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INDOOR ENVIRONMENT AND TOBACCO SMOKE EXPOSURE IN RELATION TO ALLERGIC DISEASE AND LUNG FUNCTION

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INDOOR ENVIRONMENT AND TOBACCO SMOKE EXPOSURE IN RELATION TO ALLERGIC DISEASE AND LUNG FUNCTION

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To my parents, who guided me to where I am today.

ABSTRACT

Asthma and other allergy related diseases are the most common chronic diseases in childhood, and have become a major public health concern. The rapid increase in the occurrence of these diseases, especially in high-income countries, has led to the study of the role of various environmental and lifestyle factors. The overall aim of this thesis was to evaluate the association between indoor environmental factors and the development of allergic diseases in childhood and adolescence, and more specifically, to study the association between tobacco smoke exposure during pre- or postnatal life as well as exposure to indoor mold or dampness and allergic diseases from birth to age 16 years. We used data from the Swedish prospective birth cohort BAMSE (N = 4089) and in study III data from BAMSE combined with four other European birth cohorts (N = 10860).

We found that exposure to maternal smoking during pregnancy was associated with asthma up to adolescence, especially early-transient asthma. Additionally, exposure to high doses of maternal smoking during pregnancy (≥10 cigarettes/day) was associated with persistent asthma as well as persistent rhinoconjunctivitis up to age 16 years. Based on spirometry, exposure to maternal smoking during pregnancy was also associated with lower FEV_1/FVC ratios at age 16 years. Additionally, indices from impulse oscillometry indicated increased peripheral airway resistance at age 16 years among subjects exposed to maternal smoking during pregnancy.

Exposure to secondhand smoke (SHS) during infancy was associated with overall increased risks of asthma, rhinitis, and eczema up to adolescence. However, we found suggestive evidence that the association between SHS during infancy and asthma was likely driven by exposure *in utero*. Our findings indicate that exposure to SHS during infancy, without prior exposure to maternal smoking during pregnancy, was associated with food allergen sensitization up to age 16 years. Furthermore, exposure to SHS during infancy was associated with increased risks of rhinitis *without* concomitant sensitization and eczema *with* concomitant sensitization. SHS exposure during other periods of childhood was not associated with the onset of asthma or rhinoconjunctivitis in adolescence.

Compared with non-smokers, participants who smoked daily or occasionally tended to have reduced FEV_1/FVC ratios at age 16 years, even after controlling for maternal smoking during pregnancy. Using indices from impulse oscillometry (IOS) we found increased peripheral airway resistance among adolescent smokers. These findings were corroborated when we used saliva cotinine concentrations to discriminate smokers from non-smokers.

Exposure to indicators of mold or dampness during infancy were associated with increased risk of asthma up to age 16 years, as well as an increased risk of persistent asthma. We also found suggestive evidence of an association between reported mold odor or visible mold during infancy and rhinitis up to age 16 years. No association between exposure to indicators of mold or dampness and IgE sensitization was observed.

In conclusion, findings from the studies included in this thesis suggest that exposure to maternal smoking during pregnancy is associated with asthma and measures of airway obstruction, such as reduced FEV_1/FVC ratios, up to adolescence. Exposure to SHS during infancy seems to be associated with food allergen sensitization and rhinitis up to age 16 years. Adolescent smoking is associated with reduced FEV_1/FVC ratios and increased peripheral airway resistance at age 16 years. Exposure to indicators of mold or dampness during infancy may be associated with an increased risk of asthma, and more specifically with persistent asthma up to age 16 years.

The results from this thesis can be used to help inform public health policy as well as clinicians to motivate their patients to abstain from smoking. Indoor mold and dampness is a modifiable risk factor related to the onset and persistence of asthma in children and adolescence, and further research should focus on identifying the causal agents.

LIST OF SCIENTIFIC PAPERS

I. **Thacher JD**, Gruzieva O, Pershagen G, Neuman Å, Wickman M, Kull I, Melén E, and Bergström A.
Pre- and postnatal exposure to parental smoking and allergic disease through adolescence. Pediatrics. 2014;134(3):428-34.

II. **Thacher JD**, Gruzieva O, Pershagen G, Neuman Å, van Hage M, Wickman M, Kull I, Melén E, and Bergström A.
Parental smoking and development of allergic sensitization from birth to adolescence. Allergy. 2016;71(2):239-48.

III. **Thacher JD**, Gehring U, Gruzieva O, Standl M, Pershagen G, Bauer CP, Berdel D, Keller T, Koletzko S, Koppelman GH, Kull I, Lau S, Lehmann I, Maier D, Schikowski T, Wahn U, Wijga AH, Heinrich J, Bousquet J, Anto JM, von Berg A, Melén E, Smit HA, Keil T, and Bergström A.
Secondhand tobacco smoke exposure from foetal life into adolescence and development of asthma and rhinoconjunctivitis – The MeDALL study.
Submitted manuscript.

IV. **Thacher JD**, Schultz ES, Hallberg J, Hellberg U, Kull I, Thunqvist P, Pershagen G, Gustafsson PM, Melén E, and Bergström A.
Maternal smoking during pregnancy and adolescent smoking on pulmonary function at age 16 years.
Submitted manuscript.

V. **Thacher JD**, Gruzieva O, Pershagen G, Melén E, Lorentzen J, Kull I, and Bergström A.
Mold and dampness exposure and allergic outcomes from birth to adolescence: data from the BAMSE cohort. Allergy 2017; 72: 967-974.

Studies will be referred to by their Roman numerals throughout the text.

CONTENTS

LIST OF ABBREVIATIONS

AX	Area of reactance
BAMSE	Barn, Allergy, Miljö [Milieu], Stockholm, Epidemiology
CCR	Cotinine to creatine ratio
CI	Confidence interval
FEF_{25-75}	Forced expiratory flow at 25% to 75% of FVC
FeNO	Fractional exhaled nitric oxide
FEV_1	Forced expiratory volume at one second
FVC	Forced expiratory volume
GEE	Generalized estimating equation model
GINIplus	German Infant Nutritional Intervention
IgE	Immunoglobulin E
IOS	Impulse oscillometry
ISAAC	International Study of Asthma and Allergies in Childhood
kU_A/l	Kilo Units (specified allergen IgE) per liter
LISAplus	Lifestyle-related Factors on the Development of the Immune System and Allergic Disease
L	Liters
MAS	Multizentrische Allergiestudie
MeDALL	Mechanisms for the development of allergy
OR	Odds ratio
Pa	Pascal
PEF	Peak expiratory flow
PIAMA-NHS	The Prevention and Incidence of Asthma and Mite Allergy-Natural History Study
R	Resistance
R_5	Resistance at 5 Hertz
R_{20}	Resistance at 20 Hertz
SIDS	Sudden infant death syndrome
SHS	Secondhand tobacco smoke
THS	Third-hand smoke
WHO	World Health Organization
X	Reactance

1 BACKGROUND

1.1 ALLERGIC DISEASES

1.1.1 Why study allergic diseases?

In high and middle income countries allergic diseases such as asthma, rhinitis, and eczema are common. There has been an epidemic increase in allergic diseases which has led to research efforts aiming at assessing which lifestyle and environmental factors may be responsible for this increase.[1 2] The global prevalence of asthma varies, and in Sweden the prevalence of asthma is 5-10%, while in North America, Australia, and the UK it exceeds >10%, and Asia has the lowest prevalence at <3%.[2-5] Aspects of a "westernized" lifestyle, such as diet, increased cleanliness, widespread antibiotic use, indoor and outdoor air pollution, gas cooking, obesity, tobacco smoke exposure, and dampness-related exposures, have stimulated extensive research to identify factors which may be responsible. It is important for research to identify modifiable risk factors that lead to these diseases, as they not only have substantial impact on the individuals but also on society.[6 7] Allergic diseases in general contribute to vast spending by governments and individuals to treat these chronic diseases. Additionally, lost productivity and sick days taken contribute to costs related to these diseases. Quality of life and general well-being are also reduced among individuals suffering from allergic diseases.[7-10]

Individuals living in industrialized countries spend the majority of their time indoors, roughly 80-90%.[11] Therefore, the indoor environment plays a pivotal role in health and well-being. With increased focus being placed on energy efficient practices and rising energy costs, better insulated homes can lead to greater concentrations of indoor allergens and pollutants.[12] Indoor risk factors such as exposure to environmental microorganisms, gas cooking, dust mites, building characteristics, tobacco smoke exposure, indoor mold or dampness, are suggested to influence the development of allergic diseases in children.[12-17] Tobacco smoke exposure as well as indoor mold or dampness constitute the focus of this thesis.

1.1.2 Allergic diseases and the sensitization process

Allergies are an abnormal adaptive immune response and are the most common disorders of the immune system affecting around 10-20% of the global population.[18] Immediate hypersensitivity, also known as type I hypersensitivity, is a reaction to often innocuous antigens (e.g. proteins) and is characterized by the production of allergen-specific immunoglobulin E (IgE).[18]

The sensitization process begins when an individual is exposed to an antigen (allergen) for the first time. The uptake occurs at a mucosal site (in the airway, gut, or skin) where the allergen is taken up by antigen-presenting cells (dendritic cells) and leads to activation of naïve CD4+ cells.[19 20] The naïve T cell is induced by cytokine interleukin 4 (IL4) to proliferate into a T2 helper cell (Th2). Subsequently, Th2 cells produce IL4 and IL13 that in turn simulate B cells to produce allergen-specific IgE antibodies, which bind to the surface of

mast cells and basophils, priming them for future exposure.[18 21] The individual is now considered to be "sensitized". Subsequent exposure to the allergen will be recognized by the IgE-antibodies coating the mast cells and basophils, which will induce the activation of these cells and the allergic cascade begins, stimulating the release of inflammatory mediators and histamine which causes the characteristic allergic reaction (i.e. runny nose, sneezing, and itchy/watery eyes).

1.1.3 Asthma

Asthma is a chronic inflammatory disease characterized by airway inflammation and hyperreactivity. Asthma often presents as wheezing, shortness of breath, and coughing. Chronic airway inflammation often leads to alterations in the airway epithelium including increased production of smooth muscle cells and mucus secretion. Asthma is a complex disease with many forms and phenotypes, and it is likely that each has slightly different etiology and mechanisms.[22] There is no "gold standard" for diagnosing asthma, which is based on a combination of patient history, symptoms, lung function tests, or airway provocation tests (e.g. methacholine challenge). In children under five years asthma may be challenging to diagnose, and can be misinterpreted with other conditions, such as respiratory infections that provoke wheezing.

1.1.4 Rhinitis and rhinoconjunctivitis

Rhinitis is common during childhood and adolescence, and is defined as inflammation of the nasal epithelium and characterized by nasal symptoms such as rhinorrhea, blocked and itchy nose, or sneezing.[23] Multiple forms of rhinitis exist and they often overlap. The most common is allergic rhinitis, which is driven by IgE sensitization. Rhinoconjunctivitis, is similar to rhinitis, and is also characterized by one or more of the following symptoms – nasal congestion, rhinorrhea, sneezing, and red, itchy eyes (conjunctivitis).[24]

1.1.5 Eczema

Eczema, or atopic dermatitis, is a relapsing chronic inflammatory skin disorder which presents as pruritic rash and dry skin. There are no definitive diagnostic criteria, and diagnosis is based on patient history, recognition of symptoms, and clinical features. The Williams criteria are the most commonly used and validated criteria in diagnosing eczema.[25 26]

1.1.6 IgE sensitization

IgE sensitization refers to the presence of allergen-specific IgE antibodies in the body. In general, two tests are used to identify sensitization, skin prick tests (SPT) and measuring the presence of antibodies (IgE) from a blood test. Phadiatop® and fx5® are both lab tests developed to identify mixtures of specific antibodies within blood. Higher levels of IgE in blood increases the likelihood of presenting with symptoms of allergic diseases but this is not always the case.

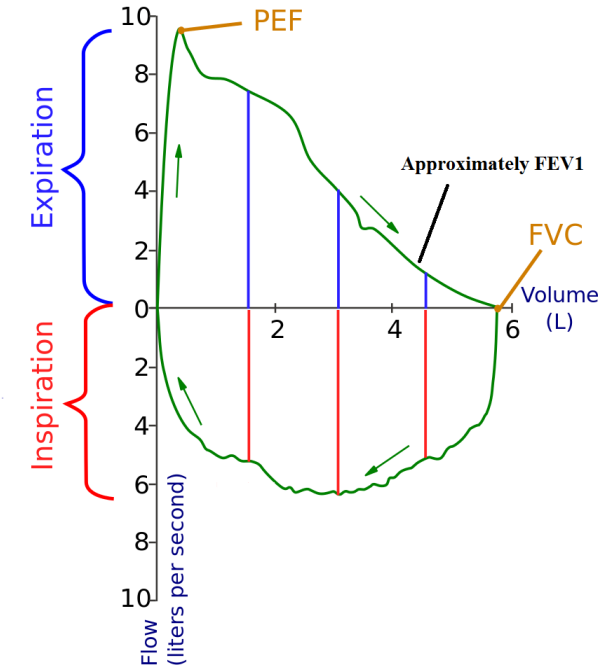
1.1.7 Lung function

1.1.7.1 Dynamic spirometry

In epidemiological studies, lung function is often assessed as an objective measurement for lung growth, development, or impairment. Dynamic spirometry is the most common pulmonary function test, and is routinely used in clinical and research settings. Spirometry measures the speed (flow) and amount (volume) of air an individual inhales or exhales over time, and generates a flow-volume loop (Figure 1[27]).[28] Using standardized methods developed by the American Thoracic Society and European Respiratory Society, spirometry provides reliable and reproducible information regarding lung capacity and function.[28] The most frequently used measures from spirometry are forced vital capacity (FVC), forced expiratory volume in one second (FEV_1), and the ratio of FEV_1/FVC.

FVC is the maximum volume of air exhaled with maximal forced effort from complete inspiration and represents the size of the lungs.[28] FEV_1 is the amount of air exhaled in one second during the FVC manoeuver and impaired values represent the degree of air flow limitation. The ratio of these two measures (FEV_1/FVC) is presented as a percent and is lower in individuals with obstructive lung disease. Other flows or volume measurements can be obtained at various time points during the FVC manoeuver such as peak expiratory flow (PEF).

Figure 1. Example of a flow volume loop. Adapted from SPhotographer.



1.1.7.2 Impulse oscillometry

Unlike dynamic spirometry, which requires maximal patient effort, impulse oscillometry (IOS) is an effort-independent pulmonary function test performed during tidal breathing. IOS provides a compliment to spirometry as it can differentiate between large and small airway effects.[29 30] IOS is a form of forced oscillation technique which means that pressure oscillations are forced upon the respiratory system and that information about the mechanical properties of the respiratory system can be derived from the "opposing forces" that the respiratory system exerts. These are resistance (R) and reactance (X). By separating data with respect to frequency of signals more detailed information about the properties of the respiratory system can be derived, such as the mean value of resistance at 5 Hertz (Hz) (R_5), resistance at 20 Hz (R_{20}), frequency dependence of resistance (R_{5-20}), and the square root of the area of reactance ($AX^{0.5}$). Higher frequencies (>20 Hz) reach the large and intermediates airways and lower frequencies (<15 Hz) penetrate the peripheral and conductive airways.

R_5-R_{20} (resistance at 5 Hz minus the resistance at 20 Hz) is considered to reflect the heterogeneous distribution of peripheral resistance and presence of small airway dysfunction.[29] The area of reactance (AX) is the integrated value of reactance with respect to frequency from 5 Hz to resonant frequency. Taking the square root of AX ($AX^{0.5}$) will linearize this variable and create a more robust reactance index than any reactance value at a particular frequency. In most cases there is good correlation between R_5-R_{20} and $AX^{0.5}$, even in a single subject. So despite that fact that R_5-R_{20} and $AX^{0.5}$ concern different physical or mechanical properties of the respiratory system they can cross-confirm each other.

1.2 TOBACCO SMOKE

1.2.1 History

The tobacco plant has been growing wild in the Americas for nearly 8000 years, and about 2000 years ago humans began chewing and smoking tobacco leaves during cultural and religious ceremonies.[31] Following his voyage to the Americas, Christopher Columbus returned with tobacco seeds to Europe, and by 1531 tobacco was grown for the first time in Europe.[31] Initially smoking was a masculine habit and became widespread during the world wars. Following World War II, smoking among women became more common, and was advertised as a sign of independence, sophistication, and being well educated.[32] Tobacco was first considered to be deleterious to health by an English author in 1602 who reported in an essay about chimney sweeps, suggesting that disease was caused by soot and tobacco smoke could have analogous consequences.[31] By the 1950's and 1960's tobacco smoking was causally linked to a wide range of medical conditions. Today we know that the burning of tobacco leaves emits more than 4500 chemicals, of which many are known to be carcinogenic and many others toxic.[33] Of the thousands of compounds emitted by burning tobacco, identifying which components influence the pathogenesis of disease is challenging. Presently cigarette smoking is a global epidemic, and is a leading preventable cause of death and disability.[33]

1.2.2 Exposure during pregnancy

Maternal smoking during pregnancy is consistently associated with numerous detrimental health effects, including but not limited to infant mortality, pre-term birth, growth retardation, respiratory infections, low birthweight, and sudden infant death syndrome (SIDS).[34 35] Despite continued health campaigns and prenatal counseling some mothers continue to smoke during pregnancy, and globally the prevalence varies from less than 10% to over 30%.[36] In high-income societies the number of pregnant women smoking is waning, but in low-income countries this number is on the rise.[37]

A fetus is exposed to the same amount of nicotine as an active smoker, which is associated with reduced uterine blood flow through vasoconstriction resulting in lower oxygen in the placenta.[38] As a result of reduced blood flow, intrauterine growth retardation leads to lower birthweight, both of which are associated with the development of the respiratory system.[39] Nicotine induces changes in lung architecture and function, and exposure to maternal smoking during pregnancy is linked with preschool wheeze, asthma, reduced lung function, and airway responsiveness in children.[40-46]

1.2.3 SHS exposure

Secondhand tobacco smoke (SHS), also referred to as passive smoking or environmental tobacco smoke, is a complex aerosol made up of carcinogenic, mutagenic, and toxic compounds. There are two types of SHS, mainstream smoke and sidestream smoke. Mainstream smoke is the tobacco smoke that is created when a smoker inhales the burning

tobacco and then exhales. Sidestream smoke is the smoke components that are released from the end of the smoldering tobacco product. Various toxic gasses, such as acrolein, benzene, carbon monoxide, and formaldehyde are found in sidestream smoke, many of which are carcinogenic and cause non-cancer health related effects.[47] Over five million deaths are attributable to smoking worldwide, and over 600,000 are linked to SHS exposure yearly.[48] According to a 2006 US Surgeon General Report, "The scientific evidence indicates that there is no risk-free level of exposure to secondhand smoke".[49]

Many children who are exposed to maternal smoking during pregnancy are also exposed to SHS postnatally, and more than half of mothers who abstain from smoking during pregnancy relapse within the first six months, and another 80-90% relapse within the first year.[37 50] SHS is one of the most critical and predominant exposures in the indoor environment, and approximately 40% of children worldwide are exposed to SHS.[51] Children are different from adults, in that their respiratory system is still developing and are more sensitive to environmental pollutants such as SHS.[47] SHS is associated with a plethora of adverse health outcomes in infants and children including SIDS, lower respiratory tract infections, asthma, rhinitis, eczema, and other chronic respiratory symptoms.[36 47 52-55] The majority of exposure to SHS occurs in early infancy and during preschool ages, when children spend substantial time in close vicinity of their parents. Neonates and children exert little control over their environment, and therefore are particularly susceptible to the harmful effects of SHS. Exposure can begin early in life, starting from exposure to maternal smoking during pregnancy and often continues into early childhood, which makes it challenging to tease apart the effects of tobacco smoke exposure in pregnancy from postpartum exposure.

