Thesis for the degree of Doctor of Medicine

Epidemiological Studies of Childhood Wheeze

Risk Factors and Long-term Outcome

Emma Goksör

UNIVERSITY OF GOTHENBURG

Department of Paediatrics
Institute of Clinical Sciences
Sahlgrenska Academy
University of Gothenburg
Gothenburg, Sweden
2011

Printed by
Ineko AB, Gothenburg, 2011

ISBN 978-91-628-8344-7

Till Erik, Peter och Mattias
- ni är orsaken jag andas

Epidemiological Studies of Childhood Wheeze Risk Factors and Long-term Outcome

Emma Goksör
Department of Paediatrics, Institute of Clinical Sciences,
Sahlgrenska Academy, University of Gothenburg

Wheezing with viral infections is common in childhood and both genetic and environmental factors have been reported to influence the risk of subsequent asthma development. The overall aim of this thesis was to study the factors influencing the risk of wheezing at preschool age and the long-term outcome following severe wheezing in early life.

In a prospective study of 5,600 children born in the region of western Sweden in 2003 questionnaires were answered at six and 12 months and at 4.5 years of age. Data were also obtained from the Swedish Medical Birth Register. Special reference was made to the effects of prenatal paracetamol exposure, antibiotic treatment during the first week of life and feeding strategies in infancy. Possible differences between multiple-trigger and episodic viral wheeze were analysed.

In a prospective study of 101 children hospitalised due to wheezing bronchitis before the age of two years, the long-term prognosis and factors influencing the risk of subsequent asthma were explored. A re-investigation at 17-20 years of age included a questionnaire and tests for allergy, bronchial hyper-responsiveness (BHR) and airway function. The study group was compared with age-matched controls.

We were able to confirm known risk factors for recurrent wheeze at age 4.5 years, such as atopic heredity, male gender, eczema and doctor-diagnosed food allergy in infancy. In addition, neonatal antibiotic treatment increased the risk, while the introduction of fish before nine months of age reduced the risk. Paracetamol exposure *in utero* increased the risk of preschool wheeze treated with inhaled corticosteroids. The risk was more pronounced among those with multiple-trigger wheeze.

An increased risk of asthma at age 17-20 years was seen in subjects with early viral wheeze. Current allergy, BHR and active smoking increased the risk of current asthma. In addition, female gender was an independent risk factor. Wheeze was more prevalent among boys in early childhood, but more boys than girls became symptom free as they grew up. Girls had more persistent asthma and relapsing symptoms during adolescence.

Signs of reduced airway function were seen in the study group and were most pronounced among females with current asthma. However, a difference was also seen among symptom free subjects. Prenatal smoke exposure was associated with reduced airway function and independently increased the risk of BHR at 17-20 years of age. On the other hand, postnatal smoke exposure was associated with becoming an active smoker, which in turn increased the risk of current asthma.

In conclusion, paracetamol exposure during pregnancy and treatment with antibiotics neonatally independently increased the risk of wheeze at age 4.5 years. The early introduction of fish had a protective effect.

Individuals with severe viral wheeze in early life run an increased risk of asthma and have signs of reduced airway function at age 17-20 years. The highest risk of asthma is seen among those with current allergy or BHR and among females. Prenatal smoke exposure increases the risk of subsequent BHR and asthma, while smoke exposure in infancy and childhood increases the risk of becoming an active smoker.

Keywords: children, wheezing, asthma, smoke exposure, gender, antibiotics, fish, paracetamol

Appended Papers

This thesis is based on the work described in the following papers:

I. Goksör E, Alm B, Thengilsdottir H, Pettersson R, Åberg N, Wennergren G.
Preschool wheeze – impact of early fish introduction and neonatal antibiotics.
Acta Paediatr. 2011 Jul 18. [Epub ahead of print]

II. Goksör E, Thengilsdottir H, Alm A, Pettersson R, Möllborg P, Erdes L, Norvenius G, Åberg N, Wennergren G.
Prenatal paracetamol exposure increases the risk of multiple-trigger wheeze at pre-school age.
Acta Paediatr. 2011 Jul 18. [Epub ahead of print]

III. Goksör E, Åmark M, Alm B, Gustafsson PM, Wennergren G.
Asthma symptoms in early childhood – what happens then?
Acta Paediatr 2006; 95: 471-8.

IV. Goksör E, Gustafsson PM, Alm B, Åmark M, Wennergren G.
Decreased airway function in early adulthood among subjects with wheezing disorder before two years of age.
Pediatr Pulmonol 2008; 43: 396-403.

V. Goksör E, Åmark M, Alm B, Gustafsson PM, Wennergren G.
The impact of prenatal and postnatal smoke exposure on future asthma and bronchial hyper-responsiveness.
Acta Paediatr 2007; 96: 1030-35.

The papers will be referred to in the text by their Roman numerals.

Contents

List of abbreviations..... 10

Introduction..... 11

Wheezing and asthma: definitions..... 12

Wheezing phenotypes..... 13
Transient and persistent wheezing..... 13
Episodic viral wheeze and multiple-trigger wheeze..... 14

Cohort studies exploring asthma and allergy development..... 15
Birth cohorts..... 15
Cross-sectional studies..... 17

Prospective studies of virus-induced bronchiolitis or obstructive bronchitis..... 18
Definitions..... 18
Finnish post-bronchiolitis study..... 20
Finnish viral wheeze study..... 21
Wheezing bronchitis follow-up..... 21
Swedish post-RSV study..... 22

Predicting persistent asthma..... 23
Asthma Predictive Index, API..... 23
Isle of Wight and PIAMA indices..... 23
Hospital API for severe viral wheeze..... 25

Identifying risk factors and protective factors..... 25
Viral infections..... 25
Heredity..... 28
Other allergic manifestations..... 30
Gender..... 31
Obesity..... 32
Smoking..... 33
Antibiotics..... 35
Paracetamol..... 36
Fish..... 37
Vitamin D..... 38
Breast-feeding..... 40
Farming and domestic animals..... 41

Aims **43**

Methods **45**

Children of Western Sweden Study: Papers **I** and **II** 45

Participants 45

Ethical approval 45

Procedures 45

Response rate 45

Questionnaire 45

Swedish Medical Birth Register (MBR) 47

Outcome variables 47

Statistics – background 48

Statistics – Papers **I** and **II** 50

Wheezing bronchitis follow-up: Papers **III**, **IV** and **V** 52

Participants 52

Ethical approval 52

Procedures 52

Questionnaire 52

Data on smoke exposure 53

Allergy testing 53

Airway function testing 53

Outcome variables 54

Statistics 55

Results and Discussion **57**

Children of Western Sweden Study: Papers **I** and **II** 57

Prevalence 57

Heredity 59

Other allergic manifestations 60

Gender 62

Smoke exposure 63

Neonatal antibiotics 64

Paracetamol 66

Fish 67

Breast-feeding 70

Other factors of interest 71

Phenotype differences 73

Representativeness of the sample 73

Strengths and weaknesses 74

Wheezing bronchitis follow-up: Papers **III**, **IV** and **V**..... 75
Asthma prevalence in adolescence..... 75
Airway function in adolescence..... 77
Virus or host..... 79
Heredity..... 80
Other allergic manifestations..... 81
Gender..... 83
Pre- and postnatal smoke exposure..... 86
Current smoke exposure..... 88
Other factors of interest..... 89
Pathogenic model..... 91
Strengths and weaknesses..... 92

General Discussion..... 95
Virus or host?..... 95
Family history and development of allergic disease..... 95
Gender..... 95
Window of opportunity..... 95
Smoking habits 1983-2003..... 96
Summary..... 97

Conclusion..... 99

Populärvetenskaplig sammanfattning..... 103

Acknowledgements..... 105

References..... 109

List of abbreviations

aOR	Adjusted odds ratio
API	Asthma Predictive Index
ARC	Allergic rhinoconjunctivitis
BHR	Bronchial hyper-responsiveness
CaCh	Cold air challenge
COPD	Chronic obstructive pulmonary disease
ECP	Eosinophil cationic protein
ERS	European Respiratory Society
EVW	Episodic viral wheeze
FENO	Fraction of exhaled nitric oxide
GI	Gastro intestinal
ICS	Inhaled corticosteroids
LCI	Lung-clearance index
MTW	Multiple-trigger wheeze
Post-BD	Post (after) bronchodilator
Pre-BD	Pre (before) bronchodilator
RSV	Respiratory syncytial virus
SPT	Skin prick test
Th_1	T-helper cell type 1
Th_2	T-helper cell type 2
V_{max} FRC	Maximum flow at functional residual capacity

Introduction

Wheezing in early childhood is common. In the salient American Tucson Study, the prevalence of wheezing with a respiratory infection was 34% during the first three years of life (Martinez *et al.* 1995). In Sweden, 20% report wheezing and about 4% medicate with inhaled corticosteroids during infancy (Alm *et al.* 2008). Wheezing is a heterogeneous disorder, with some children becoming symptom free, while others develop allergies and asthma as they grow up (Wennergren *et al.* 1997, Taussig *et al.* 2003, Piippo-Savolainen *et al.* 2004). Over the past few decades, asthma and allergies have increased in the western world (Anderson *et al.* 2007, Asher *et al.* 2006). Lately, a stagnation in the increase in asthma prevalence has been reported (Anderson *et al.* 2007, Wennergren *et al.* 2010). Huge efforts have been made to understand the underlying causes of disease and which factors influence the occurrence. Models predicting the prognosis for children with wheezing and hypotheses about the underlying mechanisms have been presented. Heredity constitutes a major risk factor for wheezing and subsequent asthma (Castro-Rodriguez *et al.* 2000, Melén *et al.* 2004). Exposure during pregnancy, to tobacco smoke, for example, maternal medication and dietary factors has been reported to play important roles (Prescott 2010). Furthermore, it has been suggested that feeding habits and environmental factors present during infancy affect the risk of subsequent allergic disease (Björkstén 1999).

To explore risk factors of wheezing in early life, a birth cohort is being prospectively followed in the "Children of Western Sweden" study. Alm *et al.* have previously reported on factors affecting the risk of wheeze treated with inhaled corticosteroids, ICS-treated wheeze, during infancy (Alm *et al.* 2008). Treatment with antibiotics during the first week of life, male gender, preterm birth and a family history of asthma or eczema increased the risk, while breastfeeding had a protective effect. One objective of this thesis was to analyse the prevalence of wheezing at age 4.5 years and the factors influencing the risk of wheezing at preschool age, in the same birth cohort.

The majority of children with wheezing in early life will have transient symptoms, but some will develop persistent asthma as they grow up (Taussig *et al.* 2003). Therefore, the long-term prognosis and factors influencing the risk of subsequent asthma are explored in a prospective study of children hospitalised due to wheezing bronchitis before the age of two years (Wennergren *et al.* 1992). In this study, Wennergren *et al.* report an increased risk of asthma at age five and 10 years (Wennergren *et al.* 1992, Wennergren *et al.* 1997). Another objective of this thesis was to analyse the prevalence of asthma at age 17-20 years and the factors influencing the risk of subsequent disease in the same cohort.

Wheezing and asthma: definitions

Wheezing can be described as a high-pitched sound, characteristic of intrathoracic obstruction usually caused by turbulent flow in narrow airways (Watts and Goodman 2007). Airway obstruction is a common symptom of lower respiratory viral infection, but can also be triggered by other factors such as allergic inflammation or exercise. Children with wheezing run an increased risk of developing asthma and allergies, but not all children who wheeze have asthma. In the many studies exploring the risk of wheeze and asthma, the frequency and sometimes severity of wheezing symptoms differ, which might affect the results and conclusions of the studies.

"Any wheeze" can be considered to be too unspecific to define children with asthma, at least in questionnaire-based studies. Recurrent wheeze has been used to identify children with more severe symptoms as a proxy for asthma (Castro-Rodriguez *et al.* 2000, Lannerö *et al.* 2006, Simoes *et al.* 2007, Beasley *et al.* 2008). However, the number of episodes required varies between studies. In the Tucson Study, as well as in a study by Sonnappa *et al.*, four or more episodes during the last year are considered to be a proxy for asthma (Castro-Rodriguez *et al.* 2000, Sonnappa *et al.* 2010), while Simoes *et al.* consider three or more episodes to be enough (Simoes *et al.* 2007). Others consider three to four episodes or more during the first two to three years of life to define recurrent wheeze (Thomas *et al.* 2006, Kummeling *et al.* 2007).

In several studies, doctor-diagnosed asthma is used instead of recurrent wheeze. This can either be an actual doctor diagnosis during a clinical examination by a physician as part of the study or, as in many questionnaire-based cohort studies, a parental report of doctor-diagnosed asthma (Lannerö *et al.* 2006, Shaheen *et al.* 2005). In addition, a report of medication with inhaled corticosteroids during the past 12 months can be regarded as a proxy for doctor-diagnosed asthma, since these children have been evaluated by a physician as being in need of asthma treatment (Alm *et al.* 2008). In some studies, both recurrent wheezing and wheezing treated with inhaled corticosteroids are used to define asthma. For example, in the re-investigation at four years of age in the BAMSE Study, asthma is defined as four or more wheezing episodes during the last year or at least one episode of wheezing and treatment with inhaled corticosteroids (Kull *et al.* 2004).

It has been suggested that these differences in definition explain the divergent results relating to the impact of risk factors and prognosis seen in different studies (Kang *et al.* 2009)

Wheezing phenotypes

Several attempts have been made to classify children with wheeze in order to better identify groups with different underlying pathophysiological mechanisms and children at risk of subsequent disease. For example, symptom patterns over time, trigger factors and risk factors present in early life have been used for different classifications (Castro-Rodriguez *et al.* 2000, Taussig *et al.* 2003, Brand *et al.* 2008, Piippo-Savolainen and Korppi 2008).

Transient and persistent wheezing

In the Tucson Study, a classification into transient wheeze (wheezing before the age of three years but not at six years of age), persistent wheeze (wheezing both before three years and at six years of age) and late-onset wheeze (no wheezing before the age of three, but wheezing at six years of age) has been suggested (Taussig *et al.* 2003). Differences in airway function, IgE levels and the prevalence of allergic heredity have been demonstrated between the groups (Taussig *et al.* 2003).

Transient wheezing was the most common syndrome, seen in more than 80 per cent of the infant wheezers. Heredity for asthma or other features associated with allergic disease, such as atopic dermatitis, eosinophilia or high IgE levels, were no more evident in transient wheezers than in children who had never wheezed (Martinez *et al.* 1995, Taussig *et al.* 2003). However, the children with a transient wheeze had reduced airway function (maximum flow at functional residual capacity, V_{max} FRC) already in infancy and, even though their airway function improved during childhood, it did not reach the levels of never wheezers (Martinez *et al*. 1995). Transient wheezers did not have any increased risk of wheezing in adolescence, but it has been speculated that these children might run an increased risk of Chronic Obstructive Pulmonary Disease (COPD) as adults (Taussig *et al.* 2003).

The children in the Tucson Study with wheezing at six years of age were classified into non-atopic and atopic wheezers.

The children with non-atopic wheeze at age six had persistent wheezing with an onset before the age of three years. The atopic wheezers presented as early persistent wheezers, with an onset before the age of three, or late wheezers, with an onset after the age of three years. Early persistent atopic wheezers and late atopic wheezers had the same prevalence of sensitisation at six years of age, but early persistent wheezers were found to have higher IgE and a reduced V_{max} FRC at age six and 11. Early onset thus indicated a poorer prognosis, with more severe disease and affected airway function (Martinez *et al.* 1995, Taussig *et al.* 2003).

Episodic viral wheeze and multiple-trigger wheeze

In a report from The European Respiratory Society (ERS) task force in 2008, the definitions based on duration of wheeze (transient, persistent and late-onset wheeze) were suggested to be of less importance when predicting the long-term outcome in a clinical setting, since the definitions are made retrospectively. Instead, a classification based on the temporal pattern of symptoms was suggested (Brand *et al.* 2008). Children with intermittent wheezing only in response to viral infections, with no wheezing in between, were classified as having episodic viral wheeze (EVW). Children with wheezing also between viral infections in response to other trigger factors such as allergens and exercise were classified as having multiple-trigger wheeze (MTW). The response to treatment is believed to differ between the two groups, with MTW being more susceptible to long-term treatment with inhaled corticosteroids. EVW is suggested to be transient with less risk of subsequent asthma, while MTW is believed to reflect chronic airway inflammation with an increased risk of developing allergic asthma (Brand *et al.* 2008). Airway function abnormalities, especially in the conductive airways, have been reported in children with MTW compared with those with EVW, independent of current symptoms or atopic status. Furthermore, children with MTW were more frequently treated with inhaled corticosteroids and had more exacerbations than children with EVW. In addition, they had a higher fraction of exhaled nitric oxide (FENO) than healthy control subjects. To summarise, these findings support the more persistent and allergic nature of MTW (Sonnappa *et al.* 2010).

However, the classification is often dependent on retrospective reports from the parents and patients have also been reported to move from one phenotype to another over time (Schultz *et al.* 2010). In addition, the differences seen in clinical features may be obvious when comparing groups but not when classifying individual patients (Sonnappa *et al.* 2010). Furthermore, the phenotypes have been suggested to reflect the severity and frequency of wheeze rather than different pathophysiological mechanisms, with MTW representing more severe disease, more susceptible to external exposure and thus triggered more frequently than the milder EVW (Garcia-Marcos *et al.* 2010).

The discussion on how to best classify the clinical types of asthma is ongoing. Recently, the term asthma endotypes was suggested to describe subtypes of asthma with different phenotypes and distinct pathophysiological mechanisms (Lötvall *et al.* 2011).

Cohort studies exploring asthma and allergy development

Birth cohorts

There are several prospective birth cohort studies exploring the development and prevalence of asthma and allergy in childhood.

The Tucson Study is a population-based un-selected birth cohort initially comprising 1,246 children followed into adulthood. Detailed questionnaires, blood samples and airway function were obtained in infancy. During the first three years of life, data were obtained during respiratory infections. Re-investigations were made throughout childhood and adolescence with the skin prick test (SPT), airway function measurements, questionnaires and blood samples. The study has identified different wheezing disorders in early life, developed the Asthma Predictive Index (API), described the outcome after early RSV-induced wheezing and reported on numerous risk factors for both early and persistent asthma and atopy (Taussig *et al.* 2003).

The National Child Development Study is a British National birth cohort of 17,414 children born in 1958. Information is available from the perinatal period and re-investigations made between age seven and 23 years. The incidence of and the risk factors for asthma and other allergic manifestations have been studied (Anderson *et al.* 1992).

The Dunedin Multidisciplinary Health and Development Study is a birth cohort initially made up of 1,037 individuals born in 1972 and 1973 in Dunedin, New Zealand. Re-investigations were performed every second year between age three to 15 years and then at age 18, 21 and 26 years. Questionnaires were distributed and assessments of airway function, BHR, reversibility and atopic sensitisation were performed from nine years of age (Sears *et al.* 2003).

In the Avon Longitudinal Study of Parents and Children (ALSPAC), almost 14,000 children enrolled during early pregnancy were also followed by questionnaires and from school age with annual clinical examinations. Detailed data on both parents were collected before the child was born and DNA from both children and parents has been collected. The study aims to continue through puberty (Shaheen *et al.* 2005, Shaheen *et al.* 2010).

The Swedish BAMSE Study is a prospective, longitudinal study of a birth cohort born between 1994 and 1996. More than four thousand eligible study subjects were identified at three months of age and have been followed with questionnaires and clinical examinations while growing up. The aim of the study is to identify risk factors for asthma and other allergic disease in childhood.

Interesting results have been reported in areas such as smoking during pregnancy, dietary factors, breast-feeding and gender differences (Kull *et al.* 2004, Melén *et al.* 2004, Kull *et al.* 2006, Lannerö *et al.* 2006, Lannerö *et al.* 2008, Kull *et al.* 2010).

The Swedish prospective, longitudinal Children of Western Sweden Study is a birth cohort of more than four thousand children born in 2003 in the region of Western Sweden and investigated at age six and 12 months and 4.5 years. The prevalence of and factors influencing the risk of wheeze and allergic manifestations in infancy and at preschool age have been reported (Alm *et al.* 2008, Alm *et al.* 2009, Alm *et al.* 2011).

The Isle of Wight Study is a birth cohort of about 1,500 children enrolled at birth and re-investigated with questionnaires up to school age. Cord blood IgE was collected at birth and an SPT was performed at age four years. The natural history of childhood asthma and the risk factors associated with the development of asthma are studied (Kurukulaaratchy *et al.* 2003).

In the Perth infant asthma follow-up study, 253 infants were recruited from a full-term normal population. Airway function and airway responsiveness were evaluated in infancy at one month of age. Re-investigations were performed at six and 12 years of age to evaluate the impact of early infant airway function (Le Souëf *et al.* 2002).

The Dutch KOALA Study is a birth cohort study consisting of one "conventional subcohort" (n=2,343) of the children of mothers recruited during pregnancy when they were enrolled in another study of pelvic girdle pain. In addition, 491 children of mothers with an "alternative" lifestyle (anthroposophic, homeopathy users and so on) were included. Re-investigations with questionnaires were performed during infancy, preschool and school age. In addition, blood samples for total and specific IgE were collected at two years of age and airway function was assessed at school age (Kummeling *et al.* 2007, Notenboom *et al.* 2011).

Some of the studies focus primarily on high-risk children, with heredity for asthma or allergy. For example, the German Multicentre Allergy Study (MAS) is a birth cohort of 1,314 healthy, mature infants followed frequently during the first two years of life and then once every year until age 13. One third of the children included had risk factors for atopy (elevated cord blood IgE and/or two atopic family members) (Illi *et al.* 2004, Illi *et al.* 2006). In addition, the Childhood Origins of Asthma (COAST) Study consists of 287 children with an increased risk of developing asthma, with at least one parent having allergy and/or asthma. The study aims to evaluate the influence of the dysregulation of

the cytokine response present at birth and the effect of lower respiratory tract viral infections on the risk of subsequent asthma. The children are therefore followed closely with clinical examinations, sampling of blood and assessment of airway function. Outcome at six and eight years of age has been published (Lemanske *et al.* 2005, Jackson *et al.* 2008, Zhang *et al.* 2010).

In addition, some of these studies include interventions, studying possible effects on asthma and allergy outcomes.

In the National Asthma Campaign Manchester Asthma and Allergy Study (MAAS), over one thousand children were recruited prenatally and divided into different risk groups depending on the atopic status of their parents and whether pets were kept at home. The high-risk infants were randomly assigned to a mite-allergen avoidance programme. In addition, data were collected prenatally, cord blood samples are available and airway function measurements have been performed in early life (3 years) (Murray *et al.* 2004, Thomas *et al.* 2006).

In the Childhood Asthma Prevention Study (CAPS), the effectiveness of avoiding house dust mites and of modifying the fat composition of the diet during the first five years of life in an initial group of 616 high-risk children is being studied (Almqvist *et al.* 2007, Almqvist *et al.* 2010). The outcome at eight years was recently reported (Toelle *et al.* 2010).

In the Prevention and Incidence of Asthma and Mite Allergy (PIAMA) Study, 855 children born to allergic mothers were enrolled in a trial evaluating the effect of mite-reducing measures in the home. In addition, 3,291 children born to both allergic and non-allergic mothers were enrolled in a so-called "natural history" study to assess the role of dietary and environmental factors in childhood allergy (Caudri *et al.* 2009, Scholtens *et al.* 2009).

Cross-sectional studies

There are also cross-sectional studies analysing the prevalence of asthma and allergy and associations with possible risk factors.

The large multi-centre (20 countries) ISAAC Study is based on questionnaires and clinical examinations with SPT and focuses on different outcomes of asthma and allergies in childhood. The study is being conducted in three phases. Phase 1 assesses the prevalence and severity of asthma and allergic disease. Phase 2 focuses on identifying aetiological factors and phase 3 is a repetition of phase 1 after at least three years. The age groups of 6-7 years, 8-12 years and 13-14 years of age have been studied (Asher *et al.* 2006, Tabak *et al.* 2006, Beasley *et al.* 2008, Nagel *et al.* 2009, Flohr *et al.* 2011).

The West Sweden Asthma Study is a questionnaire-based survey of asthma and respiratory symptoms in adults aged 16-75 years. A random sample of 30,000 of the inhabitants of the western region of Sweden was included and the prevalence of asthma and respiratory symptoms and risk factors relating to those outcomes were analysed (Eriksson *et al.* 2010, Wennergren *et al.* 2010).

