symptoms (I, II, IV)

The definitions of allergy related diseases used in study I, II and/or IV and the outcome recurrent abdominal pain at age 12 years used in study IV are presented in Table 6. All information was based on parental reports except abdominal pain at age 12 years.

Table 6 Definitions of allergy related diseases

Variable	Definition	Study
Asthma age one year	At least three episodes of wheeze after three months of age in combination with treatment with inhaled glucocorticosteroids and/or signs of suspected hyperreactivity without concurrent upper respiratory infection. (Q1)	IV
Asthma age two years	At least three episodes of wheeze after one year of age in combination with treatment with inhaled glucocorticosteroids and/or signs of suspected hyperreactivity without concurrent upper respiratory infection. (Q2)	IV
Asthma age four, eight and 12 years	At least four episodes of wheeze in the last 12 months or at least one episode of wheeze during the same time period in combination with occasional or regular treatment with inhaled glucocorticosteroids. (Q4, Q8, Q12)	I, IV
Current wheeze	Wheezing during the last 12 months (age four to six years).	II
Current asthma	Satisfying at least two out of three of the following criteria: (i) a doctor's diagnosis of asthma ever, (ii) parental-reported wheezing during the last 12 months, or (iii) asthma medication in the last 12 months, (age four to six years).	II
Eczema age one year/ Infant eczema	Dry skin, itchy rashes for ≥ two weeks at specific location (face or arms/legs extension surfaces or arms/legs flexures or wrists/ankles flexures) of rash and/or doctor's diagnosis of eczema after three months of age.	I, IV
Eczema age two years/Infant eczema	Dry skin, itchy rashes for ≥ two weeks at specific location (face or arms/legs extension surfaces or arms/legs flexures or wrists/ankles flexures) of rash and/or doctor's diagnosis of eczema after one year of age.	I, IV
Eczema age four years	Dry skin, itchy rashes for ≥ two weeks during the last 12 months at specific location (face or arms/legs extension surfaces or arms/legs flexures or wrists/ankles flexures) of rash and/or doctor's diagnosis of eczema after two years of age.	IV
Eczema age eight years	Dry skin, itchy rashes for ≥ two weeks during the last 12 months at specific location (face or arms/legs flexures or wrists/ankles or neck) of rash and/or doctor's diagnosis of eczema after seven years of age.	I, IV
Eczema age 12 years	Dry skin, itchy rashes during the last 12 months at specific location (arms/legs flexures or wrists/ankles or neck) of rash and/or doctor's diagnosis of eczema after ten years of age.	IV
Allergic rhinitis age one year	Symptoms from eye/nose following exposure to furred pets and/or pollen and/or doctor's diagnosis of allergic rhinitis from the first three months of life. (Q1)	IV
Allergic rhinitis age two, four and eight years	Symptoms from eye/nose following exposure to furred pets and/or pollen and/or doctor's diagnosis of allergic rhinitis since the previous questionnaire. (Q2, Q4, Q8)	I, IV
Allergic rhinitis at age 12 years	Symptoms from eye/nose following exposure to furred pets and/or pollen during the last 12 months and/or doctor's diagnosis of allergic rhinitis from the age of ten years. (Q12)	IV

Table 6 continues on the next page.

Table 6 Definitions of allergy related diseases and symptoms, continued

Variable	Definition	Study
Food hypersensitivity at age one, two and four years	Symptoms related to ingestion of a certain food at the time of the questionnaire: asthma, itchy eyes and/or runny nose, facial oedema, urticaria, eczema or vomiting/diarrhoea or avoidance of certain foods (at age four years) due to previous specific symptoms at ingestion. (Q1, Q2, Q4)	I, IV
Food hypersensitivity at age eight years	Symptoms related to ingestion of a certain food at the time of the questionnaire: dyspnoea, oral itch, symptoms from eyes/nose, urticaria, eczema, vomiting and/or diarrhoea or avoidance of certain foods due to previous specific symptoms at ingestion. (Q8)	IV
Food hypersensitivity age 12 years	Symptoms related to ingestion of a certain food at the time of the questionnaire: asthma or allergy, cough/hoarseness, itchy/runny/congested nose, red itchy eyes, oedema of face/lips/eyelids, feeling of laryngeal/throat swollenness, urticaria, eczema, vomiting, diarrhoea, severe abdominal pain, pronounced tiredness or reduced consciousness or avoidance of certain foods due to previous specific symptoms at ingestion. (Q12)	IV
Recurrent abdominal pain age 12 years	Recurrent abdominal pain, menstrual pain excluded, experienced every month or more often without a diagnosis of coeliac disease or inflammatory bowel disease. (Q12)	IV

2.3.5 Definitions of IgE-reactivity/allergic sensitization (I, III, IV)

2.3.5.1 IgE data in BAMSE

The blood samples drawn at four and eight years were screened with two different IgE assays. Phadiatop® screens for IgE antibodies to a mix of common aeroallergens: birch, timothy and mugwort pollen, cat, dog and horse dander, mould (*C herbarum*) and house dust mite (*D pteronyssinus*). Fx5® screens for IgE antibodies to a mix of common food allergens: cow's milk, egg white, soy bean, peanut, cod fish and wheat (ImmunoCAP™; former Phadia AB, now Thermo Fisher Scientific, Uppsala, Sweden).

Sensitization was defined as IgE antibody serum levels ≥ 0.35 kU_A/l. Food allergen sensitization data from age four years was used in study I. Information on food and aeroallergen sensitization at both ages was used in study IV.

2.3.5.2 IgE data in ENRIECO

In study III, aeroallergen sensitization was assessed in preschool (three to four years) and school age (six, seven or eight years). Children with IgE levels exceeding 0.35 kU_A/l to the allergen tested were considered sensitized. The allergens were assessed together as "any aeroallergen sensitization" and separately in three subgroups; "pet sensitization" (cat/dog dander), "house dust mite sensitization" (*D pteronyssinus*) and "pollen sensitization" (tree/grass pollen). The numbers of available allergens as well as analysis methods varied as described in Table 7.

Table 7 Overview of analysis methods, ages at testing and types of allergens across cohorts

				Any aeroallergen sensitization		
Cohort	**Analysis method**	**Cut-off value for sensitization**	**Child's age at testing**	**Pet allergens**	**House dust mite allergens**	**Pollen allergens**
ALSPAC	SPT (ALK-ABELLÒ, Hoersholm, Denmark)	Mean wheal diameter ≥2 mm	7 y	Cat dander	*Dpt*	Grass pollen mix: Bermuda, Kentucky Blue, timothy
AMICS-Menorca	UniCap (Pharmacia, Morris Plains, NJ)	>0.35 kU_A/l	4 y	Cat dander	*Dpt*	Grass pollen mix Olive tree
BAMSE	ImmunoCAP (Phadia AB/Thermo Fisher, Uppsala, Sweden)	≥0.35 kU_A/l	4 y, 8 y	Cat, dog dander	*Dpt*	Timothy, birch, mugwort
DARC	Magic Lite SQ allergy screen (ALK-ABELLÒ, Hoersholm, Denmark)	>1.43 sU/l	3 y	Cat, dog dander	*Dpt*	Timothy, birch, mugwort
	ImmunoCAP (Phadia AB/Thermo Fisher, Uppsala, Sweden)	>0.35 kU_A/l	6 y	Cat, dog dander	*Dpt*	Timothy, birch, mugwort
GINIplus	CAP-RAST FEIA (Pharmacia Diagnostics, Freiburg, Germany)	>0.35 kU_A/l	6 y	Cat,dog dander	*Dpt*	Rye, birch, mugwort
LISAplus	CAP-RAST FEIA (Pharmacia Diagnostics, Freiburg, Germany)	>0.35 kU_A/l	6 y	Cat, dog dander	*Dpt*	Timothy, birch, mugwort
MAS	ImmunoCAP (Phadia AB/Thermo Fisher, Uppsala, Sweden)	>0.35 kU_A/l	3 y, 7 y	Cat, dog dander	*Dpt*	Timothy, birch
PIAMA-NHS	RAST-like method (Sanquin Laboratories, Amsterdam, The Netherlands).	>0.35 kU_A/l	4 y, 8 y	Cat, dog dander	*Dpt*	Cocksfoot, birch

2.4 STATISTICAL ANALYSES

Statistical analyses were performed with the STATA statistical software, version 11.2 and13SE, (StataCorp, College Station, Texas, USA).

Prevalence

Prevalence of outcomes, exposures and baseline characteristics were expressed as percentages of the total number of available observations at the time of assessment.

Confidence intervals

Proportions with 95% confidence intervals were calculated for baseline characteristics, allergy-like symptoms and selected exposures between the originally enrolled children and the children included in the respective study. Proportions were compared between groups in order to evaluate differences of importance for selection and generalizability. Non-overlapping confidence intervals were considered significantly different.

The Chi-square test of independence

Comparisons of observed and expected proportions were made by the Chi-square test. This method was used for evaluating selection bias by comparing the study populations with the original cohort populations with regard to certain factors, at confounder assessment, and for detecting differences between groups with regard to outcomes and exposures.

P for trend

P for trend was calculated for categorical variables, assuming equidistant categories in study IV.

Proportional Venn-diagrams

Proportional Venn-diagrams were calculated for asthma, eczema and allergic rhinitis at age eight years separately for infant wheezers and non-wheezers as well as for markers of increased wheeze severity in study I. Venn-diagrams give a more visual overview of associations than a two-by-two table for example.

Logistic regression

Logistic regression was used to calculate odds ratios with 95% confidence intervals as estimates of relative risk/associations. Two multivariate logistic regression models were used where different adjustment variable combinations were included in study I and IV. In the first model, covariates describing background characteristics were included as adjustment variables. In the second model, further adjustments for concurrent allergy-related diseases or other symptoms associated with exposure or outcome were included in order to assess independent associations of the exposure under study. In the first out of two analysis stages, cohort-specific crude and adjusted estimates were calculated by logistic regression also in study II and III.

Confounder assessment was done by testing potential confounding factors in the logistic regression models in a univariate and stepwise manner. Besides the confounders chosen due to subject-matter knowledge, covariates that resulted in an OR change of more than 5% (study I, II, III) or 10% (study IV) when added to the regression models were considered confounders.

Meta-analysis

The cohort-specific ORs obtained by logistic regression were combined in a random effects meta-analysis model (study II and III). This model considers both within-cohort and between-cohort variation[134] as opposed to fixed effects models where the contribution of each participating study to the combined OR estimate (weighting) depends mainly on study size.

Dose-response analysis

Dose-response associations between maternal cigarette consumption during the first trimester and preschool wheeze and asthma were assessed in study II. In study III we performed dose-response analyses between the amount of cigarettes smoked daily by the mother or anyone in infancy and IgE-reactivity to aeroallergens. Before analyses, non-linearity was explored by testing the null hypothesis that the coefficient of the quadratic exposure term was equal to zero. This was done in each individual cohort and after combining data by random effects methods. A flexible logistic model was applied for each cohort using a maximum likelihood method. In a second step, the obtained estimated regression coefficients and their variance/covariance matrices were applied in a multivariate random effects meta-analysis model.

2.5 ETHICAL APPROVALS

The BAMSE project has been approved by the Regional Ethical Review Board at Karolinska Institutet, Stockholm, Sweden. Reference numbers; 93:189, 98:175, 02:240, 2007/1634-31.

All European birth cohort studies have valid Ethical approvals from their local review boards.

3 RESULTS

3.1 CHILDHOOD ASTHMA – RISK FACTORS AND CONSEQUENCES

3.1.1 Wheezing in infancy and school age asthma (I)

Among the 3,251 included children, 823 (25.3%) wheezed at least once during the two first years of life. Among these infant wheezers, 14.1% (116 children) had asthma at age eight years, compared to 3.7% (90 children) of the 2,428 infant non-wheezers ($p<0.001$). After adjustments for sex, tobacco smoke exposure and indoor dampness/mould, the risk of asthma at age eight years was almost fourfold among infant wheezers compared to infant non-wheezers, OR 3.68 (95% CI 2.74-4.96).

Severity of wheeze and asthma

The risk of asthma at age eight years increased with the number of wheezing episodes. For the 469 children that wheezed at least three times the adjusted OR for asthma at age eight years was 3.41 (95% CI 2.09-5.56), using the 354 children with less than three episodes of wheeze during the first two years of life as reference, Table 8.

Infant wheezers with inhaled steroid medication had a higher risk of school age asthma. About 30.5% of the 151 infant wheezers with prescribed inhaled steroids had school age asthma, compared to 10.4% among the 672 wheezers without this medication. The adjusted OR for school age asthma was 3.42 (95% CI 2.20-5.32) for infant wheezers with steroid inhalants compared to wheezers without steroid inhalants, Table 8.

The risk of asthma also differed between infant wheezers depending on time of wheezing debut and persistency of wheezing symptoms. The 249 infants that wheezed during the first and second year of life, and the 353 infants with a wheeze debut during the second year of life had a higher risk of school age asthma (21.3% and 14.4% developed asthma, respectively). This is to be compared to the 218 infants wheezing only during the first year out of which only 5.0% had asthma in school age. Using these infants as reference, the adjusted OR for asthma in school age for infants that wheezed both years was 5.11 (95% CI 2.51-10.41), and for infants with wheeze debut in the second year, 3.43 (95% CI 1.69-6.96), Table 8.

The characteristics above describing an increased severity of wheeze, overlapped substantially. Over 96% of the infant wheezers with at least three wheezing episodes had also used steroid inhalants or had wheezed both during the first and second year of life.