Although outside the scope of this thesis, for completeness another aspect of cigarette smoking is third-hand smoke (THS). THS is the set of chemical compounds and components that linger long after cigarettes are extinguished.[56] For example, pollutants from residual tobacco smoke can remain on surfaces, clothes, and even the smoker himself/herself in hair or on hands.[57 58] Toxins and pollutants identified to date include nicotine, phenol, cresols, naphthalene, 3-ethenylpyridine, and nitrosamines.[58] There is some research which suggests an association between third-hand smoke with respiratory and nasal symptoms.[56 58-61]

1.2.4 Prevalence of tobacco use and SHS in Sweden

In Sweden, the prevalence of smoking has declined in recent decades and nine percent of adults were daily smokers in 2015.[62] Smoking is somewhat more common among females compared to males. Maternal smoking during pregnancy has also declined in Sweden, and a survey in 2011 indicated that 5% of mothers smoked at some point during their pregnancy which was down from 9.5% in 2003.[63] Exposure to SHS has also reduced coinciding with the decline in smoking rates, and in 2001 around 11% of 12 year-olds, 9.6% of four-year-olds, and 7.5% of eight month olds had at least one parent who smoked. There are large socioeconomic differences in the prevalence of smoking. Smoking is almost three times as common among individuals without high school educations, compared to persons with a

university degree.[64] The use of smokeless tobacco (snus/moist snuff) is more common among males (19%), compared to females (4%).[64]

1.2.5 Tobacco smoke exposure in relation to allergic diseases and lung function in childhood and adolescence

1.2.5.1 Asthma

Exposure to tobacco smoking has been linked with both exacerbations as well as the development of asthma in young children.[52 65-68] Studies focusing on whether tobacco smoke exposure contributes to the etiology of asthma in young children concluded that exposure not only increased symptoms but contributes significantly to the development of wheeze and asthma.[65 66 69-71] Studies have also attempted to disentangle which exposure presents the greatest risk, maternal smoking during pregnancy or postnatal exposure to tobacco smoke. In a prospective Swedish birth cohort study, Lannerö et al. found a twofold increased odds of wheezing in children up to two years of age who had been exposed to maternal smoking during pregnancy but not thereafter.[71] Furthermore, a meta-analysis of eight European birth cohorts was able to show that maternal smoking during pregnancy alone without exposure postpartum conveyed excess risk of wheezing and asthma in preschool age children.[42] In this meta-analysis Neuman et al. also observed elevated risks of asthma in children exposed to maternal smoking during the first year of life, but this was not statistically significant. Other studies have suggested elevated risk of asthma in children exposed to parental smoking in early life.[40 67 72]

Whether the harmful effect of early exposure to tobacco smoke persists into adolescence has yet to be elucidated, and few longitudinal studies have assessed the risk of adolescent-onset asthma in relation to maternal smoking during pregnancy or SHS exposure. Of the studies that have been published, the findings have been mixed. A Danish birth cohort that follow children up to 14-18 years of age observed no significantly elevated risk of asthma in those exposed during pregnancy, postnatally, or both.[73] In an Australian birth cohort study, Hollams et al. found a nearly twofold increased odds of current asthma at 14 years of age in children who had been exposed to maternal smoking during pregnancy.[74] Similarly, a German birth cohort followed for 20 years found a 1.7 times increased odds of developing incident asthma in those exposed to maternal smoking during pregnancy.[75] They also found no association between exposure to SHS in the first three years of life and the incidence of asthma up to 20 years of age.[75] No increased risk between lifetime SHS exposure and asthma at ages 4-17 years was seen in a Dutch cohort study.[76]

1.2.5.2 Rhinitis

The literature on the effects of tobacco smoke exposure and the development of rhinitis are more inconsistent and sparse than that of asthma. A large multicenter and multi-country cross-sectional analysis studying the effects of SHS on allergic diseases, in two groups of children ages 6-7 and 13-14 years, was conducted as part of the International Study of Asthma and Allergies in Childhood (ISAAC) initiative. This study observed significantly

elevated risks of current rhinoconjunctivitis in both age groups among those subjects exposed to maternal smoking in the first year of life, as well as for current maternal or paternal smoking.[77] In a Danish cohort study, Magnusson et al. did not find significant associations between hayfever (rhinitis) and exposure to prenatal or postnatal tobacco smoke exposure.[73] Similarly, a German birth cohort study followed up to ten years of age found no significant association between maternal smoking and allergic rhinitis.[78] A recent systematic review and meta-analysis indicated slightly elevated risk of rhinitis (OR 1.09, 95% CI 1.04-1.14) in children and adolescents exposed to parental smoking.[54] A cross-sectional study from Turkey also indicated that children exposed to maternal smoking during pregnancy had increased odds of current rhinoconjunctivitis in children at age 9-11 years.[79] There is no consensus on the role tobacco smoke plays in the development of rhinitis and there is limited literature related to the long-term effects of tobacco smoke exposure and the development of rhinitis up to adolescence.

1.2.5.3 Eczema

There is a growing body of evidence linking tobacco smoke exposure and eczema. Krämer and colleagues observed an association between an objective biomarker of SHS exposure, urinary cotinine to creatine ratio (CCR), and eczema. They found that for every 100 ng mg^{-1} of CCR there was a nearly twofold increase in eczema among preschool age children.[16] A study of cord blood cotinine at birth and eczema at two years of age in Taiwanese children indicated a fivefold increased risk of atopic dermatitis in those exposed to the 75th percentile of cotinine levels.[80] Furthermore, consistently elevated risk estimates of current eczema were seen in a large cross-sectional study of 6-7 year olds and 13-14 years olds exposed to both maternal smoking in the first year as well as current parental smoking.[72] A Swedish birth cohort suggested increased risk of eczema in four year old children exposed to maternal smoking in pregnancy, parental smoking during the child's first months, or both, but only in those children with concurrent IgE sensitization.[81] Conversely, Magnusson and colleagues observed that children exposed in pregnancy had slightly reduced risk of eczema at ages 14-18.[73] Similarly, Ludvigsson and colleagues found that children exposed to parental smoking were less likely to develop atopic dermatitis.[82] In summary, the role of tobacco smoke exposure and the development of eczema remains controversial, due to limitations in study design, length of follow-up, variations in outcome definitions, and exposure assessment.

1.2.5.4 IgE sensitization

SHS exposure is associated with respiratory and allergic disease in children, but the influence of tobacco smoke exposure on IgE mediated sensitization is uncertain.[59] Exposure to tobacco smoke is suggested to have immunomodulatory effects and is presumed to play a role in the development of allergic sensitization.[59 83] Some studies have reported increased risks for any allergen sensitization[78 84], while others report increased risks only for food allergen sensitization.[85-87] Studies have also reported inverse[83 88 89] or null associations for inhalant allergens[90] and there is inconsistent data on the role of heredity.[78 83 84 91] A recent systematic review and meta-analysis in young children and adolescents indicated that SHS is associated

with higher total IgE concentrations, the presence of specific IgE to common allergens, and positive skin prick test against common allergens.[92] These findings were mostly apparent in children under seven years of age. Reasons for such conflicting results include inadequate control of confounding, the aggregation of inhalant and food allergens together, failure to consider or control for possible differing effects of SHS between those with and without family history of atopy, or maternal versus paternal smoking.[83 91] Separating the effects of maternal smoking during pregnancy and postnatal SHS exposure is difficult, but important nonetheless, since the etiological mechanisms may differ depending on timing of exposure.

1.2.5.5 Lung function

In 1986 the US Surgeon General concluded that, "available data demonstrate that maternal smoking reduces lung function in young children", and this report has been corroborated by multiple studies since.[93-95] The literature also suggests reductions in lung function related to SHS exposure in young children.[95 96] A cross-sectional study of children ages 6-15 years observed that deficits in the small airways was inversely associated with the number of cigarettes smoked by the parents.[97] In line with this, a large multinational study of children 6-12 years suggested reduced lung function measures in children exposed during pregnancy and in early childhood.[98] Similarly, in a well characterized Australian birth cohort study, maternal smoking in pregnancy was related to reduced lung function in 14 year old children.[74] Regular SHS exposure among school age children has also been linked with reduced lung function.[99] Although exposure to tobacco smoking during the perinatal period has been consistently associated with impaired pulmonary function in infants and children, it is unclear if pulmonary function is affected in adolescence. In general, lung growth and functional development continues until the mid-20's[100], and failure to reach maximal lung growth can have lasting deleterious effects.[101 102] Data on the independent effect of adolescent smoking on lung function accounting for maternal smoking during pregnancy are even more limited, especially utilizing prospectively collected data from birth to adolescence.[103 104] Furthermore, it remains unclear if interactions between exposure during pregnancy and personal smoking in adolescence exist.

1.3 INDOOR ENVIRONMENT

1.3.1 Indoor mold and dampness

Mold and moisture and can enter homes in various ways. Materials used during construction can be unsheltered from precipitation or not given ample drying time prior to construction, and moisture is then "built into the structure". Moisture can also enter homes through leaks in roofs, windows, or even leaky plumbing between walls. Occupants also generate humidity, and coupled with poor ventilation can lead to moisture related problems. Building types, construction practices, ventilation rates, and climate all influence the amount of moisture buildup in a home.

Damp environments enhance the growth and proliferation of fungal components such as spores, fungal fragments, gram-negative bacteria, volatile organic compounds (VOC), and β-glucans which are associated with allergic and non-allergic outcomes.[105 106] Excessive moisture can promote deterioration of some building materials which can release toxic substances. The presence of mold and dampness in dwellings is common, with estimates ranging from 18% to 50% this is a major indoor problem worldwide.[107 108] Consequently, a significant percentage of the globe is exposed to mold or dampness-related exposures.[109] Epidemiological studies have found increased risk of wheeze, asthma, rhinitis, and respiratory symptoms in children exposed to indoor mold or dampness.[106 110-115] Still, there remains a lack of studies with quantitative measurements of fungi and other microbiologic exposures, and studies have been inconsistent.[116] Moreover, the role of mold or dampness exposure in relation to the persistence and late-onset of allergic diseases is not fully understood.

1.3.2 Indoor mold and dampness in Sweden

In Sweden building materials, structure, and technologies have varied over time, and various building techniques are associated with different periods. A building boom occurred between 1961 and 1975, and many high-rise buildings were rapidly constructed with basements and only exhaust ventilation, and these homes have the highest reported dampness.[117 118] During the 1970's increased importance on energy conservation required increased insulation and multilayer walls, which can be prone to moisture buildup. In 1977 a self-leveling mortar was introduced, but was then no longer used after 1983, due to increased chemical emissions when this mortar encountered moisture.[119] Studies have suggested that living in homes using this mortar had higher prevalences of mucosal inflammation and asthma.[120]

1.3.3 Mold and dampness exposure in relation to allergic disease in childhood and adolescence

1.3.3.1 Asthma

There is a growing body of evidence linking mold and dampness-related agents with respiratory symptoms and asthma in children. The vast majority of evidence comes from cross-sectional and case control studies, but a meta-analysis of eight European birth cohorts

indicated that children exposed to early visible mold and/or dampness had increased risk of asthma up to three years of age.[15] This study also suggested a nonsignificantly increased risk of asthma in children ages 6-8 years and 3-10 years.[15] Another systematic review of cohort and incident case-control studies which aimed to assess the development of incident asthma, found a 1.5 increased odds of asthma in children exposed to any sign of mold or dampness.[121] However, in a German birth cohort, no increased risk of asthma at age 20 years was observed among those children exposed to mold spots at three months of age.[75] In a recently published systematic review and meta-analysis, the presence of certain species of fungi, *Penicillium*, *Aspergillus*, and *Cladosporium*, were associated with increased risk of developing asthma symptoms in children and adults.[122] A limited number of prospective studies have been conducted, and even fewer have investigated the risk of asthma as children reach adolescence as well as the persistence or late-onset disease phenotypes. Thus, it is unclear if early mold and dampness exposure in children contributes to the development of asthma up to adolescence or simply exacerbates preexisting respiratory conditions.[116]

1.3.3.2 Rhinitis

The association between residential dampness and mold exposure and rhinitis tends to provide a consistent picture of increased risks in children, with the majority of studies observing elevated effect estimates for a variety of dampness-related exposures.[15 121 123-129] In a large cross-sectional analysis of elementary and middle school age children in Italy, Simoni and colleagues found that early exposure to mold or dampness was associated with rhinoconjunctivitis.[123] Among adolescents exposed to both early and current signs of mold or dampness, risk estimates for rhinoconjunctivitis were even higher.[123] Similar results were seen in a meta-analysis of eight well characterized European birth cohorts; early visible mold exposure (0-2 years) was associated with allergic rhinitis symptoms up to 10 years of age.[15] Nevertheless, there is a lack of prospective studies which examine the long term effects of early or current dampness-related exposure, and whether they continue to contribute to the development and persistence of rhinitis.

1.3.3.3 IgE sensitization

There is scarce information on the association between IgE sensitization in relation to dampness-related exposures, and of these studies the results are inconclusive.[130] Antova and colleagues did find increased “sensitivity to inhaled allergens” in children exposed to mold ever.[125] However, this was not based on skin prick testing or the presence of IgE. Taken together, there are indications of increased risk but the evidence is too inconsistent to draw conclusions.[111 131-133] Additional studies are necessary to evaluate this association more closely.

2 AIMS

The overarching aim of this work is to study the association between tobacco smoke or the indoor environment and development of allergic diseases as well as lung function in children and adolescents. The specific aims in this thesis are as follows:

- To study the association between maternal smoking during pregnancy and allergic diseases as well as lung function up to adolescence.

- To study the association between SHS exposure during infancy or childhood and allergic diseases as well as lung function up to adolescence.

- To investigate the association between adolescent smoking and lung function at age 16 years.

- To assess the association between exposure to indicators of mold or dampness in the home and development of allergic diseases up to adolescence.

3 METHODS

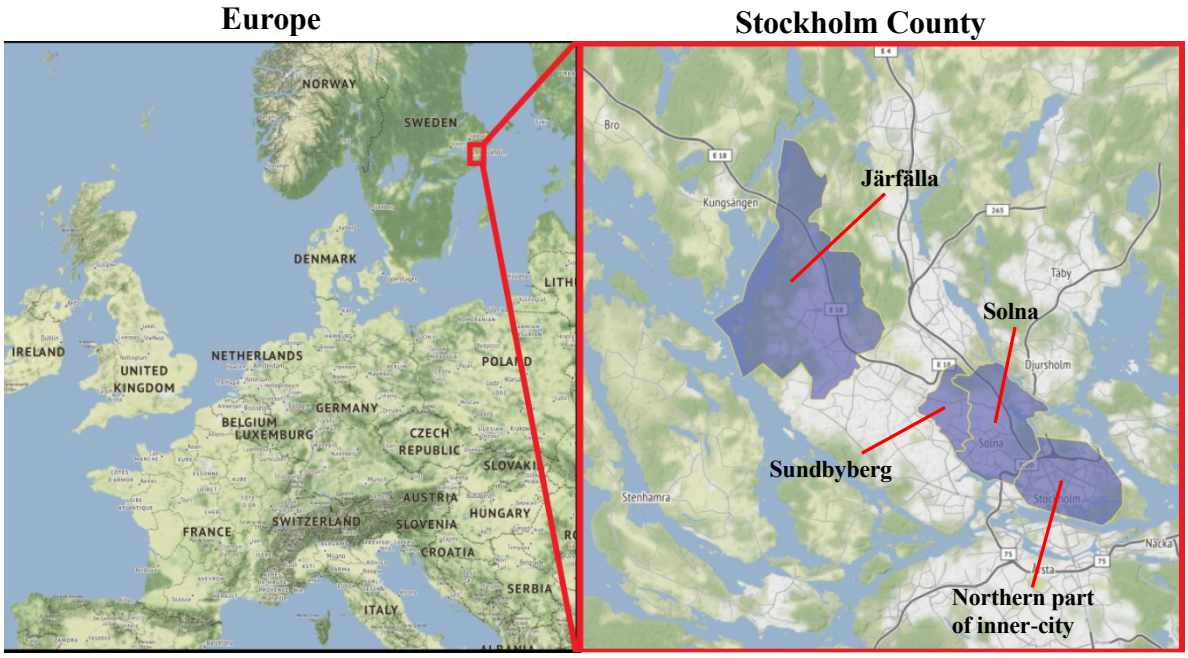
3.1 DATA SOURCES

Studies I, II, IV, and V used data exclusively from the BAMSE cohort study and study III utilized individual participant data from five European birth cohort studies (including BAMSE) which were part of the MeDALL consortium.

3.1.1 BAMSE birth cohort

The **B**arn (Children), **A**llergi (Allergy), **M**iljö (Environment), **S**tockholm, **E**pidemiologi (Epidemiology) (BAMSE) birth cohort is an ongoing prospective birth cohort study which began in February 1994. At inception, 4089 participants were recruited from four predefined areas of Stockholm (Järfälla, Solna, Sundbyberg, and parts of Stockholm inner city) (Figure 2).[134] The rational for this was to capture various exposure characteristics including, but not limited to, housing types, urban/suburban, socio-demographic characteristics, and varying levels of air pollution.

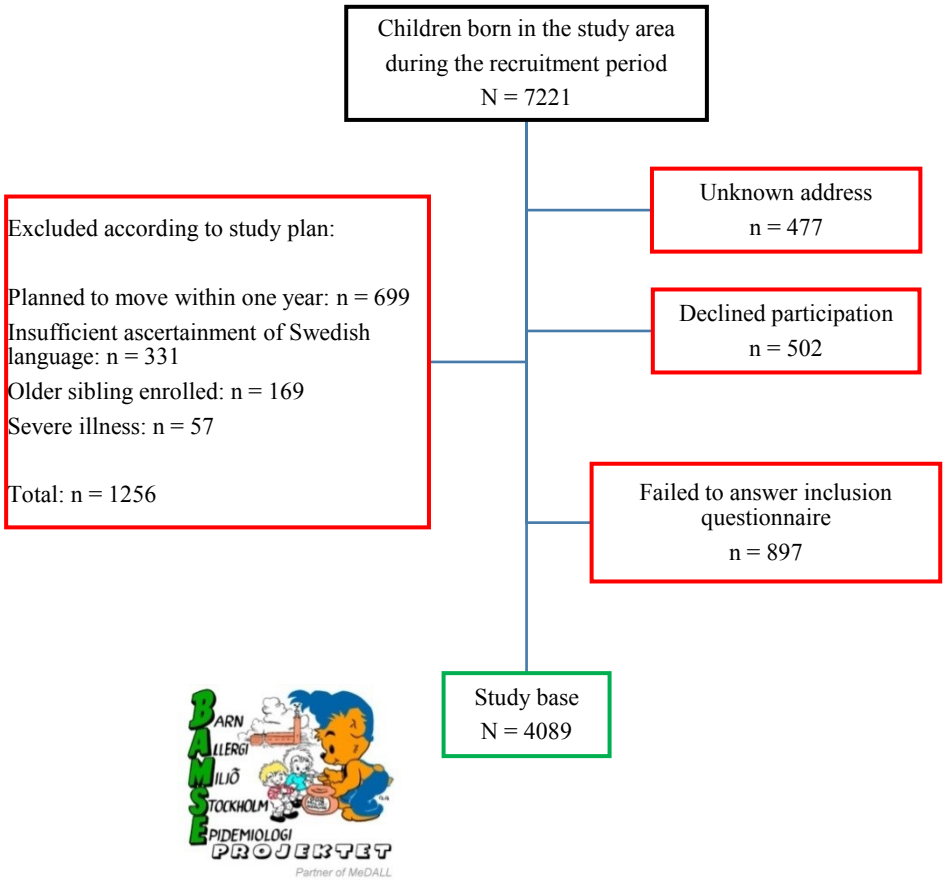
Figure 2. The four predefined recruitment areas of Stockholm County, Sweden included in the BAMSE birth cohort. Maps adapted from © OpenStreetMap contributors and created by Andrei Pyko.



3.1.1.1 Enrollment

Enrollment commenced in February 1994 and continued to 1996, and during this period 7221 children were born in the study areas. In Sweden, all newborns are invited to the children's healthcare center for a physical exam, and 99% of the children under two years of age attend.[135] During the child's first visit at the children's healthcare center, parents were invited to participate in BAMSE. Of the 7221 children eligible for the study, 477 could not be contacted, 502 declined participation, 897 never answered the questionnaire, and 1256 were actively excluded in accordance to the study plan. These 1256 actively excluded individuals were either moving within a year (n=699), did not sufficiently understand Swedish (n=331), already had a sibling enrolled (n=169), or the child was seriously ill (n=57). At the end of recruitment 4089 children were included, 75% of all eligible infants. Figure 3 summarizes this recruitment.

Figure 3. Summary of recruitment in the BAMSE birth cohort.