The Obstructive Lung Disease in Northern Sweden (OLIN) longitudinal paediatric study started in 1996 and included a cohort of 3,430 schoolchildren aged seven to eight years. Questionnaires were distributed at inclusion and annually until age 19 years. SPT was performed at inclusion and at age 11-12 and 19 years. Blood samples and tests for airway function and BHR have been performed in subsamples of the cohort (Rönmark *et al.* 2008). The study was repeated in 2006 with a new cohort aged seven to eight years (n=2,585), reinvestigated with questionnaires and SPT in 2010 (Andersson *et al.* 2010). The study aims to longitudinally study the development and incidence of asthma and allergy in children.

Prospective studies of virus-induced bronchiolitis or obstructive bronchitis

The long-term outcome after early wheezing has been studied in follow-up studies of children with severe obstructive disease in early life, *i.e.* children admitted to hospital due to virus-induced bronchiolitis or obstructive bronchitis in infancy. There are four Nordic follow-up studies, with two Finnish studies from Koupio started by Korppi and two Swedish studies started by Wennergren and Sigurs respectively (Korppi *et al.* 1986, Wennergren *et al.* 1992, Sigurs *et al.* 1995, Wennergren *et al.* 1997, Reijonen *et al.* 2000, Sigurs *et al.* 2000, Kotaniemi-Syrjänen *et al.* 2003a, Kotaniemi-Syrjänen *et al.* 2003b, Piippo-Savolainen *et al.* 2004, Hyvärinen *et al.* 2005a, Hyvärinen *et al.* 2005b, Sigurs *et al.* 2005, Routsalainen *et al.* 2010, Sigurs *et al.* 2010). Re-investigations have been made up to adolescence and adulthood. Procedures relating to the four follow-up studies are summarised in Table 1.

Definitions

Airway obstruction with wheezing is a common symptom of lower respiratory viral infection. Acute bronchiolitis has been described as a viral respiratory infection associated with bronchial obstruction, oedema and mucus, causing crepitations with or without wheezing on auscultation (Court 1973, Watts and Goodman 2007). The disease can become severe with hypoxia and hypercapnia requiring hospital care. Bronchitis is defined as a non-specific bronchial inflammation, with acute bronchitis being caused by a viral infection, sometimes associated with wheeze (Court 1973, Watts and Goodman 2007).

Table 1. Overview of the four Nordic follow-up studies of severe viral wheeze in early life.

	Started year	No. included	Male %	Age at inclusion	Bronchiolitis/wheezing bronchitis definition	First wheezing episode in	Viral agents (RSV%)	Re-investigations	Control group	Special features
Korppi Post-bronchiolitis study	1981-82	83	67%	< 24 mo. (median = 10 m)	Wheezing or prolonged expiration during respiratory infection	74%	All (40%)	2-3 y, 4.4-6 y, 8-10 y, 14.9 y, 18-21 y, 27 y	72 no atopic heredity, no wheeze < 2 y	Pneumonia group, n = 44
Wennergren Wheezing bronchitis study	1984-85	101	61%	< 24 mo. (median = 10 m)	Wheezing, retraction, rhonchi, tachypnea during respiratory infection	56%	All (28%)	3-4.5 y, 10 y, 17-20 y, 26 y (mean, ongoing)	294 Age matched at 17-20 y	-
Sigurs RSV study	1989-91	47	45%	< 12 months (mean = 116 days)	Tachypnea, prolonged expiration, dyspnea, and wheezing during respiratory infection	98%	RSV only (100%)	Mean 1 y, 3 y, 7.5 y, 13.4 y, 18 y	93 Age and gender matched, no early RSV	-
Korppi Viral wheezing study	1992-93	100	72%	> 24 months (median = 10 m)	Wheezing and respiratory distress, during acute respiratory tract infection	87%	All (31%)	1.5 m, 4 m, 8 m, 12 m, 3 y, 6 y and 11 y after admission (median age 12.3 y at last re-invest)	-	Treatment at admission: cromolyn group, budesonide group or control group

However, both bronchiolitis and bronchitis are used in different studies to define infants or older children with wheezing during respiratory infection. Most commonly, bronchiolitis is defined as wheezing during viral respiratory infections in children aged less than two years (Wennergren *et al.* 1992, Reijonen *et al.* 2000). However, in some studies, it has been suggested that bronchiolitis should only be considered in connection with viral wheeze before the age of 12 months or only respiratory infections caused by RSV (Sigurs *et al.* 1995, Schauer *et al.* 2002).

Others have defined wheezing with a viral respiratory infection as wheezing bronchitis, independent of age or viral agent (Wennergren *et al.* 1997, Thomsen *et al.* 2006). As a result, both definitions are used for children with viral wheeze (Court 1973, Elphick *et al.* 2007). In the follow-up studies described below, children with severe viral wheeze requiring hospitalisation are considered in all four studies, but somewhat different terminology is used.

Finnish post-bronchiolitis study

In the post-bronchiolitis study by the Korppi group, 130 children hospitalised due to wheezing bronchiolitis or pneumonia before the age of two years were followed until adulthood and compared with a control group (Korppi *et al.* 1986, Piippo-Savolainen *et al.* 2004, Hyvärinen *et al.* 2005a, Routsalainen *et al.* 2010).

An increased risk of asthma was seen in the bronchiolitis group in childhood and in adulthood. In the follow-up at age 18-20 years, the prevalence of doctor-diagnosed asthma was 30%, while at 27 years it was 20% among the children with early bronchiolitis. When self-reported symptoms of asthma were also included, the prevalence was 41% both in adolescence and in adulthood (Piippo-Savolainen *et al.* 2004, Routsalainen *et al.* 2010). In addition, individuals with early bronchiolitis had a reduction in airway function at age 18-20 compared with controls. Current atopy in late adolescence was associated with current asthma and BHR (Piippo-Savolainen *et al.* 2004). Furthermore, children with atopic dermatitis or elevated total serum IgE before the age of two years had more asthma in adolescence (Piippo-Savolainen *et al.* 2006).

Early predictive factors of asthma at age 18-20 years were parental asthma, early signs of atopy, early repeated wheezing and wheezing during non-RSV infection. In addition, reduced airway function in adulthood was seen in children exposed to tobacco smoke in early life (Piippo-Savolainen 2006).

Finnish viral wheezing study

The other prospective Finnish cohort study from the Korppi group has followed 100 children hospitalised due to viral wheezing before two years of age. The children have been re-investigated at 18 months and at four, six and 12 years of age (Reijonen *et al.* 2000, Kotaniemi-Syrjänen *et al.* 2003a, Kotaniemi-Syrjänen *et al.* 2003b, Hyvärinen *et al.* 2005b).

At 12 years, 40% had current asthma and 36% were using inhaled asthma medication (Hyvärinen *et al.* 2005b). Twenty-five per cent had persistent asthma from infancy to 12 years of age, with asthma on all re-investigations (Hyvärinen 2009). Subjects with teenage asthma had signs of reduced airway function (Hyvärinen *et al.* 2007).

Atopic eczema and sensitisation to inhalant allergens in infancy predicted teenage asthma (Hyvärinen *et al.* 2005b). Furthermore, an association was seen between maternal smoking during pregnancy and current bronchial hyper-responsiveness (BHR) (Hyvärinen *et al.* 2007).

At admission, one third of the cases were caused by RSV and another third by rhinovirus. As it turned out, having a rhinovirus infection at first admission was associated with elevated eosinophilic markers and atopic dermatitis in infancy. In addition, teenage asthma and atopy was more common among children with rhinovirus infection than with RSV infection at admission, but the rhinovirus infection was not an independent predictor of teenage asthma. Wheezing with rhinovirus infection was therefore regarded as a marker for those children predisposed to persistent wheeze with early allergic disease, airway inflammation and possibly affected airway function in infancy (Hyvärinen *et al.* 2005b, Hyvärinen 2009).

Wheezing bronchitis follow-up

In the Swedish prospective study by Wennergren *et al.*, 101 children hospitalised due to wheezing bronchitis before two years of age were re-investigated at preschool age and at 10 years of age (Wennergren *et al.* 1992, Wennergren *et al.* 1997). At admission, two thirds were boys and 58% of the study group had a family history of atopic disease. RSV was the most common cause of infection (Wennergren *et al.* 1992).

At preschool age, the children were re-investigated with questionnaires, clinical examination and allergy testing. More than half the study group was symptom free, while 47% reported persistent wheezing. One third of the symptomatic children were considered to have moderate to severe asthma. The most common trigger factor of preschool wheeze was respiratory infection (93%).

In about 50%, cold weather and exercise were reported to be trigger factors. Only about 15% reported pollen or furry animals as trigger factors. The predictive factors for having a persistent wheeze at preschool age were intense disease in early childhood (the need for daily medication for at least six months), early age at first wheezing episode and at admission and past or present atopic symptoms (Wennergren *et al.* 1992).

When reinvestigated at 10 years of age with questionnaires, clinical examination and allergy and airway function testing, current asthma was found in 30%. One third of the symptomatic children still had moderate to severe asthma. The predictive factors for persistent asthma at 10 years of age were intense disease in early childhood, early age at first wheezing episode and recent atopic symptoms. In addition, BHR (with a positive histamine challenge) at 10 years and exposure to tobacco smoke at home in infancy were associated with an increased risk of school age asthma (Wennergren *et al.* 1997).

Swedish post-RSV study

In the Swedish study by Sigurs *et al.*, 47 children hospitalised due to severe RSV bronchiolitis before the age of one year were followed to early adulthood. The prevalence of asthma and allergy was monitored and compared with a control group matched for age and gender. An increased prevalence of asthma, recurrent wheeze and allergic sensitisation compared with the controls was shown at age three, seven and 13 years (Sigurs *et al.* 1995, Sigurs *et al.* 2000, Sigurs *et al.* 2005).

At the re-investigation at seven years of age, children with wheezing due to RSV infection had more atopic asthma and more clinical symptoms of and sensitisation to inhaled allergens. Moreover, parental asthma, male gender and furry animals indoors increased the risk of any wheeze at age seven. Parental atopy independently increased the risk of atopic sensitisation. Having furry animals indoors was protective for sensitisation in the univariate but not the multivariate analyses (Sigurs *et al.* 2000).

At the re-investigation at age 13, the risk of asthma, recurrent wheeze or sensitisation to inhaled allergens was independently increased by early RSV-induced wheeze and parental asthma. Eighty per cent of the children with early severe RSV-induced wheeze who had asthma at 13 years of age also had current allergic sensitisation. Signs of reduced airway function were seen in the children with early RSV-induced wheeze (Sigurs *et al.* 2005).

Recently, the follow-up at 18 years confirmed the increased risk of asthma, clinical allergy and sensitisation. Reduced airway function at 18 years of age was found in the children with early RSV infection, regardless of current asth-

ma or allergy. Early severe RSV-induced wheeze and current allergic rhinoconjunctivitis (ARC) were found to be independent risk factors for asthma and recurrent wheeze at 18 years of age (Sigurs *et al.* 2010).

Predicting persistent asthma

Asthma Predictive Index, API

As previously stated, not all children who wheeze in early childhood will go on to develop asthma. Castro-Rodriguez *et al.* from the Tucson Study have proposed the Asthma Predictive Index (API), which predicts asthma at school age in children with a recurrent wheeze some time during the first three years of life (Table 2). The API consists of two major and three minor criteria of which at least one major or two minor are required for a positive asthma predictive index. The two major criteria are doctor-diagnosed parental asthma or doctor-diagnosed eczema in the child. The minor criteria are doctor-diagnosed allergic rhinitis, wheezing between colds and the presence of eosinophilia (Castro-Rodriguez *et al.* 2000).

Despite having low sensitivity of 22%, this index has a high negative predictive value, identifying children with a high likelihood of transient wheeze. The index has been used in clinical settings recommending treatment on the basis of a positive or negative index for example in the GINA and American Guidelines (Castro-Rodriguez 2011). A modified index, including allergic sensitisation to one or more aeroallergens as a major criterion and exchanging allergic rhinitis for allergic sensitisation to milk, eggs or peanuts as a minor criterion, has been suggested (Guilbert *et al.* 2004). However, it has been argued that sensitisation has less predictive value compared with eosinophilia in several studies and also that it is less reliable and more expensive in a clinical setting (Castro-Rodriguez 2011).

Isle of Wight and PIAMA indices

Other studies have proposed different predictive indices (Table 2). For example, in the Isle of Wight birth cohort, four predictive factors were suggested, *i.e.* a family history of asthma, recurrent chest infections in the second year of life, atopic sensitisation at four years of age and the absence of recurrent nasal symptoms in the first year of life (Kurukulaaratchy *et al.* 2003). In addition, the PIAMA birth cohort identified predictors of asthma at school age in children with wheezing or coughing at night before the age of four. Male gender, post-term delivery, parental education, inhaled medication, wheezing frequency, wheezing or dyspnea apart from colds, respiratory tract infections and eczema were found independently to predict asthma (Caudri *et al.* 2009).

Table 2. *Predictive indices for asthma development among children with wheezing disorder in early life.*

Asthma Predictive Index (API) from the Tucson Study	
Major criteria	
	Doctor-diagnosed parental asthma Doctor-diagnosed eczema in child
Minor criteria	
	Doctor-diagnosed allergic rhinitis in child Wheezing in between colds Eosinophilia

*One major or two minor criteria required for positive index

Modified Asthma Predictive Index (API)	
Major criteria	
	Doctor-diagnosed parental asthma Doctor-diagnosed eczema in child Sensitisation to aeroallergens
Minor criteria	
	Allergic sensitisation to milk, eggs or peanuts Wheezing in between colds Eosinophilia

Hospital Asthma Predictive Index from the Korppi group	
Major criteria	
	Doctor-diagnosed parental asthma Doctor-diagnosed eczema and/or food allergy in child Parental (especially maternal) smoking
Minor criteria	
	Sensitisation to aeroallergens Wheezing due to non-RSV infection Eosinophilia or lack of eosinophilic response during viral infection

Predictive Index from the Isle of Wight Study
Family history of asthma Recurrent chest infections in second year of life Atopic sensitisation at age 4 years Absence of recurrent nasal symptoms first year

Predictive Index from the PIAMA Study
Male gender Post-term delivery Parental education Inhaled medication Wheezing frequency Wheezing or dyspnea apart from colds Respiratory tract infections Eczema

Hospital API for severe viral wheeze

Based on the findings in the Korppi group, a further modified API applicable to children hospitalised due to viral wheeze in early life has been proposed, (Table 2). The major criteria are doctor-diagnosed parental asthma, doctor-diagnosed atopic dermatitis and/or food allergy in the child and parental (especially maternal) smoking. The minor criteria are sensitisation to inhaled allergens, wheezing due to non-RSV infection and eosinophilia or a lack of eosinophilic response during viral infection. As in the API from the Tucson Study, one major or two minor criteria are required for a positive index (Piippo-Savolainen and Korppi 2008).

Identifying risk factors and protective factors

To better understand the underlying pathophysiological mechanisms and identify possible preventive interventions, studies of risk factors and protective factors for the development of wheeze and asthma have been performed. Several factors have been identified, but their individual impact on asthma development has sometimes been the subject of debate. Interaction between different factors has been suggested and recently the importance of genetic interaction (different genotypes being differently susceptible to different factors) has been highlighted (Gilliland *et al.* 2002, Melén *et al.* 2004, Eder *et al.* 2004).

A description of the impact and suggested mechanisms of the most discussed factors follows below.

Viral infections

Triggers of wheeze

The most common trigger of obstructive symptoms, *i.e.* wheezing in early childhood, is a viral infection (Heymann *et al.* 2004). RSV is the most prevalent virus associated with severe wheezing in the first year of life (Jartti *et al.* 2004). In temperate climates, it peaks during the winter months, causing more than 60% of wheezing episodes in infants (Jartti *et al.* 2004). Eventually, all children have been affected by RSV at the age of three years (Ogra 2004). Infants aged less than six months, premature infants and infants with chronic diseases like BPD, heart disease or immunodeficiency run an increased risk of severe disease (Ogra 2004). However, other viruses have also been identified as triggers of obstructive disease in infants: rhinovirus, enterovirus, adenovirus, coronavirus, metapneumovirus, bocavirus, influenza and parainfluenza virus (Korppi *et al.* 1986, Wennergren *et al.* 1992, Jartti *et al.* 2004, Hyvärinen 2009). Rhinovirus has been reported to be the most common cause of wheeze in older children and adults, but it also causes wheeze in infants (Heymann *et al.* 2004, Jartti *et al.* 2004, Busse *et al.* 2010).

The most recent addition to viruses associated with wheezing is the bocavirus. It has been reported to present with respiratory symptoms, most commonly in children aged less than three years. Allander *et al.* found bocavirus in 19% of wheezing children, but a high incidence of co-infections with other viruses has also been reported (Allander *et al.* 2007).

Increased risk of asthma

An increased prevalence of wheeze and asthma has been reported following virus-induced wheezing in early life. In the post-bronchiolitis study from the Korppi group, an increased risk of asthma has been reported until the age of 27 years (Piippo-Savolainen *et al.* 2004, Routsalainen *et al.* 2010). Likewise, Sigurs *et al.* report an increased risk of asthma, recurrent wheeze and allergic sensitisation during childhood and early adulthood following hospitalisation due to RSV bronchiolitis during the first year of life (Sigurs *et al.* 2000, Sigurs *et al.* 2005, Sigurs *et al.* 2010). In the Tucson Study, an increased risk of wheeze up to age 11 but not at age 13 is reported following early lower respiratory tract illness due to RSV, where more than 90% experienced wheezing. No association was seen with atopic disease (Stein *et al.* 1999a).

It has been suggested that wheezing due to infection with rhinovirus during infancy increases the risk of subsequent asthma more than wheezing as a result of RSV infection (Kotaniemi-Syrjänen *et al.* 2003b, Hyvärinen *et al.* 2005b, Lemanske *et al.* 2005). In the Finnish study by the Korppi group, an increased risk of asthma during teenage following hospitalisation due to severe wheezing in early life was seen. The risk of asthma was about five times higher if the wheezing was caused by RSV, but it was 10 times higher if the wheezing was caused by rhinovirus (Hyvärinen *et al.* 2005b). This was confirmed in the COAST high-risk cohort, where wheezing caused by rhinovirus during infancy was the strongest predictor of wheezing at three years of age (Lemanske *et al.* 2005). In addition, an increased risk of asthma at six years was seen following early RSV-induced wheeze, but the risk was even higher when the wheeze was caused by rhinovirus infection, independent of atopic sensitisation (Jackson *et al.* 2008). Wennergren *et al.* report an increased asthma risk at age five and 10 years in children hospitalised due to wheezing bronchitis, irrespective of the virus that caused the infection at admission (Wennergren *et al.* 1992, Wennergren *et al.* 1997).

Age at infection and viral load

The age when infected might be of importance and it appears as if the effect of the viral infection might differ if the infection does or does not occur during a vulnerable phase of immunological development. For example, in the study by Sigurs *et al.*, almost all the subjects in the cohort were severely infected before

the age of six months (Sigurs *et al.* 1995). The immune response to viral infection has been reported to change over time. Kristjansson *et al.* report a T-helper cell type 2-like (Th_2) response to RSV but also to influenza and parainfluenza in children infected before the age of three months compared with children infected after the age of three months (Kristjansson *et al.* 2005).

Host or virus

Does the viral infection give rise to the increased risk of subsequent wheeze and asthma or is the child who wheezes already predisposed to wheezing with viral infections and to developing asthma?

In a large Danish twin study, Thomsen *et al.* conclude that a shared genetic predisposition to both severe RSV infection and asthma could explain the association. A causal link between RSV infection and asthma could be statistically rejected, while asthma causing RSV infection could not (Thomsen *et al.* 2009). However, in a recent study by Wu *et al.*, an association was found between age at winter virus peak and risk of severe bronchitis and asthma in childhood. An increased risk was found among children aged four months during the winter virus peak, suggesting developmental damage caused by the RSV infection leading to an increased asthma risk later on. However, the risk of later asthma was associated with the actual virus peak during that particular year and not the actual time when the child was infected (Wu *et al.* 2008).

Both the studies by Thomsen *et al.* and by Wu *et al.* focus on the diagnosis of RSV infection as outcome and not the symptoms the infection triggered (for example, wheeze) (Wu *et al.* 2008, Thomsen *et al.* 2009). However, it has been suggested that different symptoms during viral infection influence the subsequent risk of asthma. The Everard group reports different outcomes in children hospitalised due to RSV infection with either bronchiolitis with crepitations or only wheeze. The children with wheezing were more likely to be allergic, have more wheezing, have more severe respiratory illness and more frequently receive treatment with inhaled corticosteroids at age three years (Elphick *et al.* 2007). These findings indicate that the host response, rather than the virus causing the infection, is responsible for the increased risk of subsequent asthma (Kuehni *et al.* 2009). This notion is further supported by the reports of an increased asthma risk following wheezing in infancy not only with RSV but also with other viruses, especially rhinovirus (Lemanske *et al.* 2005, Kuehni *et al.* 2009).

Allergy and viral infection

A synergistic effect of allergic sensitisation and viral infection has been suggested (Sly 2011). Respiratory infection has been reported to be more severe

and of longer duration in asthmatics compared with healthy individuals (Corne *et al.* 2002). Furthermore, children with allergic asthma have been reported to have respiratory infections of increased duration and severity compared with children with non-allergic asthma (Olenec *et al.* 2010).

Recently, the role of viral respiratory infections in asthma was summarised in a review by Busse *et al.* (Busse *et al.* 2010). Viral infections have been reported to cause damage to the airways with the impairment of the epithelial barrier function increasing the absorption of allergens and irritants, increasing allergic inflammation. However, increased viral replication has been reported in already damaged epithelium, indicating that an epithelium affected by allergic inflammation could promote viral replication, thereby causing more severe disease This can also explain why other environmental factors like tobacco smoke that might damage the epithelium increase the risk of viral wheeze (Busse *et al.* 2010).

A change in the antiviral immune response in individuals with allergic asthma has been reported, with an impaired interferon response from mononuclear and bronchial epithelial cells. Both allergic inflammation and viral infection (rhinovirus) have been reported to affect epithelial cells to produce cytokines promoting Th_2 differentiation, thereby increasing allergic inflammation (Busse *et al.* 2010). Impaired interferon production *in vitro* has been reported already in early infancy in individuals with an increased risk of wheezing in infancy (Guerra *et al.* 2004a, Gern *et al.* 2006).

These findings suggest that some individuals with an altered immune response might be more susceptible to viral disease and prone to more severe symptoms like wheezing (Gern *et al.* 2006).

Conclusion

To summarise, the question of whether the viral infection itself gives rise to the increased risk of subsequent wheeze and asthma or whether the child responding with wheezing to viral infections is already predisposed to develop asthma is the subject of debate.

It could also be that both hypotheses are true – some viruses might cause pathological changes in the airways in susceptible individuals, thereby promoting an increased asthma risk (Jackson *et al.* 2008).

Heredity

To have a predisposition to allergic disease is referred to as being atopic. For decades it has been known that the tendency to develop asthma and allergies is more pronounced in children with parental allergic disease.

Parental asthma

A family history of asthma has been reported to increase the risk of asthma during childhood in several studies (Martinez *et al.* 1995, Rönmark *et al.* 2008, Lim *et al.* 2010). Parental asthma is regarded as an important factor for predicting asthma in childhood and is included in the API (Castro-Rodriguez *et al.* 2000). Heritability estimates vary between studies but, for example, the twin study of Koeppen-Schomerus reports 68% heritability due to parental asthma (Koeppen-Schomerus *et al.* 2001, Lim *et al.* 2010.). Moreover, in the follow-up studies of severe viral wheeze, parental asthma has been shown to affect the long-term outcome (Piippo-Savolainen *et al.* 2006, Hyvärinen 2009).

Both maternal and paternal asthma increase the risk of asthma in the child. Maternal asthma has been suggested to have a greater impact than paternal. This might be explained by non-genetic factors influencing the child *in utero* or *post partum* (Lim *et al.* 2010).