Table 8 Associations between sex, allergic heredity, characteristics of infant wheeze, and early comorbidity in relation to asthma at age eight years among children with infant wheeze, (N=823)

		Asthma at age 8 years			
		N	n	%	aOR (95 % CI)*
Total		823	116	14.1	
Sex†	Girl	341	46	13.5	1.00 (reference)
	Boy	482	70	14.5	1.10 (0.73-1.67)
Allergic heredity‡	No	508	59	11.6	1.00 (reference)
	Yes	302	54	17.9	1.53 (1.02-2.30)
Wheeze persistency	First year of life only	218	11	5.0	1.00 (reference)
	Second year only	353	51	14.4	3.43 (1.69-6.96)
	Both years	249	53	21.3	5.11 (2.51-10.41)
Wheeze episodes	≤2 episodes	354	24	6.8	1.00 (reference)
	≥3 episodes	469	92	19.6	3.41 (2.09-5.56)
Use of inhaled steroids	No	672	70	10.4	1.00 (reference)
	Yes	151	46	30.5	3.42 (2.20-5.32)
Infant eczema	No	574	62	10.8	1.00 (reference)
	Yes	249	54	21.7	2.31 (1.52-3.49)
Infant rhinorrhoea	No	726	98	13.5	1.00 (reference)
	Yes	97	18	18.6	1.32 (0.75-2.34)
Croup-like cough	No	455	54	11.9	1.00 (reference)
	Yes	368	62	16.9	1.45 (0.96-2.18)
Night cough	No	73	12	16.4	1.00 (reference)
	Yes	748	103	13.8	0.73 (0.38-1.43)
Cough during activity	No	277	29	10.5	1.00 (reference)
	Yes	543	87	16.0	1.53 (0.97-2.44)
Acute media otitis	No	368	48	13.0	1.00 (reference)
	Yes	453	67	14.8	1.07 (0.71-1.61)
Recurrent abdominal pain	No	744	97	13.0	1.00 (reference)
	Yes	77	19	24.7	2.33 (1.30-4.16)

*Adjustment made for sex, heredity, dampness or mould at home, tobacco smoke exposure and maternal age.
†Adjustment made for all potential confounders besides sex.
‡Adjustment made for all potential confounders besides heredity.
aOR, adjusted odds ratio; CI, confidence interval.

Wheeze, allergic heredity and asthma

Allergic heredity was more common among wheezing infants compared to non-wheezers, the proportions with allergic parents being 37.3% compared to 28.0% in the respective groups ($p<0.001$). Allergic heredity was associated with asthma at age eight years among infant wheezers, adjusted OR 1.53 (95% CI 1.02-2.30), Table 8.

Wheeze, comorbidity and asthma

Allergic comorbidity such as eczema and food hypersensitivity as well as symptoms from the respiratory tract (rhinorrhoea and different kinds of cough) were more common among infant wheezers compared to non-wheezers, ($p<0.001$). Among the infant wheezers, concomitant eczema was associated with higher odds of asthma at age eight years, adjusted OR 2.31 (95% CI 1.52-3.49). This was also true for food hypersensitivity, adjusted OR 1.93 (95% CI 1.22-3.04) and for doctor's diagnosed food allergy, adjusted OR 2.66 (95% CI 1.52-4.63). Furthermore, symptoms after ingestion of wheat and hens' egg was associated with asthma at age eight years, adjusted OR 3.15 (95% CI 1.08-9.20) and 2.40 (95% CI 1.15-5.00). No associations between infant rhinorrhoea or cough among infant wheezers and asthma at age eight years were seen, Table 8.

Wheeze, recurrent abdominal pain and asthma

Recurrent abdominal pain between six months and two years of life was more frequent among the infant wheezers. Among these children, 77 (9.4%) suffered from recurrent abdominal pain compared to 127 (5.2%) among the children without wheeze ($p<0.001$). Infant wheezers with parental reported recurrent abdominal pain had a higher risk of asthma at age eight years, adjusted OR 2.33 (95% CI 1.30-4.16), Table 8. Significant associations remained between recurrent abdominal pain among infant wheezers and asthma at age eight years after additional adjustment for factors related to abdominal pain in infancy, adjusted OR 2.31 (95% CI 1.21-4.43).

Combining risk factors in infant wheezers

Allergic heredity, increased severity of wheeze (at least three episodes of wheeze), infant eczema and recurrent abdominal pain were combined into four groups depending on number of coexisting risk factors. For the 92 wheezers with three or four risk factors, 38.0% had asthma at age eight years compared to 7.3% among the 151 wheezers with no risk factors who wheezed less than three times in infancy. In the group of wheezers with one (329 children) or two risk factors (236 children), 8.8% and 16.1% had asthma at age eight years, respectively.

ADDITIONAL RESULTS (I)

When does asthma start in infants with severe wheeze?

It might be argued that the infants with increased severity of wheeze already had asthma before age two years and not early asthma-like respiratory symptoms, therefore the prevalence of doctor's diagnosis of asthma and asthma according to definition at age one and two years (the definition is described in Table 6, page 25) were assessed among children up to two years of age with any wheeze as well as among the subgroups of wheezers with different characteristics of increased severity of wheeze in the two first years of life. The overlaps between infant wheeze and severity subgroups and these two definitions of asthma in infancy are described in Table 9.

About 20-30% of the 823 infant wheezers had received a diagnosis of asthma or fulfilled the definition of asthma at the same age, whereas the majority of infants with more than three episodes of wheeze, inhaled steroid use, or persistent wheeze had asthma according to definition before age two years. Besides for steroid inhalant use, the overlap was less with regard to a doctor's diagnosis of asthma in the subgroups with increased severity of wheeze.

Table 9 Proportions with doctor's diagnosis of asthma or asthma according to definition among infant wheezers and subgroups with increased severity of wheeze in the two first years of life

	Any wheeze ≥1 episodes N=823	Wheeze ≥3 episodes N=469	Use of inhaled steroids N=151	Persistent wheeze (1st and 2nd yr) N=249
	n (%)	n (%)	n (%)	n (%)
A doctor's diagnosis of asthma in the first two years of life				
No	644 (78.3%)	314 (66.9%)	33 (21.9%)	146 (58.6%)
Yes	178 (21.7%)	155 (33.1%)	118 (78.1%)	103 (41.4%)
Asthma according to definition in the first two years of life				
No	543 (66.5%)	190 (40.9%)	15 (9.9%)	97 (39.4%)
Yes	274 (33.5%)	274 (59.1%)	136 (90.1%)	149 (60.6%)

Strengthening the definition of wheeze

The results after restricting the analyses in study I to infants that wheezed at least three times during the two first years of life are displayed in Table 10 and 11. The same independent risk factors remained in these children as in the infant wheezers. In addition, having received a doctor's diagnosis of food allergy before age two years was also an independent risk factor for school age asthma.

Table 10 Associations between sex, allergic heredity, characteristics of infant wheeze and early comorbidity in relation to asthma at age eight years among children with at least three episodes of wheeze during the two first years of life (N=469)

		Asthma at age 8 years			
		N	n	%	aOR (95 % CI)*
Total		377	92	19.6	
Sex†	Girl	189	31	20.1	1.00 (reference)
	Boy	280	54	19.3	0.91 (0.56-1.48)
Allergic heredity‡	No	284	41	14.4	1.00 (reference)
	Yes	178	49	27.5	2.06 (1.27-3.34)
Wheeze persistency	1st year of life only	72	2	2.8	1.00 (reference)
	2nd year only	163	36	22.1	9.12 (2.11-39.45)
	Both years	232	53	22.8	8.91 (2.09-37.99)
Use of inhaled steroids	No	333	51	15.3	1.00 (reference)
	Yes	136	41	30.1	2.07 (1.26-3.40)
Infant eczema	No	326	45	13.8	1.00 (reference)
	Yes	143	47	32.9	3.23 (1.95-5.33)
Infant rhinorrhoea	No	400	76	19.0	1.00 (reference)
	Yes	69	16	23.2	1.16 (0.61-2.19)
Croup-like cough	No	251	41	16.3	1.00 (reference)
	Yes	218	51	23.4	1.39 (0.86-2.25)
Night cough	No	30	10	33.3	1.00 (reference)
	Yes	438	82	18.7	0.42 (0.18-0.95)
Cough during activity	No	125	22	17.6	1.00 (reference)
	Yes	343	70	20.4	0.99 (0.57-1.72)
Acute media otitis	No	194	34	17.5	1.00 (reference)
	Yes	274	51	20.8	1.08 (0.66-1.77)
Recurrent abdominal pain	No	420	76	18.1	1.00 (reference)
	Yes	49	16	32.6	2.26 (1.14-4.47)

*Adjustment made for sex, heredity, dampness or mould at home, tobacco smoke exposure and maternal age.
†Adjustment made for all potential confounders besides sex.
‡Adjustment made for all potential confounders besides heredity.
aOR, adjusted odds ratio; CI, confidence interval.

Table 11 Association between recurrent abdominal pain and factors associated with recurrent abdominal pain during the first two years of life in relation to asthma at age eight years among children with at least three episodes of wheeze during the two first years of life. Results expressed as adjusted odds ratios with 95 % confidence intervals, (N=469)

		Asthma at age 8 years				
					Model 1	*Model 2*
		N	n	%	aOR (95 % CI)*	aOR (95 % CI) †
Recurrent abdominal pain	No	420	76	18.1	1.00 (reference)	1.00 (reference)
	Yes	49	16	32.6	2.26 (1.14-4.47)	2.69 (1.21-5.96)
A doctor's diagnosis of food	No	722	72	17.1	1.00 (reference)	1.00 (reference)
allergy	Yes	47	20	42.5	3.69 (1.88-7.22)	4.08 (1.46-11.38)
Infant eczema	No	326	45	13.8	1.00 (reference)	1.00 (reference)
	Yes	143	47	32.9	3.23 (1.95-5.33)	2.96 (1.67-5.23)
Food hypersensitivity	No	356	61	17.1	1.00 (reference)	1.00 (reference)
	Yes	99	28	28.3	1.70 (0.99-2.94)	0.61 (0.25-1.50)
Symptoms at ingestion of	No	421	78	18.5	1.00 (reference)	1.00 (reference)
cow's milk	Yes	48	14	29.2	1.69 (0.83-3.42)	0.71 (0.24-2.08)
Symptoms at ingestion of	No	456	86	13.6	1.00 (reference)	1.00 (reference)
wheat	Yes	13	6	46.1	2.78 (0.84-9.24)	1.93 (0.42-8.81)
Diarrhoea	No	338	68	20.1	1.00 (reference)	1.00 (reference)
	Yes	131	24	18.3	0.83 (0.48-1.43)	0.57 (0.30-1.07)

* *Model 1*: Adjustment made for sex, heredity, dampness or mould at home, tobacco smoke exposure and maternal age.
†*Model 2*: Besides adjustment as in model a, each multivariate logistic regression model consists of recurrent abdominal pain, doctor's diagnosis of food allergy, infant eczema, food hypersensitivity, symptoms at ingestion of cows' milk and wheat, and diarrhoea.
aOR, adjusted odds ratio; CI, confidence interval.

What about the non-wheezers?

Table 12 displays the assessment of similar risk factors for school age asthma as for the infant wheezers among the 2,428 infants that did not wheeze during the first two years of life. Allergic heredity, infant eczema, croup-like cough, cough during activity, food allergy diagnosis and food hypersensitivity were characteristics and comorbidities that were associated with asthma at age eight years in this group. After additional adjustment for all comorbidities except the one under study, allergic heredity, infant eczema, croup-like cough and food hypersensitivity remained independent risk factors for school age asthma among the children without parental-reported symptoms of wheeze during infancy, (data not shown).

Table 12 Associations between sex, allergic heredity and early comorbidity in relation to asthma at age 8 years among children without infant wheeze, (N=2,428)

		Asthma at age 8 years			
		N	n	%	aOR (95 % CI)*
Total		2428	90	3.7	
Sex†	Girl	1265	42	3.3	1.00 (reference)
	Boy	1163	48	4.1	1.31 (0.85-2.00)
Allergic heredity‡	No	1737	41	2.4	1.00 (reference)
	Yes	676	49	7.2	3.22 (2.10-4.93)
Use of inhaled steroids	No	2408	89	3.7	1.00 (reference)
	Yes	20	1	5.0	1.07 (0.14-8.31)
Infant eczema	No	1856	49	2.6	1.00 (reference)
	Yes	572	41	7.2	2.47 (1.60-3.82)
Infant rhinorrhoea	No	2294	85	3.7	1.00 (reference)
	Yes	134	5	3.7	0.87 (0.34-2.21)
Croup-like cough	No	2098	329	3.2	1.00 (reference)
	Yes	329	23	7.0	2.14 (1.30-3.52)
Night cough	No	748	19	2.5	1.00 (reference)
	Yes	1677	71	4.2	1.55 (0.92-2.60)
Cough during activity	No	1460	42	2.9	1.00 (reference)
	Yes	967	48	5.0	1.64 (1.07-2.52)
Acute media otitis	No	1365	48	3.5	1.00 (reference)
	Yes	1050	41	3.9	1.09 (0.71-1.68)
Recurrent abdominal pain	No	2297	80	3.5	1.00 (reference)
	Yes	127	9	7.1	1.99 (0.96-4.10)
Dr diagnosis of food allergy	No	2881	73	3.2	1.00 (reference)
	Yes	142	16	11.3	3.16 (1.76-5.66)
Food hypersensitivity	No	2057	59	2.9	1.00 (reference)
	Yes	319	29	9.1	3.40 (2.13-5.44)

*Adjustment made for sex, heredity, dampness or mould at home, tobacco smoke exposure and maternal age.
†Adjustment made for all potential confounders besides sex.
‡Adjustment made for all potential confounders besides heredity.
aOR, adjusted odds ratio; CI, confidence interval.

3.1.2 Maternal smoking during pregnancy and preschool asthma (II)

The associations between exposure to maternal tobacco smoking during pregnancy and preschool wheeze and asthma were assessed in a pooled analysis including 21,600 European children from eight birth cohorts. An overview of periods of recruitment, numbers and proportions of included children from each cohort are provided in Table 1, page 19.

The prevalence of wheeze at age four to six years ranged from 6.2% to 14.7% across cohorts. On average, 10.4% of the children had wheezed during the last 12 months in preschool age. The corresponding prevalence range for preschool asthma was 3.4% to 13.7%, with an average proportion of 6.6% for all cohorts combined.

The children were allocated into four disjunctive categories depending on maternal smoke exposure status during pregnancy and in the first year after delivery, Table 13.