At a median age of two months, parents of the 4089 infants (2065 males and 2024 females) completed the baseline questionnaire. This questionnaire queried information on various environmental and behavioral factors including smoking history, housing characteristics, socio-demographics, and parental history of allergic disease. In 1996, an abridged questionnaire was sent to non-responders and participants who were actively excluded. To understand if and how these non-responders differed from those included, a short survey

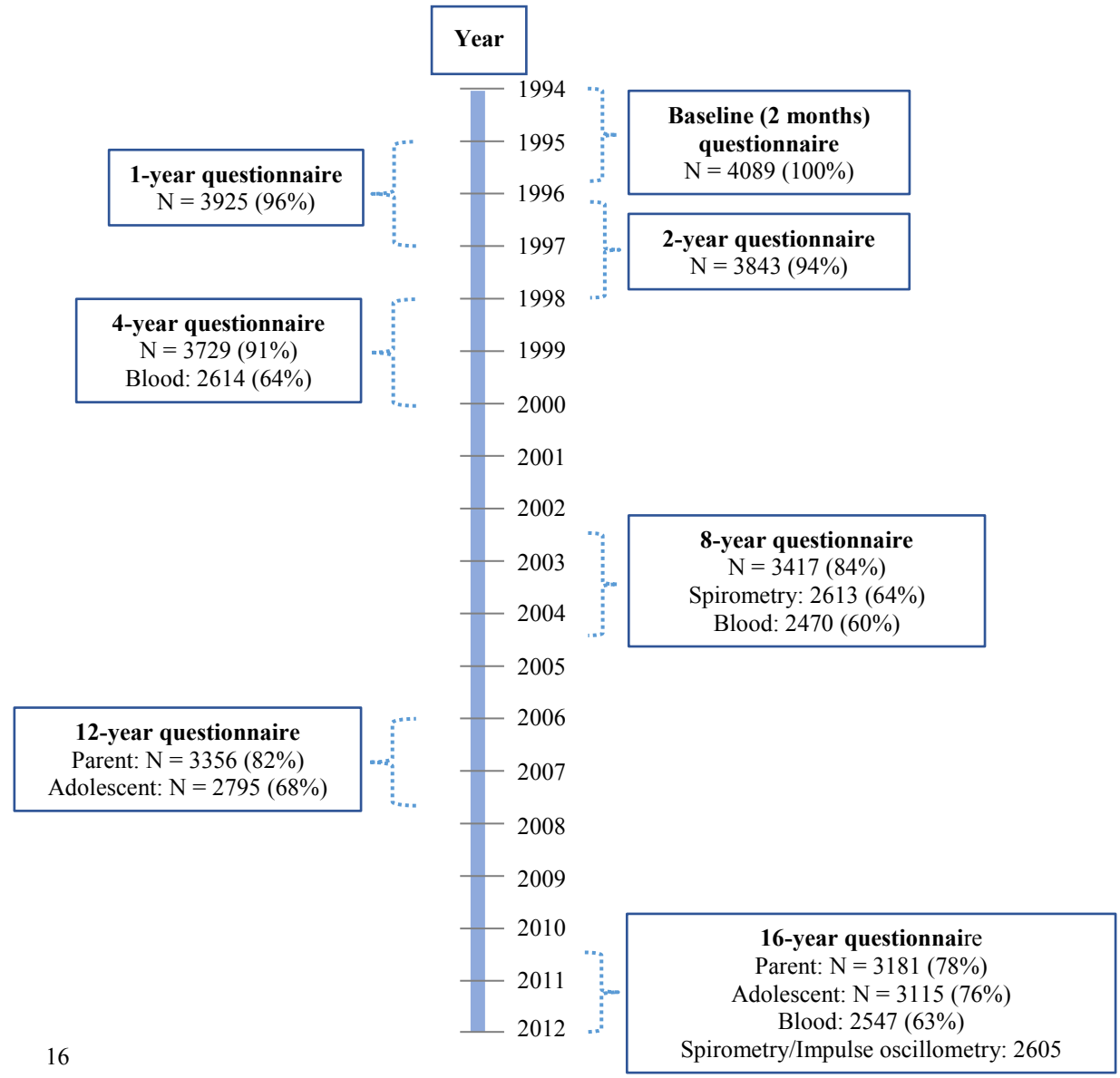
querying parental history of allergic disease, pet ownership, and tobacco smoking habits was sent out, and an overall response rate of 67% was achieved.[134] Data gathered from this questionnaire indicated that non-participants and those actively excluded were more commonly exposed to tobacco smoke than the participating families (maternal smoking – 18% vs. 9%; paternal smoking – 23% vs. 17%), but no differences were seen with regards to parental history of allergic disease.[134]

3.1.1.2 Follow-up

Subsequent follow-up questionnaires were sent out to the baseline participants (4089) at ages 1, 2, 4, 8, 12, and 16 years of age with response rates of 96%, 94%, 91%, 84%, 82%, and 78% respectively (Figure 4).

In conjunction with questionnaires, at ages 4, 8, and 16 years study subjects were invited to participate in clinical examinations. At these examinations blood draws with venipunctures and lung function testing were performed. A summary of the follow-ups is illustrated in Figure 4.

Figure 4. Flow chart of the BAMSE cohort follow-up periods and data collected.



3.1.2 The MeDALL consortium

The **Me**chanisms for the **D**evelopment of **All**ergy (MeDALL) consortium, a Seventh Framework Program European Union project, began in 2010 and was organized to generate novel knowledge on the mechanisms of the initiation of allergy.[136-138] The MeDALL consortium included 14 ongoing European birth cohorts initiated between 1990-1997. Study III included five European birth cohorts (including BAMSE) which had follow-ups into adolescence and data on exposures and outcomes. The following cohorts were included in study III, BAMSE (single-center, Sweden),[134] GINIplus (multicenter, Germany),[139] LISAplus (multicenter, Germany),[140] MAS (multicenter, Germany),[141] and PIAMA (multicenter, The Netherlands).[142] An overview of birth cohorts included in study III is presented in Table 1.

3.2 STUDY POPULATIONS

The final study population of study I comprised children who participated in three or more follow-ups, had complete information on maternal smoking during pregnancy or SHS during infancy, and had information about any allergic disease outcome from any age, which resulted in 3798 participants (93% of the baseline cohort). This inclusion criteria was chosen to promote stable and robust longitudinal analyses.

In study II we included 3316 participants (81% of the baseline cohort) who had complete information on maternal smoking during pregnancy or SHS exposure during infancy, confounders, and gave a blood sample at age 4, 8, or 16 years.

Study III was based on the MeDALL consortium and involved 10860 participants (59% of the 18451 children at recruitment) for whom information was available on maternal smoking during pregnancy and/or infancy, and asthma or rhinoconjunctivitis between ages 14-16 years (Table 1).

Table 1. Overview of included birth cohorts from the MeDALL consortium.

Cohort acronym	Country	Years of recruitment	Number of children at recruitment N	Number included in final analyses n (%)
BAMSE	Sweden	1994-1996	4089	3112 (76.1)
GINIplus	Germany	1995-1998	5991	2956 (49.3)
LISAplus	Germany	1997-1999	3094	1713 (55.4)
MAS	Germany	1990	1314	560 (42.6)
PIAMA	The Netherlands	1996-1997	3963	2519 (63.6)
Total			18451	10860

In study IV the final study population comprised participants with a valid spirometry measure, information on smoking habits at age 16 years, and covariates – 2295 participants (56% of the baseline cohort). For analyses related to saliva cotinine concentrations, participants with a valid lung function measure, a saliva cotinine sample, and covariates were included (N=1523).

The final study population of study V comprised participants who provided a baseline response to any mold or dampness indicator and participated in three or more follow-ups, which resulted in 3293 participants (81% of the baseline cohort).

3.3 EXPOSURE AND OUTCOME DEFINITIONS

3.3.1 Background characteristics and variables

Background variables for studies I-V are summarized in Table 2. The majority of baseline characteristics (BAMSE) were queried from the initial questionnaire at a median age of two months. Baseline and background characteristics from the other cohorts in study III were also gathered shortly before (PIAMA) or after birth (GINIplus, LISAplus, MAS).

Table 2. Definitions of background characteristics and covariates in studies I to V.		
Variable	**Variable definition**	**Study**
Sex	Biological sex of participant (male vs. female).	I, III, IV, V
Age	Participant's age at lung function assessment in years.	IV
Height	Participant's height at lung function assessment in centimeters.	IV
Birth weight	Birthweight of the child in grams.	III
Gestational age	Gestational age of the child in weeks.	III
Maternal age	Mother's age at birth of child (<26 years vs. ≥26 years).	V
Breastfeeding	Exclusive breastfeeding for a duration of ≥4 months (yes vs. no).	III
Parental history of allergic disease	Mother and/or father reporting doctor's diagnosis of asthma or hay fever (yes vs. no).	I, II,III, V
Socioeconomic status	Categorized base on parental occupation according to the Nordic standard occupational classification and divided into manual workers (low) and non-manual workers (high).	I, II, V
Parental education level	Based on the highest educational level of either parent. Divided into low – primary school, lower vocational education, or lower secondary education; intermediate – intermediate vocational education or intermediate/higher secondary education; high – higher vocational education or university degree.	III
Mean income	Mean income in the neighborhood and are defined on the basis of small-area market statistics from Statistics Sweden (http://www.scb.se).	I*
Early day-care attendance	Did the child attend nursery or day-care with other children at any time from ages 0, 1, and/or 2 years (yes vs. no).	III
Older siblings	The presence of at least one older sibling at the time of birth (yes vs. no).	III, V
Study center	GINIplus, LISAplus, and PIAMA were all multicenter studies. GINIplus consisted of Munich, Bavaria, Wesel, and North-Rhine-Westfalia. LISAplus consisted of Munich, Leipzig, Wesel, and Bad Honnef. PIAMA was divided into North – Groningen, Friesland, Drenthe; Central – Utrecht, Gelderland; and West – Rotterdam and surrounding municipalities.	III
Study arm	GINIplus and PIAMA had an observational and intervention study arm in these cohorts. GINIplus had an arm that delayed the introduction of solid foods[143] and PIAMA had an arm that distributed mite impermeable mattress covers.[144]	III
Cohort	Birth cohort (BAMSE, GINIplus, LISAplus, MAS, PIAMA).	III
Maternal smoking during pregnancy	Mother smoking at least one cigarette daily at any trimester of gestation (yes vs. no).	V
Parental smoking during infancy	Mother and/or father smoking at least one cigarette daily at baseline questionnaire (2 months) (yes vs. no).	V
Secondhand smoke throughout childhood	Exposure to SHS at any age after year one.	I,II,III
Mold or dampness in dwelling	Presence of signs of mold or dampness in the home at ages 0, 1, and/or 2 years (yes vs. no).	III
* Only used in sensitivity analyses.		

3.3.2 Exposure assessment

3.3.2.1 Tobacco smoke

For studies I, II, and IV tobacco smoking was assessed via questionnaires. For prenatal exposure only maternal smoking habits were queried at baseline. The number of cigarettes smoked was also ascertained for each trimester. At the 12-year follow-up fathers were asked retrospectively if they smoked during their child's pregnancy (BAMSE), but these data were only used in sensitivity analyses and some results are presented in section 4.9.1.

In BAMSE, SHS exposure was assessed at baseline, 1, 2, 4, 8, 12, and 16 years for both mothers and fathers. The number of cigarettes smoked per day was also ascertained and used in dose-response analyses. At age 16 years participants answered their own questionnaire which also gathered information on personal smoking habits. Table 3 outlines all tobacco smoke exposure related variables and definitions used in BAMSE. Smoke exposure was defined as smoking cigarettes, cigars, cigarillos, or pipe smoking.

Table 3. Definitions of tobacco smoke exposure in BAMSE (Studies I, II, and IV).

Variable	Definition	Study
Maternal smoking during pregnancy (*in utero*)	Maternal smoking ≥1 cigarette daily at any time during pregnancy.	I, II, IV
SHS during infancy	Maternal or paternal smoking ≥1 cigarette daily at baseline questionnaire.	I, II, IV
SHS at ages 1, 2, 4, 8, 12, and 16	Maternal or paternal smoking ≥1 cigarette daily at the time of the respective follow-up questionnaire.	I, II, IV
Adolescent smoking	Any smoking at age 16 years.	IV
Daily smoking	Smoking at least one cigarette per day.	IV
Occasional smoking	Smoking less than one cigarette per day.	IV

In study III each cohort defined tobacco smoking in a relatively similar manner and is summarized in Table 4. Smoke exposure was defined as smoking cigarettes, cigars, cigarillos, or pipe smoking. In all cohorts prenatal smoking was ascertained during pregnancy or shortly thereafter, and all cohorts had information on the number of cigarettes smoked by the mother for each trimester of pregnancy.

Table 4. Definitions of tobacco smoke exposure in MeDALL (Study III).

	Maternal smoking	SHS exposure				
Birth cohort	**Maternal Smoking during pregnancy**	**Infancy (first year of life)**	**When children were ages 1-2 years**	**When children were ages 4-6 years**	**When children were ages 8-10 years**	**When children were ages 14-16 years**
BAMSE	Mother smoked at least 1 cig/day during any trimester of pregnancy.	Mother, father, or others actively smoke at home at age 2 months.	Mother, father, or others actively smoke at home at age 2 year.	Mother, father, or others actively smoke at home at age 4 years.	Mother, father, or others actively smoke at home at age 8 years.	Mother, father, or others actively smoke at home at age 16 years.
GINIplus	Mother smoked during any trimester of pregnancy.	Mother actively smoking inside the home between age 5 and 12 months.	Smoking inside the home (in general) at age 2 years.	Mother, father, or others actively smoke inside the home at age 6 years.	Mother, father, or others actively smoke inside the home at age 10 years.	Mother, father, or others actively smoke inside the home at age 15 years.
LISAplus	Mother smoked during any trimester of pregnancy.	Mother, father, or others actively smoke inside the home at age 1 year.	Mother, father, or others actively smoke inside the home at age 2 years.	Mother, father, or others actively smoke inside the home at age 6 years.	Mother, father, or others actively smoke inside the home at age 10 years.	Mother, father, or others actively smoke inside the home at age 15 years.
MAS	Mother smoked during any trimester of pregnancy.	Mother, father, or others actively smoke inside the home at age 1 month.	Mother, father, or others actively smoke inside the home at age 18 months.	Mother, father, or others actively smoke inside the home at age 4 years.	Mother, father, or others actively smoke inside the home at age 9 years.	No data
PIAMA	Mother smoked during any trimester of pregnancy.	Cigarettes, pipes, cigars smoked in the house by mother, father and/or other household member at age 3 months.	Cigarettes, pipes, cigars smoked in the house by mother, father and/or other household member at age 2 years.	Cigarettes, pipes, cigars smoked in the house by mother, father and/or other household member at age 4 years.	Cigarettes, pipes, cigars smoked in the house by mother, father and/or other household member at age 8 years.	Cigarettes, pipes, cigars smoked in the house by mother, father and/or other household member at age 14 years.

3.3.2.2 Saliva cotinine

Cotinine is a widely accepted quantitative biomarker of recent uptake of nicotine which may result from active or passive smoking.[145] Saliva samples were collected when participants were 16 years of age. Saliva collection kits were distributed at the clinical examination at age 16 years along with detailed instructions. Saliva was collected in the morning and evening time (prior to dental hygiene) using sterile dental rolls (braided cotton dental rolls; Salivetter®, SARSTEDT AG & Co., D-51582 Nümbrecht). Participants were instructed to keep the roll in their mouths until it was saturated with saliva and then place it in the pre-labeled sample tube and sent directly to the laboratory via post. Samples were centrifuged and stored at -80 degrees Celsius. Evening samples were analyzed using the Salimetrics® cotinine kit (Salimetrics Europe, Ltd, Suffolk, UK) with a detection limit of 0.8 ng/ml.
The distribution of saliva cotinine concentrations in nonsmokers was positively skewed, therefore the data were log-transformed, and we assigned a value of 0.4 ng/ml, half the detection limit, to undetectable concentrations.[145] Based on prior literature a cut-off of ≥12 ng/ml was used to discriminate active smokers from nonsmokers.[146] A total of 20 evening samples had too little saliva and were excluded from analysis.

3.3.2.3 Mold and dampness

In study V indoor environmental exposures were based on parental answers at the baseline questionnaire. We defined four variables to assess mold or dampness in the home from the following questions[115]:
Mold odor—'Is there, or has there ever been, a smell of mildew in the home?'
Visible mold—'Has there been any visible mold in the home in the past year (prior to the date of the baseline questionnaire)?'
Dampness damage—'Is there, or has there ever been, any type of moisture damage (spots or similar) in the home?'
Any mold or dampness indicator—The presence of any of the three aforementioned exposure indicators.
Exposure score—The sum of mold and dampness indicators (ranging from 0 to 3).
Participants who answered 'I don't know' to the above questions were coded as missing.

3.3.3 Outcome assessment

3.3.3.1 Asthma, rhinitis, rhinoconjunctivitis, and eczema

In studies I, II, and V allergic outcomes at ages 1, 2, 4, 8, 12, and 16 years were derived based on parental answers from questionnaires at the different follow-up periods. In study I incident disease was defined as fulfilling the diagnosis of asthma, rhinitis, or eczema at the specific age without fulfilling it at any prior follow-up.
In study III, allergic outcomes were also derived based on parental answers to questions at each cohort's respective follow-up period, and at ages 14-16 years a combination of children's and parent's answers were utilized. Table 5 summarizes the studies outcome definitions.

Table 5. Definitions of allergic outcomes.		
Variable	**Definition**	**Study**
Asthma at age one year	≥3 episodes of wheeze after three months of age combined either with treatment with inhaled glucocorticosteroids or signs of suspected hyperreactivity (wheezing or severe coughing with exertion and cold weather, or disturbed coughing at night) without concurrent upper respiratory infection.	I, II, V
Asthma at age two years	≥3 episodes of wheeze after one year of age combined with treatment with inhaled glucocorticosteroids or signs of suspected hyperreactivity (wheezing or severe coughing with exertion and cold weather, or disturbed coughing at night) without concurrent upper respiratory infection.	I, II, V
Asthma at ages four, eight, 12, and 16 years	≥4 episodes of wheeze in the last 12 months or ≥1 episode of wheeze during the same time period in combination with occasional or regular treatment with inhaled glucocorticosteroids.	I, II, V
Rhinitis at age one year	Symptoms from eyes or nose after exposure to furred pets or pollen or doctor's diagnosis of allergic rhinitis from the first 3 months of life.	I, II, V
Rhinitis at age two, four, and eight years	Symptoms from eyes or nose after exposure to furred pets or pollen or doctor's diagnosis of allergic rhinitis since the previous questionnaire.	I, II, V
Rhinitis at age 12 years	Symptoms from eyes or nose after exposure to furred pets or pollen during the last 12 months or doctor's diagnosis of allergic rhinitis from the age of 10 years.	I, II, V
Rhinitis at age 16 years	Symptoms from eyes or nose after exposure to furred pets or pollen during the last 12 months or doctor's diagnosis of allergic rhinitis from the age of 12 years.	I, II, V
Eczema at age one year	Dry skin, itchy rashes for ≥2 weeks at specific location (face or arm or leg extension surfaces, or arm or leg flexures, or wrist or ankle flexures) of rash or doctor's diagnosis of eczema after 3 months of age.	I, II
Eczema at age two years	Dry skin, itchy rashes for ≥2 weeks at specific location (face or arm or leg extension surfaces, or arm or leg flexures, or wrist or ankle flexures) of rash or doctor's diagnosis of eczema after 1 year of age.	I, II
Eczema at age four years	Dry skin, itchy rashes for ≥2 weeks during the last 12 months at specific location (face or arm or leg extension surfaces, or arm or leg flexures, or wrist or ankle flexures) of rash or doctor's diagnosis of eczema after 2 years of age.	I, II
Eczema at age eight years	Dry skin, itchy rashes for ≥2 weeks during the last 12 months at specific location (face or arm or leg flexures, or wrists or ankles, or neck) of rash or doctor's diagnosis of eczema after 7 years of age.	I, II
Eczema at age 12 years	Dry skin, itchy rashes during the last 12 months at specific location (arm or leg flexures, or wrists or ankles, or neck) of rash or doctor's diagnosis of eczema after 10 years of age.	I, II
Eczema at age 16 years	Dry skin, itchy rashes during the last 12 months at specific location (arm or leg flexures, or wrists or ankles, or neck) of rash or doctor's diagnosis of eczema after 12 years of age.	I, II
Asthma at ages 4-6 years (MeDALL definition)	Positive answer to two out of three: 1) Doctor's diagnosed asthma ever (parental reported); 2) Asthma medication in the past 12 months (parental reported); 3) Wheezing in the past 12 months (parental reported)	III
Asthma at ages 8-10 years (MeDALL definition)	Positive answer to two out of three: 1) Doctor's diagnosed asthma ever (parental reported); 2) Asthma medication in the past 12 months (parental reported); 3) Wheezing in the past 12 months (parental reported)	III
Asthma at ages 14-16 years (MeDALL definition)	Positive answer to two out of three: 1) Doctor's diagnosed asthma ever (parental reported); 2) Asthma medication in the past 12 months (child reported, if	III

	available); 3) Wheezing in the past 12 months and/or breathing difficulties, where available (child reported, if available)	
Rhinoconjunctivitis at ages 4-6 years (MeDALL definition)	Positive answer to the following questions: 1) In the past 12 months problems with sneezing, or a runny, or blocked nose when child did not have a cold or flu (parental reported); 2) In the past 12 months, has this nose problem been accompanies by itchy-watery eyes (parental reported).	III
Rhinoconjunctivitis at ages 8-10 years (MeDALL definition)	Positive answer to the following questions: 1) In the past 12 months problems with sneezing, or a runny, or blocked nose when child did not have a cold or flu (parental reported); 2) In the past 12 months, has this nose problem been accompanies by itchy-watery eyes (parental reported).	III
Rhinoconjunctivitis at ages 14-16 years (MeDALL definition)	Positive answer to the following questions: 1) In the past 12 months problems with sneezing, or a runny, or blocked nose when child did not have a cold or flu (child reported, if available); 2) In the past 12 months, has this nose problem been accompanies by itchy-watery eyes (child reported, if available).	III

3.3.3.2 Clinical phenotypes

To study timing of onset, progression, and persistence of allergic diseases we classified asthma, rhinitis, and rhinoconjunctivitis into early-transient, persistent, and adolescent/late-onset clinical phenotypes in studies III and V. In study III early-transient disease was defined as having the disease only during the age interval 4-6 years, but not after (age intervals 8-10 and 14-16 years). Persistent disease was defined as having the disease of interest at the first age interval (4-6 years) and still having the disease at the latest age interval (14-16 years). Adolescent-onset phenotype was defined as being disease free at the first two age intervals (4-6 and 8-10 years) and only having the disease at the latest age interval (14-16 years).