Parental allergy

It has been suggested in the BAMSE Study and the West Sweden Asthma Study, for example, that heredity for allergies influences the risk of asthma in the offspring (Melén *et al.* 2004, Wennergren *et al.* 2010). A change in maternal immune responses during pregnancy has been reported in mothers with allergy, possibly favouring postnatal allergic sensitisation in the offspring (Breckler *et al.* 2010, Sly 2011). Furthermore, the reduced production of interferon gamma by cord blood monocytes is reported in infants with atopic heredity (Gern *et al.* 2006).

Gender and genes

It has been suggested that the impact of family history differs between boys and girls. Melén *et al.* report an interaction between male gender and parental allergic disease in children with early persistent wheezing. When analysing maternal and paternal influence separately, the effect was only seen for maternal allergic disease. This might be explained by genetic imprinting or it may be suggestive of X-linked genes, more likely to be unmasked in boys (Melén *et al.* 2004). Several genes and loci have been suggested to be of importance in the development of asthma and allergic disease. Genes important in the Th_2-mediator response have been identified, but "non-allergic" pathways, in muscle remodelling, for example, have also been shown to be associated with asthma and BHR (Ober and Yao 2011).

Other allergic manifestations

Allergic march

Numerous studies have observed the increased prevalence of wheezing and asthma in children with early eczema and other allergic manifestations such as food allergies (Kotaniemi-Syrjänen *et al.* 2003a, Guilbert *et al.* 2004, Illi *et al.* 2004, Hyvärinen *et al.* 2005b, Illi *et al.* 2006, Piippo-Savolainen *et al.* 2006, Piippo-Savolainen *et al.* 2007). The allergic march is described as a pathway, starting with eczema and food allergies in infancy, developing to wheezing and persistent asthma during preschool and school age and thereafter becoming sensitised to airborne allergens (Spergel 2010). In addition, when it comes to predicting persistent asthma in children, allergic manifestations in early life have a high predictive value. For example, the API includes doctor-diagnosed eczema as one of the major criteria and doctor-diagnosed allergic rhinitis and eosinophilia as two of the minor ones (Table 2) (Castro-Rodriguez *et al.* 2000).

Altered immune response

Already at birth, a change in the T-helper cell type 1 (Th_1) immune response has been reported in children with subsequent allergic disease (Björkstén 1999, Prescott 2010). The slower maturation of the Th_1 immune response in atopic children has been observed (Björkstén 1999, Jenmalm 1999, Sly and Holt 2011). In addition, a change in neonatal interferon gamma production has been associated with an increased risk of subsequent atopy (Tang *et al.* 1994). It could be argued that eczema and allergic sensitisation during infancy are other manifestations of the same disease as recurrent wheeze and subsequent persistent asthma, rather than a causative factor. However, the predisposed child with early allergies might respond differently to environmental exposure and life events. For example, early allergic sensitisation synergistically affects the increased risk of asthma following wheezing with viral respiratory infections in infancy (Sly 2011). A recent study by Subrata *et al.* reports an interaction between IgE antibodies in the lung and the recruitment of pro-inflammatory macrophages to the lung during viral infection (Subrata *et al.* 2009).

Impact on persistence of viral wheeze

Both early and current allergic manifestations are associated with persistent or relapsing asthma following severe viral wheezing disease in early life. In the follow-up studies from the Korppi group, both early and current manifestations of allergy were associated with asthma during teenage and in early adulthood (Piippo-Savolainen *et al.* 2004, Piippo-Savolainen *et al.* 2006, Hyvärinen 2009). However, at age 27, only current allergic manifestations were associated with adult asthma (Ruotsalainen *et al.* 2010).

In the Swedish post-RSV study, Sigurs *et al.* report that childhood sensitisation and allergic rhinitis are associated with persistent or relapsing asthma up to the age of 18 years (Sigurs *et al.* 2010).

Gender

Course of wheezing in boys and girls

Wheezing and asthma in infancy and childhood is more prevalent among boys than girls (Korppi *et al.* 1986, Skobeloff *et al.* 1992, Wennergren *et al.* 1992, Martinez *et al.* 1995, Taussig *et al.* 2003, Melén *et al.* 2004). However, the female dominance of asthma in adulthood is well known (Skobeloff *et al.* 1992, Sears *et al.* 2003). The reversal in gender distribution is seen somewhere between the age of 10 years and late adolescence (Skobeloff *et al.* 1992, Schatz *et al.* 2004). The pattern is described in the Tucson Study, where wheezing in early childhood was clearly more common in boys, with a decreasing prevalence at older age. In the case of girls, the prevalence of wheeze decreased in the early years but started to increase after the age of 10 years. At age 16, wheezing was equally common among boys and girls in the Tucson Study. Doctor-diagnosed asthma was also more common among boys at age 16, but with less difference seen in adolescence, with an increasing prevalence in girls (Taussig *et al.* 2003). Similar results were described back in 1992 by Anderson *et al.* in the British National Child Development Study, with asthma being more common in boys up to age 16, independent of current and prior atopic status. At age 23, the prevalence was higher among girls. The incidence of asthma in boys increased until the age of 16 and then started to decrease, while the incidence in females increased by almost 1% a year between age 17 and age 23 (Anderson *et al.* 1992).

Pulmonary development

The higher prevalence of wheezing in male infants might be explained by different pulmonary development in infancy in boys and girls. Young *et al.* has described lower airway function seen in boys compared with girls during the first year of life, drawing the conclusion that, in infancy, girls have larger airways in relation to absolute lung size compared with boys (Young *et al.* 2000). Pulmonary development continues through childhood into adolescence, with different growth patterns seen in females and males. At age 16, the roles are reversed, with females having more narrow airways in relation to lung size compared with boys (Merkus *et al.* 1993, Melgert *et al.* 2007).

Obesity

As discussed below, obesity has been associated with wheeze and asthma. The impact of obesity in girls has been emphasised and associated with early menarche, indicating increasing levels of oestrogen (Castro-Rodriguez *et al.* 2001).

Hormonal changes

It has been suggested that hormonal changes are important in the gender shift seen in asthma prevalence during adolescence, as summerised by Melgert *et al.* in 2007 (Castro-Rodriguez *et al.* 2001, Melgert *et al.* 2007). Salam *et al.* report an association between early menarche and asthma, indicating that the cumulative dose of female sex hormones might act pro-inflammatory (Salam *et al.* 2006). This is further supported by the fall in asthma prevalence in postmenopausal women and the premenstrual worsening described in female asthmatics. Furthermore, it has been argued that testosterone acts in an anti-inflammatory manner and thereby has a protective effect on asthma (Melgert *et al.* 2007).

Obesity

In a meta-analysis, Flaherman and Rutherford conclude that both high birth weight and overweight during childhood increase the risk of future asthma (Flaherman and Rutherford 2006). In line with this conclusion, Matricardi *et al.* report a mainly consistent link between obesity and wheezing and asthma during childhood in a review of the paediatric field (Matricardi *et al.* 2007). Several studies have excluded the possibility of asthma causing obesity. In actual fact, a significantly lower BMI in adulthood was seen among subjects with childhood asthma in the Dunedin cohort (Hancox *et al.* 2005). However, the association appears to be complex. For example, conflicting results have been reported regarding the relationship between allergic disease and obesity. Some studies report an increased risk of atopy with obesity, while others see no association or even a reduced risk of atopy (von Mutius *et al.* 2001, Matricardi *et al.* 2007, Sidoroff *et al.* 2011). In addition, obesity in asthmatics has been associated with less BHR, suggesting obesity-related symptoms mimicking those of asthma (Bibi *et al.* 2004, Matricardi *et al.* 2007).

Gender

Gender has been reported to affect the association between obesity and asthma, which is more evident among girls. In the Tuscon Study, girls with pre-pubertal obesity run an increased risk of later asthma. As mentioned above, obesity in girls has been associated with early menarche and increasing levels of oestrogen (Castro-Rodriguez *et al.* 2001). However, there are studies that also report an association between obesity and asthma in males (Gilliland *et al.* 2003, Guerra *et al.* 2004b).

Possible mechanisms

As summarised in the review by Matricardi *et al.*, there are several possible explanations supporting the association between obesity and subsequent asthma. As indicated above, gender-specific and oestrogen-dependent effects are important. Furthermore, reduced airway function has been reported in obese children. In addition, obesity has been associated with the increased production of pro-inflammatory substances such as cytokines and the hormone leptin. As mentioned above, a high birth weight might be associated with both subsequent obesity and asthma. Furthermore, maternal diet and physical activity during pregnancy might influence the outcome in offspring. Moreover, the diet during childhood appears to play an important role in the development of both obesity and asthma. For example, a high total caloric intake and low n-3 polyunsaturated fatty acids (PUFA) consumption might increase the risk (Matricardi *et al.* 2007).

Smoking

Impact of exposure to tobacco smoke

Exposure to tobacco smoke both during pregnancy and in infancy and childhood has been reported to increase the risk of wheeze and asthma (Rylander *et al.* 1993, Lødrup Carlsen *et al.* 1999, Stein *et al.* 1999b, Lux *et al.* 2000, Taussig *et al.* 2003, Lannerö *et al.* 2006). It has been suggested that maternal smoking during pregnancy is particularly important (Rylander *et al.* 1993, Lødrup Carlsen *et al.* 1999, Stein *et al.* 1999b, Lux *et al.* 2000, Lannerö *et al.* 2006). Impaired airway function in infancy and childhood has been reported following smoke exposure *in utero* (Hanrahan *et al.* 1992, Lødrup Carlsen *et al.* 1997, Gilliland *et al.* 2000). However, it has also been argued that postnatal smoke exposure affects airway function (Mannino *et al.* 2002, Piippo-Savolainen *et al.* 2006). Since very few mothers who smoke during pregnancy quit *post partum*, it is difficult to distinguish the effects of pre- and postnatal exposure (Alm *et al.* 1998, Stein *et al.* 1999b).

Genetic variations have been suggested to influence the vulnerability to smoke exposure both *in utero* and postnatally. For example, different genotypes of the gluthatione-S-transferase enzymes have been associated with altered vulnerability to smoke exposure affecting the risk of subsequent wheeze and asthma (Gilliland *et al.* 2002, Kabesch *et al.* 2004).

Active smoking

Active smoking in adolescents and adults has been reported to increase the risk of current asthma (Strachan *et al.* 1996, Sears *et al.* 2003). The social inheritance of smoking habits, *i.e.* the environment in which the child grows

up, has been reported to be closely associated with becoming an active smoker as an adult (De Vries *et al.* 2003). In addition, in a twin study by Vink *et al.*, it is concluded that the smoking initiation behaviour was explained by genetic influences in 44%, shared environment in 51% and by unique environmental factors in 5%. However, smoking dependence was explained by genetic factors in 75%, while only 25% was explained by unique environmental factors. These findings emphasise the genetic impact on continuous nicotine dependence (Vink *et al.* 2005).

Allergy and smoking

The association between smoke exposure and allergic sensitisation has been the subject of debate. Some studies report an increased risk of allergic sensitisation following prenatal smoke exposure (Kulig *et al.* 1999, Bråbäck *et al.* 2001), while others do not report any such association (Murray *et al.* 2004). Lannerö *et al.* found no association with smoke exposure *in utero* but an increased risk of sensitisation to inhalants and food allergens in children following parental smoking in early life (Lannerö *et al.* 2008).

Pathophysiology

The pathophysiological mechanisms of smoke exposure are not fully understood. However, in a recent review Rehan *et al.* report of prenatal smoke exposure causing lung hypoplasia and reduced lung growth, with fewer and larger saccules, resulting in a reduced surface area for gas exchange in the fetus in animal models (Rehan *et al.* 2009). Furthermore, a change in respiratory control and an increased risk of sudden infant death syndrome has been reported (Alm *et al.* 1998, Rehan *et al.* 2009). In addition, Rehan *et al.* reported that prenatal smoke exposure changed cell signalling in the developing alveolus, affecting surfactant synthesis and the differentiation of alveolar cells and lung fibroblasts. The increased differentiation of lung fibroblasts into myofibroblasts, a cell type associated with COPD and asthma, was reported (Rehan *et al.* 2009).

The developing immune system has been reported to be affected by prenatal smoke exposure, with the delayed maturation of the immune response at birth (Prescott 2010, Noakes *et al.* 2003). In addition to the toxic effects of nicotine on the airways, the suppression of fetal breathing movements, changing lung growth, genotoxicity and the epigenetic effects of prenatal smoke exposure, has also been suggested (Sly 2011).

Smoking regulations

In line with these and other findings of the negative effects of tobacco smoke *in utero*, attempts have been made to reduce the incidence of maternal smoking during pregnancy. In Sweden, the prevalence has gone from about 31% in the

early 1980s to around 6.7% in 2009 (Swedish National Board of Health and Welfare 2004, Lafih 2011). The prevalence is higher among those in underprivileged socioeconomic society groups and among younger women (Swedish National Board of Health and Welfare 2007).

Antibiotics

Impact on wheeze and asthma

Antibiotic treatment in infancy has been reported to increase the risk of wheeze at preschool age (Thomas *et al.* 2006, Kummeling *et al.* 2007, Marra *et al.* 2009) and the risk of asthma at school age (Droste *et al.* 2000, Kozyrskyj *et al.* 2007). A dose-response relationship between the risk of asthma and the number of antibiotic treatments has been reported (Kozyrskyj *et al.* 2007, Marra *et al.* 2009). In these studies, the possibility of reverse causation cannot be completely excluded. In a recent register-based study, Almqvist *et al.* reported an association between prescriptions for asthma medications and antibiotics in early childhood, especially strong for antibiotics used to treat respiratory infections. This highlights the possibility of reverse causation or confounding by indication (Almqvist *et al.* 2011).

Marker of asthma?

Neonatal antibiotic treatment could be a marker of asthma rather than a causative factor. Delayed immune maturation at birth is seen in infants born to parents with allergy and asthma, as indicated by cord blood cytokine levels or responses to stimulation *in vitro* (Gern *et al.* 2006, Gold *et al.* 2009, Sly and Holt 2011). Furthermore, there are data linking the delayed maturation of the immune system at birth and in early postnatal life with an increased risk of subsequent asthma in children and with an increased frequency of lower respiratory illnesses (Zhang *et al.* 2009, Sly 2011). The low production of interferon-gamma at birth or shortly after predicts recurrent wheezing later on (Guerra *et al.* 2004a) and the excessive production of IL-5 by T-cells at birth is associated with an increased risk of subsequent severe respiratory infections (Zhang *et al.* 2009). It could be speculated that delayed immune maturation at birth could increase the susceptibility not only to viral but also to other infections, leading to antibiotic treatment.

Pathophysiology

On the other hand, a causal relationship is supported by the findings of a change in mucosal immune response following antibiotic treatment. It has been suggested that the change in gastrointestinal (GI) microflora following antibiotic treatment, with long-term changes in GI colonisation, impairs the development of immunological tolerance. Changes to the mechanisms of anti-

gen presentation, with the disturbance of the T-regulatory cell response, have been discussed (Noverr and Huffnagle 2005). Furthermore, impaired gut barrier function and the Th_2 polarisation of the immune response have been seen in murine models (Oyama *et al.* 2001, Schumann *et al.* 2005). Disturbances in the intestinal environment in early life, altering the microflora of the gut and the exposure to microbes and allergens, therefore appear to increase the risk of future allergic asthma. This is further supported by findings indicating less atopic disease following probiotic administration (Kalliomäki *et al.* 2003, West *et al.* 2009).

Paracetamol

Increased risk of asthma

Several studies have reported an increased risk of asthma following paracetamol exposure both *in utero* and during infancy (Shaheen *et al.* 2005, Koniman *et al.* 2007, Beasley *et al.* 2008, Rebordosa *et al.* 2008, Garcia-Marcos *et al.* 2009, Perzanowski *et al.* 2010, Bakkeheim *et al.* 2011) and recent meta-analyses have confirmed the association (Etminan *et al.* 2009, Eyers *et al.* 2011). Furthermore, a dose-dependent effect has been described (Beasley *et al.* 2008). In addition, paracetamol use has been reported to increase the risk of other allergic manifestations such as eczema and rhinoconjunctivitis (Beasley *et al.* 2008, Bakkeheim *et al.* 2011). However, not all studies find such an association between paracetamol exposure and the risk of wheeze. In a study by Kang *et al.*, no association was found between paracetamol exposure during pregnancy and asthma in the offspring (Kang *et al.* 2009).

Pathophysiology

Paracetamol has been described as increasing oxidant-induced inflammation by lowering the glutathione levels when it is metabolised in the liver (Forrest *et al.* 1982, Nuttall *et al.* 2003, Fogarty and Davey 2005). As a result, the pulmonary antioxidant defence is diminished and airway inflammation is increased (Varner *et al.* 1998). The lowering of glutathione levels by paracetamol has also been suggested to enhance the Th_2 responses, changing antigen presentation and recognition (Nuttall *et al.* 2003, Farquhar *et al.* 2010). Unlike acetylsalicylic acid, paracetamol does not affect the COX-2 pathway, which is activated during respiratory viral infections. The COX-2 pathway increases the production of prostaglandin E2, which in turn inhibits the production of Th_1-cytokines, shifting the balance towards Th_2 (Varner *et al.* 1998). Paracetamol has been shown to have immune-modulating effects, altering the immunological response to vaccination if it is given prior to the fever reaction (Prymula *et al.* 2009). The recent findings that maternal antioxidant polymorphisms modify the association between paracetamol exposure and childhood asthma further support a causal relationship (Shaheen *et al.* 2010). However, it has

been argued that therapeutic doses of paracetamol do not affect the gluthatione levels sufficiently to change the antioxidant defence (Scialli *et al.* 2010).

Confounding

In fact, a causal relationship between paracetamol exposure and asthma has been disputed. It has been suggested that confounding by indication explains the association that is seen. Paracetamol might be a proxy for respiratory morbidity, since children with respiratory infections in early childhood often receive paracetamol (Lowe *et al.* 2010, Tapiainen *et al.* 2010). However, studies that only consider paracetamol exposure during pregnancy still find an association (Shaheen *et al.* 2005, Perzanowski *et al.* 2010). On the other hand, maternal use of paracetamol could be a proxy for maternal respiratory infection. Furthermore, paracetamol use could be a marker of a difficult pregnancy, other maternal diseases and possibly also of the intake of other medication.

Fish

Effect on asthma and allergy

A high maternal intake of fish during pregnancy and the intake of fish by the child itself during infancy have been reported to reduce the risk of later allergic manifestations in the child (Nafstad *et al.* 2003, Kull *et al.* 2006, Alm *et al.* 2009, Hesselmar *et al.* 2010, Øien *et al.* 2010, Kremmyda *et al.* 2011). In the Dutch part of ISAAC-2, an association between frequent fish intake and a reduced risk of asthma was seen at school age (Tabak *et al.* 2006). Likewise, Nafstad *et al.* report a reduced risk of asthma, allergic rhinitis and sensitisation to dust mites at four years of age following fish consumption during the first year of life (Nafstad *et al.* 2003). In addition, several studies report a protective effect as a result of fish consumption in infancy on eczema in childhood (Alm *et al.* 2009, Hesselmar *et al.* 2010, Øien *et al.* 2010). In the Children of Western Sweden Study, Alm *et al.* report a reduced risk of eczema during the first year of life following the early introduction of fish in infancy (Alm *et al.* 2009). In the Norwegian PACT Study, a reduced risk of eczema at two years of age as a result of early fish consumption was seen, but no effect on eczema in the child as a result of maternal fish consumption during pregnancy was seen (Øien *et al.* 2010). However, other studies also report a reduced risk of asthma, eczema and allergic sensitisation in the child as a result of frequent maternal fish consumption during pregnancy (Salam *et al.* 2005, Willers *et al.* 2007). The effect was more pronounced in children with maternal asthma or atopy (Salam *et al.* 2005).

Pathophysiology

Increasing the ratio of n-6 to n-3 PUFAs, due to an increased intake of linoleic acid, has been reported to influence the development of allergic disease by acting on inflammatory and immunological pathways. Fish is rich in n-3 PUFA and it has been suggested that it opposes the action of n-6 PUFA, thereby reducing the risk of allergy (Kremmyda *et al.* 2011). However, the association between the prevalence of asthma and n-3 PUFA has not been confirmed when analysing serum levels in children or adults (Bolte *et al.* 2006). However, other components of fish have also been suggested to contribute. For example, fish is a source of vitamin D, which, it has also been argued, reduces the risk of allergic disease (Holick 2008, Mullins and Camargo 2011). However, confounding or bias could explain the association with fish consumption as a marker of socioeconomic or other lifestyle factors, as well as a proxy for other dietary patterns (Almqvist *et al.* 2007).

Prevention studies

Attempts have been made to prevent asthma and atopy development in children using fatty acid supplementation. In the CAPS study, children with a heredity for asthma were randomised to fatty acid supplementation in an attempt to reduce the asthma incidence. However, no effect was seen on asthma, eczema or atopy at five years of age (Marks *et al.* 2006). These findings were confirmed in a study of the whole CAPS birth cohort which found no relationship between plasma levels of n-3 PUFA or n-6 PUFA and any of the outcomes (Almqvist *et al.* 2007).

Furthermore, no convincing effect of n-3 PUFA supplementation in individuals with already established asthma has been demonstrated (Woods *et al.* 2002). However, Nagakura *et al.* report some beneficial effects on already established asthma in children following high-dose n-3 PUFA supplementation (Nagakura *et al.* 2000). In addition, Dunstan *et al.* report an effect on neonatal cytokine responses to allergens and less severe eczema in infancy in the offspring following maternal fish oil supplementation during pregnancy in atopic mothers (Dunstan *et al.* 2003). These findings are supported by the results of Furuhjelm *et al.*, reporting less food allergy and eczema during infancy in children with atopic heredity following maternal n-3 PUFA supplementation during pregnancy (Furuhjelm *et al.* 2009)

Vitamin D

Deficiency

There are reports of immune-modulating effects of vitamin D, suggesting that vitamin D levels might play a role in the maturation of the immune system

and possibly in the pathogenesis of allergic disease (Mora *et al.* 2008, Mullins and Camargo 2011). The incidence of vitamin D deficiency is likely to have increased during the past decades as a result of the increasing amount of time spent indoors (Mullins and Camargo 2011). The major source of vitamin D is exposure to sunlight, but there are also dietary sources such as fish. In addition, foods fortified with vitamin D are available in some countries. For example, milk is fortified with vitamin D in the United States, Sweden and Finland (Holick 2008). Swedish children also receive daily supplementation with 10 micrograms of vitamin D (400 IE) during the first two years of life.

Protective effect on allergy and asthma

A reduced risk of wheeze in early childhood has been associated with higher levels of vitamin D in cord-blood and higher maternal intake during pregnancy (Camargo *et al.* 2007, Camargo *et al.* 2011). An inverse association has been found between levels of vitamin D and the risk of respiratory infections before three months of age (Camargo *et al.* 2011). Furthermore, an inverse association has been suggested between vitamin D levels and eczema and food allergies (Byremo *et al.* 2006, Sidbury *et al.* 2008, Vassallo and Camargo 2010). An increased prevalence of food allergies has been reported in children born during the winter months (Mullins *et al.* 2011) and eczema has been reported to improve with exposure to the sun and with vitamin D supplementation (Byremo *et al.* 2006, Sidbury *et al.* 2008).

However, the findings regarding vitamin D and allergic disease are not consistent and have been suggested to reflect reverse causation, since subjects severely affected by allergic disease might spend more time indoors (Hyppönen *et al.* 2004, Mullins and Camargo 2011).

Effect on immune system

The vitamin D status has been reported to affect both the innate and the adaptive immune response, explaining the suggested protective effect on wheeze, asthma and allergic disease. Increased levels of antibacterial peptides stimulating mucosal integrity have been reported with higher vitamin D levels (Vassallo and Camargo 2010). Low levels of vitamin D might therefore increase the susceptibility to infections and allergen exposure. Vitamin D has been reported to affect the adaptive immune response by favouring a Th_1-dominated response (Mora *et al.* 2008, Mullins and Camargo 2011). For example, it has been suggested that vitamin D agonists suppress allergen specific IgE, change dendritic cell maturation, induce tolerogenic dendritic cells and affect the Th_1/Th_2 cell function (Mora *et al.* 2008).

Breast-feeding

Effect on immune system?