Table 13 Prevalence of maternal smoking during pregnancy and during the first year after delivery in eight European birth cohorts comprising 21,600 children included in the pooled analyses

	No smoking (reference)	**Smoking during pregnancy only**	**Smoking during the first year only**	**Smoking during pregnancy and first year**
Birth cohort	n (%)[*]	n (%)[†]	n (%)[‡]	n (%)[§]
ALSPAC	5460 (71.2)	157 (2.1)	407 (5.3)	1,584 (20.8)
AMICS-Menorca	268 (60.8)	28 (6.3)	12 (2.7)	133 (30.2)
BAMSE	3,051 (83.1)	93 (2.5)	153 (4.2)	376 (10.2)
DARC	315 (63.6)	35 (7.1)	17 (3.4)	128 (25.9)
GINIplus	3,159 (83.3)[**]	123 (3.2)[**]	137 (3.6)[**]	375 (9.9)[**]
LISAplus	1,421 (80.7)	106 (6.0)	67 (3.8)	166 (9.4)
MAS	561 (63.6)	18 (2.0)	127 (13.9)	188 (20.6)
PIAMA-NHS	2,291 (78.1)	175 (6.0)	56 (1.9)	413 (14.1)
Total	16,526 (76.5)	735 (3.4)	976 (4.5)	3,363 (15.6)

[*] No maternal smoking during pregnancy or in the first year after delivery.
[†] Maternal smoking of at least one cigarette daily during any time of pregnancy, but no smoking during the first year after delivery.
[‡] No maternal smoking during pregnancy, but maternal smoking during the first year after delivery.
[§] Maternal smoking of at least one cigarette daily during any time of pregnancy and during the first year after delivery.
[**] Information on maternal smoking collected 4 months after delivery.

We found 735 (3.4%) children exposed to maternal tobacco smoke during pregnancy but not during the first year of life, Table 13. This group of *in utero* exposed children had an increased risk of wheeze and asthma in preschool age, with combined adjusted ORs of 1.39 (95% CI 1.08-1.77) for wheeze, and 1.65 (95% CI 1.18-2.31) for asthma, Figure 3. Adjustments were made for sex, parental education, birth weight, parental asthma and older siblings.

Excluding children exposed to tobacco smoke not only by the mother but also by the father or other persons during the first year resulted in similar combined adjusted ORs, 1.68 (95% CI 1.26-2.25) for preschool wheeze and 1.75 (95% CI 1.16-2.63) for asthma. Further restriction to children that were not exposed to maternal tobacco smoke at the time of outcome assessment resulted in combined adjusted ORs of 1.63 (95% CI 1.25-2.12) for wheeze and 1.95 (95% CI 1.34-2.85) for asthma. Similar results were observed when children exposed to tobacco smoke by any persons (i.e. not only to maternal smoking) in the first postnatal year and in preschool age were excluded, data not shown.

There was a significant dose-response effect between maternal smoking during the first trimester and risk of preschool wheeze and asthma. Every five cigarette increase in daily consumption conferred an adjusted OR of 1.18 (95% CI 1.02-1.38) for preschool wheeze and 1.23 (95% CI 1.03-1.47) for asthma. These analyses were restricted to children whose mothers did not smoke during the first postnatal year.

Figure 3A and B Associations between maternal smoking during pregnancy only (no maternal smoking during the first year of life) in relation to preschool wheeze (3A) and asthma (3B). Cohort-specific odds ratios (ORs) and 95% confidence intervals (CIs) were obtained by logistic regression adjusted for sex, parental asthma, parental education, siblings and birth weight. Combined ORs and 95% CIs derived by random effects methods. N=total number of children in each cohort, n=number of exposed cases in each cohort. ALSPAC (UK) lacked information on doctor's diagnosis of asthma and was not included in the analyses of current asthma.

Figure 3A

Maternal smoking during pregnancy (but not in the first year) in relation to preschool wheeze

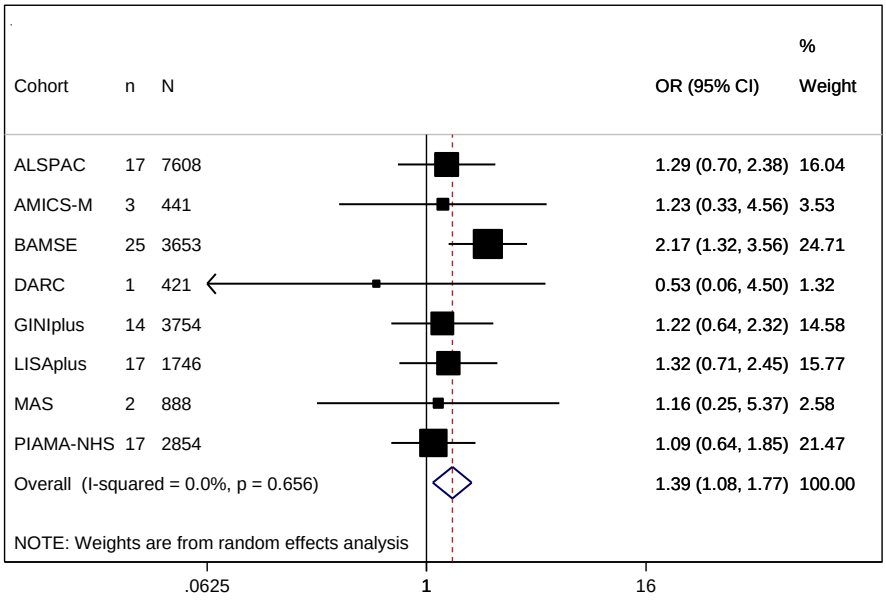
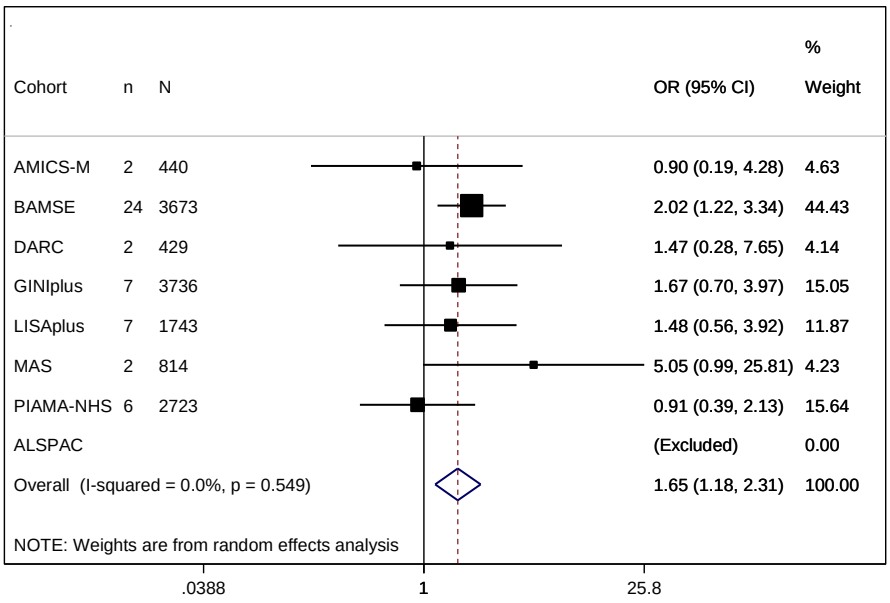


Figure 3B

Maternal smoking during pregnancy (but not in the first year of life) in relation to preschool asthma



The 3,363 children (15.6%) exposed to maternal tobacco smoke during pregnancy as well as in the first year after delivery also had a higher risk of preschool wheeze and asthma, with combined adjusted ORs of 1.25 (95% CI 1.09-1.43) and 1.30 (95% CI 1.00-1.68), respectively.

There was no increased risk for preschool wheeze or asthma for the 976 children (4.5%) exposed to maternal tobacco smoke in the first year after delivery but not during pregnancy, with combined adjusted ORs for preschool wheeze 0.91 (95% CI 0.71-1.17) and 1.20 (95% CI 0.84-1.71) for preschool asthma.

ADDITIONAL RESULTS (II)

Is the dose-response effect a marker of continued smoking throughout pregnancy?

We investigated if the observed dose-response effect based on information on amount of smoked cigarettes from the first trimester was a marker of continued smoking throughout pregnancy by further restricting the dose-response analyses to only comprise children that were not exposed to maternal tobacco smoke during the third trimester as well as during the first year after delivery. These analyses resulted in adjusted ORs of 1.30 (95% CI 0.94-1.79) for preschool wheeze and 1.44 (95% CI 1.14-1.81) for preschool asthma for every five cigarette increment in daily consumption during the first trimester.

Exclusion of birth weight as a confounder

We adjusted for birth weight as a confounder in study II. However, birth weight may be a mediator and in that case we should not have adjusted for it. Exclusion of birth weight as adjustment variable resulted in combined ORs (adjusted for sex, parental asthma, parental education and siblings) of 1.38 (95% CI 1.10-1.74) and 1.62 (95% CI 1.18-2.23) for maternal smoking during pregnancy but not in the first year and preschool wheeze and asthma, respectively. No significant heterogeneity was present between studies.

Potential interaction between pre- and postnatal smoke exposure

Pre- and postnatal smoke exposure may interact in an independent way that influences wheeze or asthma development. The pooled P-value for interaction between maternal smoking during pregnancy and maternal smoking during the first year after delivery was 0.817 for preschool wheeze as outcome. The corresponding pooled P-value for interaction for preschool asthma as outcome was 0.198.

3.1.3 Childhood asthma and recurrent abdominal pain (IV)

The association between allergy-related diseases and recurrent abdominal pain was examined in study IV. At age 12 years, 237 (9.1%) children reported having abdominal pain at least once every month. Of these, 15 (6.6%) fulfilled the definition of asthma at age one year, compared to 82 (3.5%) of the 2,373 children without abdominal pain, p=0.02.

In logistic regression analyses, asthma during the first and second year of life was significantly associated with recurrent abdominal pain at age 12 years, with ORs adjusted for sex of 2.26 (95% CI 1.27-4.04) and 1.81 (95% CI 1.07-3.05), respectively. Asthma at age 12 years was significantly associated with recurrent abdominal pain at the same age, with an OR adjusted for sex of 2.15 (95% CI 1.35-3.43), Figure 4A. However, asthma did not remain an independent risk factor for concurrent abdominal pain at age 12 years after additional adjustment for other coexisting allergy-related diseases, OR 1.66 (95% CI 0.96-2.85), Table 14.

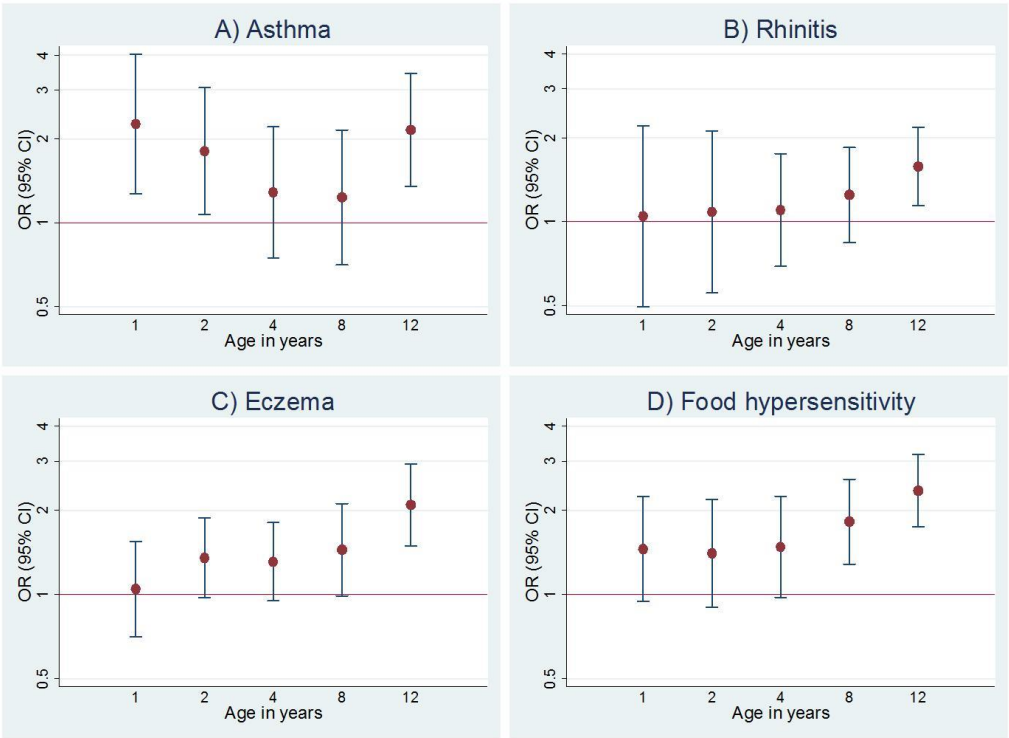


Figure 4 Allergy-related diseases during childhood and recurrent abdominal pain at age 12 years. Age-specific associations between A) asthma, B) allergic rhinitis, C) eczema, and D) food hypersensitivity and recurrent abdominal pain at age 12 years. Results are presented as adjusted odds ratios (OR) with 95% confidence intervals (95% CI), adjusted for sex.

"Total allergy burden" and recurrent abdominal pain

The risk of abdominal pain at age 12 years increased with increasing number of coexisting allergy related symptoms (asthma, allergic rhinitis, eczema and food hypersensitivity), P for trend <0.001. All allergy-related symptoms were significantly associated with concurrent abdominal pain at age 12 years, Figure 4 and Table 14 (Model I). After adjustment not only for sex but for all allergy-related symptoms except the one under study, the significant association remained for eczema, OR 1.86 (95% CI 1.27-2.74) and food hypersensitivity, OR 2.00 (95% CI 1.42-2.79). This also held true for children with a doctor's diagnosis of food allergy combined with reported food hypersensitivity and positive food sensitization at age eight years, OR 2.16 (95% CI 1.02-4.57), Table 14 (Model II).