In study V comparable definitions were used. Asthma phenotypes were defined as: early-transient asthma and was classified as having asthma at ages 1, 2, or 4 years but not again at any successive follow-ups; persistent asthma was classified as having asthma at ages 1, 2, or 4 years and then again at ages 8, 12, or 16 years; and late-onset asthma was defined as having the first incidence of asthma at ages 8, 12, or 16 years.[115]

3.3.3.3 IgE sensitization

At ages 4, 8, and 16 years, blood sampling was performed in 2614 (64%), 2470 (60%), and 2547 (62%) participants respectively. At each follow-up, sera were screened with Phadiatop® and fx5® for various inhalant and food allergens respectivly.[91] Sera that scored positive for Phadiatop® or fx5® were subsequently analyzed for allergen-specific IgE antibodies. A technical cut-off at 0.35 kU_A/l was set.

Any allergen sensitization was defined as a positive reaction to any of the tested allergens. Inhalant allergens were classified as either indoor (cat, dog, horse, and/or house dust mite) or outdoor (timothy grass, birch, mugwort, and/or mold).[91] Food allergen sensitization was defined as sensitization to any tested food allergen (cow's milk, hen's egg, soybean, peanut, cod fish or wheat). In studies II and V to explore the effect of exposure (SHS or mold/dampness) on sensitization combined with symptoms we characterized allergic vs non-allergic phenotypes by combining symptoms of asthma, rhinitis, and eczema with and without sensitization.

3.3.3.4 Lung function

Lung function testing was performed in 2605 participants (spirometry or impulse oscillometry) at age 16 years. Indices of pulmonary function were analyzed by spirometry and by IOS using the Jaeger MasterScreen-IOS system (Carefusion Technologies, San Diego, CA) and have been described in depth elsewhere.[147] For spirometry indices, multiple maximal expiratory flow volume measurements were performed by each participant and the highest values of FEV_1, and FVC were used in analyses.[148] The FEV_1/FVC ratios were expressed as percentages. Standard deviation scores for FEV_1, FVC, and FEV_1/FVC were computed taking age, sex, height, and ethnicity into account using the Global Lung Function Initiative (GLI) reference values.[149] The spirometer was calibrated daily using a 3-Liter precision syringe.

Further information on the IOS system has also been given in detail elsewhere.[29 148 150 151] In study V, IOS indices were assessed by having participants perform tidal breathing through a mouthpiece while pressure impulses were delivered from a loud speaker throughout the respiratory tract.[148] Each participant performed the maneuver at least twice and quality control checks were conducted by visual examination of waveforms at the time of the maneuver.[150] The mean value of resistance at 5 Hertz (Hz) (R_5), resistance at 20 Hz (R_{20}), frequency dependence of resistance (R_{5-20}), and the square root of the area of reactance ($AX^{0.5}$) were used in the final analyses. Daily accuracy checks for the IOS system were conducted using a reference resistance (0.20 $kPa \cdot L^{-1} \cdot s^{-1}$).[150]

Fractional exhaled nitric oxide (FeNO) is a simple noninvasive test which reflects eosinophilic airway inflammation.[152] We used an Eco Medic instrument system (ECO MEDICS AG, Dürnten, Switzerland) and single breath technique was used according to the ATS and ERS guidelines.[153] FeNO was expressed as parts per billion (ppb).

3.4 STATISTICAL ANALYSES

The majority of statistical analyses were performed with STATA (release 12; Stata Corp., College Station, TX, USA). In study III R, version 3.2.2 (R Core Team, 2012) was also used.

3.4.1 Prevalence and incidence

We used prevalence and incidence to summarize the frequency of exposure, outcomes, and sociodemographic characteristics in our study populations. Prevalence and incidence were presented as frequency or percent of total.

3.4.2 Chi square test

The chi square test, also written as X^2, is used to determine whether there are significant differences between expected frequencies and the observed frequencies between categorical variables. In the context of this thesis the chi square test was used to compare the complete baseline cohort with selected study populations with regards to sociodemographic characteristics and potential confounding factors.

3.4.3 Logistic regression

Logistic regression is a statistical method used to assess associations between two variables when the dependent variable (outcome) is binary. In general, logistic regression was used in studies I-V to identify potential confounding factors by conducting stepwise logistic regression. In studies I, II, III, and V final covariates were selected if they lead to more than 5% change in odds ratio (OR) or were selected based on putative risk factors. In study IV, covariates were selected if they changed the β coefficient by 10% and likelihood ratios test was significantly different (p-value <0.05) from a simpler model. The magnitude of associations are represented as odds ratios (OR) with corresponding 95% confidence intervals (CI).

In study I final covariates included sex, parental history of allergic disease, and socioeconomic status. In some analyses we also adjusted for parental smoking throughout childhood which was conducted to account for any SHS after infancy. Furthermore, we tested if there was any effect modification by sex or allergic heredity with exposure to maternal smoking during pregnancy or parental smoking during infancy by including interaction terms.

Study II included parental history of allergic disease and socioeconomic status as final covariates. As with study I we also adjusted some models for SHS throughout childhood. We tested if there was any significant interaction between SHS in infancy and parental allergic disease. Additionally, we stratified by maternal smoking during pregnancy.

In study III, sex, parental history of allergic disease, parental educational level, older siblings, study centers (GINIplus, LISAplus, and PIAMA), and intervention versus observational study arms (GINIplus and PIAMA) were selected based on subject-matter knowledge and covariates selected based on model testing included daycare attendance and breastfeeding. To separate the effects of maternal smoking during pregnancy from other postnatal SHS exposure, in some models we adjusted for SHS throughout childhood (age intervals 1-2, 4-6, 8-10, and 14-16 years).

Confounders selected in study IV included age, sex, and height and were selected using a stepwise linear regression model. We also tested a mutually adjusted model which included smoking during pregnancy, SHS during infancy, SHS exposure at age 16 years, and adolescent smoking at age 16 years to assess any independent effects. Furthermore, we stratified our analyses by sex and by wheeze in the last 12 months.

The final covariates included in study V consisted of sex, socioeconomic status, parental history of allergic disease, maternal smoking during pregnancy, parental smoking during infancy, maternal age, and older siblings. We also stratified our analyses by building construction period due to various building materials and techniques utilized at different time periods.

3.4.4 Multinomial logistic regression

Multinomial logistic regression is a statistical method similar to logistic regression, but instead of having a binary dependent variable this model can predict the probabilities of different possible outcomes of a categorically distributed dependent variable. Multinomial logistic regression was used in studies II, III, and V.

3.4.5 Generalized estimating equations (GEE)

Used in studies I, II, III, and V, GEE modeling was the most prevalent statistical method used in this thesis. GEE models are a type of mixed model that are used in the analysis of longitudinal data.[154] We used an unstructured correlation matrix, and in studies I and II an interaction term with time was included to assess the effect of exposure over time.

3.4.6 Meta-analysis

Random effects meta-analysis is a statistical method which is used to combine effect estimates from different studies, and in the case of study III effect estimates from cohort specific GEE analyses. One can combine estimates using fixed or random effects. A fixed-effect meta-analysis estimates a single effect that is assumed to be common to every study, whereas random-effect meta-analysis estimates the mean of a distribution of effects and takes into account within-cohort and between-cohort variation.[155]

3.4.7 Linear regression and quantile regression

Linear regression is a statistical approach used to assess the association between a continuous dependent variable with an independent variable (exposure). Quantile regression, sometimes referred to as regression on the median, is used when data are skewed and is robust to outliers. Both linear and quantile regression were used in study IV.

3.5 ETHICAL APPROVAL

The BAMSE project was approved by the regional ethics committee at Karolinska Institutet, Stockholm, Sweden (reference numbers – 93:189; 98:175; 02:420; and 2007/1634-31).

The birth cohorts included in study III have also been approved by their respective ethical review boards.

4 RESULTS

4.1 PREVALENCE OF TOBACCO SMOKE EXPOSURE IN BAMSE

In BAMSE the prevalence of maternal smoking during pregnancy was 12.7% and parental smoking during infancy was somewhat higher at 20.8%. Figure 5 presents the prevalence of exposure to tobacco smoking during pregnancy to age 16 years in BAMSE. Parental smoking slowly declined from around 20% at birth to 14% at age 16 years. Figure 6 illustrates the overlap between maternal smoking during pregnancy and parental smoking during infancy. In BAMSE paternal smoking habits were not queried at baseline, however this question was asked at the 12 year follow-up and 17% (n=566) indicated they smoked during the mothers' pregnancy.

At age 16 years 280 (12.2%) of adolescents were smoking, 102 (4.4% of the study population) smoked daily and 178 (7.8% of the study population) smoked occasionally. The mean age of smoking onset (≥1 cigarette/week) was age 14.7 years. The prevalence of smoking was comparable between males and females. However, of the 102 (4.4%) participants who used smokeless tobacco (snus/moist snuff) only five were females. Nearly two thirds of those who used smokeless tobacco were also active smokers. Saliva cotinine concentrations were higher among males than females (geometric mean 0.21 ng/ml versus 0.13 ng/ml), and is likely explained by the higher smokeless tobacco use among males.

Figure 5. Prevalence of tobacco smoke exposure in BAMSE.

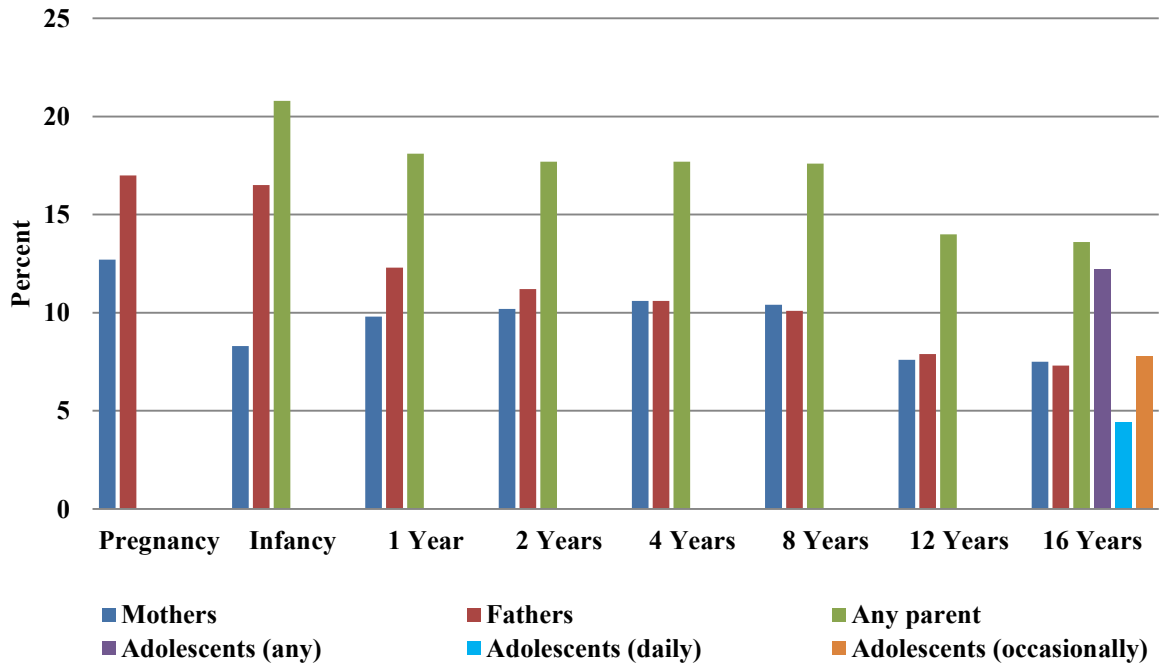
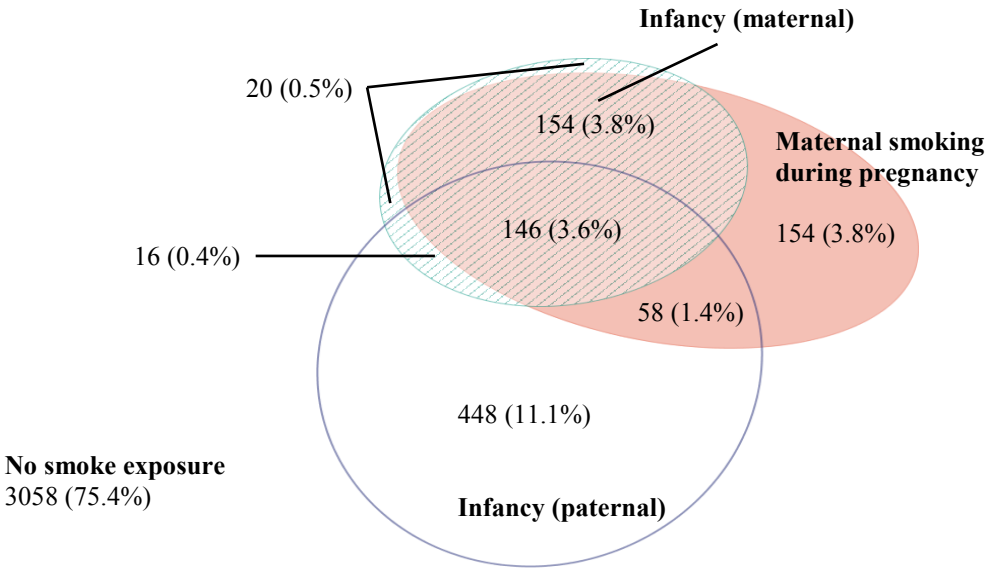


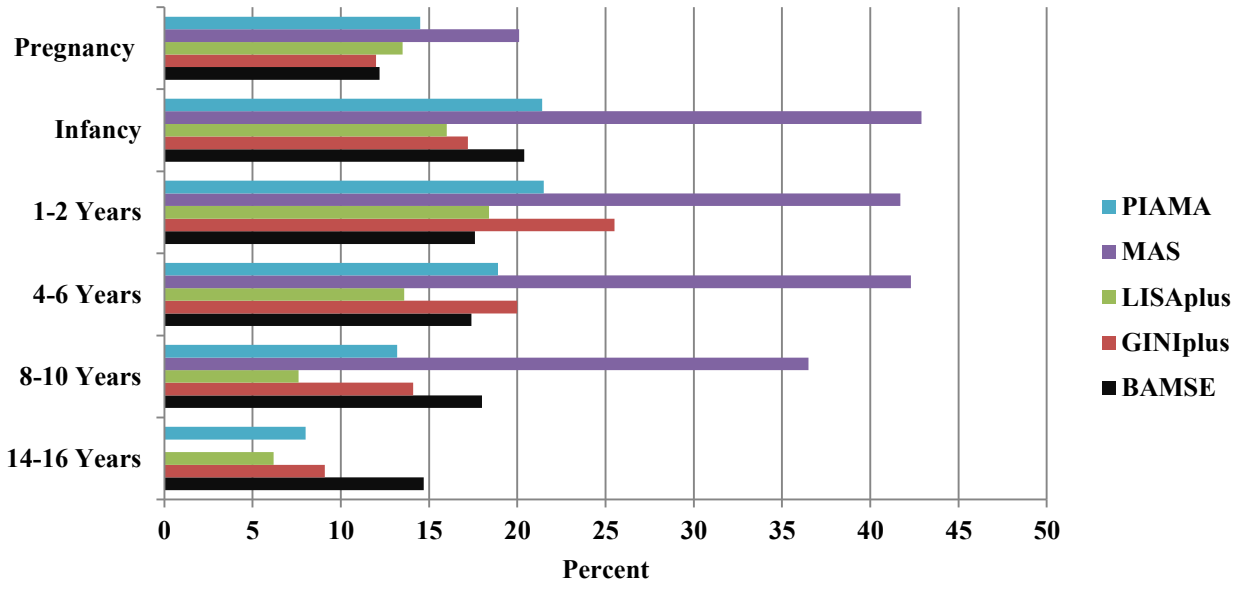
Figure 6. The overlap between tobacco smoke exposure during pregnancy and infancy in BAMSE.



4.2 PREVALENCE OF TOBACCO SMOKE EXPOSURE IN MEDALL

The prevalence of maternal smoking during pregnancy ranged from 12-20%, and exposure to SHS during infancy ranged from 16-43% across the cohorts included in study III (Figure 7). The prevalences of SHS exposure from ages 1-16 years are also presented in Figure 7 below.

Figure 7. Prevalence of tobacco smoke exposure in MeDALL.



4.3 PREVALENCE OF ALLERGIC DISEASES AND SENSITIZATION

4.3.1 Prevalence of allergic diseases in BAMSE

The prevalences of asthma, rhinitis, and eczema in BAMSE are presented in Figure 8. Over ages 1 to 16 years asthma averaged around 6%, while rhinitis became increasingly more prevalent as children became older, and by age 16 years 25.4% had rhinitis. Eczema was more common in pre-school ages but tended to decline as participants became older, and at age 16 years 9.8% had eczema.

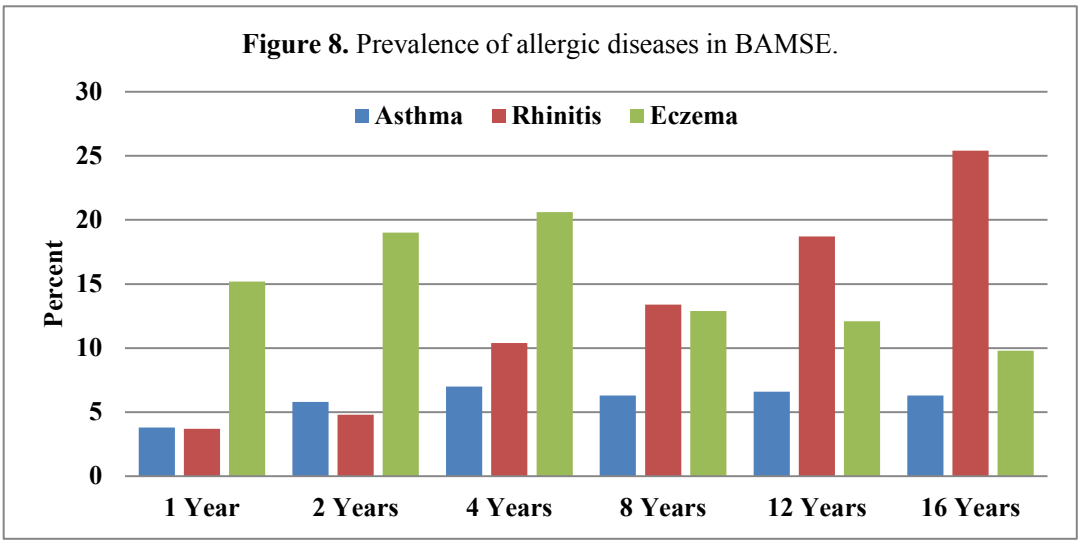


Figure 8. Prevalence of allergic diseases in BAMSE.