It has been suggested that breast milk contains components active in providing protection from infections and components stimulating the maturation of the neonatal immune system. The transfer of cell-mediated immunity and cytokines through breast milk has been observed (Björkstén 1999). In addition, a change in the composition of n-3 PUFA has been reported in the milk of mothers of infants with allergic disease during the first year of life (Duchén *et al.* 1998, Björkstén 1999). It is possible that breast-feeding could affect the developing immune system and its response to potential allergens (Björkstén 1999).

Protective for wheeze

A protective effect by breast-feeding on wheezing disorder up to preschool age has been reported but not necessarily on allergic asthma later in life (Wright *et al.* 2001, Snijders *et al.* 2007, Elliott *et al.* 2008, Scholtens *et al.* 2009, Kull *et al.* 2004). The ISAAC-2 Study has recently reported less non-atopic wheeze at age 8-12 years in breastfed children but no association with allergic manifestations (Nagel *et al.* 2009). However, in the BAMSE Study a protective effect was seen on asthma with allergic sensitisation, but not for non-allergic asthma, at eight years (Kull *et al.* 2010).

Eczema and breast-feeding

The possible effect of breast-feeding on eczema is the subject of debate. In the BAMSE cohort, Kull *et al.* report a reduced risk of eczema at four years following breast-feeding for four months or more (Kull *et al.* 2005). In the KOALA Study, the suggested protective effect on eczema at two years of age was modified by maternal allergic status (Snijders *et al.* 2007). Recently, Flohr *et al.* reported that no protective effect of exclusive breast-feeding on eczema at school age could be seen in the ISAAC-2 (Flohr *et al.* 2011). In a Cochrane report, Kramer and Kakuma conclude that no convincing risk reduction is seen for eczema or allergic disease (Kramer and Kakuma 2002).

Conclusion

For this reason, breast-feeding does not appear to protect against the development of allergic disease, but it might influence the susceptibility to early respiratory infections, thereby explaining the reduced prevalence of wheezing (Duijts *et al.* 2009).

Farming and domestic animals

Farm environment

Several studies report that a farm environment has a protective effect on asthma and allergies. A reduced prevalence of asthma, hay fever and eczema has been observed in adult farmers, with a dose-dependent effect depending on the number of years of farm exposure (Douwes *et al.* 2007). Children of farmers have been reported to have a reduced risk of asthma, wheeze and hay fever (Von Ehrenstein *et al.* 2000). In the West Sweden Asthma Study, a lifelong protective effect on allergic rhinitis as a result of growing up on a farm during the first five years of life is seen (Eriksson *et al.* 2010). Growing up on a farm also had a protective effect on doctor-diagnosed asthma in adolescence (Wennergren *et al.* 2010). In addition, farm exposure *in utero* has been reported to affect the risk of asthma, hay fever and eczema in the child (Douwes *et al.* 2008, Roduit *et al.* 2011).

Distinct farm exposure has been reported to have different effects on the subsequent risk of asthma and wheeze in children. For example, keeping pigs, farm milk consumption and spending time in animal sheds had a protective effect on asthma diagnosis. On the other hand, keeping hares or rabbits increased the risk of asthma, while keeping sheep increased the risk of wheeze (Ege *et al.* 2007). Recently, Ege *et al.* reported of a clear inverse relationship between the range of microbial exposure and the risk of asthma. This was true both for bacteria and fungi with a reduced prevalence of asthma seen with a wider diversity of exposure (Ege *et al.* 2011).

Genetic variation has been reported to modulate the protective effect of farming environment. For example, polymorphism of the toll-like receptor type 2 has been suggested to alter the susceptibility to asthma and allergies in children of farmers (Eder *et al.* 2004).

It has been suggested that exposure to micro-organisms or components of microbes such as endotoxins explains the protective effect of a farming environment, by shifting the immune response towards a Th_1-dominated balance (Schaub *et al.* 2006, Stern *et al.* 2007). This notion is supported by the increased levels of endotoxins found in the mattress dust of farmers and the inverse association between endotoxin levels and the prevalence of allergic rhinitis and atopy (Braun-Fahrländer *et al.* 2002). The protective effects of farm exposure on atopic sensitisation and the regulation of the innate immune system have also been observed following prenatal exposure, with an increasingly protective effect as the number of farm animal species encountered increases. Prenatal exposure appeared to have a greater impact than later exposure during childhood (Ege *et al.* 2006).

Domestic animals

Exposure to domestic animals has been reported to affect the risk of subsequent allergic disease. Hesselmar *et al.* reported a reduced risk of sensitisation to cats in children exposed to cats in the home (Hesselmar *et al.* 1999). This has been confirmed in other studies (Platts-Mills *et al.* 2001, Almqvist *et al.* 2010) and has also been reported to apply to dogs (Perzanowski *et al.* 2002, Almqvist *et al.* 2010). However, in the BAMSE Study an increased risk of sensitisation and severe asthma at preschool age was reported following early exposure to cat and tobacco-smoke in young asthmatic children (Melén *et al.* 2001). In addition, it has been suggested that the protective effect can be at least partly explained by allergic families choosing not to keep a pet (Almqvist *et al.* 2003), but there are results supporting a true protective effect (Ownby *et al.* 2002). More animals in the home appear to have a stronger protective effect (Ownby *et al.* 2002). Recently, Wegienka *et al.* confirmed the findings of a protective effect on allergic sensitisation also at age 18 as a result of early exposure to domestic animals. The protective effect appears to be allergen specific, with cat exposure having a protective effect on sensitisation to cats and dogs to dogs and so on. In addition, the first year of life appears to be the critical period for exposure in order to have a protective effect on subsequent allergic disease (Wegienka *et al.* 2011). It is argued that the protective effect is due to the development of tolerance toward specific allergens rather than being endotoxin mediated. It is argued that a high level of oral exposure during a critical period of life can be regarded as "sublingual desensitisation", stimulating tolerance and thereby prohibiting subsequent sensitisation through the skin or nose (Erwin *et al.* 2011).

Aims

The overall aim of this thesis was to study factors influencing the risk of wheeze at preschool age and the long-term prognosis until early adulthood following severe wheezing in early childhood.

The specific aims of the thesis were:

To study factors influencing the risk of wheeze at 4.5 years of age with special emphasis on the impact of neonatal antibiotics, early fish introduction and paracetamol exposure during pregnancy (Papers I and II)

To study whether the risk factors for multiple-trigger wheeze and episodic viral wheeze differed at preschool age (Paper I)

To describe the prevalence of asthma in early adulthood following hospitalisation due to virally induced obstructive disease before the age of two years (Paper III)

To study the factors influencing the risk of asthma in early adulthood, following hospitalisation due to virally induced obstructive disease before the age of two years (Paper III)

To describe the airway function in early adulthood following hospitalisation due to virally induced obstructive disease before the age of two years (Paper IV)

To study the factors influencing the risk of having reduced airway function in early adulthood following hospitalisation due to virally induced obstructive disease before the age of two years (Paper IV)

To study the impact of prenatal and postnatal smoking on asthma in early adulthood, following hospitalisation due to virally induced obstructive disease before the age of two (Paper V).

Methods

Children of Western Sweden Study, Papers I and II

Participants

Data were obtained from a prospective, longitudinal cohort study of children born in the region of western Sweden in 2003. The region has 1.5 million inhabitants, one sixth of the Swedish population. It comprises urban, rural and coastal areas and the largest city is Gothenburg, with 500,000 inhabitants. From the total birth cohort of 16,682 infants, the random sample comprised 8,176 families (50% of the birth cohort).

Ethical approval

The study was approved by the Ethics Committee at the University of Gothenburg.

Procedures

After written informed consent was obtained, the parents answered questionnaires when the children were six and 12 months and 4.5 years. The procedures are described in further detail in the flow chart in Figure 1.

Response rate

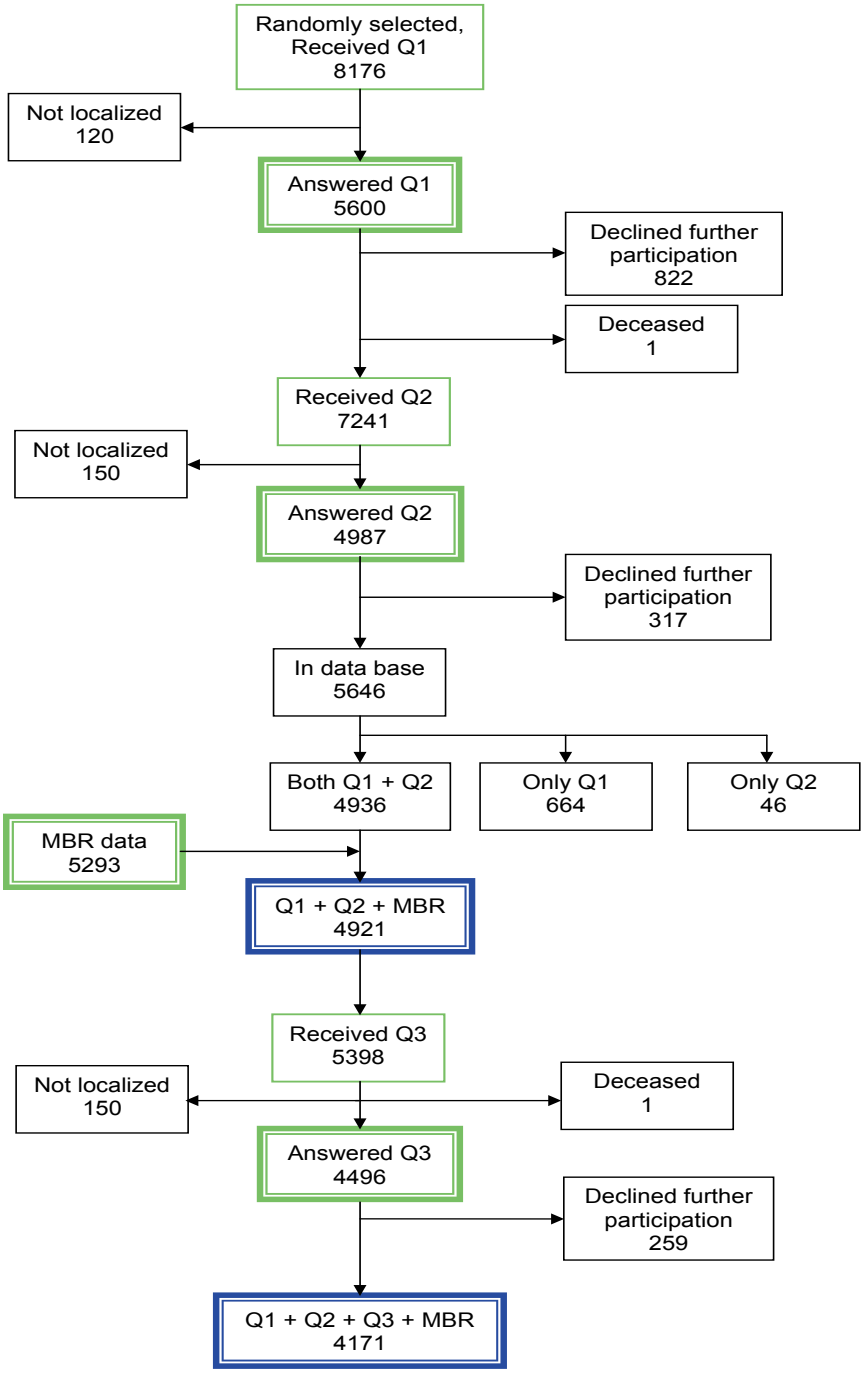
For the families that were initially contacted, the response rate at six months of age was 68% (5,600/8,176). At 12 months of age, it was 61% (4,987/8,176), which equals 69% (4,987/7,241) of the distributed questionnaires at 12 months or 88% (4,936/5,600) of the questionnaires also answered at six months.

At 4.5 years, questionnaires were distributed to the responders at six and/or 12 months, except for those who had declared that they no longer wished to participate. The response rate at 4.5 years was 55%, *i.e.* 4,496 of the families that were initially contacted. This equals 83% of the 5,398 questionnaires distributed at 4.5 years of age. After supplementation with data from the Swedish Medical Birth Register (MBR), the database consists of 4,171 infants with a full data set (all three questionnaires and the MBR).

Questionnaires

The questionnaires were based on the Swedish version of the ISAAC questionnaire and the questionnaire from the Swedish BAMSE Study. Questions relating to the outcome variables and important covariates are summarised in Appendix 1 (Table S1, Paper I).

Figure 1. *Flow chart of the Children of Western Sweden Study (yearly birth cohort of 2003: n=16,682). Q1 indicates questionnaire 1 at 6 months of age; Q2, questionnaire 2 at 12 months of age and Q3, questionnaire 3 at 4.5 years of age. The non-responders are not indicated in the figure.*



Information regarding pregnancy and postnatal factors was collected at six months of age. Information regarding the duration of breast-feeding, introduction of different foods and health and disease during the first year of life was collected at 12 months. At 4.5 years of age, questions were asked about current health and disease, family, environment and feeding habits.

Admission to a neonatal ward during the first week of life and treatment with broad-spectrum antibiotics during this period were recorded from the six-month questionnaire.

Information on the consumption and introduction of fish was collected at 12 months of age. Questions were asked about when fish was introduced, the frequency of fish consumption and the type of fish of choice.

Data on the maternal intake of medical drugs during pregnancy were obtained from the six-month questionnaire, with the following question: "Did the mother take any medication during pregnancy? If yes, please specify… ". If paracetamol use was specified, this was denoted as prenatal paracetamol exposure and compared with no prenatal paracetamol exposure.

Swedish Medical Birth Register (MBR)

Supplementation with data from the MBR was made for subjects who had answered the six- or 12-month questionnaires and was available for 5,293 individuals. Information regarding pregnancy and delivery was obtained and was the basis of information about the following parameters: gender, gestational age, caesarean section, Apgar score, maternal age, small for gestational age and large for gestational age.

Outcome variables

As primary outcome variables, recurrent wheeze and inhaled corticosteroid (ICS)-treated wheeze at 4.5 years of age were used.

Recurrent wheeze was defined as children with three or more episodes of wheezing during the last 12 months (Simoes *et al.* 2007). However, information was available on no wheeze, one to two episodes of wheeze and more than three episodes of wheeze during the last 12 months, making it possible to perform a trend analysis and a multinomial regression analysis using the number of wheezing episodes as the outcome variable.

ICS-treated wheeze was defined as wheeze treated with ICS during the last 12 months (Alm *et al.* 2008). In Sweden, many of the children with repeated wheezing are treated with inhaled corticosteroids, regardless of whether the

wheezing is multiple triggered or episodic viral. The responders to this treatment might have fewer than three episodes of wheezing during the last 12 months and would thus be lost in the group with recurrent wheeze. Furthermore, children with ICS-treated wheeze have been evaluated by a physician and found to be in need of treatment. Accordingly, the definition of ICS-treated wheeze can be regarded as a proxy for doctor-diagnosed asthma.

Among the children with recurrent or ICS-treated wheeze, the phenotypes of episodic viral wheeze (EVW) and multiple-trigger wheeze (MTW) were analysed. These terms have been suggested by the ERS Task Force on wheezing disorders in preschool children (Brand *et al.* 2008). MTW denotes children who wheeze both during and between viral episodes, while EVW denotes children who wheeze intermittently and are well between viral episodes.

Statistics – background

Definitions

The establishment of a causal relationship is complex and, in this context, epidemiological studies can contribute with the identification of plausible associations that need to be further explored. To be causal, the association must not be explained by artefact or confounding and the exposure has to precede the outcome (Abramson and Abramson 2001). In addition, there are several factors that may strengthen or weaken the possibility of causality, based on the "Bradford Hill criteria", Table 3 (Hill 1965).

Reverse causation can occur if an association is seen between an exposure and outcome and the exposure is considered to be causal, when in fact the outcome is the cause of the exposure.

Table 3. *Causal criteria according to Hill.*

Criterion
1. Strenght
2. Consistency
3. Specificity
4. Temporality
5. Biological gradient
6. Plausibility
7. Coherence
8. Experimental evidence
9. Analogy

A factor is considered to be a confounder if it is associated with both the outcome and the exposure and if it is not an effect of the exposure (Rothman 2002).

Confounding by indication occurs if the effect of an exposure is not due to the exposure itself but is in fact due to the reason for that exposure.

Interaction between two factors on an outcome is seen when their combined effect deviates from additivity, when acting synergistically, for example.

Multivariate analysis – variable selection

In epidemiology, the multivariate analysis is used to assess the strength and significance of the relationship between different variables and the outcome while controlling for confounding (Abramson and Abramson 2001). Models can be used to evaluate the causal role of different factors (Rothman 2002). In addition, the prediction of risk based on the effects of multiple factors can be estimated and the prognostic value of different variables can be explored (Altman 1999, Abramson and Abramson 2001). The resulting estimates are unconfounded and are thus independent of the other variables in the model (Rothman 2002).

There are different approaches when deciding which factors to consider in the multivariate analysis. Commonly, clinically and intuitively relevant factors should be considered in the model (Hosmer and Lemeshow 2000, Abramson and Abramson 2001). In addition, a univariate analysis of each variable might be helpful in identifying factors for the model (Hosmer and Lemeshow 2000). The factors can be selected based on the significance (p-value) or strength of the association (OR, point estimate) to exposure and/or outcome (Abramson and Abramson 2001). In addition, their contribution to the model or their effect on the strength of the association between the exposure and outcome can be used ("change in estimate") (Hosmer and Lemeshow 2000, Abramson and Abramson 2001). However, regardless of the approach, factors should be kept if past experience tells us that it is important for the outcome (Altman 1999). Factors might not appear as confounders if analysed separately, but confounding might become evident when analysed collectively, thereby supporting the inclusion of clinically relevant factors in the model (Hosmer and Lemeshow 2000, Abramson and Abramson 2001).

The number of factors in the model should be minimised to create a stable and more easily generalised model. The larger the model, the greater the standard error and the more dependent the model is on the observed data. The number of variables that can be included depends on the sample size, number of cases in each outcome group and number of total variables (Hosmer and Lemeshow 2000).

The significance is dependent on the size of the sample; for example, large effects may be non-significant in small samples and unimportant effects significant in large samples (Abramson and Abramson 2001). In a larger sample, the chance of significance is therefore also increased for weak predictors.

When using significance to identify possible candidates for the model (without *a priori* selection based on clinical relevance), a liberal level of significance has been suggested (Hosmer and Lemeshow 2000, Abramson and Abramson 2001). If the significance level is too strict, important factors might not be identified and, if it is too liberal, unimportant factors may be included (Hosmer and Lemeshow 2000). However, when identifying candidate variables from pre-identified relevant factors, using significance levels as low as 0.01 to identify strong predictors has been suggested (Abramson and Abramson 2001).

Statistics – Papers I and II

In the statistical analysis, two-by-two tables with the χ^2 test and binary logistic regression were used. Odds ratios (OR) were estimated with 95% confidence intervals (CI).

The multivariate models aimed to adjust for possible confounding regarding the primary predictors that were analysed (neonatal antibiotics, the early introduction of fish and paracetamol exposure during pregnancy respectively). In addition, strong predictors of the outcome were included to ensure a stable model.

Candidate factors were identified from literature in the field and from clinical experience, see Appendix 1 (Table S2 in Paper I). To identify reasonably strong predictors, the selection of variables for the multivariate models was preceded by univariate analyses of the candidate factors and a significance level of < 0.01 was chosen for inclusion (Table 1 in Paper I). Factors with implied clinical importance, as well as plausible confounders for the primary predictors, were included, regardless of their significance.

A level of 0.01 was used to identify strong predictors as a sensibly conservative level, given the large sample size and large number of factors to consider. We believe that this has led to stable, parsimonious models.

The stability of the model was confirmed in an extended multivariate model including only factors with $p < 0.1$ in the univariate analysis, Appendix 1 (Table S3, Paper I). Factors were included based only on the level of significance in the univariate analyses. As a result, known confounders and predictors with clinical relevance might not be included. However, the strongest predictors were naturally also included in this extended model and the results were similar, with only minor changes in the point estimates.

Paper I

In the multivariate model for recurrent wheeze (Table 2 in Paper I), adjustments were made for maternal smoking during pregnancy and any breast-feeding for four months or more, as these factors have previously been shown to affect the risk of childhood asthma (Rylander *et al.* 1993, Kull *et al.* 2004, Kull *et al.* 2010). Parental level of education was included as a marker of socioeconomic status. In addition, the strong predictors identified in the univariate analysis ($p<0.01$) were included.

Furthermore, the results of the multivariate analyses were confirmed using an extended model including factors with $p < 0.1$ in the univariate analyses, Appendix 1 (Table S3 in Paper I).

Paper II

The multivariate model used in Paper II included plausible confounding factors for the association with paracetamol exposure and factors identified as strong predictors based on previous analyses of risk factors for preschool wheeze in the same cohort (Paper I), as summarised in the Table 1 footnote in Paper II.

In addition to the strong predictors ($p<0.01$) identified in the univariate analyses, we included factors previously shown to influence the risk of childhood wheeze, such as maternal smoking during pregnancy and breast-feeding (Rylander *et al.* 1993, Kull *et al.* 2004, Kull *et al.* 2010). Maternal antibiotic use during pregnancy was included as partially reflecting maternal respiratory infection. An Apgar score of < 7 at 5 min was included as a proxy for postnatal vulnerability, while parental educational level was included as a marker of socioeconomic status.

We also performed the multivariate analysis for exclusive prenatal paracetamol exposure compared with no medication exposure during pregnancy. Exclusive paracetamol exposure was defined as exposure to paracetamol but not to any other medication during pregnancy.

In addition, the results of the multivariate analyses were confirmed using an extended model including factors with $p < 0.1$ in the univariate analyses, Appendix 2 (Table S1 in Paper II).

The attributable fraction of paracetamol exposure, AF, was calculated using the formula: *AF = (the proportion of cases exposed to the factor) * (OR – 1)/OR* (Coughlin *et al.* 1994).

Wheezing bronchitis follow-up, Papers III, IV and V

Participants

Between March 1984 and November 1985, 101 consecutive children aged less than two years who were hospitalised due to wheezing bronchitis were included in a prospective study to determine the characteristics and prognosis of wheezing in early childhood. Details relating to the first hospital stay and earlier re-investigations at preschool age and at age 10 years have been published previously (Wennergren *et al.* 1992, Wennergren *et al.* 1997). Plausible long-term risk factors such as family atopy, passive smoking and gender were registered at first admission. Family atopy was defined as asthma, allergic rhinoconjunctivitis, food allergy or atopic eczema in parents or siblings.

To clarify the outcome in early adulthood, the cohort was re-investigated at the age of 17-20 years. For comparison, 401 controls from the Gothenburg population matched for age were randomly sampled from the national population register.

Ethical approval

The study was approved by the Ethics Committee at the University of Gothenburg.

Procedures

A recent health history was obtained using a questionnaire. In the cohort, 88% (89/101) completed the form, while 73% (294/401) did so in the control group (Paper III). Two additional responses were added to the cohort in Paper V, giving a response rate of 90% (91/101).

In the cohort, 62% (55/89) were willing to undergo specific objective testing and were tested for allergic sensitisation, airway function and bronchial hyperresponsiveness. There were no significant differences in baseline characteristics between the subjects in the cohort available for follow-up and the 12 missing subjects. In the control group, 28% (82/294) were tested in a manner similar to that used in the cohort.

Questionnaire

Questions regarding current symptoms of asthma and allergy (eczema, rhinitis and food allergy), asthma medication, furry pets at home, current own and passive smoking were included in the questionnaire. In addition, the cohort was asked for permission to include information from the Swedish Medical Birth Register (MBR) regarding maternal smoking during pregnancy.

Data on smoke exposure

Data on maternal smoking during early pregnancy (gestational week 8-12, first trimester) were obtained from the MBR, after obtaining written consent. The strata were nil, one to nine, or more than nine cigarettes/day. These data were appended to the other data. Data on maternal smoking during pregnancy were obtained in 67 individuals. Of these 67 subjects, 44 had been available for testing, see Appendix 3 (Figure S1, Paper V).

Data on post-natal smoke exposure were noted at first admission and defined as passive smoking at home in infancy. Moreover, data on passive smoking at home at 10 years of age were available from previous re-investigations (Wennergren *et al.* 1997). Questions on current active and passive smoking were included in the questionnaire in early adulthood.