Table 14 Allergy-related diseases at different ages and risk of recurrent abdominal pain at 12 years

Exposure	OR (95%CI) Model I[1]	OR (95%CI) Model II[2]
Asthma 1yr	2.26 (1.27-4.04)	2.33 (1.29-4.24)
Asthma 2yrs	1.81 (1.07-3.05)	1.83 (1.07-3.13)
Asthma 4yrs	1.29 (0.75-2.21)	1.36 (0.77-2.41)
Asthma 8yrs	1.23 (0.71- 2.15)	0.93 (0.50-1.74)
Asthma 12yrs	2.15 (1.35-3.43)	1.66 (0.96-2.85)
Allergic rhinitis 1yr	1.05 (0.50-2.20)	0.81 (0.37-1.76)
Allergic rhinitis 2yrs	1.08 (0.55-2.12)	0.88 (0.44-1.77)
Allergic rhinitis 4yrs	1.10 (0.69-1.75)	1.10 (0.66-1.84)
Allergic rhinitis 8yrs	1.25 (0.84-1.85)	0.87 (0.54-1.39)
Allergic rhinitis 12yrs	1.58 (1.15-2.17)	0.96 (0.65-1.43)
Eczema 1yr	1.05 (0.71-1.55)	0.94 (0.62-1.42)
Eczema 2yrs	1.35 (0.98-1.88)	1.24 (0.88-1.75)
Eczema 4yrs	1.31 (0.95-1.81)	1.28 (0.89-1.85)
Eczema 8yrs	1.44 (0.99-2.11)	1.32 (0.89-1.96)
Eczema 12yrs	2.09 (1.49-2.92)	1.86 (1.27-2.74)
Food hypersensitivity 1yr	1.45 (0.94-2.24)	1.48 (0.94-2.33)
Food hypersensitivity 2yrs	1.40 (0.90-2.19)	1.27 (0.80-2.03)
Food hypersensitivity 4yrs	1.48 (0.98-2.24)	1.26 (0.80-1.99)
Food hypersensitivity 8yrs	1.82 (1.28-2.58)	1.80 (1.20-2.69)
Food hypersensitivity 12yrs	2.35 (1.74-3.17)	2.00 (1.42-2.79)
IgE food allergy, 4yrs[3]	3.34 (1.64-6.82)	2.19 (0.91-5.26)
IgE food allergy, 8yrs[3]	2.63 (1.50-4.61)	2.16 (1.02-4.57)

[1]Model I: Logistic regression adjusted for sex.
[2] Model II: Logistic regression adjusted for sex and all other allergic diseases than the exposure under study. Exposure and adjustment variables from the same age of the child.
[3]Food hypersensitivity *and* sensitisation to food *and* a doctor's diagnosis of food allergy.

ADDITIONAL RESULTS (IV)

Recurrent abdominal pain in the two first years of life and at age 12 years

Since children reporting recurrent abdominal pain at age 12 years may have had recurrent abdominal pain already in the two first years of life, we compared the prevalence of recurrent abdominal pain at age 12 years among children with and without recurrent abdominal pain in infancy. The prevalence of recurrent abdominal pain at age 12 years was somewhat higher among children who had parental reported abdominal pain in the two first years of life compared to those who did not (14% vs. 8%, p=0.009). However, most (86%) of the children with parental reported recurrent abdominal pain in the two first years of life did not report recurrent abdominal pain at age 12 years.

3.2 CHILDHOOD IgE- REACTIVITY – RISK FACTORS AND CONSEQUENCES

3.2.1 Early second hand tobacco smoke exposure and aeroallergen sensitization (III)

We investigated the association between maternal smoking during pregnancy and infancy and pet, house dust mite and pollen sensitization in the offspring up to age eight years. The analyses were based on 14,613 children from eight European birth cohorts. An overview of periods of recruitment and numbers of included children for each cohort are provided in Table 1, page 19.

In preschool age, 4,204 children from five cohorts were included in the pooled analyses. The prevalence of aeroallergen sensitization ranged from 12.5% to 18.9%. Sensitization to pollen dominated in most cohorts, followed by house dust mite sensitization.

Maternal smoking during pregnancy was not associated with any aeroallergen sensitization, with combined ORs 1.04 (95% CI 0.83-1.31) in preschool age and 1.05 (95% CI 0.85-1.30) in school age. Moderate heterogeneity was present at school age, (I^2=62.2%, p=0.015). We observed no associations between maternal smoking during pregnancy and sensitization in preschool or school age for any of the subgroups pets, house dust mite or pollen (data not shown).

No significant associations were observed between maternal or any SHS exposure in infancy and aeroallergen sensitization in preschool age, with combined adjusted ORs 1.07 (95% CI 0.82-1.40) and 0.90 (95% CI 0.65-1.25), respectively, Figure 5. Moderate heterogeneity was present between studies in the assessment of any SHS exposure (I^2=47.4%, p=0.107).

When aeroallergen subgroups were analysed separately, there was a statistically significant association between any SHS exposure and pet sensitization in preschool age, combined adjusted OR 1.40 (95% CI 1.02-1.90). However, the association was non-significant for maternal SHS exposure, combined adjusted OR 1.38 (95% CI 0.92-2.06). No significant associations were observed for house dust mite or pollen sensitization in preschool age (data not shown).

Figure 5 Associations between maternal (5A) and any (5B) SHS exposure in infancy and any aeroallergen sensitization in preschool age. Individual ORs with 95% CI obtained by logistic regression adjusted for sex, parental allergy and parental education. Overall OR with 95% CI derived by random effects methods. n=total number of exposed cases, N=total number of included children.

Figure 5A

Maternal SHS exposure in infancy and any aeroallergen sensitization in preschool age

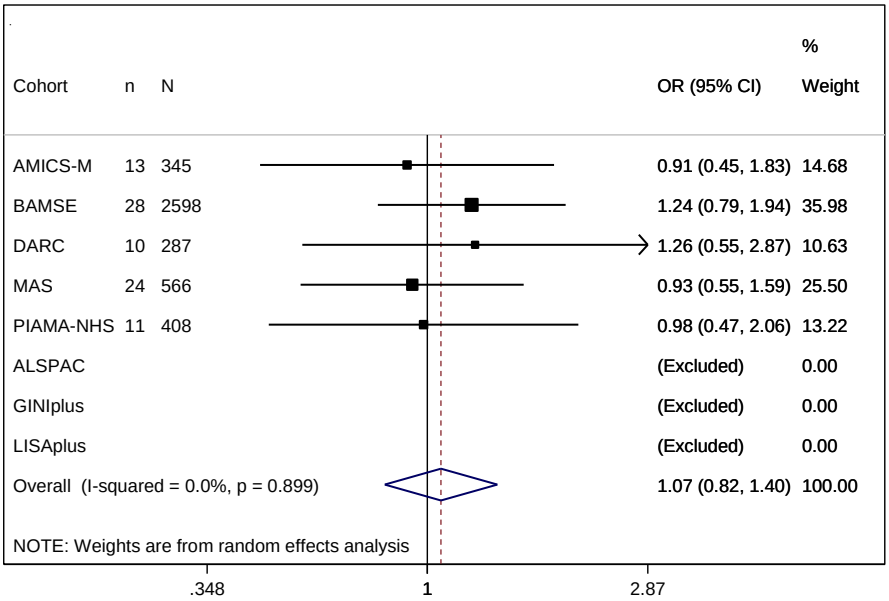
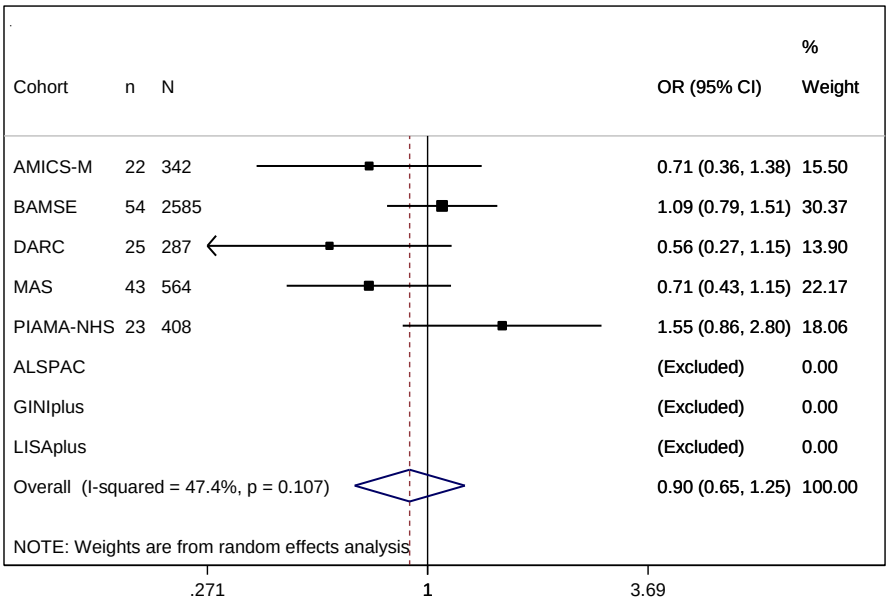


Figure 5B

Any SHS exposure in infancy and any aeroallergen sensitization in preschool age



In school age, 13,279 children from seven cohorts were included in the analyses. The prevalence of school age aeroallergen sensitization was higher compared to in preschool age, with prevalence's ranging from 14.8% to 34.5% across cohorts. Again, pollen was the dominating sensitizing aeroallergen, followed by house dust mite sensitization.

There were no significant associations between maternal or any SHS exposure in infancy and any aeroallergen sensitization in school age, with combined adjusted ORs of 0.99 (95% CI 0.80-1.23) and 1.00 (95% CI 0.85-1.18), respectively. Moderate heterogeneity was present between studies (I^2=55.3%, p=0.037 and I^2=52.1%, p=0.051), Figure 6. Similar non-significant associations were seen when assessing the three aeroallergen subgroups as separate outcomes, data not shown.

Figure 6 Associations between maternal (6A) and any (6B) SHS exposure in infancy and any aeroallergen sensitization in school age. Individual ORs with 95% CI obtained by logistic regression adjusted for sex, parental allergy and parental education. Overall OR with 95% CI derived by random effects methods. n=total number of exposed cases, N=total number of included children.

Figure 6A

Maternal SHS exposure in infancy and any aeroallergen sensitization in school age

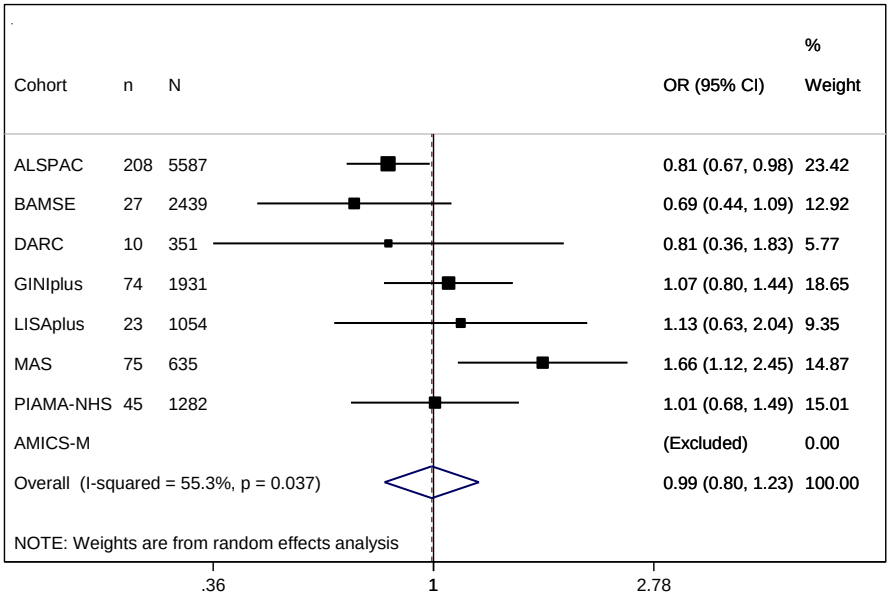
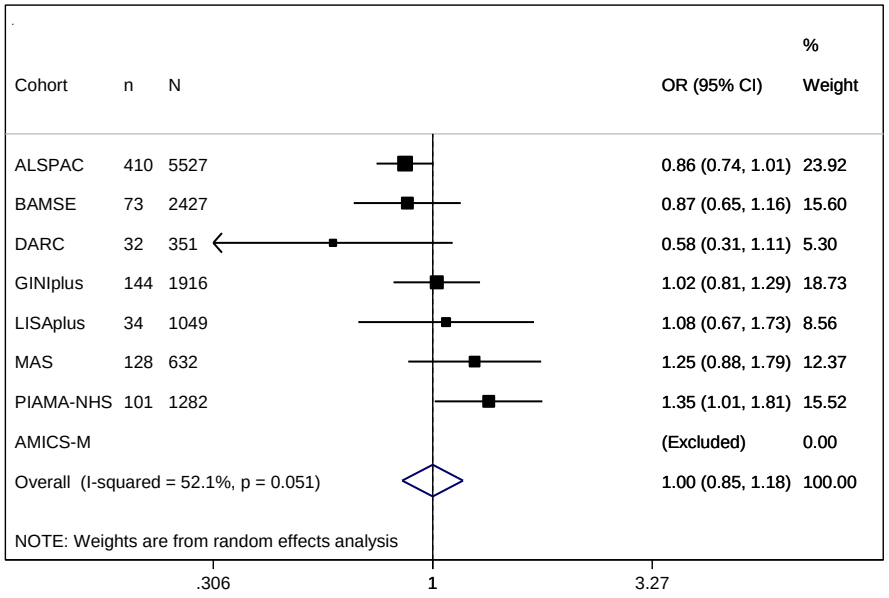


Figure 6B

Maternal SHS exposure in infancy and any aeroallergen sensitization in school age



3.2.2 Recurrent abdominal pain and IgE-reactivity (IV)

Aeroallergen sensitization and recurrent abdominal pain

A subgroup of 2,285 children had available blood samples on IgE-reactivity to common airborne allergens (birch, timothy or mugwort pollen, cat, dog or horse dander, mould or house dust mites) at four or eight years. The proportions of 12-year-olds sensitized to airborne allergens at four and/or eight years were comparable among the 237 children with abdominal pain compared to the 2,373 children without abdominal pain, with proportions sensitized at four years of 18% and 14% (p=0.17), at eight years 26% and 25% (p=0.78) and at both years 32% and 30% (p=0.63), respectively. In logistic regression analyses, sensitization to common aeroallergens at ages four and/or eight years was not associated with abdominal pain at age 12 years, Table 15.