4.3.2 Prevalence of sensitization in BAMSE

The prevalence of sensitization to any allergen at ages 4, 8, and 16 years were 24.1%, 34.8%, and 45.9% respectively, and the overlap between any sensitization at these ages is illustrated in Figure 1, Manuscript II. Sensitization became more prevalent and few children lost their sensitization. In addition, Figures 9 and 10 illustrate the overlap of sensitization to inhalant allergens and food allergens at ages 4, 8, and 16 years respectively. In general, inhalant allergen sensitization became increasingly more prevalent, whereas participants tended to lose their food allergen sensitization.

Figure 9. Proportional relationship of sensitization to inhalant allergens at ages 4, 8, and 16 years among participants in BAMSE.

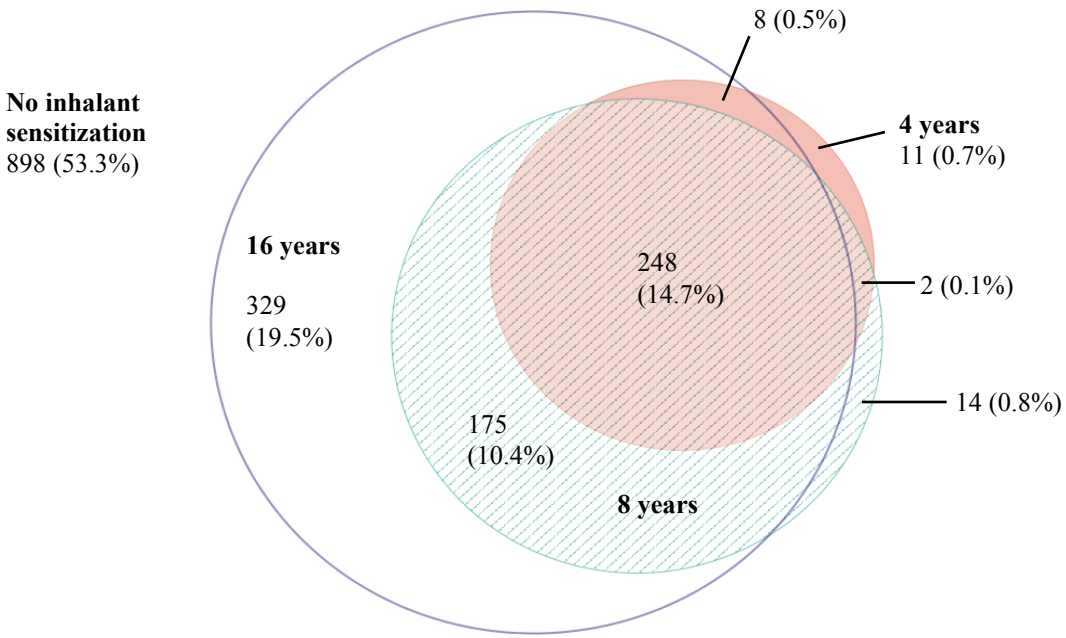
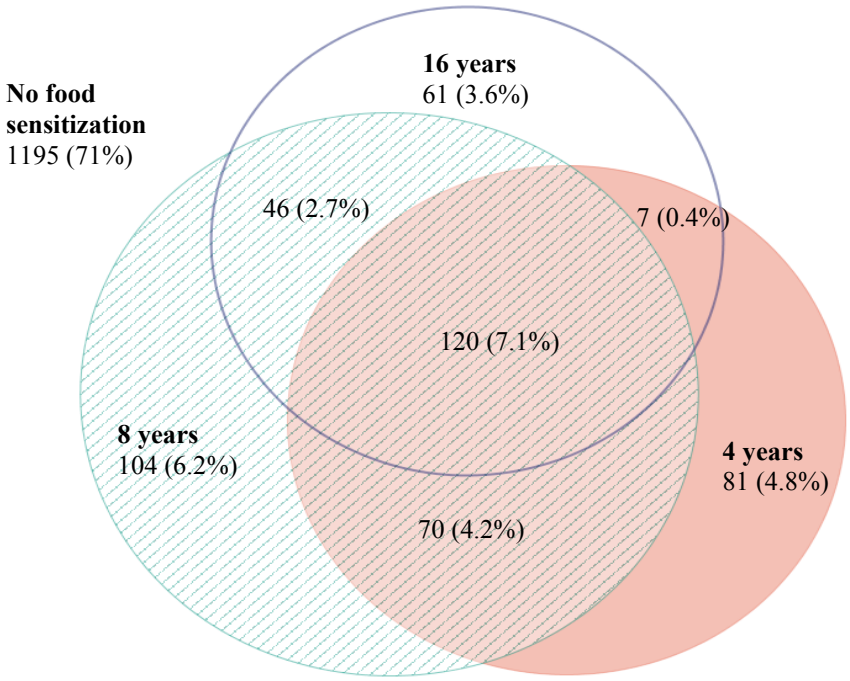


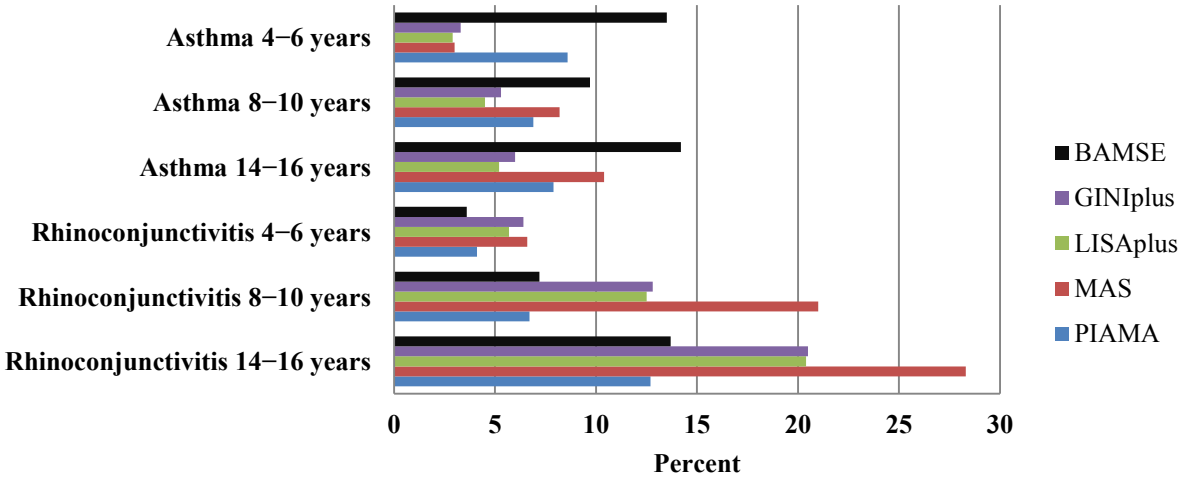
Figure 10. Proportional relationship of sensitization to food allergens at ages 4, 8, and 16 years among participants in BAMSE.



4.3.3 Prevalence of allergic diseases in MeDALL

The prevalence of asthma and rhinoconjunctivitis varied across the cohorts included in study III. BAMSE consistently had the highest prevalence of asthma and the German cohorts (GINIplus, LISAplus, and MAS) had higher prevalences of rhinoconjunctivitis (Figure 11), but in all cohorts rhinoconjunctivitis increased with age. The prevalence of asthma phenotypes were similar at around 4%, and the prevalence of rhinoconjunctivitis phenotypes were highest for adolescent-onset (12.2%) and lowest for early-transient (1.6%).

Figure 11. Prevalence of asthma and rhinoconjunctivitis in MeDALL.



4.4 LUNG FUNCTION SUMMARY STATISTICS

Table 6 below summarizes the anthropometric and lung function characteristics of participants with lung function data at age 16 years. Generally, males had larger lung volumes than females, as expected, but had lower airway resistance.

Table 6. Anthropometric and lung function characteristics among children at age 16 years in BAMSE.

	16 years					
Variable	**Males**			**Females**		
	N*	**Mean**	**SD**	**N***	**Mean**	**SD**
Age, years	1092	16.7	0.4	1203	16.7	0.4
Height, meters	1092	1.8	0.07	1203	1.7	0.06
FEV_1, milliliters	1052	4501.8	644.5	1175	3484.9	443.6
FEV_1, z-score	1051	-0.03	0.96	1175	-0.04	0.90
FVC, milliliters	969	5382.7	778.6	1140	4037.6	526.0
FVC, z-score	968	0.15	0.95	1140	0.15	0.88
FEV_1/FVC, %	929	83.8	6.6	1112	86.5	6.1
FEV_1/FVC, z-score	929	-0.30	0.98	1112	-0.36	0.95
Saliva cotinine level (ng/ml)‖	706	0.21	10.0	817	0.13	7.0
Impulse oscillometry	**N***	**Median**	**IQR**	**N***	**Median**	**IQR**
R_5, $(Pa*L^{-1})*s$	1051	325.0	95.0	1143	400.0	100.0
R_{20}, $(Pa*L^{-1})*s$	1051	310.0	75.0	1143	375.0	85.0
R_5-R_{20}, $(Pa*L^{-1})*s$	1051	15.0	45.0	1143	20.0	55.0
$AX^{0.5}$, $(Pa*L^{-1})$	1050	160.0	130.0	1143	270.0	195.0
FeNO (ppb)	1081	16.0	14.1	1188	12.8	10.4

* Total number of subjects.
‖ Geometric mean.

4.5 MATERNAL SMOKING DURING PREGNANCY IN RELATION TO ALLERGIC DISEASES AND LUNG FUNCTION

4.5.1 Allergic diseases

In studies I and III we observed consistent associations between maternal smoking during pregnancy and asthma up to adolescence. In study I we found that children exposed to maternal smoking during pregnancy had increased overall risk of asthma up to age 16 years (OR = 1.45; 95% CI, 1.15–1.83) (Figure 12). Moreover, following adjustment for parental smoking throughout childhood, the risk of prevalent and incident asthma remained elevated (see manuscript I, Table 2). Likewise in study III, results from meta-analyses also suggested increased risk of asthma up to ages 14-16 years (OR = 1.19; 95% CI, 0.98-1.43) (Figure 13). More specifically, in study III we observed increased risk of early-transient asthma (OR = 1.71; 95% CI, 1.21-2.41), which is in line with study I where the highest risks of asthma were seen in early childhood.

Figure 12. Association between maternal smoking during pregnancy in relation to asthma, rhinitis, and eczema during childhood and adolescence in BAMSE. Adjusted for sex, parental allergic disease, and socioeconomic status. ○ Asthma; ● Rhinitis; ● Eczema.

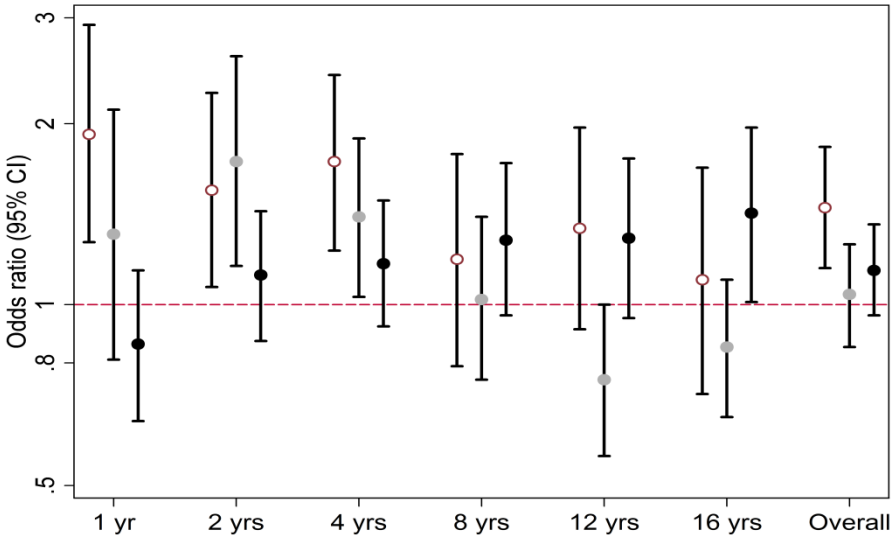
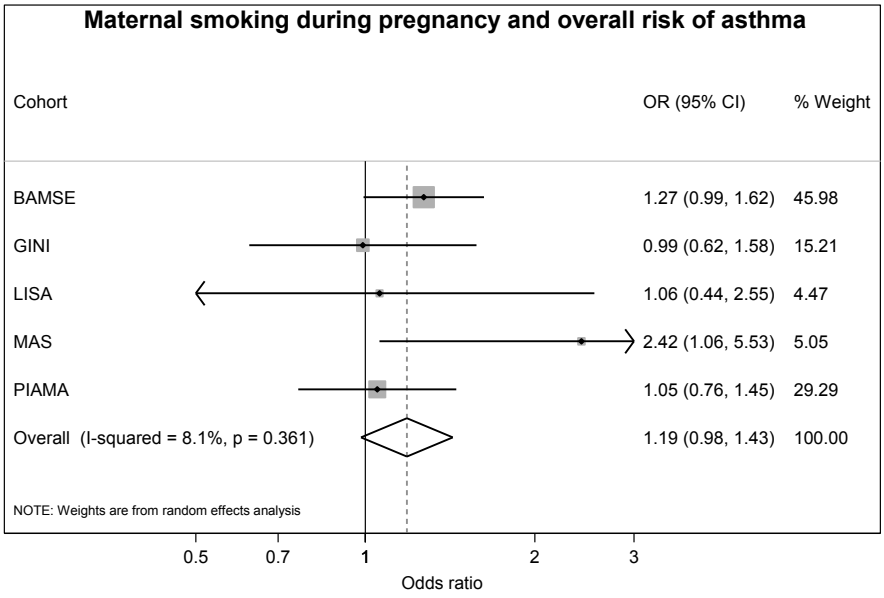


Figure 13. Associations between maternal smoking during pregnancy and prevalent asthma up to 14 to 16 years of age in MeDALL. Cohort specific ORs and 95% CIs were obtained by GEE adjusted for sex, parental education level, parental allergy, older siblings, breastfeeding, study center, intervention arm, and early day-care attendance.



A significant dose-response relationship for increasing number of cigarettes smoked during pregnancy and risk of asthma was seen in study I, and children exposed to ≥10 cigarettes per day during any trimester of pregnancy had increased risk for asthma up to age 16 years (OR = 1.68; 95% CI, 1.23-2.29). Similarly, in study III children exposed to high doses of maternal smoking during pregnancy (≥10 cigarettes/day) had significantly increased risks of both early-transient asthma and persistent asthma (OR = 2.07; 95% CI, 1.60-2.68 and OR = 1.66; 95% CI, 1.29-2.15, respectively). Additionally, in study III we also observed evidence for persistent rhinoconjunctivitis among participants exposed to maternal smoking during pregnancy (OR = 1.77; 95% CI, 1.20-2.59).

No consistent associations between maternal smoking during pregnancy and IgE sensitization, rhinitis, or eczema were observed in studies I and II.

4.5.2 Lung function

In study IV, participants exposed to maternal smoking during pregnancy had significantly reduced FEV_1/FVC ratios at age 16 years (-1.1% (95% CI, -2.0 to -0.2)). This reduction in FEV_1/FVC ratios remained after mutually adjusting for adolescent smoking, SHS during infancy, and SHS smoke at age 16 years (-1.0% (95% CI, -2.1 to 0.02)), albeit not statistically significant. Indices from IOS suggested increased airway resistance among individuals exposed to maternal smoking during pregnancy. Compared to unexposed participants, those exposed to maternal smoking during pregnancy had significant increases in R_5 (13.1 $Pa \cdot L^{-1} \cdot s$ (95% CI, 1.4 to 24.9)), R_{5-20} (9.2 $Pa \cdot L^{-1} \cdot s$ (95% CI, 2.8 to 15.6)), and $AX^{0.5}$ (21.8 $(Pa \cdot L^{-1} \cdot s)^{0.5}$ (95% CI, 3.9 to 39.7)) at age 16 years.

4.6 SHS EXPOSURE AND ALLERGIC DISEASES

4.6.1 Allergic diseases

In studies I and III we explored the association between exposure to SHS during infancy with asthma, rhinitis, rhinoconjunctivitis, and eczema. In BAMSE, exposure to parental smoking during infancy was associated with an overall increased risks of asthma (OR = 1.23; 95% CI, 1.01-1.51), rhinitis (OR = 1.18; 95% CI, 1.01-1.39), and eczema (OR = 1.26; 95% CI, 1.09-1.45) up to age 16 years (Figure 14). Similarly, in study III we observed combined elevated risks of overall asthma among participants exposed to SHS during infancy (OR = 1.15; 95% CI, 1.00-1.31). However, due to the high correlation between maternal smoking during pregnancy and SHS exposure during infancy, in sensitivity analyses we attempted to disentangle these exposures. Because we had increased numbers in study III, we categorized exposure into maternal smoking during pregnancy only, parental smoking during infancy only, and exposure to both. We found evidence suggesting that the association between SHS exposure during infancy and asthma risk was mainly driven by exposure during pregnancy (Figure 15).

In study III SHS exposure during infancy was not associated with overall risk of rhinoconjunctivitis up to adolescence (OR = 1.05; 95% CI, 0.92-1.19), nor was SHS during

infancy associated with phenotypes of asthma or rhinoconjunctivitis. We also examined whether exposure to SHS during different periods of childhood (infancy, 1-2, 4-6, 8-10, or 14-16 years) was associated with the risk of adolescent-onset asthma and rhinoconjunctivitis, but following adjustment for maternal smoking during pregnancy, no significant associations were apparent.

Figure 14. Associations between SHS exposure during infancy in relation to asthma, rhinitis, and eczema during childhood and adolescence in BAMSE. Adjusted for sex, parental allergic disease, and socioeconomic status. ○ Asthma; ● Rhinitis; ● Eczema.

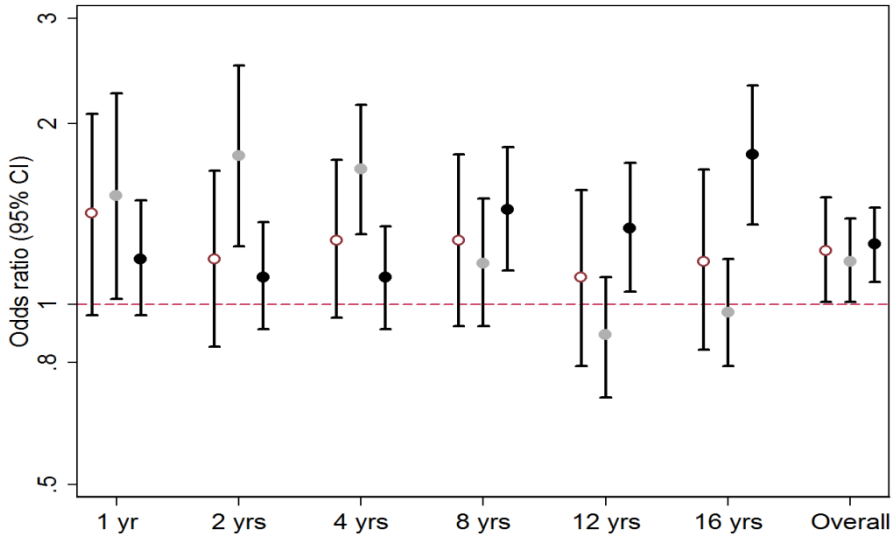
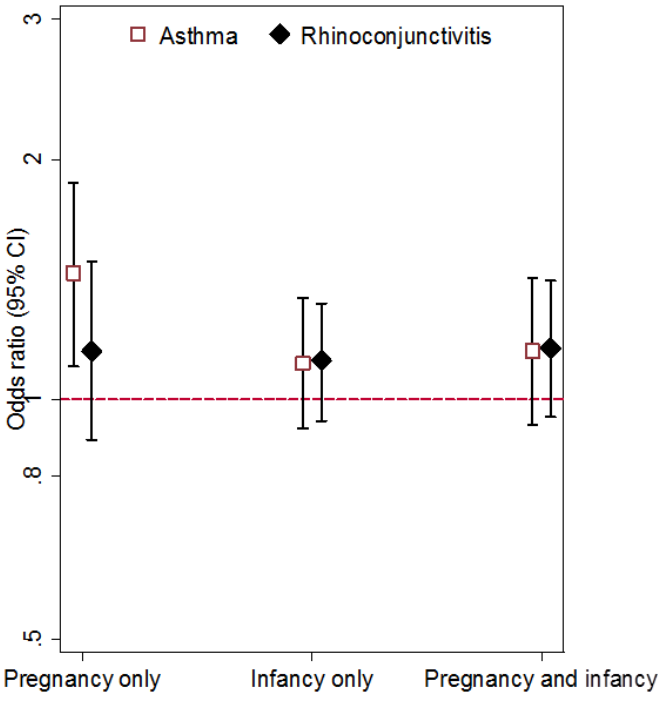


Figure 15. Associations between maternal smoking during pregnancy only, SHS exposure during infancy only, and both in relation to asthma and rhinoconjunctivitis up to age 14-16 in MeDALL.