Allergy testing

The allergy tests included a skin prick test (SPT, Soluprick® extracts ALK, Copenhagen, Denmark), specific IgE in serum (Phadiatop®, Pharmacia Diagnostics, Uppsala, Sweden) and an eosinophil count in the blood. The allergens included in the SPT were birch, grass, mugwort, horse, dog, cat, rabbit, house dust mites (*D. pteronyssinus* and *D. farinae*) and moulds (*Alternaria* and *Cladosporium)*. The allergens included in the Phadiatop® test were birch, grass (timothy), mugwort, horse, dog, cat, house dust mite (*D. farinae, D. pteronyssinus)* and mould (*Cladosporium)*.

Allergic sensitisation was defined as a positive SPT or Phadiatop®. The presence of current allergic sensitisation, allergic rhinoconjunctivitis or atopic eczema was denoted as current allergy.

Airway function testing

Airway function measurements (Table 4), included spirometry to record FEV_1, FVC, FEV_1/FVC ratio, MEF_{50}, MEF_{25} and R_{5Hz} using the forced oscillation technique (FOT, IOS, Erich Jaeger AG, Würzburg, Germany). Spirometry was performed and evaluated according to the ATS standards (American Thoracic Society 1995).

Bronchial responsiveness was assessed using a dry, cold air hyperventilation challenge (CACh). Before the challenge, three maximum forced expirations were performed. The largest FEV_1 was noted. The subjects then hyperventilated dry, cold (-15°C) air with 5% CO_2 at 75% of predicted maximum voluntary ventilation (resting FEV_1 x 26 (l/min)) over four minutes using a respiratory heat exchange system (Jaeger RHES®, Erich Jaeger AG, Würzburg, Germany).

Table 4. *Airway function measurements. List of abbreviations.*

Airway function measurements	
FEV_1	Forced expiratory volume in 1 second
FVC	Forced vital capacity
FEV_1/FVC	Ratio of forced expiratory volume in 1 second over forced vital capacity
MEF_{50}	Maximum expiratory flow at 50% of the FVC
MEF_{25}	Maximum expiratory flow at 25% of the FVC
R_{5Hz}	Airway resistance measurements at 5Hz

Spirometry and airway resistance measurements were performed pre-bronchodilation (pre-BD), two, five and 10 minutes after CACh and then again after the inhalation of a nebulised β_2-agonist, *i.e.* post-BD. A maximum post-challenge decrease in FEV_1 of $\geq$ 10% over the subsequent ten minutes was regarded as pathological and classified as evidence of bronchial hyper-responsiveness (Gustafsson and Kjellman 2000).

Airway function parameters were presented as percentages of mean predicted values compared with gender-specific and height-related reference values (Solymar *et al.* 1980). The FEV_1/FVC ratio was also presented as an absolute numerical value. Findings of either FEV_1, FEV_1/FVC ratio, MEF_{50} or MEF_{25} below the lower limits of normality ($\leq$ -1.96 RSD) in an individual were regarded as an indicator of abnormal airway function, pre- or post-BD respectively (Piippo-Savolainen *et al.* 2004, Piippo-Savolainen *et al.* 2006). This was done to allow for a sensitive dichotomous test for evaluating the risk of abnormal airway function in early adulthood.

To ensure the validity of the reference values, we also analysed the material with reference values for older individuals (> 20 years of age) (Hedenström *et al.* 1985, Hedenström *et al.* 1986).

Outcome variables

Current asthma was defined as wheezing or asthma and/or the need for anti-asthmatic treatment (β_2-agonists or inhaled corticosteroids) during the preceding 12 months. Asthma was classified as mild, moderate or severe, according to the classification system of the Swedish Society of Paediatric Allergology (www.barnallergisektionen.se). Children with mild asthma had mild and infrequent asthma symptoms and symptom control with a β_2-agonist alone. Individuals with moderate asthma needed $\leq$ 400 µg/day of inhaled corticosteroids plus a β_2-agonist or leukotriene antagonist. Severe asthma required > 400 µg/day of inhaled corticosteroids, plus a long-acting β_2-agonist and/or leukotriene antagonist.

Statistics

Univariate

Factors predicting the presence of asthma were analysed using two-by-two tables with the χ^2 test, independent samples *t*-test and binary logistic regression.

When analysing airway function as a continuous parameter, the one-way analysis of variance was used, after ensuring the normality of the data. Factors predicting the presence of abnormal airway function (categorical variables) were analysed using two-by-two tables with the χ^2 test and binary logistic regression. The frequency of subjects with evidence of abnormal airway function was thus analysed in relation to possible risk factors.

Multivariate

The multivariate analyses of factors predicting asthma (Paper III) in early adulthood were conducted in three different models, as indicated in Table 5a, including factors that were statistically significant ($p<0.05$) in the univariate analysis. The first model included the current factors associated with asthma in early adulthood. The second model included all significant factors present both currently and in infancy. Finally, a model including only the factors present in infancy was performed.

When analysing the risk of having at least one abnormal airway function parameter pre- or post-BD (Paper IV), the factors included in the multivariate models were identified as significant in the univariate analysis ($p<0.05$) for airway function parameters (continuous variables) or abnormal airway function using logistic regression (categorical variables) both pre- and post-BD. In addition, current active smoking was included as a risk factor for current asthma. The multivariate analyses for abnormal airway function were performed for the cohort and the whole study group (cohort and control group), including all factors (both current and infantile) or only infantile factors as indicated in Table 5b.

When analysing the impact of pre- and postnatal smoke exposure (Paper V) on outcomes in early adulthood (asthma, BHR, active smoking and allergic sensitisation), the multivariate model included the factors present in infancy (Table 5c).

Table 5. *Overview of the multivariate models and outcomes used in Papers III (Table 5a), IV (Table 5b) and V (Table 5c).*

Table 5a

Multivariate models for asthma	
Current factors	Allergy BHR Active smoking Gender
All factors	Allergy BHR Active smoking Gender Family history of atopy Early passive smoking
Infantile factors	Gender Family history of atopy Early passive smoking

Table 5b

Multivariate model for abnormal airway function	
Cohort **Infantile factors**	Gender Prenatal smoking Intense disease in early life
Cohort **All factors**	Gender Prenatal smoking Intense disease in early life Current asthma Current allergic sensitisation Current BHR Current active smoking
Study group **Infantile factors**	Gender Severe wheezing in early life (belonging to the cohort)
Study group **All factors**	Gender Severe wheezing in early life (belonging to the cohort) Current asthma Current allergic sensitisation Current BHR Current active smoking

Table 5c

Multivariate models for pre- and postnatal smoke exposure	
Outcomes	Asthma BHR Allergic sensitisation Active smoking
Adjustments	Family history of atopy Gender Pre- and postnatal smoking respectively

Results and Discussion

Children of Western Sweden Study, Papers I and II

Prevalence – Papers I and II

Recurrent wheeze

At 4.5 years of age, 20% reported at least one episode of wheezing, while 5.5% had had a recurrent wheeze (*i.e.* ≥ 3 episodes of wheezing) during the last year. Of these, 75% reported treatment with asthma medication, 55% treatment with inhaled corticosteroids and 55% reported doctor-diagnosed asthma. Of the children with recurrent wheeze at preschool age, 43% had MTW, while 57% had EVW.

ICS-treated wheeze

ICS-treated wheeze was reported in 6% and, by definition, all these children had treatment with asthma medication and inhaled corticosteroids. An asthma diagnosis was reported in 73%. Of the children with ICS-treated wheeze at preschool age, 41% had MTW, while 59% had EVW.

Proxy for asthma

Recurrent wheeze denotes children with repeated symptoms and can be regarded as a proxy for asthma (Castro-Rodriguez *et al.* 2000, Simoes *et al.* 2007). As might be expected, the children with recurrent wheeze in our study report less treatment with asthma medication and less doctor-diagnosed asthma than children with ICS-treated wheeze. This supports the reasoning that ICS-treated wheeze is a proxy for doctor-diagnosed asthma, since these children have been evaluated by a physician as being in need of asthma treatment.

Duration of wheeze

As illustrated in Figure 2, 67% reported no wheezing during the first 4.5 years of life in our study. Transient wheezing (at least one episode of wheezing during infancy but not at preschool age) was reported by 13%. Late-onset wheeze (no wheezing in infancy but at least one episode of wheezing at preschool age) was seen in 12.5%, while persistent wheeze (at least one episode of wheezing reported both in infancy and at preschool age) was reported in 7.5% of the total cohort. Of the children who reported at least one episode of wheezing during the first year of life, 63% were symptom free during the past 12 months at preschool age (transient wheezing). Only 15% had ICS-treated wheeze at preschool age. Of children who reported ICS-treated wheeze during infancy, about 50% were symptom free at preschool age. However, 40% also reported ICS treatment at preschool age. This is illustrated in Figure 3.

Figure 2. *Type of wheeze from infancy to age 4.5 years in the Children of Western Sweden Study. Transient wheeze indicates at least one episode of wheezing during infancy but not at preschool age. Late-onset wheeze indicates no wheezing in infancy but at least one episode of wheezing at preschool age. Persistent wheeze indicates at least one episode of wheezing reported both in infancy and at preschool age.*

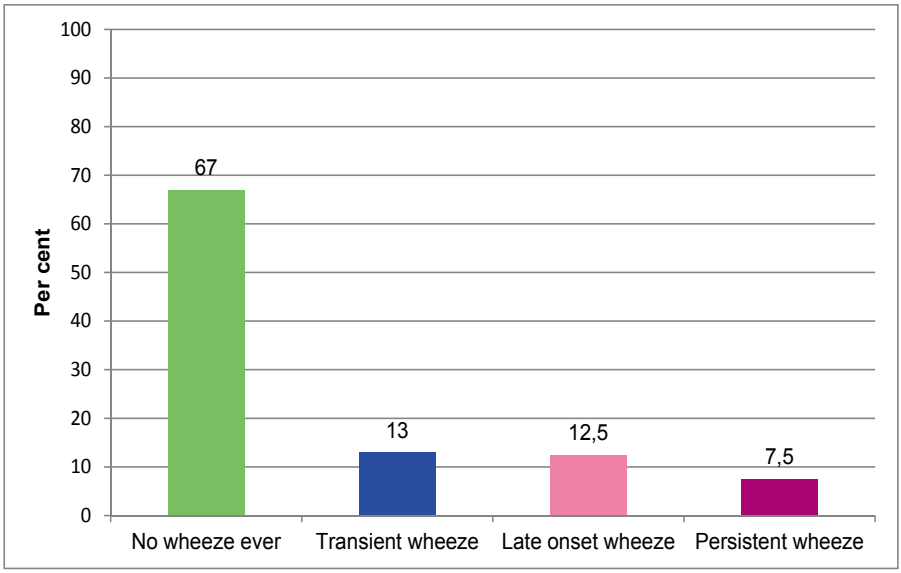
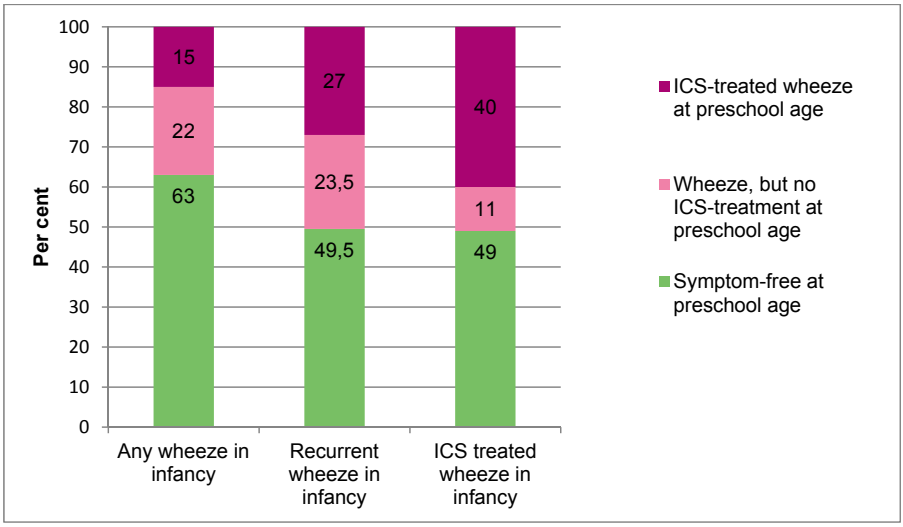


Figure 3. *Outcome at preschool age in children with reported wheezing in infancy in the Children of Western Sweden Study. The course of wheezing is shown for children with any wheeze (i.e. at least one episode of wheezing during the first year of life), recurrent wheeze (i.e. 3 or more episodes of wheezing during the first year of life) and for ICS-treated wheeze in infancy. The outcomes at preschool age are shown as symptom free and wheeze with and without ICS treatment during the past 12 months at age 4.5 years.*



Corresponding prevalence

The prevalence of wheeze at 4.5 years in our cohort is comparable to the prevalence in other birth cohort studies. The Swedish BAMSE Study reports a prevalence of asthma of 7% at four years of age, defined as four or more episodes of wheeze during the last 12 months or at least one episode of wheezing during the same period if treated with inhaled corticosteroids, *i.e.* recurrent and/or ICS-treated wheeze during the last year (Kull *et al.* 2004). The corresponding figure in our study is 8.4%. In the OLIN Study the prevalence of doctor-diagnosed asthma at seven to eight years was 5.7% in 1996 and 7.4% in 2006, but less severe respiratory symptoms were seen among asthmatics in 2006 (Rönmark *et al.* 2008, Andersson *et al.* 2010). Similarly, the prevalence of asthma (doctor-diagnosed or use of asthma medication) at six to seven years was 6.7% in the KOALA Study (Notenboom *et al.* 2011). While in the ISAAC Study (phases one and three), the prevalence of any wheeze during the past 12 months in six to seven year olds in the Swedish material was 10% (Asher *et al.* 2006).

Heredity – Paper I

Increased risk of wheeze

A family history of atopic disease and asthma (eczema, allergic rhinoconjunctivitis or asthma) increased the risk of recurrent wheeze at preschool age as presented in Paper I (Table 2, Paper I). The effect was even more pronounced among children with MTW, supporting the notion that these children are more prone to develop allergic disease and persistent asthma (Brand *et al.* 2008). No significant effect of parental atopic disease was seen among children with EVW.

Parental asthma, eczema and rhinoconjunctivitis

When analysing the impact of parental asthma, eczema and rhinoconjunctivitis separately, the effect on recurrent wheeze at preschool age was not significantly increased for parental rhinoconjunctivitis, while the effect was similar for parental asthma or eczema, as shown in Table 6. However, for viral episodic wheeze, the effect of parental asthma was most pronounced, while parental eczema appeared to have a greater impact on MTW. This offers further support for the hypothesis relating to a higher allergic predisposition in children with MTW.

In the investigation at 12 months of age in the same cohort (Alm *et al.* 2008), maternal asthma and having a sibling with asthma or eczema increased the risk of ICS-treated wheeze during infancy. Moreover, in other studies, a family history of asthma or allergic disease has been reported to increase the risk of

asthma during childhood (Martinez *et al.* 1995, Castro-Rodriguez *et al.* 2000, Melén *et al.* 2004, Lim *et al.* 2010, Wennergren *et al.* 2010). In the BAMSE Study, an effect of parental allergic disease (asthma and/or allergy to airborne allergens) on the risk of wheezing at four years of age is reported (Melén *et al.* 2004).

In addition, the West Sweden Asthma Study reports an equal impact of parental asthma and allergies on the risk of asthma in the offspring, with a synergistic effect when both factors were present (Wennergren *et al.* 2010).

Table 6. *Parental asthma, eczema and rhinoconjunctivitis analysed simultaneously in the same model for recurrent wheeze at preschool age. In addition, results are presented for recurrent multiple-trigger and episodic viral wheeze. Unpublished data.*

	Recurrent wheeze	Multiple-trigger wheeze	Episodic viral wheeze
	aOR; 95% CI	aOR; 95% CI	aOR; 95% CI
Parental asthma	**1.7; 1.2-2.5**	1.4; 0.8-2.5	**1.9; 1.1-3.2**
Parental eczema	**2.0; 1.4-2.8**	**3.0; 1.7-5.1**	1.5; 0.996-2.4
Parental rhinoconjunctivitis	1.0; 0.7-1.4	1.4; 0.8-2.4	0.9; 0.5-1.3

Adjustments were made for parental education level, male gender, smoking during pregnancy, maternal medication during pregnancy, gestational age < 37 weeks, caesarean section, treatment with antibiotics during the first week of life, breast-feeding 4 months or more, doctor-diagnosed food allergy during first year of life, eczema during the first year of life, the introduction of fish before 9 months of age and fish once a month or more at 1 year of age.

Other allergic manifestations – Paper I

Allergic manifestations in infancy

Doctor-diagnosed food allergy or eczema during infancy independently increased the risk of recurrent wheeze, with a more pronounced effect on MTW (Table 2, Paper I). This is illustrated in Figure 4, which shows the prevalence of early allergic manifestations among children with recurrent wheeze at preschool age. The same was true for ICS-treated wheeze (unpublished data, data not shown). These findings are supported by many other studies reporting an association between early atopic disease and subsequent wheezing and asthma (Castro-Rodriguez *et al.* 2000, Kotaniemi-Syrjänen *et al.* 2003a, Guilbert *et al.* 2004, Illi *et al.* 2004, Illi *et al.* 2006, Hyvärinen *et al.* 2005b, Piippo-Savolainen *et al.* 2006, Piippo-Savolainen *et al.* 2007).

Allergic manifestations at preschool age

Current allergic manifestations were also more prevalent among children with recurrent wheeze at preschool age and this was clearly seen in the children with MTW, see Table 7 (unpublished data). For example, more than 20% of the children with MTW reported current doctor-diagnosed food allergy, while about 15% reported doctor-diagnosed allergic rhinitis at preschool age. The corresponding figures for EVW were 5.1% and 1.5%, which was about the same as for the children without any recurrent wheeze at preschool age. The increased prevalence of other allergic manifestations both in infancy and at preschool age among the children with MTW supports the notion that these children are more prone to develop allergic asthma as they grow up (Brand *et al.* 2008). In addition, this fits well with the concept of an "allergic march", starting with eczema and food allergies in infancy, followed by the development of asthma and allergic rhinitis when approaching school age (Spergel 2010).

Figure 4. *Prevalence of eczema and food allergy in infancy among children with and without recurrent wheeze at preschool age in the Children of Western Sweden Study. The results are also shown for multiple-trigger wheeze (MTW) and episodic viral wheeze (EVW) among the children with recurrent wheeze.*

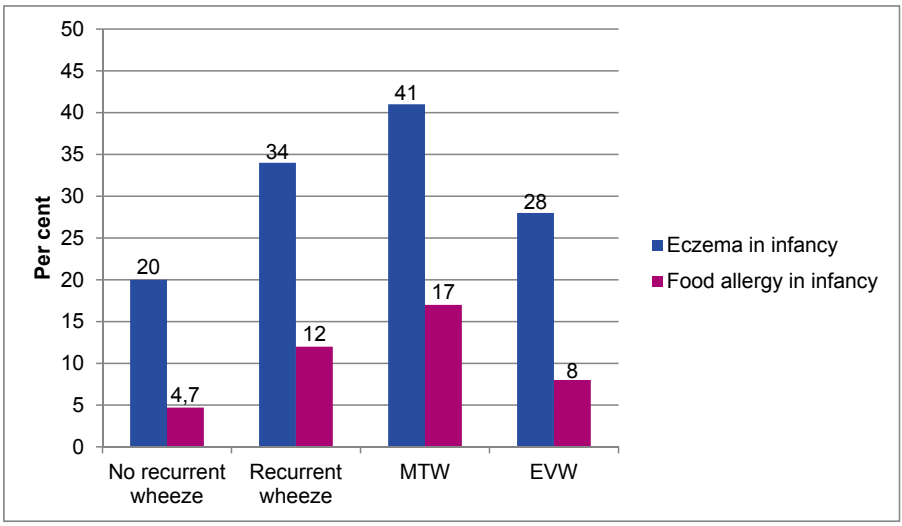


Table 7. *Prevalence of current allergic manifestations in children with no recurrent wheeze compared with children with recurrent wheeze at preschool age in the Children of Western Sweden Study. The results are also shown for multiple-trigger wheeze (MTW) and episodic viral wheeze (EVW) among the children with recurrent wheeze.*

 # indicates a reported doctor diagnosis.

indicates treatment with topical corticosteroids.

*(*p<0.05, **p<0.01 and ***p<0.001, chi-square test).*

	Food allergy#	Allergy test positive	Peanut allergy#	Eczema#	Eczema treated ##	Eczema ever	Symptoms of allergic rhinitis	Allergic rhinitis#
No recurrent wheeze	4.0%	7.6%	0.7%	8.3%	14.6%	30.0%	4.4%	1.4%
Recurrent wheeze	13.0%***	27.0%***	2.5%***	14.0%**	24.0%***	51.0%***	25.0%***	7.0%***
MTW	23.0%***	43.0%***	5.0%***	19.4%***	28.0%***	57.0%***	47.0%***	14.6%***
EVW	5.1%	13.2%*	0.7%	9.5%	20.4%	44.5%**	8.8%*	1.5%

Gender – Paper I

Increased risk of wheeze

Male gender increased the risk of recurrent wheeze at preschool age, but the effect was slightly less pronounced than that seen in infancy for any wheeze and for ICS-treated wheeze (Alm *et al.* 2008). When analysing children with MTW and EVW separately, significance was not reached, but the effect was somewhat more pronounced among children with EVW at preschool age (Table 2, Paper I).

It is well known that wheezing in childhood is more common among boys (Korppi *et al.* 1986, Skobeloff *et al.* 1992, Wennergren *et al.* 1992, Martinez *et al.* 1995, Melén *et al.* 2004). In the BAMSE Study, male gender was a risk factor for both wheeze and sensitisation at the age of four years (Melén *et al.* 2004). We found a slightly more pronounced effect of gender on EVW and a diminishing impact at preschool age compared with infancy. These findings fit well with the reasoning that the increased prevalence in boys is explained by the different airway development seen in boys and girls. As discussed above, male infants have been reported to have more narrow airways in relation to their absolute lung size than girls, explaining the higher incidence of wheeze (Young *et al.* 2000). When the pulmonary development continues as the child grows, the differences in the prevalence of asthma between boys and girls is

first reduced and then switched to an increased prevalence in girls compared with boys, starting in adolescence (Skobeloff *et al.* 1992, Taussig *et al.* 2003, Schatz *et al.* 2004, Melgert *et al.* 2007).

Smoke exposure – Paper I

Prevalence

The prevalence of smoking during pregnancy in this cohort was 9.6%. This is highly comparable to the prevalence of 9.6% in maternal smoking in early pregnancy in western Sweden in 2003 reported by the National Board of Health and Welfare (Alm *et al.* 2008).

No significant effect on preschool wheeze

We found no significant effect of smoke exposure during pregnancy or in infancy on the risk of recurrent wheeze at preschool age (Table 2, Paper I). In addition, we saw no impact of exposure to snuff or nicotine supplementation during pregnancy or passive smoking in infancy (maternal or paternal) on the risk of wheeze (unpublished, data not shown). However, smoke exposure during pregnancy increased the risk of ICS-treated wheeze in the univariate analysis (OR 1.6; 1.1-2.3) but did not remain significant in the multivariate analysis (aOR 1.5; 0.9-2.4).

In the follow-up at 12 months of age, an increased risk of at least one episode of wheeze during infancy was seen with smoke exposure *in utero*, but it did not increase the risk of ICS-treated wheeze during the first year of life (Alm *et al.* 2008).

There are many studies reporting an increased risk of wheeze and asthma in children exposed to tobacco smoke, especially during pregnancy (Rylander *et al.* 1993, Lødrup Carlsen *et al.* 1999, Stein *et al.* 1999b, Lux *et al.* 2000, Taussig *et al.* 2003, Lannerö *et al.* 2006). Both Rylander *et al.* and the BAMSE birth cohort report an effect of maternal smoking during pregnancy on the risk of wheezing bronchitis, wheeze ever and asthma (Rylander *et al.* 1993, Lannerö *et al.* 2006). Moreover, in the ALSPAC Study, an increased risk of wheeze with smoke exposure during pregnancy was seen (Lux *et al.* 2000). One possible explanation of why we did not find a clear impact of smoking during pregnancy on wheezing at preschool age is the change in smoking habits in recent decades, with fewer heavy smokers (see General Discussion).