Table 15 Associations between sensitization to common airborne and food allergens at age four and/or eight years and recurrent abdominal pain at age 12 years among the 2,285 children with available blood samples.

Exposure	OR (95%CI) Model I[1]	OR (95%CI) Model II[2]
Airborne sensitization 4yrs	1.46 (0.96-2.21)	1.17 (0.74-1.83)
Airborne sensitization 8yrs	1.16 (0.81-1.66)	1.04 (0.71-1.53)
Airborne sensitization 4 or 8yrs	1.25 (0.89-1.74)	1.04 (0.72-1.49)
Food sensitization 4yrs	1.87 (1.28-2.74)	1.77 (1.17-2.68)
Food sensitization 8yrs	1.37 (0.94-1.98)	1.35 (0.91-2.00)
Food sensitization 4 or 8yrs	1.65 (1.19-2.27)	1.63 (1.15-2.29)

[1]Model I: Logistic regression adjusted for sex.

[2]Model II: In case of positive sensitization we adjusted for sex and the other sensitization. Exposure and adjustment variables from the same age of the child.

Food sensitization and recurrent abdominal pain

At age four and/or eight years, IgE-reactivity to common food allergens (cow's milk, egg white, soy bean, peanut, cod fish and wheat) were tested in 2,285 children. Food sensitization at age four years and at age four or eight years was more common among the 12-year-olds with abdominal pain than among those without abdominal pain, with proportions of 23% and 38% compared to 14% and 27% (p=0.002, p=0.004), respectively. There was a significant association between food allergen sensitization at age four years and abdominal pain at age 12 years after adjustment for sex, OR 1.87 (95% CI 1.28-2.74), Table 15 (model I). The significant association remained after having further adjusted for aeroallergen sensitization, OR 1.77 (95% CI 1.17-2.68), Table 15 (model II). Similar significant associations were seen for food sensitization age four or eight years, with OR 1.65 (95% CI 1.19-2.27) Table 15 (model I), and OR 1.63 (95% 1.15-2.29) Table 15 (model II). After stratification for parental reported food hypersensitivity at the time of positive food sensitization (i.e. at four or eight years), a significantly increased risk of recurrent abdominal pain at 12 years was only observed among the children whose parents had not reported food hypersensitivity, OR 1.67 (95% CI 1.06-2.62) compared to children with parental-reported food hypersensitivity, OR 1.13 (95% CI 0.59-2.17).

4 DISCUSSION

4.1 CHILDHOOD ASTHMA – RISK FACTORS AND CONSEQUENCES

4.1.1 Wheezing in infancy and school age asthma (I)

About 25% of all children wheezed at least once during their two first years of life. These children had an almost fourfold risk of asthma at age eight years compared to non-wheezers. Among the infant wheezers, four independent risk factors were identified for having asthma at age eight years. The risk factors were heredity, increased severity of wheeze, infant eczema and recurrent abdominal pain. Among wheezing infants with three or four of these risk factors, 38% had asthma at age eight years, compared to 7% among wheezers without risk factors.

The observed prevalence of wheeze in infancy is similar to other studies[90, 135, 136].Wheezing in infancy is a known risk factor for asthma development, and the augmented risk observed among wheezing children has been shown previously[32, 91, 137]. Moreover, the independent associations between heredity, increased severity of wheeze, and comorbidity of eczema and school age asthma have been confirmed by others and are well established[31, 87, 138]. However, few prospective studies have reported (or assessed) recurrent abdominal pain as a potential risk factor for childhood asthma and this finding is discussed in the following section.

4.1.2 Recurrent abdominal pain and childhood asthma (I, IV)

In study I, recurrent abdominal pain was reported an independent risk factor for asthma at school age in the group of children that wheezed at least once during the two first years of life, whereas a significant association between asthma in the two first years of life and recurrent abdominal pain at age 12 years was observed in study IV. Asthma at age 12 years was also related to an increased risk of concurrent abdominal pain. However, after adjustments for other allergy-related diseases under study (allergic rhinitis, eczema and food hypersensitivity), asthma did not remain significantly associated with abdominal pain at 12 years.

Both study I and study IV indicate an association between asthma and recurrent abdominal pain. However, the temporal association between the two conditions remains unclear. Questions regarding recurrent abdominal pain were not available in the four and eight year the follow-ups, contributing to the difficulties evaluating temporality. In additional analyses in this thesis, recurrent abdominal pain remained an independent risk factor for school age asthma after restriction of analyses to children that wheezed at least three times in the two first years of life (Table 10 and 11, page 35-36). Since the majority of these children satisfied the definition of asthma at age two years (Table 9, page 34), recurrent abdominal pain in infancy may be a consequence of asthma and not a risk factor for developing asthma as suggested in study I. We also performed additional analyses investigating the relation between parental reported recurrent abdominal pain during the first two years of life and recurrent abdominal pain at age 12 years (page 44). Although there was a significant

association between recurrent abdominal pain in the two first years of life and at age 12 years (p=0.009), the vast majority (86%) of children with parental reported recurrent abdominal pain during the two first years of life did not report recurrent abdominal pain at age 12 years. Thus, this additional information is not useful for assessing temporality of recurrent abdominal pain.

Few previous studies have been able to address the temporal relation between recurrent abdominal pain and asthma especially in paediatric populations, and the existing results are conflicting. Two studies support the conclusion from study I, reporting recurrent abdominal pain to precede symptoms of asthma in children. One was a cohort study where a history of recurrent abdominal pain was significantly associated with wheeze and asthma up to age seven years but not thereafter[113], the other a case-control study where the majority of asthmatics reported symptoms of recurrent abdominal pain having occurred before those of asthma[59]. In contrast, a U.K. study following a national sample of children from age seven to eleven suggested that wheeze may precede abdominal pain[50]. Two retrospective studies reported a significant increase in IBS (irritable bowel syndrome, a specific phenotype of abdominal pain[139]) incidence among people who already had asthma[53, 54], which corresponds with the conclusion of study IV. However, clinical symptoms of atopic diseases including asthma were not sufficient for IBS occurrence in a selected group of children aged three to 13 years with severe allergy[79].

Regardless temporality, the higher than expected prevalence of concurrent abdominal pain observed in 12-year-olds with asthma (study IV) as well as in wheezing infants (study I) compared to asymptomatic children is supported by numerous studies[48, 50, 53-62, 76, 77, 140], whereof five include children. This relation emphasizes the potential role of a common pathogenetic origin or shared susceptibility to common factors.

There are several hypotheses regarding mutual mechanistic origins between recurrent abdominal pain/IBS and asthma. For example, a generalized abnormality of both bronchial and intestinal smooth muscle cells and/or a disturbance of smooth muscle regulation by the autonomic nervous system have been suggested, but results are conflicting[58, 78, 141]. This could be a plausible explanation to the occurrence of wheeze and recurrent abdominal pain and relation to school age asthma in study I. However, no independent association remained between asthma and concurrent abdominal pain in the 12-year-olds after adjustment for other allergy-related diseases in study IV, indicating that there may be other explanations or that this mechanism is not sufficient.

Conversely, it has been suggested that gastrointestinal side effects related to anti-asthma treatment explains the higher prevalence of recurrent abdominal pain/IBS in asthmatics, i.e. the association is entirely due to confounding by medication. However, this hypothesis has not been confirmed[54, 56, 57]. Constipation and GER (gastroesophageal reflux) are common causes of abdominal pain among infants and toddlers[142], but also among older children although to a lesser extent[143]. GER and constipation can be accompanied by wheezing[144, 145] and IgE mediated or cell mediated cow's milk hypersensitivity can trigger these symptoms in

infants[146]. Moreover, the prevalence of GER is higher among asthmatic children compared to non-asthmatics and can provoke attacks of asthma[147], and the relation between GER and asthma is well recognized[148]. Residual confounding by these abdominal pain-related comorbidities cannot be excluded in study I or IV due to lack of information.

Another hypothesis is that these conditions have a common underlying inflammatory process[58], since the respiratory and gastrointestinal tracts have analogous mucosal-associated lymphoid tissue[149]. This inflammation could result in barrier defects in the gastrointestinal tract, increasing the risk of disturbed motility and pain sensitivity[67, 80]. Thus, the relation between wheeze and recurrent abdominal pain in infancy and asthma in study I as well as the significant association between asthma and concurrent abdominal pain at age 12 years could be due to a shared low-grade inflammation in the respiratory and gastrointestinal tract. Furthermore, one study reported a lower risk of IBS onset among asthma patients with per oral corticosteroid medication which has systemic anti-inflammatory effects[53], although this was not confirmed in another study[54].

A relationship between inflammation and gut motility or visceral pain perception has been proposed[70, 74]. Mast cells are central in allergic inflammation and cause hyperreactive airways in allergic asthma. An accumulation of activated mucosal mast cells in the gut have been observed in some IBS patients[70, 74]. Mediators secreted by these mast cells may interact with sensory nerve endings and promote visceral hyperresponsiveness that lead to symptoms of recurrent abdominal pain/IBS[74]. It is therefore plausible that a mutual immunologic dysregulation could be part of the explanation of the association between asthma or wheeze and recurrent abdominal pain in study I and IV. However, before we can extend the "united airways" to the "united mucosa" more mechanistic studies are needed[56].

The question of whether recurrent abdominal pain should be considered a risk factor or a consequence of asthma remains to be answered. However, the finding of an increment in risk of abdominal pain with increasing number of allergy-related diseases in the 12-year-olds in study IV strengthens the hypothesis of a common immune dysregulation, maybe combined with a primary neuromuscular disorder producing both respiratory and gastrointestinal hyperresponsiveness.

In additional analyses, recurrent abdominal pain in infant non-wheezers showed elevated odds ratios for school age asthma although not statistically significant (Table 12, page 37). These results are based on few cases and the hypothesis that symptoms of recurrent abdominal pain and infant wheeze originate from abnormal smooth muscle function in the bronchi and gut cannot be evaluated. Moreover, food hypersensitivity was an independent risk factor in non-wheezers but we cannot exclude that some parents reported this symptom due to recurrent abdominal pain. Croup-like cough and cough during activity were significantly associated with asthma and bronchial hyperresponsiveness may cause cough. Moreover, misclassification of wheezing as croup may explain the association to asthma. The significant association between cough and asthma in non-wheezers also serves as a good

example of the fact that asthma is more than wheeze. It is not unusual that asthma underlies symptoms of prolonged cough or diffuse tiredness in children.

4.1.3 Maternal smoking during pregnancy and preschool asthma (II)

We found an association between maternal tobacco smoking during pregnancy and wheeze and asthma at four to six years of age in the offspring, even among the *in utero* exposed children that were not exposed to maternal smoking in the first year after birth. Moreover, the likelihood of developing wheeze and asthma increased in a dose-dependent manner in relation to the daily amount of consumed cigarettes during the first trimester of pregnancy.

The increased risk for wheeze and asthma in children exposed to maternal tobacco smoke *in utero* has been reported by others[27, 107-109, 111, 116, 150, 151]. In contrast to other studies, our study population was sufficiently large to enable assessment of potential independent associations between prenatal smoke exposure and wheeze or asthma since 735 (3.4% of the study population) children whose mothers smoked only during pregnancy could be identified. Furthermore, trimester-specific smoke exposure data allowed for assessment of the risks of early but not late foetal smoke exposure.

Increased risks of wheeze and asthma were present among the 496 children exposed to maternal smoking during the first, but not during the third trimester, and not in the first postnatal year. However, this early risk was not confirmed in a later study from the Generation R birth cohort, where the influence of maternal smoking during the first trimester only was insufficient whereas maternal smoking throughout pregnancy significantly increased the risk of preschool wheeze[27].

Maternal smoking influences the growth and development of the foetal respiratory system [106, 152]. For example, nicotine and carbon monoxide in cigarettes reduce blood flow and delivery of oxygen and nutrients to the foetus[152], resulting in impaired overall foetal cell growth and development including that of the respiratory system. Lung development commences in the fourth week of pregnancy and by the early second trimester the conductive airways down to the terminal bronchioles have been formed[153]. This branching morphogenesis is a critical part in lung development[153], and may therefore be a period when the foetus is especially susceptible to environmental insults such as tobacco smoke. Thus, the significant association between early foetal smoke exposure and preschool wheeze and asthma in our study may be explained by timing of exposure with a particularly critical period of lung development.

Although thousands of compounds are found in tobacco smoke, nicotine seems to be the most harmful agent with regard to foetal lung development and maturation[101]. This substance passes the placental barrier readily[154]. The developing lung expresses nicotinic acetylcholine receptors sensitive to nicotine. This interaction probably underlies many effects of maternal smoking on lung development by altering the lung structure in ways that influence lung function in the offspring[155] although the exact mechanisms are still unclear.

The observed association between maternal smoking in early pregnancy and wheeze or asthma is reinforced by the significant dose-response relation between daily amount of smoked cigarettes during the first trimester and preschool wheeze and asthma. It can be argued that this relation is explained by continued smoking throughout pregnancy although our assessments were restricted to children unexposed to maternal smoking during the first year. However, more than two thirds of the mothers that smoked during pregnancy but not after delivery succeeded to quit before the third trimester. Furthermore, after exclusion of children exposed to maternal smoking during the third trimester from analyses (additional results, page 41), the significant dose-response effect remained for asthma, but not for wheeze. However, decrements in subgroup sizes increase statistical uncertainty.

Maternal smoking during pregnancy is an established determinant of foetal growth and risk of low birthweight[116]. Birth weight may therefore be considered a mediator of the effect of foetal tobacco smoke exposure for childhood asthma. However, when we explored the potential mediating effect of birth weight in our analyses, we observed comparable odds ratios in models with and without birth weight as an adjustment variable (additional results, page 41). This is supported by previous studies reporting none or only a small fraction of foetal tobacco smoke exposure effects on asthma development to be mediated through foetal growth[27, 116]. In addition, the main influence of tobacco smoke exposure on foetal growth occurs during the third trimester[156, 157]. Thus, the association between tobacco smoke exposure and respiratory morbidity in the offspring is probably mostly independent of the association between tobacco smoke exposure and foetal growth. In hindsight, maybe adjusting for birth weight was unnecessary.