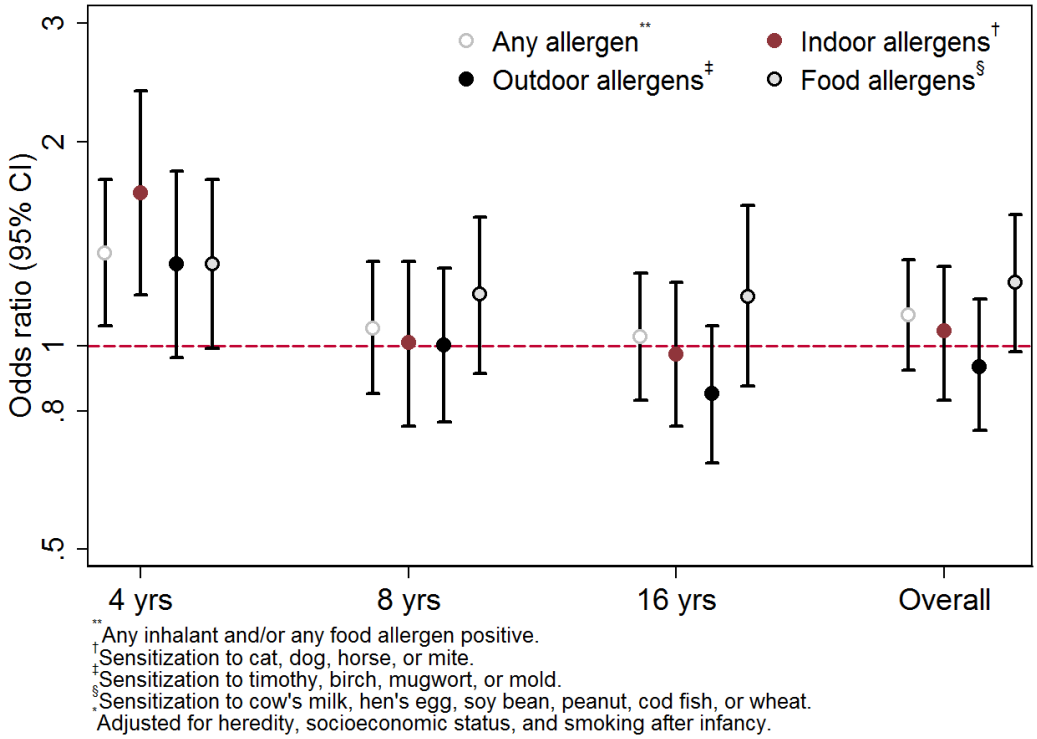
*OR and 95% CI were obtained by a GEE model adjusted for sex, parental education level, parental allergy, older siblings, breastfeeding, study center, intervention arm, and early daycare attendance.

4.6.2 IgE sensitization

In study II we evaluated the association between tobacco smoke exposure and the development of sensitization. Participants exposed exclusively to SHS exposure during infancy had increased risk of food allergen sensitization at age four years (OR = 1.47; 95% CI, 1.08-2.00), and nonsignificantly increased risks at ages eight (OR = 1.34; 95% CI, 0.99-1.79) and 16 years (OR = 1.32; 95% CI, 0.93-1.86). At age four years there was an increased risk of indoor allergen sensitization among participants exposed exclusively to SHS exposure during infancy (OR = 1.50; 95% CI, 1.00-2.24), but was not apparent at later ages.

In longitudinal analyses, we found that participants exposed during infancy had a borderline overall increased risk of food allergen sensitization up to age 16 years (OR = 1.24; 95% CI, 0.98-1.56) (Figure 16). At ages 4, 8, and 16 years there was a tendency for increased risks for food allergen sensitization. In analyses of single allergens we observed positive associations for all food allergens with the exception of cod fish (see Manuscript II, Table 2). In addition, dose response analyses indicated that children exposed to ≥10 cigarettes per day had increased risk of food allergen sensitization (see Manuscript II, Table 3).

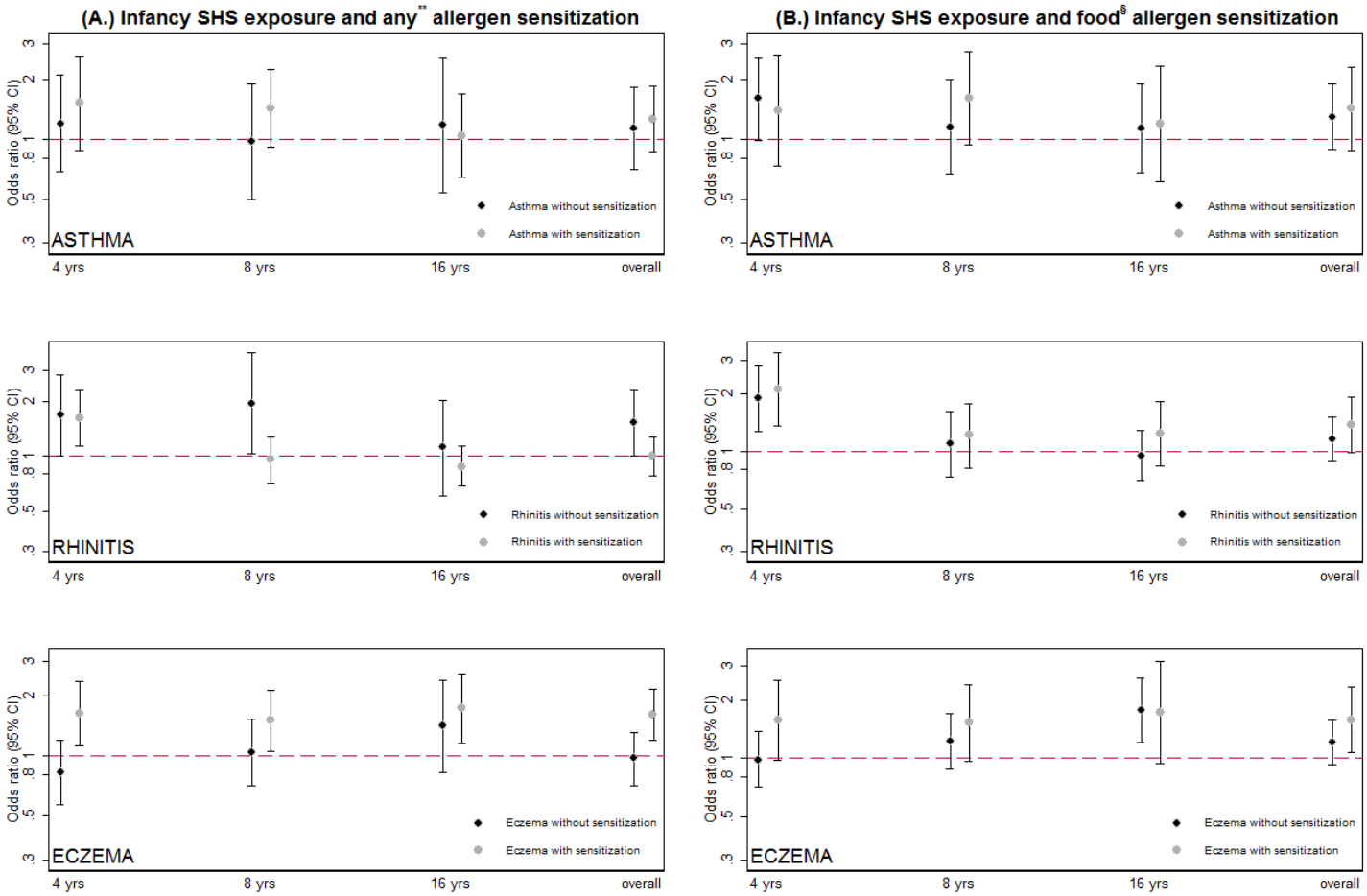
Figure 16. Association between exposure to SHS during infancy and the risk of allergic sensitization during the first 16 years of life in BAMSE.



We were curious to see if this association was only present for participants with allergic heredity, so we stratified our findings by those with and without parental history of allergic disease. We found no statistically significant differences between those participants with (OR = 1.36; 95% CI, 0.90-2.06) and without (OR = 1.17; 95% CI, 0.88-1.57) allergic parents.

Being sensitized does not necessarily mean you present with symptoms of allergic disease. Therefore, we combined any sensitization and food allergen sensitization with symptoms of asthma, rhinitis, and eczema (Figure 17). In these analyses we observed no associations with exposure to SHS during infancy and asthma with or without sensitization. However, we observed an increased risk for rhinitis among nonsensitized participants (OR = 1.53; 95% CI, 1.01-2.31) and an overall increased risk of eczema in those participants with any sensitization (OR = 1.62; 95% CI, 1.20-2.18).

Figure 17. Association between SHS exposure during infancy and the risk of any or food allergen sensitization and allergic diseases in BAMSE.



**Any inhalant and/or any food allergen positive.
§Sensitization to cow's milk, hen's egg, soy bean, peanut, cod fish, or wheat.
* Adjusted for heredity, socioeconomic status, and smoking after infancy.

4.7 ADOLESCENT SMOKING IN RELATION TO LUNG FUNCTION AT AGE 16 YEARS

4.7.1 Adolescent smoking and lung function at age 16 years

The association between adolescent smoking and lung function are presented in Table 7. Compared with non-smokers, participants who indicated they smoked daily or occasionally had reduced FEV_1/FVC ratios at age 16 years (-0.9% (95% CI, -1.8 to -0.1)). In a model combining maternal smoking during pregnancy, SHS during infancy, SHS exposure at 16 years, and adolescent smoking, FEV_1/FVC ratios were non-significantly lower among adolescent smokers at age 16 years (-0.8% (95% CI, -1.7 to 0.1)). Using indices from IOS we found that adolescent smoking was associated with a significant increase in R_{5-20} (6.5 Pa·L–1·s (95% CI, 0.7 to 12.2)) at age 16 years, which is indicative of increased airway resistance in the peripheral airways. However, no associations were seen for R_5, R_{20}, or $AX^{0.5}$.

Table 7. Differences in lung function between exposed and unexposed participants to tobacco smoke and lung function at age 16 years in BAMSE.

		FEV_1 (ml)		**FVC (ml)**		**FEV_1/FVC (%)**	
	n	**Diff.***	**95% CI**	**Diff.***	**95% CI**	**Diff.***	**95% CI**
Participants smoking							
Non-smokers	2015	Reference		Reference		Reference	
Adolescent smoking†	280	-12.6	-69.3;44.2	16.6	-50.1;83.3	-0.9	-1.8;-0.1
Occasional smokers	178	-34.4	-103.6;34.8	17.2	-64.1;98.4	-1.1	-2.1;-0.04
Daily smokers	102	26.3	-64.3;116.9	15.6	-91.2;122.3	-0.7	-2.1;0.6

* Calculated by linear regression on the mean adjusted for sex, age, and height.
† Daily or occasional smoking.

4.7.2 Saliva cotinine levels and lung function

When we excluded smokeless tobacco users and used a cut-off of ≥12 ng/ml to discriminate adolescent smokers from non-smokers, we correctly classified 97.6% of daily smokers. Our finding of a lower FEV_1/FVC ratios among active smokers was corroborated when using this cut-off. We observed a decline in FEV_1/FVC ratios of -1.5% (95% CI, -2.5 to -0.4) compared to children with a saliva cotinine concentration of <12 ng/ml.

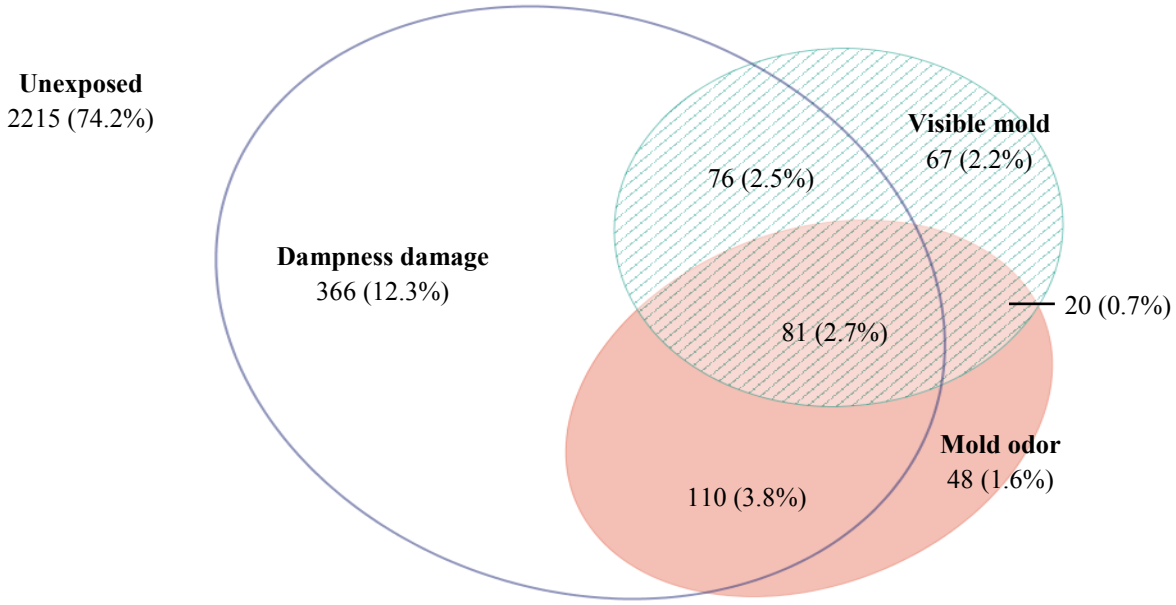
4.8 MOLD AND DAMPNESS EXPOSURE AND ALLERGIC DISEASES

4.8.1 Asthma, rhinitis, and sensitization

In study V we examined if indoor dampness or mold was associated with increased risk of asthma, rhinitis, and sensitization in childhood or adolescence.

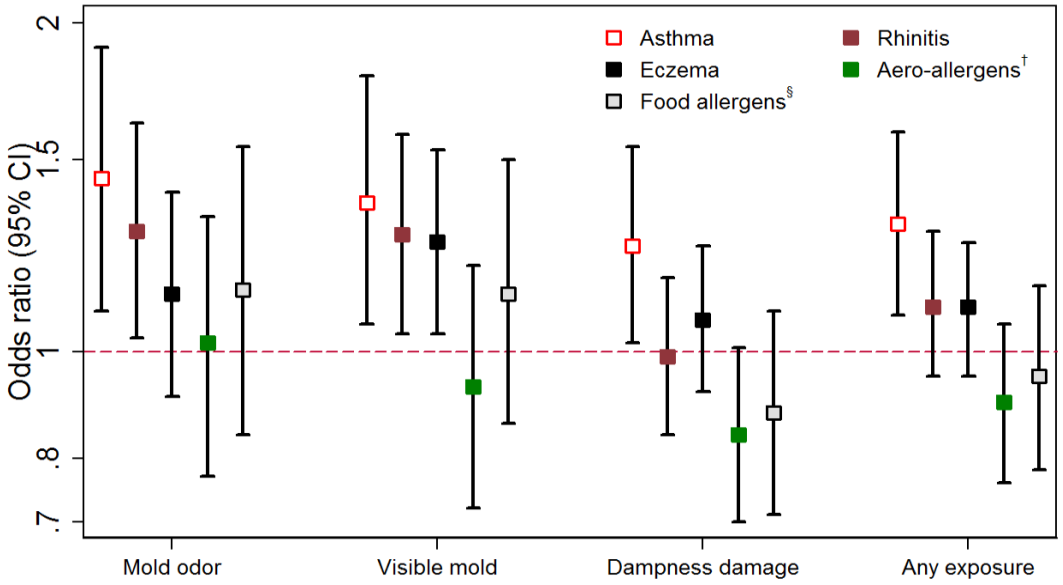
Using questionnaires from baseline (two months) we ascertained how many participants were exposed to mold odor, visible mold, or dampness damage. Of the 3798 included participants, 299 (9.1%) were exposed to mold odor, 325 (8.6%) to visible mold, 758 (23.5%) to dampness damage, and 967 (30.4%) to any mold or dampness indicator at baseline. Figure 18 below illustrates the overlap between these exposures.

Figure 18. Distribution and proportional relationship of mold odor, visible mold, and dampness damage exposure at baseline in BAMSE (n=2983).



In longitudinal analyses from birth to age 16 years, participants exposed to mold odor, visible mold, or dampness damage during infancy had an overall increased risk of asthma (Figure 19). An increased risk was seen for rhinitis, but only in participants exposed to mold odor (OR = 1.29; 95% CI, 1.03-1.62) or visible mold (OR = 1.28; 95% CI, 1.04-1.58).

Figure 19. Associations between exposure to mold or dampness during infancy and the risk of asthma, rhinitis, eczema, aero-allergen or food allergen sensitization up to age 16 years in BAMSE.



* Odds ratios (OR) and 95% confidence intervals (CI) obtained from GEE adjusted for sex, parental history of allergy, socioeconomic status, maternal smoking during pregnancy, parental smoking in the first two months, maternal age <26 years, and presence of siblings.
†Sensitization to cat, dog, horse, mite, timothy, birch, mugwort, or mold.
§Sensitization to cow's milk, hen's egg, soy bean, peanut, cod fish, or wheat.

Participants exposed to an increasing number of mold or dampness indicators (exposure score) showed a significant trend for increased odds of asthma (p_{trend}=0.002) (Table 8). And a similar, however non-significant trend was observed for rhinitis (p_{trend}=0.14).

No overall or dose dependent associations with indicators of mold or dampness during infancy and IgE sensitization to airborne or food allergens were observed. However, an increased risk for non-allergic asthma (OR = 1.80; 95% CI, 1.27–2.55) and non-allergic rhinitis (OR = 1.41; 95% CI, 1.03–1.93) were observed in participants exposed to any mold or dampness indicator during infancy.

Interestingly, we observed somewhat higher risks of rhinitis in participants living in homes built after 1975 compared with those homes constructed before 1961 (Table 4, manuscript V). This could have to do with building construction practices and materials during various periods, as oil prices increased in 1974, and a focus on energy efficiency became increasingly important.[156] For example, the introduction of self-leveling mortar from 1977 to 1983 has since shown to emit chemicals such as ammonia and sulfhydryl compounds in the presence of moisture.[115 117] However, we observed no significant interaction between exposure to mold or dampness indicators and asthma or rhinitis.

Table 8. Severity of exposure to mold or dampness indicators[*] in relation to overall asthma, rhinitis, and sensitization among children in BAMSE.