Neonatal antibiotic treatment – Paper I

Increased risk of wheeze

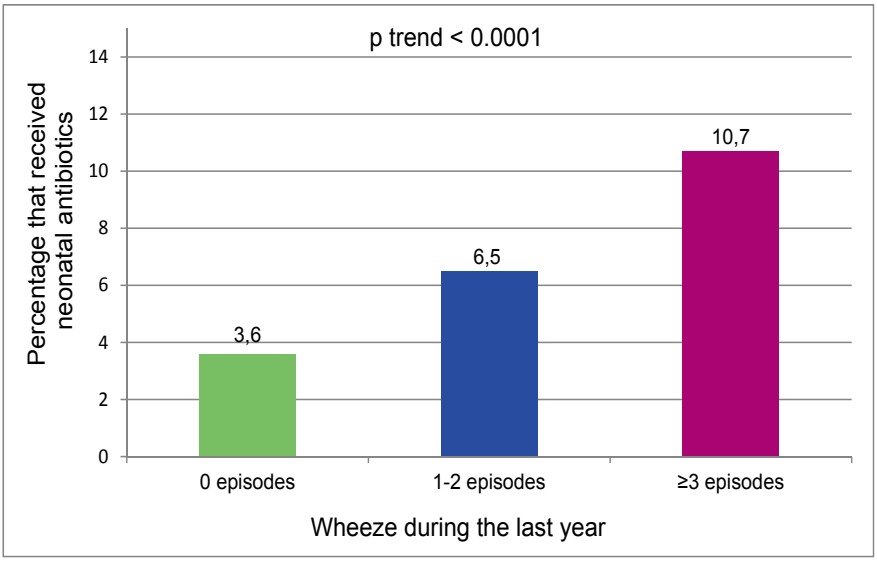
Broad-spectrum antibiotics in the first week of life increased the risk of recurrent wheeze during the last 12 months at age 4.5 years (adjusted OR 2.2; 95% CI 1.3-3.8) and of MTW (aOR 2.8; 1.3-6.1). The effect was comparable to that seen on ICS-treated wheeze at one year of age, as reported earlier in the same cohort (aOR 2.8; 1.4-5.6) (Alm *et al.* 2008). Our results are in line with findings in other studies that show an increased risk of preschool wheeze in children treated with antibiotics in infancy (Thomas *et al.* 2006, Kummeling *et al.* 2007, Marra *et al.* 2009). Other studies have reported a dose-response relationship, but our data did not allow for that analysis (Kozyrskyj *et al.* 2007, Marra *et al.* 2009). However, the impact of neonatal antibiotics could be seen more clearly in children with more frequent wheeze, as shown in Figure 5 (chi square for linear trend $p < 0.0001$). This was confirmed in a multinomial logistic regression using "no wheeze" as the reference category. The adjusted OR for the predictor neonatal antibiotics was 1.4 (0.89-2.2) for "1-2 episodes of wheeze" vs. "no wheeze" and 2.4 (1.3-4.2) for "$\geq$ 3 episodes of wheeze" vs. "no wheeze".

Reverse causation

In our study, we have only considered broad-spectrum antibiotic treatment during the first week of life to minimise the risk of reverse causation. However, a new-born child that is predisposed to develop asthma might also be more susceptible to neonatal infections due to a change in immune response already at birth (Guerra *et al.* 2004a, Zhang *et al.* 2009, Sly 2011), making antibiotic treatment a proxy for asthma rather than a causative factor.

Delayed immune maturation is more common in children with a family history of asthma and atopy (Gold *et al.* 2009, Sly and Holt 2011). However, we were unable to demonstrate any interaction between atopic heredity or parental asthma respectively and neonatal antibiotic treatment in our study (Paper I). In addition, the effect of neonatal antibiotic treatment was still independently significant when controlling for parental asthma separately in the multivariate analysis. To minimise the influence of susceptibility to infections at birth, due to delayed immune maturation, we adjusted for caesarean section and preterm birth, as markers of postnatal vulnerability. Furthermore, we performed the multivariate analysis also controlling for Apgar < 7 at 5 min, as a proxy for postnatal vulnerability with stable results for all included variables.

Figure 5. *Percentage of children in the Children of Western Sweden Study that received antibiotics during the first week of life among children with no wheeze, 1-2 episodes of wheeze or 3 or more episodes of wheeze at preschool age. P for trend < 0.0001.*



Patho-physiological mechanism

The neonatal immune response is dominated by a Th_2 response, while the Th_1 immune function matures postnatally, stimulated by microbial exposure (Björkstén 1999, Schaub *et al.* 2008, Prescott 2010). As a result, colonisation and infections might be of importance. Colonisation begins in the first week of life, with some fluctuations until three months of age. When established, the gut flora is fairly stable, with only temporal changes with antibiotic treatment, for example (Björkstén 1999). This indicates that disturbances in early life might have a greater impact on the composition of the gut flora and greater immune modulating effects than later disturbances. This reasoning supports our findings that antibiotic treatment during the first week of life increases the risk of childhood wheeze.

The composition of the gut flora has been reported to differ between children in countries with a different prevalence of allergies. For example, Estonian children were more intensely colonised and had a higher lactobacilli content than Swedish children (Sepp *et al.* 1997, Sepp *et al.* 2000). In addition, a change in the composition of the gut flora with more coliform flora was found in atopic children in both countries (Björkstén *et al.* 1999). Our results are compatible with this reasoning, showing a more pronounced effect of neonatal antibiotic treatment in children with MTW, a phenotype more prone to develop allergic ("true") asthma (Brand *et al.* 2008).

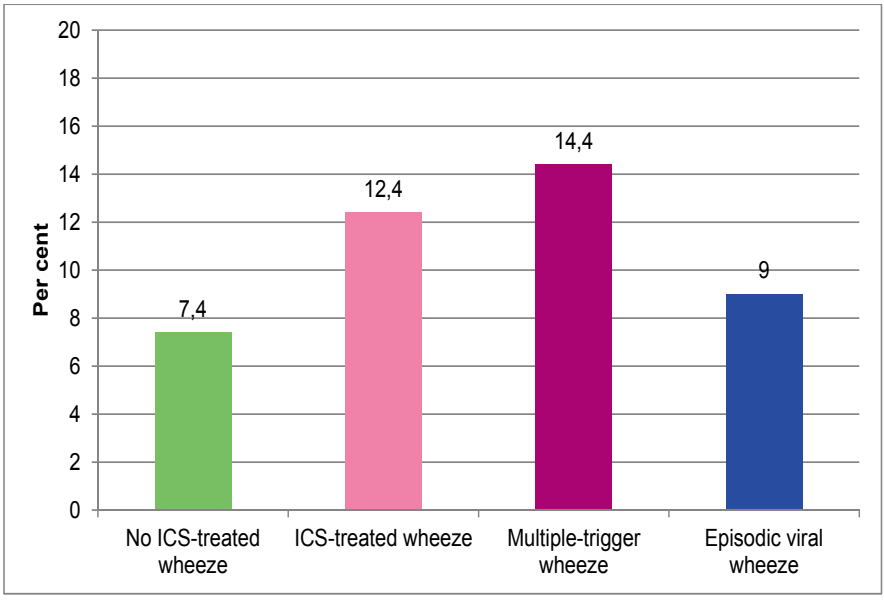
Paracetamol – Paper II

Prevalence

Medicines had been taken during pregnancy by 28.4% of the mothers. Paracetamol had been taken by 7.7%, while exclusive paracetamol use was reported in 5.3%.

The prevalence of prenatal paracetamol exposure in children with ICS-treated wheeze was significantly higher than in children without ICS-treated wheeze, as illustrated in Figure 6. The subgroup of children with MTW had an even higher prevalence, while it was less pronounced among the children with EVW (Figure 6 and Table 1, Paper II).

Figure 6. *Prevalence of prenatal paracetamol exposure among children with and without ICS-treated wheeze at preschool age in the Children of Western Sweden Study. The results are also shown for multiple-trigger wheeze (MTW) and episodic viral wheeze (EVW) among the children with ICS-treated wheeze.*



Increased risk of wheeze

In line with findings in other studies, we found that the risk of ICS-treated wheeze was independently increased by paracetamol (aOR 1.6; 95% CI 1.01-2.6) (Shaheen *et al.* 2005, Koniman *et al.* 2007, Beasley *et al.* 2008, Rebordosa *et al.* 2008, Garcia-Marcos *et al.* 2009, Perzanowski *et al.* 2010, Bakkeheim *et al.* 2011). Recent meta-analyses have confirmed the association (Etminan *et al.* 2009, Eyers *et al.* 2011) and a dose-dependent effect has been described (Bea-

sley *et al.* 2008). Our data did not allow for any analysis of a dose-response relationship. Within the ICS-treated group, the effect was significant for MTW (aOR 2.4; 1.2-4.8) but not for EVW (aOR 1.1; 0.5-2.3). The results were similar using the extended model, as seen in Appendix 2 (Table S1, Paper II) and when analysing the effect of exclusive paracetamol exposure during pregnancy (Paper II). The attributable fraction for paracetamol exposure in ICS-treated wheeze was 4.7% (95% CI 0.1-7.6) and 8.4% (2.4-11.4) in ICS-treated MTW.

Confounding

As we consider paracetamol exposure only during pregnancy in our study, the possible confounding of the respiratory morbidity of the child itself can be disregarded. However, maternal use of paracetamol could be a proxy for maternal respiratory infection, which we were not able to adjust for. However, we did adjust for maternal antibiotic use and maternal asthma, since asthmatic mothers might run a higher risk of respiratory infections.

The association might also be confounded by the possibility of asthmatic mothers taking less aspirin/NSAID and more paracetamol instead, making paracetamol use a proxy for asthma in the mother. We therefore adjusted for asthma, eczema and rhinoconjunctivitis in the mother in our study.

In addition, it might be argued that paracetamol use in pregnancy is a marker of a difficult pregnancy, increasing the vulnerability of the child. We therefore adjusted for caesarean section, prematurity and a low Apgar score at five minutes. In addition, paracetamol use could be a proxy for other diseases and possibly also for the intake of other medication. However, in our study, the effect of paracetamol was not altered when considering only exclusive paracetamol exposure, excluding the use of other medication, including asthma and allergy medication.

Fish – Paper I

Protective effect on wheeze

The introduction of fish before the age of nine months reduced the risk of recurrent wheeze (aOR 0.6; 0.4-0.8). This protective effect was not significant for wheeze during the first year of life in the same cohort (Alm *et al.* 2008). However, the risk of eczema in infancy was reduced (aOR 0.76; 0.62-0.94) (Alm *et al.* 2009). In addition, a protective effect by early fish introduction has been demonstrated in allergic rhinitis at preschool age in the same cohort (aOR 0.49; 0.29-0.82) (Alm *et al.* 2011). These findings are in line with the results of other studies (Nafstad *et al.* 2003, Kull *et al.* 2006, Hesselmar *et al.* 2010, Øien *et al.* 2010, Kremmyda *et al.* 2011).

Type of fish

Fatty fish like salmon, mackerel and herring are supposedly richer in n-3 PUFA than white fish. Other studies have reported that the protective effect of fish on wheeze might be independent of the type of fish ingested, indicating that the effect cannot be ascribed to n-3 PUFA alone (Marks *et al.* 2006, Øien *et al.* 2010). In our study, the most commonly consumed type of fish in infancy (Paper I) was white fish (79%), followed by salmon (17%), flat fish (3%) and herring/mackerel (1%). Since the question used in our questionnaire was not precise enough, the data did not allow conclusions to be drawn about the influence of different types of fish on asthma development.

Confounding

Confounding by indication or bias could influence the association that was seen. For example, a family history of asthma or early allergic manifestations in the child, such as eczema or food allergy, could affect the timing of the introduction and possible avoidance of different foods like fish. In our study, fish was introduced one to two weeks later in children with atopic heredity, eczema or doctor-diagnosed food allergy during infancy. However, these variables were all adjusted for in the multivariate analysis and we found no significant interaction between them and early fish introduction. In addition, socioeconomic factors and other lifestyle factors could influence the dietary pattern. We adjusted for parental educational level and factors known to vary with socioeconomic status, such as breast-feeding and maternal smoking during pregnancy. In the extended multivariate model ($p<0.1$ in the univariate analyses), adjustments were made for maternal age at birth and paternal employment at six months.

Window of opportunity

The protective effect we found following the early introduction of fish was independent of atopic heredity, the educational level of the parents and allergic disease during infancy. These findings are in line with the reports from the BAMSE Study, the LISA Study Group and the Australian CAPS Study, which found no evidence supporting the delayed introduction of solids in order to prevent allergic disease and asthma (Kull *et al.* 2006, Mihrshahi *et al.* 2007, Zutavern *et al.* 2008). In addition, the American Academy of Paediatrics concludes that there is "little evidence that delaying introduction of complementary foods beyond 4 to 6 months of age prevents the occurrence of allergic disease" (Greer *et al.* 2008). In fact, there is growing support for the notion that regular, early exposure to food proteins during a "window of opportunity" in infancy stimulates the development of tolerance to food allergens. This "window of opportunity" supposedly occurs between four and six months of age and delayed exposure might even increase the risk of food allergy (Prescott

et al. 2008). This reasoning is supported by the findings by du Toit *et al.* that the prevalence of peanut allergy is reduced in Jewish children in Israel, where peanuts are introduced during early weaning in infancy, compared with Jewish children in the UK, who avoided peanuts in line with current recommendations (Du Toit *et al.* 2008).

Possible interaction

There was some indication of a combined effect between neonatal antibiotics and early fish introduction. This is illustrated in Figure 7 where the prevalence of recurrent wheeze in late fish users exposed to neonatal antibiotics was more than five times the prevalence of early fish users not exposed to neonatal antibiotics. In children who were exposed to neonatal antibiotics but were early fish users or late fish users not exposed to neonatal antibiotics, the prevalence of recurrent wheeze was only twice as high. In addition, the indication of a combined effect was also evident in a stratified logistic regression, as shown in Figure 8. However, the interaction did not reach significance (p=0.084).

Figure 7. *Prevalence of recurrent wheeze at preschool age among children with or without neonatal antibiotic treatment (No Ab or Ab) and with late or early introduction of fish (Late fish/Early fish) respectively. The results indicate a synergistic effect if exposed to both antibiotic treatment and the late introduction of fish. However, there was no statistically significant interaction (p=0.084).*

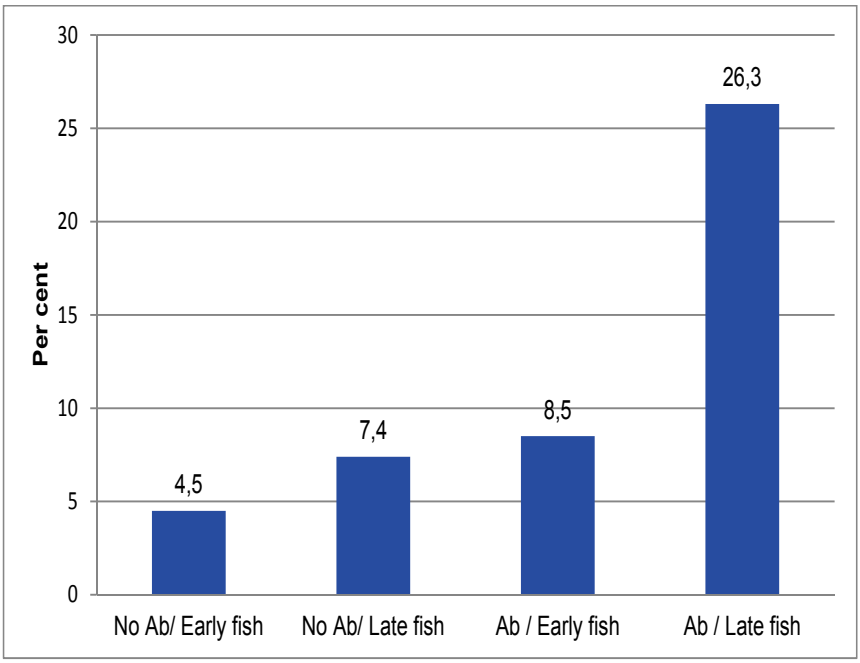
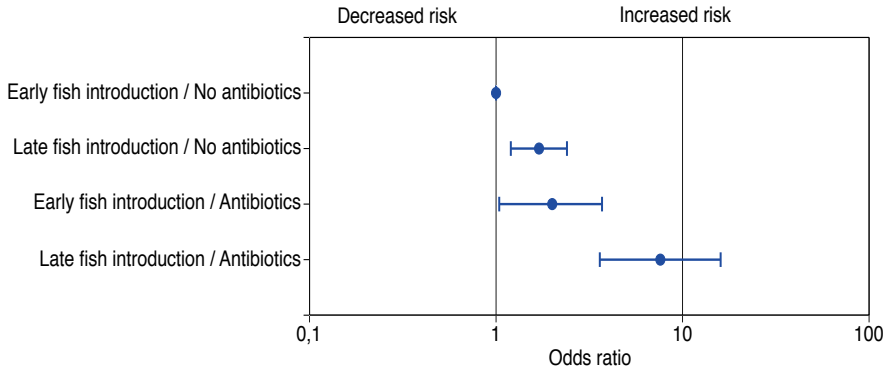


Figure 8. *The risk of recurrent wheeze for children with or without neonatal antibiotic treatment and with the late or early introduction of fish respectively. The results indicate a synergistically increased risk of recurrent wheeze if exposed to both antibiotic treatment and the late introduction of fish. However, there was no statistically significant interaction (p=0.084).*



Breast-feeding – Paper I

Possible protective effect

We found indications of a protective effect, close to significance, by breast-feeding for four months or more on recurrent wheeze at preschool age (aOR 0.7; 0.5-1.04), Table 2 in Paper I. However, in the extended model (including factors with $p<0.1$ in the univariate analyses), the protective effect was statistically significant (aOR 0.6; 0.4-0.96), as seen in Appendix 1 (Table S3, Paper I). These findings are supported by other studies which found an inverse association between breast-feeding and wheezing at preschool age (Wright *et al.* 2001, Elliott *et al.* 2008, Scholtens *et al.* 2009, Kull *et al.* 2004). In addition, Alm *et al.* found a similar impact of breast-feeding on any wheeze and on ICS-treated wheeze during infancy in the same cohort (Alm *et al.* 2008).

Episodic viral wheeze most affected

Interestingly, in our study, we note a pronounced risk reduction in children with EVW (aOR 0.6; 0.3-0.9, extended model), while no protective effect was seen in children with MTW (aOR 0.8; 0.4-1.5, extended model), Appendix 1 (Table S3, Paper I). This indicates a risk reduction in children with non-atopic disease, which might be due to breast-feeding reducing the susceptibility to infections, resulting in fewer respiratory infections and thereby less wheezing (Duijts *et al.* 2009). In addition, no obvious effect is seen in the children with a predisposition for atopic disease (MTW), in line with previous findings of no protective effect by breast-feeding on subsequent allergic disease (Kramer and Kakuma 2002, Nagel *et al.* 2009, Flohr *et al.* 2011).

Other factors of interest – Paper I

Wheezing with viral infections

Wheezing with viral infections in early life has been associated with an increased risk of subsequent wheeze and asthma (Taussig *et al.* 2003, Piippo-Savolainen *et al.* 2004, Hyvärinen *et al.* 2005b, Sigurs *et al.* 2010). In particular, RSV and rhinovirus have been put forward as especially important pathogens in this context (Stein *et al.* 1999a, Kotaniemi-Syrjänen *et al.* 2003b, Hyvärinen *et al.* 2005b, Lemanske *et al.* 2005, Sigurs *et al.* 2010).

In our cohort, we had no specified information about early viral infections. However, wheezing in early childhood is most frequently triggered by viral infections, as is also shown in our data. More than 95% of children with wheezing at preschool age reported having a cold (viral infection) as a trigger factor for wheeze. As mentioned above, 63% of children with recurrent wheeze were classified as having EVW (wheeze triggered only by colds) at preschool age.

Day care and older siblings

Ball *et al.* report day care attendance and having more siblings as being associated with an increased risk of wheezing LRI in early childhood. However, the risk of asthma, wheeze and atopy at school age was reduced. Exposure to other children therefore increased the risk of wheeze in early life but was protective for asthma and wheeze in school-age children (Ball *et al.* 2000, Taussig *et al.* 2003). In our study, attending day care during the first year of life was reported in about 20% of the children, but this did not influence the risk of wheeze in infancy or at preschool age (unpublished, data not shown). Having two or more siblings slightly increased the prevalence of any wheeze and recurrent wheeze in infancy, but it did not affect the risk of recurrent wheeze at preschool age, Table 8 (unpublished data). The effect of exposure to other children therefore appeared to follow the same pattern as that reported in the Tucson Study.

Table 8. *Prevalence of wheeze in infancy and at preschool age among children with fewer than 2 siblings or 2 or more siblings reported at 6 months of age. The results indicate an association between number of siblings and risk of wheeze in infancy but not with risk of wheeze at preschool age.*

	Any wheeze in infancy, %	Recurrent wheeze in infancy, %	Recurrent wheeze at preschool age, %
Fewer than two siblings	20	5.3	5.3
Two or more siblings	25	7.6	5.7
p-value (Fisher's two-sided)	0.001	0.014	0.664

Obesity

At this time, the potential impact of overweight and obesity on subsequent wheeze and asthma during childhood has not been evaluated in this study.

Vitamin D

We saw no effect by vitamin D supplementation on the risk of wheeze at preschool age. We had data on vitamin D supplementation (yes or no) at 12 months of age and only 282 children (5.7%) were reported not to receive their recommended supplementation. Our data did not allow for any analysis of the dose or length of the supplementation. It was therefore not possible to perform an analysis of the actual vitamin D status of the child.

Rural residence

Information on rural residence was requested in the six-month questionnaire. Around 20% (1,155) of the children were reported to have a rural residence (unpublished data). At preschool age, we included a question about whether the family lived on a farm and 5.3% (240) reported doing so (unpublished data). However, neither a rural residence in infancy nor living on a farm at 4.5 years of age was associated with less risk of wheezing at preschool age (unpublished data).

A protective effect on asthma and allergic disease following exposure to a farm environment has been reported (Ege *et al.* 2007, Eriksson *et al.* 2010, Wennergren *et al.* 2010, Roduit *et al.* 2011). Exposure during pregnancy and the first year of life might be of greater importance than subsequent exposure influencing the maturing immune system (Ege *et al.* 2006, Roduit *et al.* 2011). In addition, certain kinds of farming environment, such as different kinds of stock and the way they are kept, might influence the outcome (Ege *et al.* 2007). Our information on a farming environment does not provide specific information regarding the timing and length of exposure or the kind of animals that were kept on the farm, for example. This might explain why we do not see an effect on preschool wheeze.

Furry animals in the home

No association between having a furry animal in the home in infancy and subsequent wheeze was seen in our study (Paper I, data not shown). This did not change when adjusting for the possibility of allergic families avoiding furry pets (a question was included in the questionnaire regarding the reason for not having pets). The protective effect of domestic animals that has previously been described has mostly been shown in relation to allergic sensitisation. The effect has been reported to be allergen specific, with, for example, exposure

to cats reducing the risk of subsequent sensitisation to cats (Platts-Mills *et al.* 2001, Ownby *et al.* 2002, Almqvist *et al.* 2010, Wegienka *et al.* 2011).

Phenotype differences

Our results indicate different presentations of allergic manifestations for EVW and MTW, as mentioned above and illustrated in Figure 4 and Table 7. MTW is associated with a higher prevalence of other allergic manifestations such as eczema, food allergy and allergic rhinitis both in infancy and at preschool age. In addition, our results show some differences in risk factors for MTW and EVW (Table 2, Paper I and Table 1, Paper II). To summarise, these findings support a more allergic nature of MTW, suggesting a higher risk of the development and persistence of allergic asthma. On the other hand, the nature of EVW appears to be more non-atopic.

Multiple-trigger wheeze

In line with the Asthma Predictive Index, the effect of atopic heredity, as well as food allergy in infancy, was more pronounced in children with MTW (Castro-Rodriguez *et al.* 2000). In addition, the association with antibiotic treatment during the first week of life was only significant for MTW. This indicates a greater impact on subsequent allergic asthma development in line with the findings of Droste *et al.,* who report an effect of antibiotics early in childhood only in children with atopic heredity (Droste *et al.* 2000). Furthermore, paracetamol exposure during pregnancy increased the risk of MTW, but not of EVW, within the ICS-treated group. This may suggest a link between paracetamol exposure and the development of allergic asthma.