We had no information on paternal or other sources of SHS during pregnancy and therefore potential associations between maternal SHS exposure during pregnancy and wheeze or asthma were not assessed. Although the influence from maternal smoking during pregnancy on wheeze and asthma is probably stronger than that of paternal exposure[27, 109], the lack of paternal prenatal smoking data is a limitation. An association between paternal smoking during pregnancy and childhood wheeze and asthma has been reported[158]. Therefore, we may have underestimated the magnitude of the risk of foetal smoke exposure.

Another potential explanation to our finding of increased risks of early *in utero* smoke exposure on preschool wheeze and asthma may be differences between mothers that quit smoking early compared to late in pregnancy with regards to health, lifestyle or other uncontrolled factors. If these factors are related to the outcome there may be residual confounding. Furthermore, reporting inaccuracies may exist since smoke exposure and outcome data were collected through parental questionnaires. For example, pregnant women might under report smoking although validation studies have concluded that self-reported smoking by pregnant women can be trusted[115, 159, 160]. Non-differential exposure misclassification due to under reporting would attenuate the true association.

Mothers who quit smoking early in pregnancy might become more attentive to respiratory symptoms in their offspring due to the past smoking history, reporting symptoms and perhaps seeking health care more often compared to mothers who continue to smoke. This over

reporting would lead to an overestimation of the relative risk for this particular group. On the other hand, this could adhere to all mothers that succeeded to quit regardless time point of cessation during pregnancy. Contradictory to this theory, a significantly increased risk of preschool wheeze and asthma was seen also among the children whose mothers smoked both before and after delivery. In addition, under reporting of symptoms and underutilization of health care for respiratory symptoms among smoking parents may be even more feasible due to the social stigmatization surrounding smoking, particularly in the context of children and health[161, 162]. This would result in an underestimation of the true association.

The lack of associations between maternal smoking during the first year after birth and preschool wheeze or asthma needs to be addressed. Postnatal tobacco smoke exposure is difficult to assess due to its indirect nature compared to direct maternal foetal exposure. Parents that report active smoking may avoid exposure of the infant/child due to the known health hazards. In addition, the importance of parental smoke exposure decreases with time as growing children spend less time at home, which is the commonest source of childhood SHS exposure[96]. Disease-related modification of exposure, i.e. parents changing their smoking habits when their children show symptoms of respiratory disease ought to be limited due to the prospective study design. However, very early respiratory symptoms may to some extent increase parental avoidance, contributing to an underestimation of the relative risk of SHS on wheeze and asthma.

In contrast to foetal cord blood exposure, the infant is exposed to tobacco smoke via the airways. Critical time windows when the airways of the infants are more vulnerable to SHS as well as duration of SHS exposure may be important factors for respiratory disease development[117]. The induction time in our study may be too short. Other known respiratory symptoms besides wheeze such as frequent cough, phlegm and lower respiratory tract infections that can be triggered by SHS may influence lung function in a longer perspective. What is certain is that infants are vulnerable to SHS since lung structures and the immune system continue to develop during the first year after birth and the defence mechanisms are still relatively weak[96]. Furthermore, results from a recent systematic review and meta-analysis show consistent evidence of a modest association between SHS and childhood asthma[163].

SHS exposure during infancy is difficult to investigate separately from prenatal exposure since these exposures are closely related. Maybe the mothers in our analyses who started smoking in the first postnatal year differed in some important way that is related to the outcome? One might speculate that they had less asthma or IgE-reactivity, and as a consequence these strong predisposing factors for asthma may not have been present in their children.

Since pre-and postnatal smoke exposure are closely correlated and affect the incidence of wheeze and asthma in the offspring, analysing potential interaction between these two exposures may be of value. The additional analyses in this thesis showed no signs of significant interaction (additional results, page 41).

4.2 CHILDHOOD IgE-REACTIVITY – RISK FACTORS AND CONSEQUENCES

4.2.1 Recurrent abdominal pain and IgE-reactivity (IV)

There was an increased risk of abdominal pain at 12 years in children sensitized to food allergens at four or eight years. This association remained significant after adjustment for sensitization to airborne allergens. Stratified analyses revealed that the augmented risk at 12 years was confined to children whose parents had not reported food hypersensitivity in their children at the time of testing positive to food allergens. Sensitization to airborne allergens at four or eight years was not associated with abdominal pain at 12 years.

Although 20 to 65% of patients with recurrent abdominal pain/IBS self-report induction or worsening of gastrointestinal symptoms after ingestion of specific foods[65, 75, 164], results regarding an actual impact of food hypersensitivity on abdominal pain/IBS are contradictory[64, 75, 80, 81, 164-167]. However, some studies among adults have suggested a causative role of IgE mediated food hypersensitivity in a subgroup of IBS patients that often have other signs of atopy[80, 166, 167]. Research regarding food sensitization as a potential risk factor for recurrent abdominal pain in children is lacking, but a retrospective study has suggested that cow's milk allergy constitutes a risk factor for the development of functional gastrointestinal disorders in children[66], and a relation between food hypersensitivity and intestinal dysmotility has been demonstrated in paediatric populations[145, 168, 169].

The finding of a significant association between unrecognized food hypersensitivity in children sensitized to common foods and recurrent abdominal pain later in childhood is intriguing. It can be speculated that these children continued to ingest foods that they were sensitized to, and that this chronic allergen exposure may have contributed to the development of recurrent abdominal pain. This corresponds to the previously postulated hypothesis that IgE mediated food hypersensitivity plays a causative role for IBS in a subgroup of vulnerable subjects[62, 65, 80, 167]. It would have been valuable to have data on sensitization to foods among the 12-year-olds. Due to lack of this information, we do not know to which extent food sensitization changed over the years and if this change differed between the children with and without known food hypersensitivity as well as with or without recurrent abdominal pain at age 12 years.

Another limitation is that the parental-reported food hypersensitivity may not have corresponded to the tested food items at age four and eight years, thus we cannot be sure that these children actually avoided the foods they were sensitized to. In addition, sensitization does not equal clinical allergy and although the cut-off of $\geq$0.35 kU_A/l is commonly used when assessing sensitization, a positive as well as negative test result does not equal presence and absence of clinical IgE mediated allergy, i.e. symptoms may occur below the threshold value and clinical tolerance may be present although still testing positive. In post hoc analyses, a variable of food allergy was created combining reported food hypersensitivity and concomitant positive food sensitization with a doctor's diagnosis of food allergy between one and 12 years. Children with food allergy at age four or eight years had significantly more

abdominal pain at age 12 years. It is therefore reasonable to believe that an association between IgE mediated food hypersensitivity, food sensitization and food hypersensitivity and recurrent abdominal pain exists in children. However, the question regarding causality remains unanswered since we cannot rule out that recurrent abdominal pain existed before the age of 12 years.

The association between positive sensitization/food hypersensitivity and recurrent abdominal pain is biologically plausible. Mediators of activated mast cells are known key players in allergic inflammation. Mast cells are found in increased numbers through the gut mucosa of IBS patients[62, 74], and mediators such as tryptase contribute to visceral hyperalgesia[62, 73]. The severity of pain perception in IBS patients has been directly correlated to the close proximity between nerve fibres and mast cells[74]. Mast cells also interact with epithelial cells, enhancing permeability of the epithelial barrier as well as mucosal inflammation[62, 70]. Mucosal mast cells can be activated by a number of stimuli including allergens and can thereby be linked to both allergy and IBS[62]. Tobin et al observed that patients with an allergic background had more severe gastrointestinal symptoms, a higher number of mucosal mast cells and high intestinal tryptase release[62]. In conclusion, mast cell activation can be involved in both allergy and IBS, with additive or synergistic effects on symptoms of abdominal pain [62].

On the other hand, the barrier defects seen in IBS patients may enhance entry of allergens through the mucosa and promote sensitization with subsequent occurrence of adverse reactions and not the other way around. In an intact mucosal barrier only small quantities of antigen or pathogens cross beyond the epithelium[165]. This could for example be one explanation to the high incidence of food allergy in young children whose mucosal barrier is immature. Whether allergy or sensitization is a risk factor or consequence of barrier defects and/or inflammation in IBS thus remains to be determined.

Airborne allergens are probably also partly ingested with the possibility to bind and activate mast cells in the gut[170]. We saw no association between airborne sensitization and recurrent abdominal pain, and adjustment for airborne sensitization did not influence the association between food sensitization and recurrent abdominal pain. The lack of association in our study indicates that in children aged eight to 12 years, pollen allergen cross-reactivity with food may not be an important trigger of abdominal pain. However, the incidence of sensitization to airborne allergens and clinical symptoms of cross-reactive allergy such as the oral allergy syndrome has not reached its peak at this age and may be a more important factor later in life although an association was not confirmed in an adult study[167].

One potential methodological weakness in our study may be the use of serum IgE antibody data only. Serum IgE antibodies or SPT may not be optimal methods for assessing food sensitization in subjects with food hypersensitivity dominated by gastrointestinal symptoms. It has been suggested that provocation tests directly on the colon mucosa may be a more sensitive method[171].

4.2.2 Tobacco smoke exposure in infancy and IgE-reactivity (III)

There were no apparent associations between tobacco smoke exposure during pregnancy or infancy and aeroallergen sensitization later in childhood, except between SHS exposure in infancy and pet sensitization in preschool age, where a tendency towards an increased risk including a significant dose-response effect was observed.

Our results showing no associations between early SHS exposure and aeroallergen sensitization during childhood corresponds with previous studies that assessed aeroallergen sensitization separately from food allergens[127, 129, 130, 172]. However, when aeroallergen subcategories were analysed, there was a significant association between any SHS exposure in infancy and pet sensitization in preschool age including a significant-dose response effect. There are plausible biological explanations to this association. Pet allergens reach the airway mucosa with ease due to their more volatile nature compared to house dust mite allergens. Two potential mechanisms of how SHS could promote aeroallergen sensitization have been suggested. SHS may interact directly with the allergen and promote the allergenic capacity[122, 123, 173, 174]. SHS also have direct irritant effects on the airway mucosa, causing inflammation[120] that may facilitate the penetration of aeroallergens and increase the risk of sensitization. Hypothetically, air pollution from other sources than SHS could augment the risk of aeroallergen sensitization by similar irritant effects. However, this was not confirmed in a recent pooled analysis assessing the association between traffic-related air pollution and sensitization where several of the cohorts from this study were included[175].

A recent meta-analysis of published studies concluded that a moderately increased risk of food or aeroallergen sensitization was present among children exposed to household tobacco smoking, and that this risk was confined foremost to preschoolers. The associations were stronger in cohort studies compared to case-control studies[28]. However, we found no previous studies supporting the finding of an association between SHS in infancy and preschool pet sensitization. Lannerö et al. have previously observed this association in the BAMSE cohort[29] but this agreement of results may be explained by the substantial contribution to the pooled estimate by BAMSE in our analyses. Another possible explanation that deserves mentioning is the problem that arises with multiple comparisons. Since many statistical comparisons were made in this study, the risk of falsely rejecting a true null hypothesis may be an explanation to this isolated finding.

Some studies have reported interactions between SHS exposure and inherited predisposition for allergy, but the observations are conflicting. Augmented risks have been found in children with[110, 127] as well as without[128] an allergic predisposition. We found no evidence of effect modification by parental allergy on SHS exposure. The discrepant results may have several explanations. For example, differences in avoidance behaviour may exist between the allergic and non-allergic parents and symptoms of atopy such as infantile eczema may be recognized earlier by allergic smoking parents, possibly leading to a disease-related modification of exposure in this group. In addition, the definitions of exposures and allergic heredity are heterogeneous with varying numbers and types of parental allergies included in the

definitions of allergic heredity across studies. Moreover, immune responses to SHS may differ with regard to sensitization status. Murine models have shown immunosuppressive effects in already sensitized mice[121, 123, 176] whereas primary sensitization has been demonstrated in mice without prior sensitization[122, 173, 174].

In contrast to study II, we were not able to study independent associations of prenatal and postnatal tobacco smoke exposure due to lack of sufficient statistical power in study III. Adjustments for pre- and postnatal tobacco smoke exposures in the respective regression models did not reveal any independent associations. Nevertheless, the impact of tobacco smoke on the foetal immune system may differ from that of postnatal exposure due to differences in immune maturation as well as routes of exposure.

4.3 STRENGTHS AND LIMITATIONS

The major strength of this thesis is that all studies were based on data from birth cohorts with prospective designs where exposures were assessed before disease onset. The exposure and disease assessments were detailed and follow-ups were conducted at several occasions. The cohorts were based on population-based samples, rendering results that apply to the general paediatric population.

Study I and IV were based on data from the BAMSE birth cohort. This cohort contains a comparatively large unselected group of children enabling analyses of subgroups and allowing for stratification. The high initial participation rate and limited loss to follow-up reduce selection bias and increase generalizability of results.

Study II and III comprised of data from eight European birth cohorts from the ENRIECO project. All cohorts were initially designed to study the development of asthma and allergies in childhood and individual participant data were harmonized before the analysis stage which reduces between-study heterogeneity. The pooling process created a large study population with high statistical power and allowed for assessment of associations between tobacco smoke exposure and asthma or IgE-reactivity in various populations. Furthermore, the large study size in study II enabled us to study separate risks of pre- and postnatal smoke exposure on the development of wheeze and asthma, and also to perform sensitivity analyses by restriction and exclusion of groups with characteristics that may have confounded associations.

One weakness is that all exposures and outcomes were based on questionnaire data except for IgE-reactivity. Other potential weaknesses that have to be considered in observational studies are discussed in the following section.

4.4 METHODOLOGICAL CONSIDERATIONS

Epidemiological studies may be afflicted by errors that affect precision, validity and generalizability of the results. The two main categories consist of random error and systematic error[177].

4.4.1 Random error

Random errors are the result of unexplained variability in the data and occur in all observational studies with finite study populations. This variability is reflected by the width of the confidence intervals and can be reduced foremost by increasing the size of the study population. Confidence intervals were computed for all associations in the studies of this thesis in order to provide accurate information about the precision of the investigations.