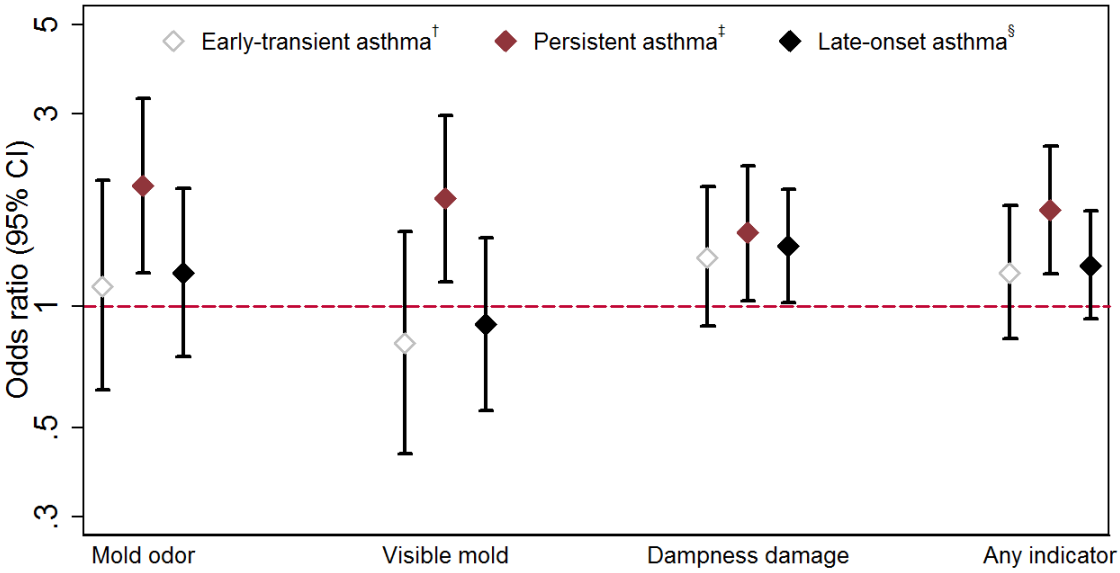
	Exposure score (crude model[†])	Exposure score (adjusted model[‡])
	OR (95% CI)	**OR (95% CI)**
Asthma		
No mold or dampness indicator	Reference	Reference
1 indicator	1.24 (1.00-1.55)	1.16 (0.93-1.44)
2 indicators	1.47 (1.09-2.00)	1.37 (1.01-1.86)
3 indicators	2.00 (1.26-3.16)	1.73 (1.10-2.74)
p-value for trend	<0.01	<0.01
Rhinitis		
No mold or dampness indicator	Reference	Reference
1 indicator	1.09 (1.02-1.21)	1.03 (0.87-1.22)
2 indicators	1.24 (0.97-1.59)	1.18 (0.92-1.52)
3 indicators	1.43 (0.96-2.13)	1.23 (0.82-1.85)
p-value for trend	0.01	0.14
Aeroallergen sensitization[§]		
No mold or dampness indicator	Reference	Reference
1 indicator	0.94 (0.78-1.14)	0.94 (0.77-1.13)
2 indicators	0.83 (0.62-1.11)	0.85 (0.63-1.14)
3 indicators	1.01 (0.63-1.62)	0.90 (0.55-1.48)
p-value for trend	0.30	0.23
Food allergen sensitization[¶]		
No mold or dampness indicator	Reference	Reference
1 indicator	0.87 (0.70-1.09)	0.87 (0.69-1.09)
2 indicators	0.99 (0.71-1.38)	1.02 (0.73-1.42)
3 indicators	1.33 (0.80-2.22)	1.22 (0.72-2.07)
p-value for trend	0.93	0.99

[*] Mold odor, visible mold, or dampness damage.
[†] Crude odds ratio (OR) and 95% confidence intervals (CI) obtained from GEE.
[‡] Odds ratio (OR) and 95% confidence intervals (CI) obtained from GEE adjusted for sex, socioeconomic status, parental allergic disease, maternal smoking during pregnancy, parental smoking during infancy, maternal age <26 years, and presence of siblings.
[§] Sensitization to cat, dog, horse, mite, timothy, birch, mugwort, or mold.
[¶] Sensitization to cow's milk, hen's egg, soybean, peanut, cod fish, or wheat.

4.8.2 Phenotypes of asthma

We found that participants exposed to any mold or dampness indicator during infancy had an increased risk of persistent asthma (OR = 1.73; 95% CI, 1.20-2.50). Similar results were seen for mold odor, visible mold, and dampness damage separately (Figure 20). Exposure to dampness damage was also significantly associated with an increased risk of late-onset asthma (OR = 1.41; 95% CI, 1.02-1.95).

Figure 20. Exposure to mold or dampness indicators during infancy and early-transient, persistent, and late-onset asthma in BAMSE.



* Odds ratios (OR) and 95% confidence intervals (CI) obtained from logistic regression adjusted for sex, parental allergic disease, socioeconomic status, maternal smoking during pregnancy, parental smoking in the first two months, maternal age <26 years, and presence of siblings.
† Early-transient asthma defined as asthma occurrence at age 1, 2, or 4 years but not at age 8, 12, or 16 years.
‡ Persistent asthma defined as asthma occurrence at age 1, 2, or 4 years and again at age 8, 12, or 16 years.
§ Late-onset asthma defined as first occurrence of asthma at age 8, 12, or 16 years.

4.9 ADDITIONAL RESULTS

4.9.1 Paternal smoking during pregnancy and allergic diseases

There is literature supporting an association between paternal smoking during pregnancy (i.e. maternal SHS exposure during pregnancy) and asthma in children[40 157], and such data were collected at the 12 year follow-up in BAMSE. In explorative analyses we found that exposure to paternal smoking during pregnancy was associated with asthma up to age 16 years even among those unexposed to maternal smoking during pregnancy (OR = 1.39; 95% CI, 1.07-1.80) (Table 9). Similarly, in a mutually adjusted model we observed an overall increased risk of asthma up to age 16 years in participants exposed to paternal smoking during pregnancy (Table 10). We observed no association between exposure to paternal smoking during pregnancy and rhinitis. An increased risk of eczema among participants exposed to paternal smoking during pregnancy and eczema was seen, however this disappeared following adjustment for parental smoking during infancy.

Table 9. Overall associations between paternal smoking during pregnancy and allergic diseases and sensitization stratified by maternal smoking during pregnancy (BAMSE).

	No maternal smoking during pregnancy	**Yes maternal smoking during pregnancy**
Paternal smoking during pregnancy	OR (95% CI)	OR (95% CI)
Asthma*	1.39 (1.07-1.80)	1.15 (0.72-1.83)
Rhinitis*	1.15 (0.94-1.42)	1.19 (0.81-1.75)
Eczema*	1.25 (1.03-1.50)	1.00 (0.70-1.44)
IgE sensitization†		
Any allergen‡	0.89 (0.67-1.18)	1.15 (0.75-1.79)
Inhalant allergens‖	0.91 (0.67-1.23)	1.35 (0.84-2.19)
Food allergens¶	1.02 (0.73-1.44)	0.96 (0.55-1.68)

* OR and 95% CIs obtain from GEE adjusted for adjusted for sex, parental allergic disease, and socioeconomic status.
† OR and 95% CIs obtain from GEE adjusted for adjusted for sex, parental allergic disease, SHS exposure during infancy, and socioeconomic status.
‡ Any inhalant and/or food allergen sensitization.
‖ Sensitization to cat, dog, horse, mite, timothy, birch, mugwort, or mold.
¶ Sensitization to cow's milk, hen's egg, soybean, peanut, cod fish, or wheat.

Table 10. Overall associations between maternal and paternal smoking during pregnancy and allergic diseases and sensitization in a mutually adjusted model (BAMSE).

Paternal smoking during pregnancy*	**OR (95% CI)**
Asthma*	1.33 (1.05-1.67)
Rhinitis*	1.16 (0.97-1.39)
Eczema*	1.19 (1.01-1.41)
IgE sensitization†	
Any allergen‡	0.98 (0.78-1.24)
Inhalant allergens‖	1.04 (0.80-1.33)
Food allergens¶	1.03 (0.77-1.37)
Maternal smoking during pregnancy**	
Asthma**	1.32 (1.03-1.72)
Rhinitis**	0.92 (0.74-1.14)
Eczema**	1.06 (0.87-1.29)
IgE sensitization††	
Any allergen‡	0.95 (0.75-1.21)
Inhalant allergens‖	0.94 (0.73-1.22)
Food allergens¶	0.93 (0.69-1.24)

* OR and 95% CIs obtain from GEE adjusted for adjusted for sex, maternal smoking during pregnancy, parental allergic disease, and socioeconomic status.
† OR and 95% CIs obtain from GEE adjusted for adjusted for sex, parental allergic disease, maternal smoking during pregnancy, SHS exposure during infancy, and socioeconomic status.
‡ Any inhalant and/or food allergen sensitization.
‖ Sensitization to cat, dog, horse, mite, timothy, birch, mugwort, or mold.
¶ Sensitization to cow's milk, hen's egg, soybean, peanut, cod fish, or wheat.
** OR and 95% CIs obtain from GEE adjusted for adjusted for sex, paternal smoking during pregnancy, parental allergic disease, and socioeconomic status.
†† OR and 95% CIs obtain from GEE adjusted for adjusted for sex, parental allergic disease, paternal smoking during pregnancy, SHS exposure during infancy, and socioeconomic status.

5 DISCUSSION

5.1 STRENGTHS AND LIMITATIONS

Every epidemiological study has strengths and limitations. The most noteworthy strength of the materials used in this thesis is the use of prospective birth cohorts. Data collected in a prospective manner prevents many pitfalls which affect epidemiological studies. When prospectively collecting data on exposure prior to an outcome you can be relatively certain that exposure occurred prior to disease onset and that disease status did not influence reporting of exposure, however this is not necessarily the case for allergic diseases. Another noteworthy strength is the number of individuals included in the BAMSE cohort and pooled data from other birth cohorts. A large sample size allowed the analysis of subgroups and various disease phenotypes. Loss to follow-up, also known as drop-out, is comparably low in BAMSE which retained 78% of the original cohort at the 16 year follow-up.

The majority of the data in these studies were collected via questionnaire which has the potential for error and is the main limitation in this thesis. This is expanded on later (section 5.2.2).

5.2 METHODOLOGICAL CONSIDERATIONS

With all epidemiological studies it is critical to consider whether observed findings are precise, valid, and generalizable. Two major pitfalls in epidemiologic research that threaten a study's validity are bias and confounding. Bias can be divided into random and systematic error. Systematic error can occur in the design, measurement of the exposure, measurement of the outcome, or in the selection or follow-up of participants.[158] Validity is the lack of systematic error, and can be divided into internal validity and external validity (generalizability). Internal validly refers to the legitimacy of inferences made to the source population, and external validity pertains to the validity of the inferences made to people outside that population.[159] More of external validity below (section 5.2.5).

5.2.1 Systematic error – selection bias

Selection bias can occur during the recruitment of study participants and/or the follow-up of these participants. Selection bias arises if study participants are more or less likely to be selected due to factors related to exposure, outcome, or both.[158] At the recruitment stage for BAMSE, 75% of all eligible children were included. The survey assessing non-responders found that parental tobacco smoking was more prevalent among non-responders compared to the 75% included in BAMSE, but no other significant differences in background characteristics concerning known risk factors for allergy-related disease were obsereved.[134] A low recruitment rate does not necessary impact the association between exposure and disease, but has more influence on external validity (see section 5.2.5).

In study III, participation rates varied across included cohorts and not all cohorts provided data on nonparticipants. By design, PIAMA and MAS were risk-enriched, and these cohorts

purposely included more children with higher allergy risk. In GINIplus no differences with regards to socioeconomic status or parental history of allergic diseases between nonparticipants and the final cohort were reported.[160]

Loss to follow-up is bias occurring during the follow-up of study participants. Response rates in BAMSE have been consistently high, with 78% of study participants completing questionnaires at age 16 years. To assess if selection bias occurred due to loss to follow-up, in each study we compared the selected study populations with the original participants in relation to background characteristics and outcomes. In general, the selected study populations were comparable regarding distribution of background characteristics. In study III participation rates varied, and at ages 14-16 years participation rates ranged from 42.6% (MAS) to 76.1% (BAMSE). Overall, nonsmoking parents with higher education were more likely to participate up to age 14 to 16 years. It is crucial to limit loss to follow up in prospective cohort studies, as this can bias associations between exposure and outcome and impact the validity of the study.

5.2.2 Systematic error – information bias (misclassification)

Information bias or misclassification occurs when the measurement of exposure or outcome in participants is erroneous. Misclassification can be either differential or nondifferential. Differential misclassification occurs when the classification error *depends* on the values of other variables and nondifferential misclassification is classification error that does *not* depend on the values of other variables.[159]

All exposure and outcome information in this thesis were based on answers from parental and/or participant questionnaires, except for studies II and IV, which used IgE levels based on blood samples or lung function, respectively. Therefore, misclassification of exposure or outcome to some degree is unavoidable.

Misclassification of tobacco smoking is possible due to underreporting. The negative health effects of tobacco smoking are well known, thus underreporting is possible in studies I-IV. Nevertheless, validation studies have found that self-reported data are often comparable with cord blood, urinary cotinine, or indoor air nicotine measurements.[161-163] Due to the nature of prospective birth cohort studies, the collection of exposure information prior to disease onset reduces the risk of differential misclassification. In other words, parents are not influenced by the disease status of their child at the time of reporting, at least for exposures like maternal smoking during pregnancy or in the first year of life. In general, misclassification due to underreporting can potentially result in an underestimation of the true effect.

In study IV we used self-reported smoking habits and smokeless tobacco use among 16 year old participants as well as saliva cotinine samples. We used a cut-off of 12 ng/ml to discriminate active smokers from non-smokers and correctly classified 97.6% of daily smokers. This cut-off was based on prior literature, and there is no “gold standard” level. Therefore, it is likely that some exposure misclassification occurred. Nevertheless, we used

the saliva cotinine levels more as a confirmatory marker of what we observed in self-reported data, and the effect we observed could be an underestimation of the true association.

In study V exposure to indicators of mold or dampness were also assessed by questionnaires, and some exposure misclassification is expected. In a nested case-control study, a home visit was conducted for 540 participants where an inspector assessed the presence of mold or dampness when the child was between one and two years of age. The correlation between parental reported and inspector noted signs of mold or dampness was somewhat low.[12] The home visit was conducted in the first winter season (October–March) following the child's recruitment into a case-control study, and mold or dampness in the home may have changed from the time the parent completed the questionnaire. Additionally, parents with allergic disease more frequently reported indicators of mold or dampness than parents without allergic disease, which could contribute to differential exposure misclassification. However, in study V we found that risk estimates were elevated both in children with and without parental allergic disease.[115]

In epidemiological research how you choose to define your outcome is critical to avoiding misclassification. A too inclusive definition often includes those that really do not have the disease whereas a too stringent definition may exclude those with a more mild form of the disease. Therefore, finding an ideal definition can be challenging. This brings up the concept of sensitivity and specificity. The sensitivity of an outcome measurement is the probability that a subject who truly has the outcome is classified as such, and specificity is the probability that a subject not having the outcome will be classified as such.[159]

Defining asthma is difficult, and there are no definitive diagnostic criteria or gold standard. What makes defining asthma even more challenging is the dynamic nature of the disease, with many forms and phenotypes. We used the same definition for asthma in studies I, II, and V which was stricter than the definition used in study III. The definition of asthma in all studies was based on a combination of reported symptoms, asthma medication in the last 12 months, or doctor diagnosed asthma. Study III's definition was agreed upon by a panel of experts in the MeDALL consortium and was chosen partly to accommodate different questions from the various cohorts and to facilitate harmonization of data.[164] We observed minor regional differences in asthma prevalence which may reflect differing diagnostic criteria, as doctor diagnosed asthma was one of three criteria used to define asthma. The somewhat lower prevalence of asthma in the German cohorts (GINIplus, LISAplus, and MAS) might be explained by different diagnostic conventions between countries.[165] The definition of asthma used in BAMSE was modified based on the ISAAC questionnaires which have been validated and are found to be accurate.[5]

Defining IgE sensitization was based on IgE antibodies in blood, and is not subject to reporting bias. IgE levels in blood correlate well with skin-prick tests (SPT) and specific IgE antibodies measured by the CAP-RAST test.[166] The decision to use 0.35 kU_A/l was based on the literature and is widely used as a cut-off. Symptoms of allergic disease and IgE sensitization are correlated; however it is important to remember that they are not

synonymous. In other words, a subject can have a high IgE antibody levels but not present with any clinical symptoms and vice versa.

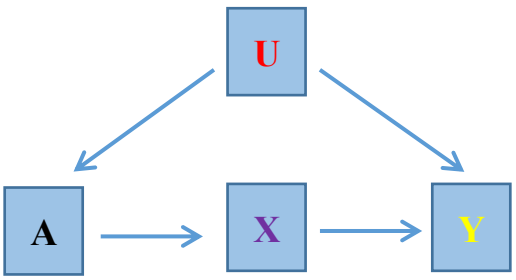
Lung function was assessed by dynamic spirometry and impulse oscillometry (IOS) according to the ATS/ERS guidelines by well trained and experienced staff. These outcome measures are likely only to be affected by random error, and any misclassification is likely to be non-differential and would lead to a dilution of any true association.

Misclassification of outcome could occur if mothers who quit smoking during early pregnancy may be more aware of respiratory symptoms in their offspring. These mothers may more readily seek healthcare or report symptoms differently than mothers who continue to smoke. Such reporting could potentially lead to an overestimation of the association. However, the reverse could also occur. Mothers who continue to smoke both during pregnancy and infancy may be less likely to report symptoms and seek healthcare less frequently than non-smoking mothers.[167 168] Such reporting would lead to an underestimation of the true association.

5.2.3 Confounding

In observational studies one can never be entirely certain if the observed association is not due to uncontrolled or residual confounding. Confounding occurs when a factor (exposure, intervention, treatment, etc.) outside the studied exposure and outcome is related to both the exposure and the outcome.[158] In other words, a confounding factor must fulfil three criteria: 1) must be a risk factor for disease, 2) must be associated with the exposure under study, and 3) must not be affected by the exposure or the disease.[159] A commonly drawn directed acyclic graph (DAG) to illustrate this concept is below (Figure 21). For example, socioeconomic status could confound the association between maternal smoking during pregnancy and asthma because socioeconomic status is associated with both maternal smoking during pregnancy and asthma.[169 170] Therefore we adjusted for socioeconomic status in studies I, II, and III. Maternal smoking during pregnancy is associated with low birthweight and may be considered a potential mediator of the association between maternal smoking during pregnancy and allergic outcomes.[171] In studies I, II, III, and IV we explored the effect of birthweight but found no significant modifying effects.

Figure 21. Example of a DAG. Where **A** represents exposure, **Y** the outcome, **U** is a confounder, and **X** is a mediator between **A** and **Y**.



In the design process one must collect information on all potential confounding factors, which can later be used in the analysis phase and controlled for. In the BAMSE birth cohort and in the other cohorts included in study III, extensive questionnaires gather information on known and potential confounders. Additionally, repeated follow-up questionnaires continued to gather such information.

The selection and control of confounders can be done in various ways. The selected confounders for studies I-V are presented in section 3.4. In general we selected covariates based on *a priori* knowledge as well as testing the influence of potential confounders on the effect estimate. The most common way to control for confounding is to include it in a multivariate model.

A residual confounding factor that could influence the associations we found in the studies included in this thesis is paternal smoking during pregnancy. There are studies that suggest an association between paternal smoking during pregnancy and childhood asthma.[172] We did not collect this information at baseline but this was asked at the 12 year follow-up. If paternal smoking during pregnancy is associated with allergic diseases, we likely underestimated effect of fetal exposure to tobacco smoke during pregnancy.

Another source of confounding could be related to how we defined socioeconomic status (SES). Tobacco smoking is related to health outcomes as well as socioeconomic status. In studies I, II, IV, and V we defined socioeconomic status according to the Nordic standard occupational classification and Swedish socioeconomic classification and was based on parental occupation.[173] There is the potential that this did not capture true socioeconomic status and that residual confounding remained. To address this, in sensitivity analyses we checked if mean income in the neighborhood affected our results but the effects were negligable.[161]

5.2.4 Random error

Random error, as the name implies, occurs by chance and largely affects the precision of risk estimates. Precision of an estimate is the lack or random error. The single most effective way to reduce random error and increase precision is to increase sample size. The width of 95% confidence intervals is one way of visualizing your precision; wide intervals indicate low precision and narrow ones suggests high precision.

5.2.5 External validity – generalizability

Generalizability refers to the ability to make inferences as they pertain to participants within your study population and generalize them to individuals outside the study population.[159] To do this one must consider how the source population was selected. In BAMSE, 75% of eligible children born in Stockholm were included, and the only difference was seen in smoking rates. Thus it is reasonable to believe our sample was representative, and generalizable to Sweden, although not representative regarding tobacco smoke exposure.[134] Moreover, because BAMSE and the other cohorts included in study III (GINIplus and

LISAplus) were population-based and had high follow-up rates it is reasonable to assume that results can be generalized to the pediatric populations in high and middle income countries. There is also no obvious reason why tobacco smoke exposure or mold or dampness exposure would affect European children in different ways than other children around the world. If the underlying biological mechanisms are true there is no explanation why our findings would not be relevant for other populations. Due to the lower participation rate of smoking parents at recruitment (BAMSE), the impact of tobacco smoke exposure in the general population could possibly be slightly higher. High risk cohorts, such as MAS and PIAMA, may hamper generalizability as they are less comparable to the general population.

5.2.6 Weighing the evidence – causality

When drawing conclusions in epidemiological research and establishing causality consideration should be given to the following criteria adapted from Bradford Hill[174]:

Strength of the association – stronger associations are more likely to be causal.

Evidence of dose-response relationship – do higher doses of exposure have stronger effects on the outcome?