Episodic viral wheeze

The effect of male gender and the duration of breast-feeding appeared to be more pronounced among children with EVW, even though it was only close to significance. The protective effect of breast-feeding was more emphasised in children with EVW and this supports the reasoning that breast-feeding reduces susceptibility to infections (Duijts *et al.* 2009). In addition, we can see a more pronounced effect of caesarean section among the EVWs, even though it was only close to significance. Caesarean section might be a marker of postnatal vulnerability, with perhaps affected airway function and susceptibility to respiratory infections and wheezing (Thavagnanam *et al.* 2008).

Representativeness of the sample

As presented in Appendix 1 (Table S4, Paper I), some differences were found between the responders and non-responders to the follow-up at 4.5 years. Among the non-responders, the frequency of parents with a low educational

level and mothers smoking during pregnancy and preterm birth was higher. There was also a slightly lower prevalence of atopic heredity and breast-feeding for four months or more among non-responders. No other significant differences were found. In addition, it can be noted that, even though the differences were statistically significant, the numerical differences were small. Furthermore, as previously reported by Alm *et al.*, the material appears to be largely representative of the population. A comparison was made of gestational age, birth weight, maternal age, smoking during pregnancy and mode of delivery between our cohort and data for the Swedish birth cohort of 2003 from the National Board of Health and Welfare and very similar figures were found (Alm *et al.* 2008).

Strengths and weaknesses

Outcome measures

The weaknesses of this study are those inherent in questionnaire-based studies, *i.e.* there can always be some uncertainty about the validity of answers. The outcome variables were based on parental reports of symptoms and diagnoses. No objective measures of atopy or asthma were available. However, our questionnaire is based on the Swedish part of the ISAAC Study and the Swedish BAMSE Study. For example, we have used the Swedish term for wheeze ("*pipande eller väsande"* breathing) which was used in both those studies. There is good agreement between the Swedish and English wordings. The Swedish word "*pipande*" means whistling or wheezing, *"eller"* means or and "*väsande*" means hissing or wheezing. Like the English word "wheezing", "*väsande*" is onomatopoetic.

Recurrent symptoms of wheezing in this age group (preschoolers) can be considered to represent asthma, which is supported by the high prevalence of current asthma medication among children with recurrent wheeze in our study (75%). Furthermore, 73% of children with ICS-treated wheeze reported an asthma diagnosis, confirming the notion that ICS-treated wheeze is a proxy for doctor-diagnosed asthma.

Antibiotic treatment

The question about antibiotic treatment was asked retrospectively (six months *post-partum*) and no hospital charts were available to confirm indication or dosage.

Medication during pregnancy

The question about medication during pregnancy was also asked retrospectively (six months *post-partum*) and did not relate to paracetamol use in particular. As a result, information on dose or time of intake was not available.

However, it might be argued that the mothers who reported paracetamol use during pregnancy in our study considered their use substantial enough to report. Furthermore, we do not have any information on paracetamol use by the child itself during the first years of life. This has been reported to increase the risk of subsequent asthma and could influence the outcome at preschool age (Beasley *et al.* 2008).

Possible bias

As expected, the responders at 4.5 years were somewhat more diligent and health conscious than the non-responders. This could result in some bias in the sample, but this is difficult to avoid in cohort studies.

Strengths

One strength of the study is the large size of the birth cohort. In all, we have data from all three questionnaires for more than 4,000 children. We consider our multivariate model to be stable and reliable, adjusting for the plausible confounders available. This is supported by the similar results using an extended multivariate model that included factors with $p < 0.1$ in the univariate analysis. As reported earlier, the material appears to be largely representative of the population (Alm *et al.* 2008).

Wheezing bronchitis follow-up, Papers III, IV and V

Asthma prevalence in adolescence – Paper III

In the re-investigation at 17-20 years of children hospitalised due to wheezing bronchitis before the age of two years, 43% (38/89) reported asthma in the preceding 12 months. Our findings correspond well with the prevalence reported in other re-investigations following early viral wheeze, as summerised in Table 9. Piippo-Savolainen *et al.* in the Korppi group report an asthma prevalence of 30 to 41% at age 18-20 years, depending on the definition (Piippo-Savolainen *et al.* 2004), and Sigurs *et al.* report a prevalence of 39% at age 18 years (Sigurs *et al.* 2010).

Higher than control group

The asthma prevalence of 43% was significantly higher compared with the prevalence of 15% in the control group. There were some indications that asthmatics were over-represented in the control group, which would indicate an even lower asthma prevalence in the general population. Belonging to the cohort, *i.e.* having been hospitalised due to early wheezing, was an independent risk factor for asthma at age 17-20 years in the total study group. The risk of having asthma was four times higher in the cohort compared with the control group.

Table 9. Outcome at last re-investigation in the four Nordic follow-up studies of severe viral wheeze in early life.

	Response rate latest re-investigation	Outcome, asthma definition	Asthma prevalence, study group	Asthma prevalence, control group	Secondary asthma definition	Prevalence, secondary definition, study group	Prevalence, secondary definition, control group
Korppi Post-bronchiolitis study	65% (54/83) 18-21 y 71% (59/83) 27 y	Doctor-diagnosed asthma: maintenance medication or symptoms and PEF monitoring	30% (16/54) 20% (12/59)	11% (5/45) 5% (2/39)	"Wider definition": previous diagnosis and wheeze or cough during past 12 months	41% (22/54) 41% (24/59)	11% (5/45) 10% (4/39)
Wennergren Wheezing bronchitis study	88% (89/101) 17-20 y	Wheeze/asthma symptoms and/or asthma medication during past 12 months	43% (38/89)	15% (44/294)	-	-	-
Sigurs RSV study	98% (46/47) 18 y	≥ 3 episodes of doctor-diagnosed wheeze past 12 months	33% (15/46)	7% (6/92)	Recurrent wheeze: ≥ 3 episodes parent reported wheeze past 12 months	39% (18/46)	9% (8/92)
Korppi Viral wheezing study	81% (81/100) 12.3 y	Maintenance medication or wheezing or cough and positive exercise challenge	40% (32/101)	-	-	-	-

Mild, moderate and severe asthma

Of the subjects with asthma in the cohort, two thirds were classified as mild and one third as moderate to severe asthma. A similar distribution was found among the subjects reporting asthma at preschool and school age in the cohort. This was also the case among the asthmatics in the control group at age 17-20 years, as indicated in Figure 1, Paper III.

Course of asthma

We can see an increase in asthma prevalence in adolescence compared with the asthma prevalence of 30% reported in the re-investigation at 10 years, indicating a relapse of wheezing symptoms in some individuals (Wennergren *et al.* 1997). The course of wheezing from early childhood to adolescence is illustrated in Figure 2, Paper III, indicating a group of children with a symptom free period during childhood but with relapsing symptoms in adolescence. Only 12% of the cohort reported persistent asthma, *i.e.* classified as asthmatics in all three re-investigations. Of the subjects who were free from asthma in adolescence, more than half had already become symptom free before the first re-investigation at preschool age. The remainder became symptom free at a later age.

Airway function in adolescence – Paper IV

Asthmatic females in the cohort most affected

We found signs of reduced airway function in the subjects in the cohort, *i.e.* individuals hospitalised due to wheezing bronchitis before the age of two years. The expiratory flow measurements were particularly affected (FEV_1/FVC, MEF_{25} and MEF_{50}). When comparing the cohort with the control group, a reduction was seen both pre- and post-BD in the cohort (Table 1, Paper IV). In addition, abnormal airway function was found in 31% in the cohort vs. 16% in the control group ($p = 0.040$). Furthermore, subjects with asthma had signs of reduced airway function (Table 2 in Paper IV), but the symptom free subjects in the cohort also had lower FEV_1/FVC than symptom free subjects in the control group. The reduction in airway function was most pronounced in asthmatic females in the cohort, as indicated in Figure 1, Paper IV. Females in the cohort had lower airway function than males, with a higher prevalence of abnormal airway function. In addition, the airway function measurements of females in the cohort differed from those of female controls.

Having bronchial hyper-responsiveness at age 17-20 was associated with somewhat affected airway function measurements. In addition, subjects with allergic sensitisation in the cohort had signs of reduced airway function compared with subjects with allergic sensitisation in the control group.

Furthermore, an intense disease in infancy was associated with lower MEF_{50} in early adulthood and, as reported in Paper V, a reduced post-BD FEV_1/FVC was found in subjects exposed to smoke *in utero*.

Risk factors for abnormal airway function

In our study, the risk of abnormal airway function in early adulthood within the cohort was independently increased by female gender and prenatal smoke exposure. Female gender was also an independent risk factor in the whole study group, as was hospitalisation due to wheezing, *i.e.* belonging to the cohort. In addition, bronchial hyper-responsiveness independently increased the risk of abnormal airway function post-BD (Table 4, Paper IV). The risk factors of importance are summarised in Figure 2, Paper IV.

Other studies

Our findings of a reduction in airway function in early adulthood following early wheezing are consistent with other studies (Piippo-Savolainen *et al.* 2004, Sigurs *et al.* 2010). In the post-bronchiolitis study by the Korppi group, Piippo-Savolainen *et al.* report signs of reduced airway function affecting expiratory flow measurements in particular, in the post-bronchiolitis group compared with controls. Abnormal airway function (same definition as in our study) was seen in 36% of the bronchiolitis group compared with 11% in the control group (Piippo-Savolainen *et al.* 2004). Moreover, in the follow-up of viral wheezing, the Korppi group report reduced FEV_1/FVC and MEF_{25} in subjects with teenage asthma (Hyvärinen *et al.* 2007).

Sigurs *et al.* report reduced airway function, with lower FEV_1/FVC and FEF_{25-75}, at 18 years of age in children with early RSV infection, regardless of current asthma or allergy. In addition, greater airway hyper-responsiveness and bronchodilator response was seen within the RSV group. An increase in the lung clearance index (LCI) indicating abnormal ventilation distribution, was seen in subjects with current asthma, but it did not differ between the RSV cohort and the controls (Sigurs *et al.* 2010). Already at 13 years of age, signs of reduced airway function (lower FEV_1/FVC ratio and FEF_{75}) in the children with RSV-induced wheeze were seen in subjects both with and without asthma (Sigurs *et al.* 2005).

In line with the findings in the post-bronchiolitis study by the Korppi group, we found affected airway function pre-BD (Piippo-Savolainen *et al.* 2004). In addition, we were able to see a reduction post-BD, indicating not only an increase in airway tone but also possible structural changes to the small airways in the cohort. These post-BD findings were most pronounced among females in the cohort and following prenatal smoke exposure.

Reference values

The differences that were seen were confirmed using reference values for older individuals as well (> 20 years), ensuring the validity of the reference values reported by Solymar *et al.* (data not shown, Solymar *et al.* 1980, Hedenström *et al.* 1985, Hedenström *et al.* 1986). In addition, no difference in height was seen when comparing females in the cohort and the control group, with and without asthma (data not shown). The difference that was seen was therefore not height related. A true difference is further supported by the findings of a difference using both spirometry and the FOT method (data not shown).

Virus or host

Impact of viral agent

At admission, a virus infection could be verified in 40 of the 101 subjects in the cohort. Of these, RSV was identified in 28% of the total cohort but in half the children admitted during the winter/spring season (Wennergren *et al.* 1992). The outcome at neither preschool age nor school age differed between those with or without an RSV infection at admission (Wennergren *et al.* 1992, Wennergren *et al.* 1997). In addition, no association was found between RSV infection at admission and atopic disease at school age (Wennergren *et al.* 1997). In line with these findings, we found no difference in asthma prevalence or airway function at age 17-20 years between subjects with and without a verified RSV infection at admission (Paper III and Paper IV) – in other words, those with wheezing from a non-RSV infection had a similarly high prevalence of asthma.

Even though a virus infection could not be verified in more than 40/101 individuals, we believe that the majority of infections at admission were caused by a virus (Heymann *et al.* 2004, Jartti *et al.* 2004). For example, the diagnostic technique for identifying rhino- and coronaviruses was lacking at the time of admission. The majority of non-diagnosed cases most definitely had a rhinovirus aetiology. Our findings indicate that hospitalisation due to viral wheezing in early life increases the risk of subsequent asthma and impaired airway function even in early adulthood. However, the type of viral agent causing the infection associated with wheezing did not influence the risk of subsequent disease, supporting the notion of a host predisposed to wheeze with viral infection.

Other studies

Sigurs *et al.* report an increased risk of asthma, allergic sensitisation and affected airway function until the age of 18 years in children with early severe RSV-induced wheezing. However, the comparison in this study was made with controls who were healthy at admission (Sigurs *et al.* 2010). So, our findings do not contradict those of Sigurs *et al.*, who found an increased risk of

asthma in children with early severe RSV-induced wheezing compared with healthy controls.

However, there are some indications that different viral agents affect the risk of subsequent asthma more than others. In the Finnish follow-up study of viral wheeze from the Korppi group, a higher risk of teenage asthma was found among those with wheezing caused by rhinovirus compared with RSV (Hyvärinen *et al.* 2005b). This was confirmed in the COAST Study, which reported that rhinovirus-induced wheeze in early life was a stronger predictor of asthma at six years than early wheezing caused by RSV (Jackson *et al.* 2008). A host also responding with wheeze to rhinovirus, which generally has less wheeze-eliciting properties, runs a higher risk of subsequent asthma, indicating a more vulnerable host, predisposed to wheezing with viral infections.

Virus or host

The increased risk of asthma following early viral wheeze could be due to damage to the airways and induced changes in the immune system as a result of the viral infection (Busse *et al.* 2010). However, it might also be that individuals predisposed to asthma have increased susceptibility to viral infections and a higher risk of wheezing once infected (Lemanske *et al.* 2005, Kuheni *et al.* 2009, Thomsen *et al.* 2009).

Perhaps the answer is a combination of the two proposed mechanisms. Some individuals might have an underlying vulnerability and an increased risk of viral wheeze. For example, a change in immune response with defective interferon-gamma production has been seen already neonatally in individuals at higher risk of subsequent atopic disease and wheeze (Tang *et al.* 1994, Guerra *et al.* 2004a, Gern *et al.* 2006). In addition, the viral infection might affect the airway epithelium and immune response more severely in some individuals. For example, an underlying allergic inflammation promotes viral replication and more severe viral infection. In addition, increased allergic inflammation is seen with viral infection (Busse *et al.* 2010). The susceptibility and predisposition of the host are therefore important, but the environmental factors that are encountered, such as viral infections, may also affect the outcome.

Heredity

Family history of atopy

The prevalence of atopy in parents or siblings was reported in 57% (58/101) of the cohort at admission (Wennergren *et al.* 1992). Having a family history of atopy was defined as asthma, allergic rhinoconjunctivitis, food allergy or atopic eczema in parents or siblings. In the re-investigation at 17-20 years, an increased risk of current asthma was seen in subjects with a family history

of atopy in the univariate analysis (Table 1 in Paper III), but it did not reach significance in the multivariate analysis (aOR 2.7; 0.9-7.5) (Table 3 in Paper III). However, family atopy was associated with having current allergy in early adulthood, which was an independent risk factor for current asthma.

A family history of both asthma and allergies has been reported to increase the risk of subsequent asthma in the offspring (Castro-Rodriguez *et al.* 2000, Melén *et al.* 2004, Lim *et al.* 2010, Wennergren *et al.* 2010). It has been suggested that maternal asthma has a greater impact (Lim *et al.* 2010). However, in our study, the data on atopy in parents or siblings at admission are somewhat blunt and do not allow for any detailed analyses of different heredity patterns.

Other follow-up studies

In the follow-up studies of severe viral wheeze in early life, the impact of family history on the long-term prognosis varies. In the Korppi group, Piippo-Savolainen *et al.* report an increased risk of asthma at age 18-20 in subjects with parental asthma but not with parental atopy or eczema (Piippo-Savolainen *et al.* 2006). Likewise, in the follow-up of viral wheeze, the Korppi group report that parental asthma is more common among subjects with teenage asthma, but the association did not reach significance. No association was seen with parental atopy (Hyvärinen *et al.* 2005b). Sigurs *et al.* report no independent impact of a family history of asthma or atopy on the risk of asthma at age 18 years. However, there were some indications of an interaction between parental asthma and early RSV infection, with an increased risk of both current asthma and allergy (Sigurs *et al.* 2010).

Other allergic manifestations

Current allergy

At the re-investigation at 17-20 years (Paper III), 60% of the cohort reported current allergy (sensitisation, ARC or eczema). Current allergy was independently associated with current asthma in early adulthood and was more prevalent with more severe asthma (Table 1, Figure 3, Paper III). However, atopy at admission or at the previous re-investigations did not predict the outcome at age 17-20 years. Nor was the eosinophilic count at admission or at age 17-20 years associated with asthma in early adulthood.

Previous re-investigations

At admission, atopic symptoms other than wheezing were reported in 25% and of those more than 70% had eczema. In the preschool follow-up, past or present atopic disease increased the risk of preschool asthma. However, atopic symptoms evident on admission did not by themselves increase the risk

of asthma at preschool age. Nor did the eosinophilic count or the total IgE at admission (Wennergren *et al.* 1992). At school age, atopic disease in recent years (current or from the age of six years) was strongly associated with current asthma. However, early atopic disease at the first re-investigation did not predict asthma at school age. The eosinophilic count at admission predicted atopic disease, but not asthma, at school age (Wennergren *et al.* 1997).

Other studies

As discussed above, early allergic disease and eczema are known to affect the risk of subsequent wheeze and asthma (Castro-Rodriguez *et al.* 2000, Guilbert *et al.* 2004, Hyvärinen *et al.* 2005b, Piippo-Savolainen *et al.* 2006, Piippo-Savolainen *et al.* 2007).

In the post-bronchiolitis study from the Korppi group, allergic sensitisation in early adulthood was associated with current doctor-diagnosed asthma and BHR (Piippo-Savolainen *et al.* 2004). In addition, an association was found between having either eczema or an elevated total serum IgE before the age of two years and asthma in early adulthood. However, the association was not seen when the factors were analysed separately (Piippo-Savolainen *et al.* 2006).

In the follow-up of severe viral wheeze, Hyvärinen *et al.* found that specific IgE for inhaled allergens, though uncommon in infancy, and early atopic eczema predicted teenage asthma. However, total IgE did not independently predict teenage asthma, but was associated with an increased risk of persistent asthma following severe viral wheeze (Hyvärinen *et al.* 2005b, Hyvärinen 2009). Furthermore, eosinophil cationic protein (ECP) in infancy correlated with teenage asthma as a marker of early airway eosinophilia (Hyvärinen *et al.* 2010).

Sigurs *et al.* report an increased prevalence of ARC and sensitisation, but not eczema, in the RSV group compared with the controls at age 18 years. In the multivariate analysis, current ARC was an independent risk factor for current asthma. The subjects with persistent or relapsing asthma until age 18 years had the highest prevalence of current ARC and sensitisation, with the same pattern already evident at age seven years (Sigurs *et al.* 2010).

Immune response during viral infection

A difference in the immune response during viral infections has been reported between children with persistent and transient wheeze. In children with transient wheeze, the same pattern as in non-wheezing children was seen, with a reduction in circulating eosinophils during acute viral infection. However, this response was not seen in children with persistent wheeze, where no reduction in eosinophils was seen during the acute phase of viral infection (Martinez *et*

al. 1998). As stated above, we found no clear association between eosinophilic levels at first admission (during viral infection) and asthma during childhood or adolescence. However, a higher eosinophilic count was seen among children with atopic disease at age 10 years (Wennergren *et al.* 1997).

In accordance with our findings, the two Finnish studies from the Korppi group found no association with the eosinophilic count at the first admission during viral infection with later wheeze until adult age (Hyvärinen *et al.* 2005b, Piippo-Savolainen 2006, Ruotsalainen *et al.* 2010). However, eosinophilic levels outside infection predicted asthma until school age but not thereafter (Piippo-Savolainen 2006, Hyvärinen *et al.* 2010). In addition, children with a primary infection caused by RSV had a lower eosinophilic count during the acute phase than if the infection was caused by other viruses (Piippo-Savolainen 2006). RSV aetiology was associated with a more favourable outcome and the findings are in line with the reports from the COAST Study (Hyvärinen *et al.* 2005b, Lemanske *et al.* 2005).

Gender

Gender distribution

At admission in early childhood, the cohort was dominated by boys (61%), reflecting the increased risk of wheezing among boys in infancy and early childhood (Korppi *et al.* 1986, Skobeloff *et al.* 1992, Wennergren *et al.* 1992, Martinez *et al.* 1995, Melén *et al.* 2004). In adolescence, a shift is seen, with asthma being more prevalent among females at age 17-20 years (Figure 9).

The shift in gender distribution took place between the re-investigations at 10 and 17-20 years. This is consistent with the previously described gender distribution of asthma seen during adolescence, resulting in an increased prevalence of asthma in females in adulthood (Anderson *et al.* 1992, Skobeloff *et al.* 1992, Schatz *et al.* 2004). However, this gender shift is not seen in the post-bronchiolitis studies of the Korppi and the Sigurs groups (Piippo-Savolainen *et al.* 2006, Hyvärinen *et al.* 2005b, Sigurs *et al.* 2010). Sigurs *et al.* report no gender difference in asthma prevalence at age 18 years (40% vs. 38%, in males and females respectively) (Sigurs *et al.* 2010).

Course of wheezing

The course of wheezing and asthma in our cohort also differed between males and females. Persistent asthma (asthma in all re-investigations) was seen in only 8% of the boys, while 21% of the girls had persistent asthma. Until school age, the prevalence of asthma decreased in both girls and boys, with boys becoming symptom free somewhat earlier than girls. Transient wheeze (already symptom free at preschool age) was seen in 40% of the boys but only 20% of the girls.

Similar to the pattern seen in the Tucson Study, the prevalence of asthma in boys steadily decreased until early adulthood in our study (Taussig *et al.* 2003). In addition, girls had a higher incidence of relapsing symptoms during adolescence, with an increased prevalence of asthma seen in early adulthood. Almost 40% of the girls reported relapsing asthma after a symptom free period at preschool and/or school age. The course of asthma in boys and girls in the cohort is illustrated in Figure 10 and Figure 11.

Airway function

As mentioned above, airway function was reduced in females in the cohort, compared with males in the cohort and females in the control group. The reduction was especially pronounced in females with current asthma in the cohort (Figure 1, Paper IV).

Female gender was an independent risk factor for asthma in early adulthood both in the cohort and in the total study group (cohort and control). In addition, the risk of abnormal airway function in early adulthood was independently increased in females.