Study I and IV consisted of 3,251 and 2,610 children and the confidence intervals in the main analyses were rather narrow, reflecting good precision. The main focus in study I was on disease development in the subgroup of 823 infant wheezers and although the initial

population sample was large, some subgroup sizes became small rendering statistical uncertainty. In recent years, research collaborations such as ENRIECO have become frequent, and the very large study populations render markedly increased precision. Study II and III included individual participant data from eight cohorts and consisted of 21,600 and 14,613 children, respectively.

4.4.2 Systematic error

Systematic errors (or bias), do not depend on study size or chance. These errors may occur at all stages in the research process, resulting in reduced validity of the observed results. The most common types of bias are addressed here.

4.4.2.1 Selection bias

Selection bias stems from the procedures used to select subjects and from factors that influence study participation[177]. It occurs when nonparticipants and/or subjects lost to follow-up differ from those remaining in the study in ways that affect the association between the studied exposure and disease. Selection bias at recruitment influences the extent to which the findings can be generalized to populations outside the study population.

The BAMSE cohort included 75% of all eligible children. The associations between exposure and disease are usually unknown among the nonparticipants and the presence of selection bias must be inferred. In the BAMSE cohort a survey was sent out to the excluded and the non-responders and the answers revealed no significant differences regarding characteristics such as allergic heredity or pet ownership. However, parental tobacco smoking was more prevalent in this group[131].

In ENRIECO, the proportion of recruited children ranged from around 55% to 95% of the target populations and some cohorts have provided information on the nonparticipants. For example, ALSPAC observed an underrepresentation of characteristics associated with lower socioeconomic status among the included children[178]. Nonparticipants were similar with regard to maternal age, birth weight and gestational age in DARC[179]. GINIplus reported no differences regarding socio-economy or allergic heredity between nonparticipants/very early drop-outs and the study population[180].

Loss to follow-up is another source of selection bias. The response rates have remained comparatively high in the BAMSE cohort throughout the years, with 82% of the original cohort still participating at 12 years of age and selection regarding important characteristics has remained low[47]. The children that provided blood samples do not differ significantly from the original BAMSE cohort regarding important background characteristics such as sex and parental allergy.

Response rates in the other ENRIECO cohorts at age six to eight years range from 49% (ALSPAC) to 95% (AMICS-Menorca) of the original study populations, with mean response

rate around 86%. Overall, no selection of greater importance has been reported although non-smoking parents with a higher education remain in the studies to a greater extent.

The inclusion criteria in the separate studies may also introduce selection bias due to differences between included and excluded children with regard to background characteristics or symptoms. In study I and IV there were no significant differences except for a slightly higher socioeconomic status among the included children. Furthermore, parents to children included in study II and III had a higher educational level and smoked less. The children included in study III were breast fed longer and their parents were more allergic. If these biases are related to the outcomes in any of the studies in this thesis they may have attenuated the observed differences.

4.4.2.2 Information bias

The collected information about or from study subjects may be erroneous, resulting in misclassification of exposure or disease. This misclassification can be differential or non-differential, referring to the mechanism of misclassification. Misclassification of exposure is non-differential if unrelated to the occurrence or presence of disease and differential if the exposure is different for those with and without disease. In the same way, misclassification of disease is non-differential if unrelated to exposure, otherwise it is differential[177]. All information in the studies regarding exposures and allergy-related symptoms was based on parental questionnaires, except for the questions regarding recurrent abdominal pain that were answered by the 12-year-olds themselves in study IV.

Misclassification of exposure is probably non-differential in most cases due to the prospective study designs, although later exposures may be have been modified by early disease onset. For all allergy-related diseases there is probably some non-differential misclassification due to the delicate balance between sensitivity and specificity, i.e. missing cases with too strict outcome definitions or classifying non-cases as allergic with more inclusive definitions.

The wheeze definition was in accordance with the ISAAC (International study of asthma and allergies in childhood) study criteria[8] in study I. The definitions of wheeze varied somewhat across cohorts included in study II, but all contained similar descriptions of the characteristic respiratory symptoms known as wheeze occurring in the last 12 months.

Asthma is difficult to measure in epidemiological studies because there are no definitive diagnostic criteria or universal gold standard[181]. Furthermore, it is a dynamic disease with alternating periods of exacerbations and quiescence of symptoms. In study I and IV we used a definition combining wheeze symptoms and asthma medication in the last 12 months since we wanted to predict chronic disease with clinical relevance. Asthma medication can be considered a proxy for a physician-diagnosis of asthma (which is sometimes considered the gold standard) since it is issued if the child has been diagnosed with asthma after evaluation by a physician. Combining medication with current symptoms ensures that the child is currently suffering from the disease and has not outgrown symptoms, which is common

before school age. In study II, two of three criteria had to be fulfilled out of the following; current wheezing symptoms, current asthma medication or a doctor's diagnosis of asthma ever. This definition has the same advantage as the one used in study I and IV, considering the fact that asthma is a dynamic disease by capturing current cases.

The definition of eczema used in study I and IV has been validated in the BAMSE children up to age two years and the results showed a 92% sensitivity and 100% specificity compared to a diagnosis of eczema after clinical examination by a dermatologist[182]. A similar definition was used at later ages and although not validated, the characteristic symptoms and localization of eczematous lesions should be easier to recognize in older children.

Allergic rhinitis in study IV was defined based on symptoms from eyes and/or nose at pollen or pet exposure with or without a doctor's diagnosis of allergic rhinitis. A Finnish validation study concluded that diagnosis-based questions regarding allergy were suitable for risk-factor studies due to good specificity and high positive predictive value, and symptom-based questions were adequate when screening for disease because they were highest in sensitivity[183]. The combination of symptoms and physician diagnosis should therefore have good sensitivity and specificity. Since symptoms at allergen contact was required the risk of falsely classifying symptoms of common colds as allergic rhinitis decreased.

The definitions of asthma, eczema and allergic rhinitis have been used in previous peer-reviewed studies[47, 184, 185].

Food hypersensitivity was assessed in study I and IV. The gold standard for verifying suspected food hypersensitivity is by food challenges. This has not been done in all BAMSE children with suspected food reactions since it is time consuming and costly. The prevalence of subjective food hypersensitivity in young children has been reported to be up to 35% of which only a few percent are verified in food challenges[186] and the majority of food sensitized children do not have any corresponding symptoms[187]. Moreover, to ask parents and children to perform food challenges could cause anxiety and jeopardize future participation in the study. In post hoc analyses in study IV, a variable for food allergy was created where suspected symptoms of food hypersensitivity, a physician diagnosis and positive food sensitization were combined. This variable probably had a higher sensitivity compared to the food hypersensitivity definition with regard to IgE mediated food allergy.

Recurrent abdominal pain in study I was defined solely as presence or absence and this definition has not been validated. Knowledge about intensity, frequency and symptoms associated to abdominal pain in infants and toddlers such as GER or constipation would have been of benefit. The smaller the infant the more difficult it is for parents to disentangle symptoms from the gut from other reasons of discomfort. It cannot be excluded that other factors associated with school-age asthma such as early wheeze-like respiratory morbidity may have confounded the association.

In contrast to the other outcomes that were based on parental reports, recurrent abdominal pain in 12-year-olds had the advantage of being self-reported. The definition was not entirely in accordance with the paediatric ROME III[139] or Apley criteria[188], but similar prevalence's of abdominal pain observed in study IV have been obtained in studies using the established definitions. The BAMSE cohort involves researchers from various research areas with different interests. What to include in each follow-up must be considered carefully. Lengthy questionnaires or embarrassing questions for 12-year-olds to answer (for example on stool characteristics) may reduce response rates. Questions regarding gastrointestinal symptoms were left out from the shortened questionnaire that was used in telephone interviews or mailed to families that had not answered the web based questionnaire.

Misclassification may have occurred regarding recurrent abdominal pain in study I since some parents may be more attentive to adverse symptoms in their children, over reporting wheeze as well as recurrent abdominal pain in their infants. This would make the relation between these symptoms appear stronger than it is. However, over reporting of symptoms would probably be unrelated to the outcome, having no influence on the estimated association to school age asthma unless these parents share some other characteristic that is associated with the outcome. One such factor could be parental allergy, but we did adjust for this. In the same way, children with allergic diseases may report more symptoms in general, resulting in stronger associations between recurrent abdominal pain and asthma or other allergy-related diseases at age 12 years in study IV.

The tests used for detecting IgE-reactivity to aeroallergens (study III, IV) and foods (study I, IV) have shown good correlation to SPT and IgE antibodies measured by the CAP-RAST test[189]. Although the cut-off value of 0.35 kU_A/l is widely used, positive and negative values do not equal presence or absence of clinical symptoms. However, the aim in study III was to assess the modulating effect of SHS on foetal and infant immune systems and in study IV to investigate if immune dysregulation causing allergy/sensitization also has a role in the development of recurrent abdominal pain and in this respect the cut-off can be considered purposeful. Moreover, it enables comparison of results with previous existing studies.

Information on parental tobacco smoking, the main exposure in study II and III, was based on parental self-reports and the lack of objective measures is a limitation. Under reporting of smoking may be present do to the social stigma associated with smoking as well as parental awareness of potential health hazards related to *in utero* and postnatal smoke exposure. After delivery, avoidance behaviour may significantly reduce direct exposure of the child although parents report active smoking. Moreover, parents may not smoke the same amount every day and the avoidance patterns may vary at different time points. This misclassification of exposure would be non-differential. However, if smoking parents modify the exposure due to very early symptoms in the child or if these parents report smoke exposure differently than parents of healthy children, the magnitude of the association will appear weaker than it is.

Although parental smoking at home is the main source of SHS exposure in children, significant exposure to SHS in other environments may be present and we have probably underestimated the true prevalence of smoke exposure. This also applies to the reference group. Although this group consisted of mothers or fathers reporting not to smoke, the concentration of metabolites of nicotine in urine and hair in children from smoke-free homes indicate that they also are exposed to SHS to some extent and therefore are not truly unexposed[112].

4.4.2.3 Confounding

Confounding occurs when other factors than the studied affect the relation between exposure and outcome, making associations seem stronger or weaker than they are. Confounding can be controlled for if accurate information is available about these factors. However, influence of unknown or unmeasured factors (residual confounding) can never be entirely excluded. Multivariate regression models were used in all studies which enabled for adjustment for several confounders.

We lacked information on certain important factors related to abdominal pain in infancy thus residual confounding may exist in study I. The same concerns the relation between allergy-related diseases and recurrent abdominal pain in study IV. Some factors differ between infants (study I) and 12-year-olds (study IV) due to differences in maturity and age dependent comorbidities to abdominal pain. For example, infantile colic has been identified as an early life factor for frequent wheezing and asthma[190]. Infantile colic is often perceived as attacks of abdominal pain by parents. However, since this condition remits around age three months and infants below six months were not included in the definition of recurrent abdominal pain, confounding by infantile colic is less probable in study I. Furthermore, we had no information on common abdominal pain-related comorbidities such as constipation and GER in study I and IV, thus residual confounding may exist.

Food hypersensitivity is another potential confounder to recurrent abdominal pain in study I. Food hypersensitivity is common in infancy, with the majority remitting before the age of three years[44]. Food hypersensitivity, particularly symptoms at ingestion of cow's milk and wheat were related to recurrent abdominal pain in study I. However, food hypersensitivity did not completely explain the association between recurrent abdominal pain in the wheezers and school age asthma. Food hypersensitivity at age eight and 12 years and food allergy at four or eight years were significantly associated with recurrent abdominal pain at age 12 years in study IV. This could imply that recurrent abdominal pain in the infant wheezers also can be due to food allergy. Unfortunately, data on IgE antibodies to common food allergens were not available from the first two years of life making this impossible to evaluate. Recurrent abdominal pain in infancy was not significantly associated with elevated IgE levels to food allergens at age four years ($p=0.89$). However, IgE mediated food hypersensitivity is more often accompanied by wheeze in contrast to cell mediated hypersensitivity and lack of IgE data in infancy is a limitation in study I[44].

We did not adjust for heredity of recurrent abdominal pain although IBS has a strong familial trend, with the association being the strongest if one parent has IBS[191]. Although heredity contributes to IBS, social learning has an equal or greater influence[191] and this factor is difficult to account for in epidemiological studies.

In study IV many potential confounders were tested but only sex led to a more than 10% change in OR. Adjustments were made for all allergy-related diseases besides the one under study, taking into account the frequent coexistence of allergic symptoms.

Mental stress is a potential confounder in study IV. Children with multiple allergy-like diseases may experience stress due to their physical health status and this stress may contribute more than immune dysregulation to recurrent abdominal pain. The observed "dose-response" pattern of allergy burden on recurrent abdominal pain could in this case be explained by an increment in stress with increasing allergy burden. It can also be speculated that parents to wheezing infants experience more stress and that these emotions cause discomfort in the infant that is interpreted as recurrent abdominal pain in study I.

Tobacco smoke exposure, an established risk factor for infant wheeze and asthma[114], was associated with recurrent abdominal pain ($p=0.018$) in study I but not in study IV. However, the significant association remained between recurrent abdominal pain in wheezers and school age asthma after adjustment for maternal tobacco smoke exposure in study I.

In study I and II with childhood asthma as outcome, adjustments were made for known or suspected risk factors for respiratory allergy and factors that influenced the estimates. Study II and III included eight cohorts that all provided information derived from different questionnaires. All cohorts had collected similar information on the most important known confounders of asthma and atopy. However, heterogeneity across cohorts was quite large regarding questions concerning breast feeding. This may mirror the existing differences in policies regarding economic support and duration of parental leaves between countries. For example, Swedish children were exclusively breast fed longer which may be due to generous economic support and comparatively long parental leaves.

An important source of bias when evaluating the associations between tobacco smoke exposure and different health outcomes is socioeconomic status. This can be defined in many ways for example as parental occupation, mother's age at delivery, household income, residential characteristics etc. Although educational systems vary between countries they correlate well to years of schooling. Therefore the numbers of educational years were harmonized across studies into three categories referring to high, middle or low parental educational level, counting the parent with the highest educational level as representative for the socioeconomic status of the family in study II and III.