Biological plausibility – is there evidence from both human and animal studies?

Temporality – exposure should occur prior to disease onset.

Specificity – is the association observed specific to a certain disease or particular individuals? In the case of smoking this criteria is less important because smoking is associated with multiple health outcomes.

Consistency – how consistent is the evidence across studies? One study alone cannot establish causality but can contribute to the evidence base.

Experiment – do experimental or intervention studies provide evidence of a causal relationship?

Analogy – are analogous exposures also causally associated with disease?

For example, we can ask the question, is tobacco smoking during pregnancy causally associated with asthma? Using the Bradford Hill criteria to assess the causality between maternal smoking during pregnancy and asthma our results achieve nearly all requirements. Specifically, we observed consistently elevated risk estimates, does-response associations, biological plausibility suggested by previous research, experimental studies using animal models support this association, consistency across other studies, and analogous associations are observed with other environmental exposures such as traffic related air pollution. Taken together this supports the hypothesis that maternal smoking during pregnancy is causally associated with asthma. One must keep always keep in mind that a single epidemiological study can never constitute full proof of causality.

5.3 MAIN FINDINGS AND GENERAL DISCUSSION

5.3.1 Maternal smoking during pregnancy in relation to allergic disease and lung function

Taken together we found that maternal smoking during pregnancy was associated with increased risk of asthma up to adolescence, and especially for early-transient asthma. We also found evidence for persistent asthma up to adolescence in participants exposed to high doses of maternal smoking during pregnancy. Additionally, we observed that highly exposed individuals during pregnancy were also at risk of persistent rhinoconjunctivitis. On the other hand, we found no association between maternal smoking during pregnancy and allergic sensitization or asthma with concomitant sensitization (i.e. allergic asthma). Exposure to maternal smoking during pregnancy was also associated with lower FEV_1/FVC ratios and increased airway resistance at age 16 years, and suggests that exposure during pregnancy may have persistent effects on lung function up to adolescence. These measures also indicate early signs of airflow obstruction.

Our finding of an association between maternal smoking during pregnancy and asthma development in early childhood is consistent with earlier studies.[42 65 67 175] However, less is known about the risk of asthma as children reach adolescence, and the varying phenotypes related to onset and progression. In our studies we observed an increased risk of asthma up to adolescence which has been reported in other birth cohort studies.[175 176] Earlier studies have also indicated that it is likely maternal smoking during pregnancy, and more importantly exposure during the first trimester that influences asthma development in children up to preschool age.[42] It remains challenging to distinguish the effects of maternal smoking during pregnancy from adolescent smoking because many mothers that smoke during pregnancy continue to do so following delivery.[37] Nevertheless, Neuman et al. effectively showed that maternal smoking during early pregnancy alone appears to increases the risk of wheeze in early childhood, a finding that is in line with our results.[42] To our knowledge, study III was the first to study the onset, progression, and persistence of asthma up to adolescence. We found evidence of increased risk of persistent asthma among participants exposed to high doses of maternal smoking during pregnancy.

Study IV is one of few prospective studies that assessed exposure to maternal smoking during pregnancy and concurrently accounted for exposure to adolescent smoking in relation to lung function in adolescents. Our finding for decreased FEV_1/FVC ratios in children exposed to maternal smoking during pregnancy corroborates some prior studies[176-178], whereas other studies have found declines in FEV_1 or FVC which was not observed in our study.[179 180] Our results confirm and extend those from the Isle of Wight cohort which also found reduced FEV_1/FVC ratios among adolescents (mean age 18 years) exposed to maternal smoking during pregnancy.[177] Additionally, in a cohort of 519 participants, Guerra et al. observed an early accelerated decline in FEV_1/FVC ratios in 26 year olds, but only in subjects exposed to both adolescent smoking and maternal smoking during pregnancy.[103] We also observed significant increases in peripheral airway resistance in adolescents exposed to maternal smoking during pregnancy. This is supported by previous studies which found reduced forced

expiratory flow at 25% to 75% of FVC (FEF_{25-75}) which is suggestive of airflow in small airways.[177 180]

Maternal smoking during pregnancy can impact a developing fetus in numerous ways. Compounds such as carbon monoxide, nicotine, cyanide, and sulfide are all known to cross the placental barrier.[181] Fetal lung organogenesis begins in the fourth week of pregnancy and by the second trimester the majority of the conductive airways and terminal bronchioles are formed.[182] Nicotine, the main constituent of tobacco smoke freely passes the placental barrier, and is linked with altered lung and alveolar architecture that can influence lung function.[39 183 184] Carbon monoxide, another byproduct of cigarette smoking, diminishes blood flow and the delivery of oxygen and nutrients to the fetus, which in turn can lead to intra-uterine growth retardation and impair the development of the respiratory system.[181] Maternal smoking during pregnancy is also associated with DNA methylation in offspring and particularly in genes associated with asthma.[35] Therefore, the association we find for both asthma and altered lung function among participants exposed to maternal smoking during pregnancy could be partly explained by these mechanisms. Collectively, this suggests that maternal smoking during pregnancy acts on the developing lung to influence the development of asthma and impact lung function up to adolescence.

Although outside the scope of this work, we did observed suggestive evidence that paternal smoking during pregnancy may influence the development of asthma up to adolescence. This association has also been observed in other cohort studies in Europe and Canada among children up to seven years.[40 157]

5.3.2 SHS during infancy in relation to allergic disease

SHS exposure during infancy, without prior exposure during pregnancy, was associated with increased risk of food allergen sensitization at four years, with similar risk estimates at ages eight, and 16 years. Significant dose-response associations with an increasing number of cigarettes smoked was also seen. When we combined symptoms of asthma, rhinitis, and eczema with sensitization we found that SHS exposure during infancy increased the risk of eczema with sensitization. In line with this, we observed a somewhat increased risk of eczema among children exposed to SHS during infancy, which has been corroborated by others.[16 77 185] SHS exposure during infancy was also associated with an overall risk of rhinitis up to adolescence with significant dose-response effects, and SHS exposure during infancy was mainly linked with rhinitis without sensitization. However, the risk estimates were highest in preschool age children and were less apparent as children became older.

The literature is mixed when it comes to the association between exposure to tobacco smoke and sensitization. We observed an association between SHS during infancy and sensitization to food allergens which is in agreement with some studies in preschool age children[85 86] but not all.[186 187] Some studies have only reported increased risk of any sensitization among those with parental history of allergy,[78] whereas others have found higher total IgE concentrations, but have not been able to identify specific types of allergens.[188] Furthermore, some studies

have not distinguished between maternal smoking during pregnancy and SHS exposure postpartum. In study II we found no association between maternal smoking during pregnancy and sensitization, which is consistent with another birth cohort study based in Australia.[176] Given the various methodologies it is difficult to draw strong conclusions.

The mechanisms behind exposure to SHS during infancy and sensitization to food allergens are not fully understood and are likely multifactorial. Some literature suggests that exposure to maternal smoking during pregnancy could preclude sensitization, presumably through an immunosuppressive effect, and that exposure postpartum influences the risk of allergic senstization.[33 189 190] On the other hand exposure to SHS during infancy could induce inflammation of mucosal surfaces and increase antigen penetration leading to sensitization.[191] Another proposed mechanism, the dual allergen exposure hypothesis, suggests that exposure through the skin leads to sensitization, while exposure to allergens through consumption leads to tolerance – a process that may be facilitated by SHS expousre.[192] Moreover, SHS exposure on the skin may impact sensitization due to damage to the proteins, lipids, and skin barrier allowing the passage of allergens through the skin.[193 194] This mechanism could also explain our findings of an increased risk or eczema in subjects exposed to parental smoking during infancy.

We observed increased risk of asthma up to adolescence in subjects exposed to SHS during infancy, and has been reported by others.[65 68] However, as described earlier, disentangling the effects from maternal smoking during pregnancy from SHS exposure during infancy is challenging due to the high correlation between these exposures. Nevertheless, it seems plausible that exposure during infancy also confers increased risk of asthma, even though the majority of risk seems to be from exposure during pregnancy.

The observed association between SHS during infancy and rhinitis up to adolescence is similar to other studies, but not all.[73 77 195 196] The association we observed tended to subside as children reached adolescence, which could explain why other studies up to adolescence did not find an association between SHS exposure and rhinitis.[73 197] Nevertheless, SHS exposure could influence the development of rhinitis through a combination of nasal irritation or obstruction as well as impairment in mucociliary clearance.[198]

5.3.3 SHS throughout childhood in relation to allergic disease

We consistently observed no significant associations between exposure to SHS during childhood or adolescence and asthma, rhinitis, rhinoconjunctivitis, or eczema. One possible explanation is that the perinatal period is a critical time window of development, and children are particularly sensitive to environmental exposures during this period. Analogous associations have been reported for traffic related air pollution as well as farming environments; in that early life exposure plays a crucial role for outcomes in later life.[14 199] During preschool ages children spend the majority of time in close vicinity of their parents and therefore more highly exposed during this vulnerable period. Parents may also try to

actively avoid exposing their children to SHS; for example 94% of parents in BAMSE reported smoking outdoors (on the balcony), near a window, or under the kitchen fan.[71]

In studies I, II, and III, we adjusted our results for "exposure throughout childhood and adolescence" but this only slightly attenuated our results. The PIAMA cohort also found no association between SHS exposure and asthma from ages 4 to 17 years.[76] Nevertheless, some cross-sectional studies have found associations between SHS exposure during childhood with asthma and respiratory symptoms in school age.[52 200] A major limitation of these studies is the inability to adequately account for exposure during pregnancy, as this information is prone to recall bias. Moreover, exposure to SHS later in childhood is not always independent of symptoms of the child, since some parents may quit smoking if their child is diagnosed with asthma.[201]

5.3.4 Adolescent smoking and lung function

In study IV we observed a suggestive association between adolescent smoking and lower FEV_1/FVC ratios and increased peripheral airway resistance, independent of maternal smoking during pregnancy. Our findings of lower FEV_1/FVC ratios among adolescent smokers were corroborated when we used saliva cotinine concentrations to discriminate smokers from nonsmokers. Taken together, this suggests that even after a short duration of smoking, signs of airflow obstruction in adolescents were already apparent.

To our knowledge study IV is the only study to assess lung function in adolescence using two methods to measure lung function and a biomarker of nicotine uptake. We observed lower FEV_1/FVC ratios among adolescent smokers which is in agreement with previous studies in adolescence[202 203], but not all.[100 103] It was challenging to distinguish the effects of maternal smoking during pregnancy from adolescent smoking because 20% (n = 58) of adolescent smokers were also exposed to maternal smoking during pregnancy. Nevertheless, when we controlled for maternal smoking during pregnancy, risk estimates for adolescent smoking remained elevated. Although risk estimates were no longer statistically significant, we believe this is likely due to insufficient power. Additionally, we used saliva cotinine concentrations in relation to lung function to provide a unique assessment of actual nicotine uptake and to confirm our finding of reduced FEV_1/FVC ratios in smoking participants.

Alveolarization continues into early adulthood, and humans do not reach peak lung volume until their early 20's.[202] It is probable that smoking in adolescence negatively impacts lung growth, and exposure to tobacco smoke in adults is causally associated with COPD, lung cancer, pneumonia, and chronic bronchitis.[204 205] Smoker's lungs show diffuse changes affecting the airway lining, epithelium, and bronchiole arcitecture.[206] Although a one to two percent-unit decline in FEV_1/FVC ratio may be small, impairments in lung function at an early age can limit the peak development in adulthood, and potentially set a course for COPD or other lung related disease in later life.

5.3.5 Mold and dampness in relation to allergic disease

We found that exposure to any indicator of mold or dampness during infancy was associated with an increased risk of asthma up to adolescence, particularly persistent asthma. Our results also suggested that exposure to any indicator of mold or dampness was associated with non-allergic asthma (asthma without IgE sensitization). We observed increased risk of rhinitis, but only among children exposed to mold odor and visible mold. Similar to asthma, we found that exposure increased the risk of non-allergic rhinitis.

Studying the association between reported indicators of mold or dampness in a home can be challenging, as reporting is subjective, and studies use various methodologies to assess exposure. Even so, there is consistent epidemiological evidence indicating that factors related to dampness in a home are associated with asthma risk in children.[15 109 110] The literature is less consistent for rhinitis, but there is evidence suggesting that exposure to mold or dampness is associated with increased rhinitis risk.[15 126 130] Exposure to compounds related to molds such as β-1-3-glucans, microbial volatile organic compounds, extracellular polysaccharides, and mycotoxins can cause recurrent irritation and immune activation of the respiratory tract.[109 110 114] After repeated exposure and extended inflammation, inflammatory-related diseases can develop, such as asthma and rhinitis. Because we found increased risks of non-allergic asthma and rhinitis, it is plausible that the relevant compounds cause a more irritative effect rather than allergic inflammatory changes in the respiratory tract.[115]

As mentioned earlier, it is challenging to subjectively identify and quantify mold and dampness exposure. Parental reported mold or dampness is subject to bias, as described in section 5.2.2. Although home inspection is a more objective and standardized way of assessing household mold or dampness, this is often costly, time consuming, and not practical in large populations. Furthermore, intra-inspector variation is not uncommon, and mold or dampness damage within walls can be overlooked.[207 208] Nevertheless, studies utilizing inspector reported indicators of mold and dampness have reported similar results to ours.[209] Finally, these indicators of mold or dampness themselves do not cause an effect, but are markers of chemical and microbial processes in a home that influences health, and to date the compounds responsible for asthma and rhinitis are not fully elucidated.

6 CONCLUSIONS

Taken together, our findings indicate that the perinatal period may be critical for the influence of tobacco smoke exposure as well as mold or dampness on allergy related diseases and/or lung function. From the separate studies it may be concluded that:

- Maternal smoking during pregnancy appears to increase the risk of asthma during childhood and adolescence, especially early-transient asthma. Heavy exposure during pregnancy (≥10 cigarettes/day) may be associated with persistent asthma and persistent rhinoconjunctivitis. Furthermore, exposure to maternal smoking during pregnancy seemed to be associated with early signs of airway obstruction expressed as lower FEV_1/FVC ratios and increased airway resistance at age 16 years.

- SHS exposure during infancy may be associated with rhinitis up to age 16 years, with highest risks in children under 12 years of age, as well as with IgE sensitization to food allergens up to age 16 years, and eczema combined with sensitization.

- Adolescent smoking tended to be associated with reduced FEV_1/FVC ratios and increased peripheral airway resistance at age 16 years, suggesting development of airflow obstruction from only a short duration of smoking.

- Exposure to indicators of mold or dampness in the home during infancy appears to increase the risk of asthma, specifically persistent asthma up to age 16 years, as well as of rhinitis up to age 16 years, particularly in subjects without IgE sensitization. Identification of the causal agents remains elusive and further studies are needed.

7 FUTURE DIRECTIONS AND PUBLIC HEALTH IMPLICATIONS

Tobacco smoke exposure is causally linked to several detrimental health effects and there are no safe levels of maternal smoking during pregnancy or SHS. Despite continued health campaigns, tobacco smoking remains a pervasive problem, and the use of new tobacco products such as e-cigarettes and smokeless tobacco has increased.[210 211] Many smokers try their first cigarette in early adolescence and establish habits that can persist into adulthood.[202] From a public health perspective the prevention or cessation of smoking should be paramount. Various smoking cessation campaigns have been met with mixed results worldwide. Banning smoking in public places such as bus stops, restaurants, and by building entrances is suggested to improve the health of non-smokers and reduce smoking rates, but has not been shown in all studies and remains controversial.[48 212] Services such as counseling, support, and nicotine replacement therapy should continue to be provided. In high and middle income countries most of the population are aware of the negative health effects from smoking. Making access to smoking more difficult may be an effective strategy in reducing smoking rates.

Findings from this thesis can be used by health professionals to motivate mothers to quit smoking before conceiving, and for parents to abstain from smoking around their children to potentially prevent allergic disease, sensitization, and lung function impairments. Continued research is needed to assess the impact of e-cigarettes and other nicotine delivery systems. Additionally, the follow-up of individuals into adulthood to evaluate the persistence, new-onset, severity of symptoms, and exacerbations related to tobacco smoking is needed from long-term cohort studies, such as BAMSE. Intervention studies are also necessary to identify the most cost-effective ways to curb smoking rates and prevent adolescents from taking up smoking. Studies are also necessary to evaluate who is at risk to take up smoking and types of individuals that have difficulty quitting to inform future preventive strategies.

Epidemiologic research with qualitative and quantitative assessment of dampness and dampness-related agents must be conducted, as the evidence on the development of incident asthma as well as rhinitis remains inconclusive. Future research should focus on identifying the causal agents through cell or animal models, and the use of personal monitoring devices. Although epidemiological studies consistently observe associations between home dampness and respiratory outcomes the causal agents and biological mechanisms are not completely understood. This makes public health policy or intervention programs challenging to implement. Moreover, the development and use of validated metrics are necessary and to identify health-relevant thresholds of agents generated by indoor mold or dampness.

8 SVENSK SAMMANFATTNING

Astma och andra allergiska sjukdomar är de vanligaste kroniska sjukdomarna i västvärlden och förekommer hos cirka 30 procent av befolkningen. Den ökade förekomsten av dessa sjukdomar har lett till studier av hur miljöfaktorer och livsstil kan påverka risken för allergisjukdomar. Det övergripande målet med denna avhandling var att undersöka sambandet mellan faktorer i inomhusmiljön – som exponering för miljötobaksrök och fukt eller mögel i bostaden – och utveckling av allergisjukdomar från födseln till 16 års ålder. Studierna har genomförts med hjälp av data från en populationsbaserad födelsekohort (BAMSE) där 4089 barn följts från födseln upp till 16 års ålder. I en av delstudierna användes data från BAMSE tillsammans med data från fyra andra europeiska födelsekohorter, där totalt 10860 barn inkluderades.

Våra studier visade att barn vars mammor rökte under graviditeten hade en ökad risk för astma upp i tonåren, där riskökningen var särskilt uttalad i förskoleåldern. Barn till mammor som rökt 10 cigaretter per dag eller mer hade även en ökad risk för kvarstående astmasymtom och rinit (hösnuva) upp till 16 års ålder. Barn vars mammor rökte under graviditeten hade även en nedsatt lungfunktion (vilket visade sig genom lägre FEV1/FVC mätt med spirometri) samt en ökning i luftvägsmotståndet i de små luftvägarna (mätt med impulsoscillometri).

Exponering för miljötobaksrök (definierat som att mamma eller pappa rökte) under spädbarnstiden var associerat med en ökad risk för astma, rinit och eksem upp till 16 års ålder. Våra resultat tyder dock på att riskökningen för astma till största delen kan förklaras av mammans rökning under graviditeten. Resultaten visar även att exponering för miljötobaksrök under spädbarnstiden var associerat med en ökad förekomst av allergiantikroppar mot födoämnen hos barn som inte exponerats för mammas rökning under graviditeten. Exponering för miljötobaksrök senare under barndomen var inte kopplat till någon ökad risk för nyinsjuknande i astma eller rinit i tonåren.

Jämfört med icke-rökare, hade studiedeltagare som rökte vid 16 års ålder (ibland eller dagligen) en nedsatt lungfunktion, även efter att vi tagit hänsyn till om de exponerats för mammas rökning under graviditeten. Vi mätte även förekomsten av kotinin i saliv hos studiedeltagarna vid 16 års ålder för att kunna skilja rökare från icke-rökare. De som hade förhöjda nivåer av kotinin i saliven (som tyder på egen rökning) hade en nedsatt lungfunktion jämfört med dem med lägre nivåer.

Exponering för fukt och mögel i bostaden under spädbarnstiden var kopplat till en ökad risk för astma upp till 16 års ålder, liksom en ökad risk för kvarstående astmasymtom. Våra resultat tyder även på att förekomst av mögellukt eller synligt mögel i bostaden under spädbarnstiden kan öka risken för rinit upp till 16 års ålder. Däremot såg vi inget samband mellan förekomst av fukt och mögel i bostaden och nivåer av allergiantikroppar i blodet.

Sammanfattningsvis visar resultaten i denna avhandling att faktorer i inomhusmiljön, som exponering för miljötobaksrök och fukt eller mögel i bostaden tidigt i livet, kan påverka utvecklingen av allergisjukdomar upp tonåren. Barn vars mammor rökte under graviditeten

hade även en nedsatt lungfunktion vid 16 års ålder. Vi såg även tendenser till lungfunktionsnedsättning hos rökande tonåringar. Resultaten från studierna kan ligga till grund för preventiva hälsoinsatser i befolkningen.

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