4.4.3 Generalizability

Generalizability can be viewed as the extent to which results from epidemiologic studies can be inferred to populations outside the one actually studied[177]. It can be assumed that the observed associations of this thesis are generalizable to European (study II and III) and Swedish (study I and IV) children from urban and suburban environments. Due to some degree of selection regarding socio-economy and parental smoking, the role of SHS exposure in the general paediatric population may be underestimated. The proportion of included children with and without allergic predisposition was determined in the study design in MAS and for a subgroup of participants in PIAMA-NHS, and the observed prevalence of parental allergy may not reflect that of the general paediatric population in the respective catchment areas[192, 193].

4.5 CLINICAL IMPLICATIONS AND FUTURE PERSPECTIVES

The results from study I consolidate previous knowledge regarding early factors associated with childhood asthma development, namely infant wheeze, increased severity of wheeze symptoms, concomitant eczema and allergic heredity. A risk factor that has not been reported extensively in this context is recurrent abdominal pain. Although residual confounding may have been present in the study, recurrent abdominal pain as perceived by the parents in their wheezing infants was associated with asthma at school age. Asking about recurrent abdominal pain is easy in the clinical setting and may be of prognostic value, although further studies are needed to confirm the association. Future research from prospective studies may reveal whether early gastrointestinal symptoms such as abdominal pain in wheezing infants reflect a lifelong tendency for abnormal function at both the gastrointestinal and bronchial mucosal surfaces or not.

An association between allergy-related symptoms and recurrent abdominal pain at age 12 years was observed in study IV, especially with regard to eczema and food allergy/hypersensitivity. Based on these results, clinicians should have a low threshold for considering potential allergy as contributing to symptoms of recurrent abdominal pain in their paediatric patients. Therapeutic options may differ between atopic and non-atopic children with abdominal pain. However, which these might be remains to be determined.

The observed association between allergy-related diseases and recurrent abdominal pain in study I and IV further reinforces the need of future prospective studies focusing on asthma and allergies to include questions on gastrointestinal symptoms and use established definitions of paediatric abdominal pain. Gathering of information regarding confounding factors and detailed follow-ups for evaluation of disease temporality and potential causal effects is merited.

Due to the intriguing finding of an association between food sensitization in four or eight year old children without reported food hypersensitivity and recurrent abdominal pain at age 12 years, a future research implement in BAMSE would be to investigate if these children differ regarding food sensitization, food hypersensitivity and recurrent abdominal pain at 16 years of age compared to the food sensitized children with known food hypersensitivity at age four or eight years. This is possible due to available data on sensitization and abdominal pain in the 16-year follow-up. The results may or may not strengthen the hypothesis that chronic mucosal inflammation can cause symptoms of both allergy and recurrent abdominal pain and that undetected food sensitization may precede symptoms of abdominal pain. More mechanistic studies of the gastrointestinal tract of allergic versus non-allergic subjects are also needed in order to increase knowledge on how specific immune reactions related to allergy may involve the gut and in particular in which ways the intestinal mucosa is affected.

A significant association was seen between prenatal exposure and preschool wheeze or asthma in study II, even in children whose mothers quit smoking early in pregnancy. This information can be used by health care professionals in order to motivate women not to start smoking or to quit before conceiving to prevent asthma in their children.

Although an independent association between early prenatal smoke exposure and development of preschool wheeze and asthma was observed in study II, no clear associations were seen between pre- or postnatal tobacco smoke exposure and aeroallergen sensitization in study III. It would be interesting to investigate if *in utero* smoke exposure foremost increases the risk of a particular phenotype of asthma. The non-atopic asthma phenotype usually has a better prognosis compared to atopic asthma. Further assessments of the same outcomes beyond preschool age could provide valuable information on long-term prognosis.

5 CONCLUSIONS

Based on the results from the studies, *in utero* tobacco smoke exposure, allergic heredity and comorbidity as well as increased severity of symptoms in infant wheezers are associated with an increased risk of childhood asthma. Furthermore, allergy-related diseases and food sensitization are associated with recurrent abdominal pain at age 12 years. On the contrary, no convincing associations exist between early life tobacco smoke exposure and aeroallergen IgE-reactivity or between aeroallergen IgE-reactivity and abdominal pain at age 12 years.

The separate studies in this thesis suggest the following conclusions:

- The risk of having asthma at age eight years is almost fourfold among the group of children that wheezed at least once during the two first years of life compared to the non-wheezers. Risk factors independently associated with school age asthma in these infant wheezers were: increased severity of wheeze, infant eczema, recurrent abdominal pain and allergic heredity. Recurrent abdominal pain as a risk factor has been sparsely reported before and residual confounding may be present.

- Maternal tobacco smoking during pregnancy is associated with an increased risk of wheeze or asthma in the offspring at preschool age. An augmented risk is present even among children that are not exposed late in pregnancy or after birth. This information is important when motivating young women to stop smoking before getting pregnant to prevent asthma in their children.

- There are no apparent associations between maternal tobacco smoking during pregnancy or SHS exposure in infancy and sensitization to pets, house dust mite, pollen or all aeroallergens combined in preschool or school age.

- An association between SHS exposure in early infancy and pet sensitization in preschool age including a significant dose-response effect was observed but this isolated finding needs to be replicated.

- Allergy-related diseases at the age of 12 years are associated recurrent abdominal pain at the same age. Food sensitization and food allergy at age four or eight years is associated with recurrent abdominal pain at 12 years. Although causal relationships cannot be determined in this study, the results support the hypothesis that allergic disease and recurrent abdominal pain have common immunologic pathways.

6 SAMMANFATTNING PÅ FINLANDSSVENSKA

Bakgrund

Antalet barn som insjuknar i allergisjukdomar har ökat under de senaste decennierna, speciellt i länder där den industriella utvecklingen nått långt. Samtidigt har nivåerna av mätbara IgE-antikroppar mot allergiframkallande ämnen också ökat. Idag har upp till vart tredje barn eller ungdom i Europa någon form av allergisjukdom och astma är den vanligaste kroniska barnsjukdomen i västvärlden. Under senare år har man kunnat skönja en avplaning avseende antalet nyinsjuknanden i astma i vissa länder.

Anledningen till den ökade förekomsten av allergisjudomar är multifaktoriell och komplex, bestående av ett samspel mellan arv och miljö. Även tidpunkten för exponering för olika miljöfaktorer spelar stor roll och extra känsliga stadier förefaller vara fosterlivet och nyföddhetsperioden.

Det finns ett tidssamband mellan ökad allergiförekomst och en förändrad livsstil med högre levnadsstandard, ökad renlighet, förändrad kost och bättre sjukvård. En hypotes är att vi exponeras för mindre infektioner och bakterier tidigt i livet och att detta leder till att vårt immunförsvar tenderar att reagera på ett allergiskt sätt om en underliggande sårbarhet finns. Därtill har astmaförekomsten ökat i takt med den urbanisering som medfört en ökad utsatthet för miljöföroreningar såsom tobaksrök och trafikavgaser. Ett samband har påvisats mellan mammans rökning under graviditeten och astma hos barnet, men det har varit svårt att helt särskilja effekterna av rökning under och efter graviditeten då många mammor fortsätter röka efter att barnet fötts. Säkra samband har inte kunnat styrkas vad gäller tobaksröksexponering tidigt i livet och förekomst av IgE-allergiantikroppar hos barnet.

Allergiska symptom följer ofta ett typiskt mönster i barndomen. Under de första levnadsmånaderna debuterar ofta eksem och vissa spädbarn blir (oftast övergående) allergiska mot mjölk- eller äggprotein. Under andra halvan av första levnadsåret kan astmaliknande pipande och väsande andningsbesvär debutera men endast en minoritet av dessa barn utvecklar astma. Hösnuva och astma blir successivt mer vanligt i skolåldern och tonåren samtidigt med en ökad förekomst av pollen- och pälsdjursallergier. Många försök har gjorts för att identifiera de barn med tidiga astmaliknande symptom som löper en högre risk att utveckla astma men hittills finns ingen metod som är av kliniskt värde.

Det finns studier som tyder på att allergi kan ge upphov till symptom från andra organ än de som vi vanligen förknippar med sjukdomen. Huvudvärk, migrän, muskelvärk och återkommande buksmärta har visat sig vara vanligare hos allergiska än icke-allergiska individer i vissa studier. En teori är att allergisk inflammation som uppstår i ett organ kan spridas till andra vävnader som har ett gemensamt immunologiskt ursprung, till exempel mellan luftvägarnas och tarmens slemhinnor. Tidigare studier avseende sambandet mellan allergiska symptom och buksmärtor har oftast varit små och de flesta har haft vuxna studiepopulationer. Därtill har resultaten inte varit entydiga.

Syfte

Syftet med denna avhandling var att:
Studie I. Undersöka vilka egenskaper och symptom hos barn med tidiga andningsbesvär som ökade risken för ha astma i åttaårsåldern.
Studie II. Studera sambandet mellan mammans rökning under graviditeten och astma hos barnet i fyra- till sexårsåldern.
Studie III. Undersöka sambandet mellan exponering för tobaksrök i fosterlivet eller nyföddhetsperioden och förekomst av allergiantikroppar mot pälsdjur, dammkvalster eller pollen hos barnet i förskole- och tidig skolålder.
Studie IV. Studera om det finns ett samband mellan allergiska symptom eller förekomst av allergiantikroppar under uppväxten och återkommande buksmärta hos barnen vid 12 års ålder.

Metod

Studie I och IV baseras på data från BAMSE-projektet (Barn, Allergi, Miljö, Stockholm, Epidemiologi) där 4,089 barn födda mellan 1994 och 1996 följts regelbundet med enkäter och blodprovstagning från cirka två månaders ålder. I studie I användes information från föräldraenkäter då barnen var ett, två och åtta år och blodprovstagning vid fyra år. I studie IV användes information från föräldraenkäter vid ett, två, fyra, åtta och 12 år samt data från blodprovstagning vid fyra och åtta år. Barnen själva svarade på enkätfrågor om buksmärta vid 12 år. I studie I kunde 3,251 barn inkluderas och i studie IV var antalet barn 2,610.

Studie II och III baseras på data från åtta europeiska studier (totalt 32,774 barn) varav en var BAMSE. Alla barn hade följts från födelsen och alla kohorter startades på 90-talet. I studie II kunde 21,600 barn inkluderas i analyserna och i studie III 14,613 barn. Adekvat information om rökning under graviditeten och första levnadsåret var ett krav i båda studierna samt tillgänglig information om andningsbesvär och astma mellan fyra och sex år (studie II) och allergiantikroppar mellan tre och fyra år eller sex, sju eller åtta år (studie III).

Resultat (ett urval)

En fjärdedel av BAMSE-barnen hade tidiga astmaliknande andningsbesvär, och av dessa barn hade 14 procent astma vid åtta års ålder. I gruppen barn utan andningsbesvär hade knappt fyra procent utvecklat astma vid samma ålder. Faktorer hos barnen med tidiga andningsbesvär såsom ärftlighet för astma eller allergi, mer uttalade andningsbesvär (mer än tre episoder med andningsbesvär, behandling med inhalationskortison eller besvär både första och andra levnadsåret), samtidigt eksem och återkommande buksmärta medförde en signifikant ökad risk för astma vid åtta år.

Cirka nio procent av 12-åringarna uppgav att de hade återkommande buksmärta minst varje månad och då var menstruationssmärta inte medräknad. Ett signifikant samband kunde påvisas mellan samtliga allergirelaterade symptom vid samma ålder och återkommande buksmärta vid 12 år. Dessutom ökade sannolikheten för återkommande buksmärta med ökande antal samtidigt förekommande allergiska symptom. Ett samband mellan tidig astma (ett och två år) och återkommande buksmärta vid 12 år observerades också. Förhöjda IgE-antikroppsnivåer mot vanliga födoämnen vid fyra och åtta år, födoämnesöverkänslighet och födoämnesallergi var också associerade till buksmärta vid 12 år.

Vi kunde identifiera 735 mammor som rökte under graviditeten men som slutade innan barnet föddes. Trots rökstoppet fanns en signifikant ökad risk för andningsbesvär och astma hos barnet när det var fyra till sex år gammalt. Risken ökade också med antalet rökta cigaretter under första trimestern av graviditeten i denna grupp av mammor. Vi kunde även konstatera att den signifikant ökade risken för andningsbesvär och astma återfanns hos förskolebarnen till mammor som rökte under första men inte under tredje trimestern och inte heller börjat röka igen efter att barnet fötts.

Vi kunde inte påvisa några säkra samband mellan mammans rökning under graviditeten eller rökning nära spädbarnet under de första månaderna efter födelsen och förekomst av IgE-antikroppar mot pälsdjur, dammkvalster eller pollen hos barnen när de var tre till fyra eller sex till åtta år gamla. Det fanns ett samband mellan rökning nära spädbarnet och IgE-antikroppar mot pälsdjur vid tre till fyra års ålder, men detta samband kvarstod inte vid sex till åtta års ålder.

Slutsatser

De faktorer som medför en ökad risk för astmautveckling i barndomen hos barnen med astmaliknande andningsbesvär tidigt i livet är en ökad svårighetsgrad av dessa besvär, ärftlighet och samtidigt eksem. Dessa resultat är i samklang med liknande studier.

Det signifikanta sambandet mellan allergirelaterade sjukdomar, allergisk sensibilisering och återkommande buksmärta stärker hypotesen om att det kan föreligga ett gemensamt immunologiskt ursprung mellan allergi och buksmärta, och att en låggradig inflammation kan finnas i tarmen hos barn med allergiska symptom.

Mammas rökning under graviditeten ökar risken för andningsbesvär och astma hos barnet i förskoleåldern även om hon slutat röka under graviditeten. Denna information är viktig att delge de kvinnor som planerar att bli gravida, så att de kan motiveras till att sluta röka innan graviditeten.

Inga säkra samband kan påvisas mellan rökning i graviditeten eller nära det nyfödda barnet och ökad förekomst av IgE-antikroppar mot pälsdjur, dammkvalster eller pollen i förskole- eller tidig skolålder i denna avhandling.

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