Figure 9. *Gender distributions among subjects with asthma among children with severe viral wheeze before the age of two. Asthma was more prevalent among boys until school age. A shift is seen in adolescence, with asthma becoming more prevalent among females.*

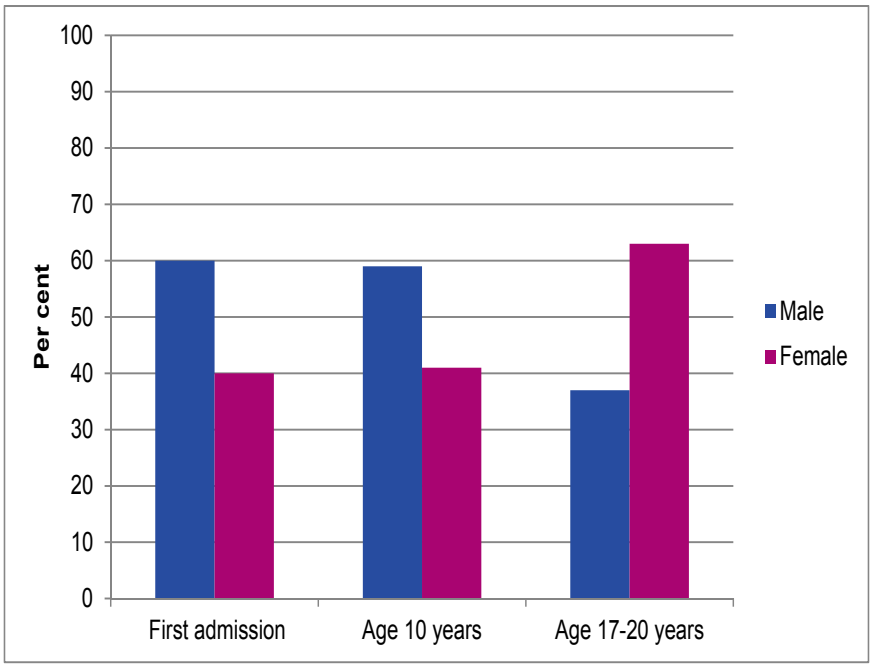


Figure 10. *Course of asthma from early childhood to late adolescence among males and females respectively, in children hospitalised due to early viral wheeze. Transient wheeze (symptom free already at preschool age) and wheeze in remission (symptom free from school age or in adolescence) was more prevalent among males, while females had more persistent (asthma at all re-investigations) and relapse of asthma (symptom free period at preschool and/or school age) in early adulthood.*

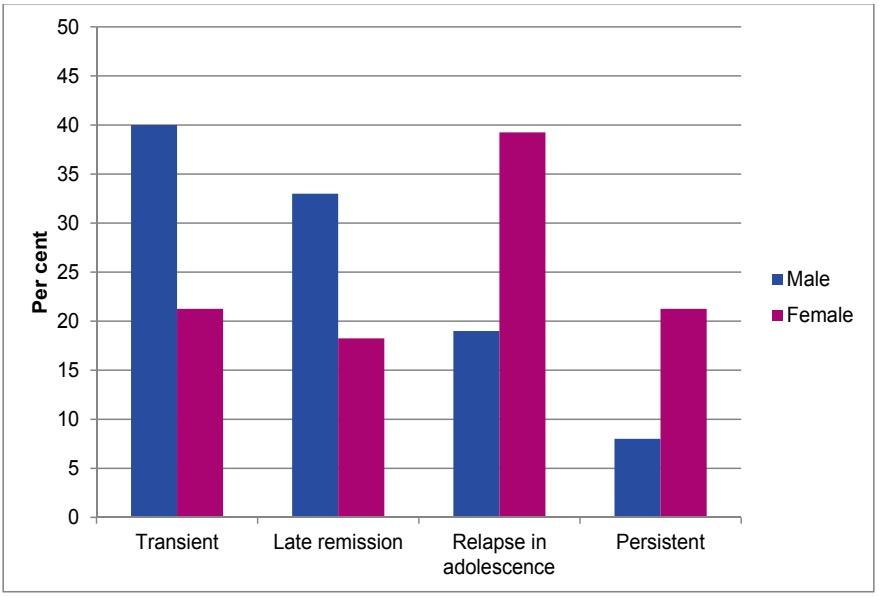
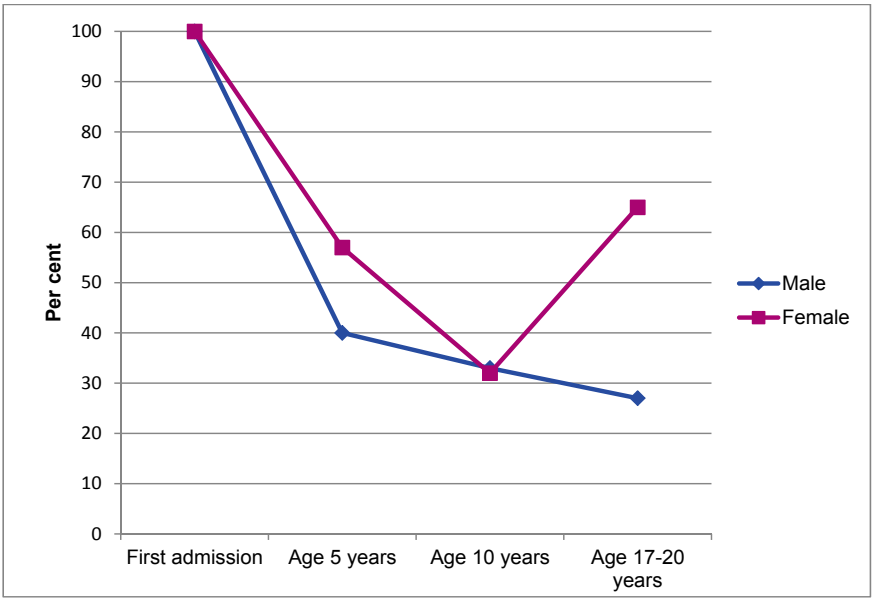


Figure 11. *Prevalence of asthma at the re-investigations among males and females respectively. Both males and females had a reduced prevalence of asthma during preschool and school age. However, females had a high rate of relapse of symptoms during adolescence, while males remained in remission.*



Pre- and postnatal smoke exposure

Prevalence

Postnatal smoking in the home by at least one parent was reported in 66% (60/91) of the cohort. Prenatal smoke exposure was not asked about at admission, but it was gathered from the MBR, with 55% (37/67) reporting maternal smoking during pregnancy. In line with findings in previous studies, very few mothers who smoked during pregnancy stopped smoking after giving birth (Alm *et al.* 1998, Stein *et al.* 1999b). In fact, 87% of the subjects in our cohort that were exposed *in utero* were also exposed postnatally. Only 30% of the cohort was not exposed either *in utero* or in infancy, Appendix 3 (Figure S2 in Paper V). The prevalence of smoke exposure during pregnancy in our cohort was higher than the average pregnant population during that time, 55% vs. 32-34%, reported by the Swedish National Board of Health and Welfare (Swedish National Board of Health and Welfare, 2004).

Increased risk of asthma

An increased risk of asthma following postnatal smoke exposure was obvious in the re-investigation at 10 years of age (Wennergren *et al.* 1997). However, even at preschool age, the prevalence of postnatal smoke exposure was higher among asthmatics compared with symptom free individuals, 73% vs. 58% (Wennergren *et al.* 1992). Eighty-four per cent of the children not exposed to smoke during infancy were symptom free at school age. However, current passive smoking at school age did not significantly increase the risk of school age asthma (Wennergren *et al.* 1997). On the other hand, passive smoking at school age did increase the risk of asthma in early adulthood (Paper V). Passive smoking at school age and in early adulthood was clearly associated with smoke exposure both pre- and postnatally, Appendix 3 (Table S1A and S1B in Paper V).

Both pre- and postnatal smoke exposure were independent risk factors for asthma at age 17-20 years, Appendix 3 (Table S3A in Paper V). The more severe the asthma, the more frequently subjects were exposed to smoke *in utero* and during infancy (p (trend) = 0.010). However, we found no dose-response effect between subjects exposed to more or less than nine cigarettes per day. Our findings are consistent with previous reports of an increased risk of subsequent wheezing and asthma in childhood following smoke exposure both *in utero* and in infancy (Rylander *et al.* 1993, Lødrup Carlsen *et al.* 1999, Stein *et al.* 1999b, Lux *et al.* 2000, Lannerö *et al.* 2006).

Prenatal smoke exposure and airway function

We found an independent association between prenatal smoke exposure and bronchial hyper-responsiveness in early adulthood, Appendix 3 (Table S3B in Paper V). Prenatal smoke exposure was also associated with a reduced FEV_1/FVC post-BD, while postnatal smoke exposure did not correlate with any spirometry findings (Paper V). In the multivariate analysis of infantile risk factors for abnormal airway function in the cohort, prenatal smoke exposure was found to be an independent risk factor post-BD (Paper IV).

In previous studies, prenatal smoke exposure has been reported to be associated with impaired airway function in infancy and childhood (Hanrahan *et al.* 1992, Lødrup Carlsen *et al.* 1997, Gilliland *et al.* 2000). In the Korppi group, Hyvärinen *et al.* found an association between teenage BHR and maternal smoking during pregnancy but not smoke exposure in infancy (Hyvärinen *et al.* 2007). However, it has been argued that postnatal smoke exposure also affects the risk of wheeze, which is supported by the findings that asthmatic children exposed to tobacco smoke have more severe asthma symptoms and poorer airway function than unexposed asthmatic children (Mannino *et al.* 2002).

In addition, Piippo-Savolainen *et al.* report a decrease in airway function in early adulthood following smoke exposure during the first two years of life (Piippo-Savolainen *et al.* 2006). However, given their close relationship, it is difficult to distinguish the effect of pre- and postnatal smoke exposure from one another and the possibility that the effect on adult airway function is due to prenatal smoke exposure cannot be ruled out (Alm *et al.* 1998, Stein *et al.* 1999b).

Postnatal smoke exposure and active smoking

In our study, postnatal smoke exposure during infancy was an independent risk factor for active smoking at age 17-20 years, Appendix 3 (Table S3C in Paper V). Active smoking has been associated with current asthma in our (Paper III) and other studies (Strachan *et al.* 1996, Sears *et al.* 2003, Hedman *et al.* 2011). This supports the findings of a social inheritance and perhaps a genetic inheritance of the predisposition to become addicted to tobacco smoke (De Vries *et al.* 2003, Vink *et al.* 2005).

Smoking cessation

Notably, we found that individuals with continuous passive smoke exposure from infancy to early adulthood had the highest prevalence of asthma at age 17-20 and that individuals in whom passive smoke exposure had been discontinued during childhood had a lower prevalence, as illustrated in Figure 12 and in Table 2 in Paper III. Parental smoking cessation during childhood

is therefore beneficial and affects the subsequent risk of asthma (P (trend) = 0.031). This is reassuring for parents who have smoked during pregnancy or the infancy of their child.

Allergic sensitisation

We were unable to confirm any effect by either pre- or postnatal smoke exposure on allergic sensitisation in early adulthood. Previous studies have reported contradictory results relating to the impact of early smoke exposure and later sensitisation, with some studies finding an association and others not (Kulig *et al.* 1999, Bråbäck *et al.* 2001, Murray *et al.* 2004, Lannerö *et al.* 2008).

Conclusion

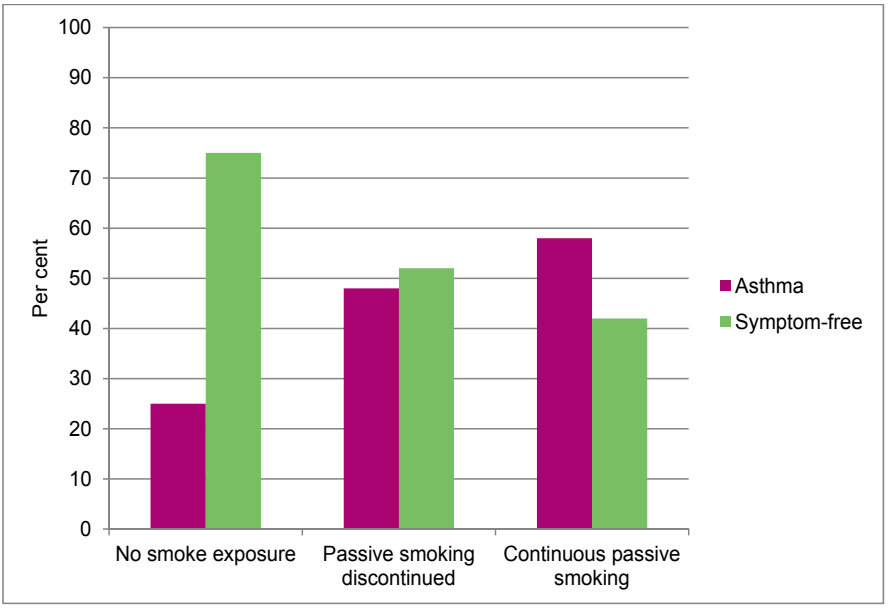
To summarise, prenatal smoke exposure appears to be associated not only with asthma but also with BHR and impaired airway function, in early adulthood. Postnatal smoke exposure also increased the risk of asthma in adulthood but appeared to be linked to the risk of becoming an active smoker oneself. This is discussed in more detail below.

Current smoke exposure

Active smoking was reported by 27% of the cohort and 21% of the control group. Interestingly, subjects with current asthma in the cohort had a higher prevalence of active smokers compared with symptom free subjects (41% vs. 18%). The same tendency was seen in the control group, but it was not as pronounced (27% vs. 20%) (Tables 1, 4a and 4b in Paper III). Active smoking was therefore associated with current asthma in early adulthood but did not remain significant in the multivariate analysis (Paper III). Current passive smoking in the home was over-represented in the cohort compared with the control group, 49% vs. 29% (Table 4a, Paper III), but it did not increase the risk of current asthma.

The risk of current asthma has been reported to be increased in active smokers in adolescence and adulthood (Strachan *et al.* 1996, Sears *et al.* 2003, Hedman *et al.* 2011). As discussed above, it has been suggested that both social and genetic inheritance influence the risk of smoking initiation (De Vries *et al.* 2003, Vink *et al.* 2005). The impact of genetic inheritance on the risk of smoking dependence has been emphasised (Vink *et al.* 2005). Individuals exposed to smoke at home in infancy and during childhood run a higher risk of becoming smokers themselves, in addition to the plausible damage done by the exposure itself to the developing airways.

Figure 12. *Smoke exposure and the risk of asthma in early adulthood in children hospitalised due to early severe viral wheeze. The results are shown for subjects with no smoke exposure from infancy to early adulthood, for subjects where passive smoke exposure had been discontinued during childhood and for continuous passive smoke exposure from infancy to early adulthood. p (trend) = 0.031.*



Other factors of interest

Furry animals

At admission, 32 families reported having furry animals in the home. No significant effect of having furry animals in infancy was seen on asthma at preschool age, 10 years of age or in early adulthood. However, among the subjects exposed to furry animals at home during infancy, 65% were symptom free at age 17-20 years, compared with 56% symptom free among non-exposed individuals and 57% in the total study population. So, if anything, a protective effect by furry animals on adolescent asthma was indicated.

Since most reports of a protective effect from exposure to furry animals relate to allergic sensitisation (Hesselmar *et al.* 1999, Platts-Mills *et al.* 2001, Ownby *et al.* 2002, Perzanowski *et al.* 2002, Almqvist *et al.* 2010, Wegienka *et al.* 2011), the effect on current allergy at age 17-20 years was also analysed in our

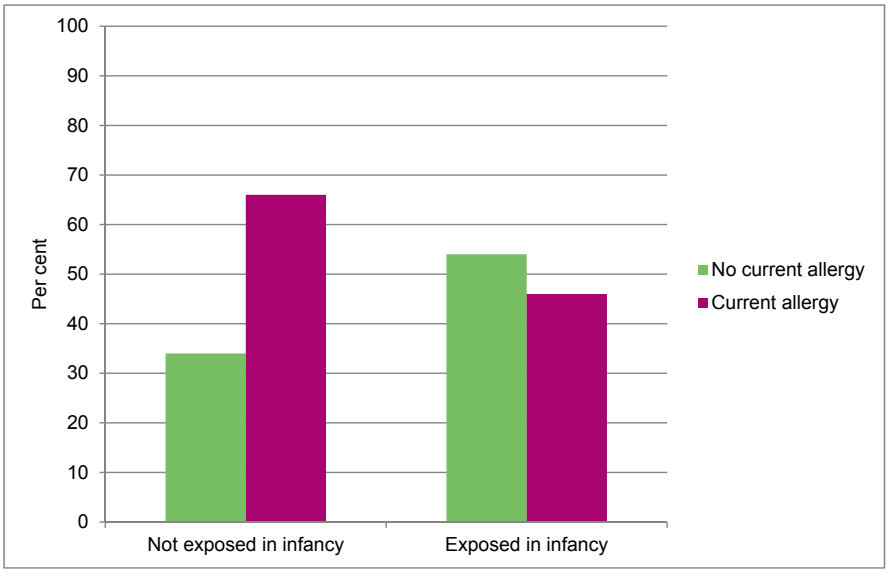
study (unpublished data). In line with previous studies, our results indicated a protective effect by exposure to furry animals in infancy on atopic disease in early adulthood, as illustrated by Figure 13. However, the association did not reach significance and was not apparent when different allergens were analysed separately. This might be due to low power and the fact that the exposure in infancy could not be analysed for different animals. It has been suggested that the protective effect reported by others is allergen specific and dose dependent (Ownby *et al.* 2002).

Furthermore, we were not able to adjust for atopic families avoiding furry animals in our study. However, it has been argued that this does not explain the protective effect reported in other studies (Ownby *et al.* 2002). In line with the findings by Wegienka *et al.* that the first year of life appears to be a critical period for exposure and that exposure later in life is of less importance, we can see no effect from current exposure to furry pets in early adulthood on either asthma or allergy (Wegienka *et al.* 2011).

Other kinds of exposure

So far, the potential effect of obesity on asthma has not been assessed in this study. No information was available on exposure to antibiotics, paracetamol or a farming environment in infancy. In addition, no data on the vitamin-D status in infancy or the length of breast-feeding was available either.

Figure 13. *Prevalence of current allergy at age 17-20 years in subjects not exposed and exposed to furry animals at home during infancy. The prevalence of current allergy tended to be lower in exposed subjects compared with non-exposed subjects, but the difference did not reach significance (p=0.101).*



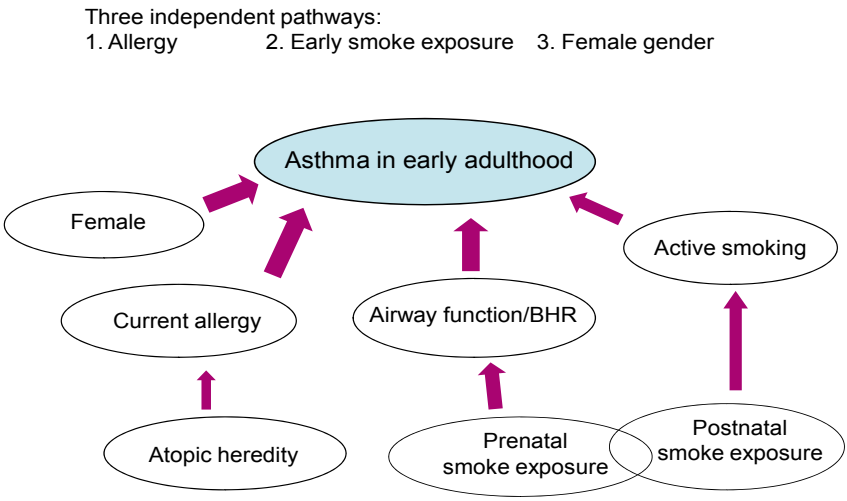
Pathogenic model

Construction

From the different associations seen with asthma at age 17-20 years in the cohort, we have constructed a pathogenic model (Figure 14) designed to sum up our results (Papers III, IV and V).

The model is based on the associations seen between family atopy and current allergy in early adulthood, between prenatal smoke exposure and current hyper-responsiveness and postnatal smoke exposure and current active smoking. In addition, we noted the association of asthma in early adulthood with current allergy, hyper-responsiveness and active smoking. We concluded that current allergy, hyper-responsiveness and active smoking could be regarded as intermediate variables between the infantile factors (family atopy, pre- and postnatal smoke exposure) and current asthma.

Figure 14. *A pathogenic model indicating three independent pathways increasing the risk of asthma in early adulthood.*



Three pathways leading to asthma

The pathogenic model consists of three pathways leading to asthma in early adulthood. One pathway from female gender, one allergic pathway from family atopy via the development of allergy and a third pathway from early smoke exposure via hyper-responsiveness and active smoking. The early smoke ex-

posure can be divided into pre- and postnatal exposure, with prenatal exposure affecting the risk of asthma via hyper-responsiveness and postnatal exposure affecting the risk of asthma via active smoking. The pathogenic model illustrated in Figure 14 combines the models proposed in Papers III, IV and V.

Previous findings

The proposed pathogenic model fits well with previous findings in other studies. As discussed above, a gender shift is seen in asthma prevalence between males and females during adolescence (Skobeloff *et al.* 1992, Taussig *et al.* 2003, Schatz *et al.* 2004). Wheezing and asthma in infancy is more prevalent in boys, while females have a higher prevalence of adult asthma (Korppi *et al.* 1986, Skobeloff *et al.* 1992, Wennergren *et al.* 1992, Martinez *et al.* 1995, Sears *et al.* 2003, Melén *et al.* 2004). It therefore makes sense that females in the cohort run a higher risk of asthma in early adulthood.

The family history of atopy is a known risk factor for the development of allergy and asthma (Castro-Rodriguez *et al.* 2000, Melén *et al.* 2004, Wennergren *et al.* 2010, Lim *et al.* 2010). In addition, the risk of asthma is higher in individuals with other atopic manifestations (Castro-Rodriguez *et al.* 2000, Guilbert *et al.* 2004, Illi *et al.* 2004, Illi *et al.* 2006).

Furthermore, there is substantial evidence relating to the negative effect of smoke exposure both in pregnancy and during infancy on future wheeze and asthma (Rylander *et al.* 1993, Lødrup Carlsen *et al.* 1999, Stein *et al.* 1999b, Lux *et al.* 2000, Lannerö *et al.* 2006). Prenatal smoke exposure has been reported to have a harmful effect on airway function at birth and in childhood (Hanrahan *et al.* 1992, Lødrup Carlsen *et al.* 1997, Gilliland *et al.* 2000). The reports of the effects of postnatal smoke exposure are somewhat divergent, but they have been reported for both wheezing and airway function (Mannino *et al.* 2002, Piippo-Savolainen *et al.* 2006). In addition, passive smoke exposure at home in childhood has been associated with a higher risk of becoming a smoker when growing up (De Vries *et al.* 2003) and active smoking is a known risk factor for current asthma (Strachan *et al.* 1996, Sears *et al.* 2003, Hedman *et al.* 2011).

Strengths and weaknesses

The long observation period and the good consistency are the strengths of the study. The cohort has been followed from before the age of two, with a response rate of 88% in early adulthood. The same trained allergy nurse has performed the tests at all re-investigations.

The small size of the cohort is a limitation. Only 55 of them were tested and this yields low power to some of the analyses made. Notably, if biologically plau-

sible, the significant effects found in a small set of material probably represent effects of importance. However, caution is advisable when generalising the findings, since the external validity might be small. It should be remembered that the cohort consists of a subgroup with severe wheezing in childhood.

In the subgroup of controls that were tested, we noted a higher prevalence of both females and asthmatics than in the total control group. Asthma, allergy and perhaps affected airway function could therefore be over-represented among the tested controls. However, we can still see a difference compared with our cohort that might be underestimated due to the constitution of the tested subgroup of the controls.

The tested individuals in the cohort did not differ from the non-tested ones, apart from slightly more self-reported allergic symptoms among the tested individuals.

The prevalence of smoking during pregnancy and in infancy in the cohort was higher compared with the general population at that time. This might reflect the negative impact of smoke exposure increasing the risk of severe wheeze, but it might also be a marker of some bias.

A more detailed questionnaire at admission would naturally have been desirable. For example, our primary data did not differentiate between pre- and postnatal smoke exposure. However, these data could be found in the MBR to some extent.

General Discussion

Several studies have reported an increased risk of subsequent asthma following wheezing in early childhood. This is also seen in our studies, with wheezing symptoms in infancy predicting recurrent wheeze and asthma during childhood and adolescence. Our results add to the studies that attempt to predict the children with wheeze who will develop asthma.

Virus or host?

Our studies confirm viral infections as the most common trigger of wheezing in early life. In the follow-up of severe viral wheeze, we can see an increased risk of later asthma in these children. Our results indicating that the asthma risk in adolescence is not dependent of viral agents support the view of a susceptible host being more prone to respond with wheezing during viral infection.

Family history and the development of allergic disease

The impact of atopic heredity and own allergic manifestations is evident in both studies, in line with the results seen in other studies. Persistent asthma during childhood is strongly influenced by genetics and is associated with the development of atopic disease following the "allergic march", as described above.

Gender

The gender distribution among children and adults with wheeze and asthma follows a clear pattern. It is more common among boys in early childhood, while the incidence in girls increases during adolescence and asthma in adults is more common among females. Our results fit well with this pattern, with boys being over-represented among preschool wheezers. In addition, we can see a gender shift in adolescence with allergic asthma, which is subsequently more prevalent in females.

Window of opportunity

It has been suggested that the environment *in utero* and exposure during early life are important for the development of subsequent asthma and allergic disease, influencing the developing immune system. In the Children of Western Sweden Study, we consider some of these kinds of exposure, such as paracetamol exposure during pregnancy and antibiotic treatment in the neonatal period, confirming an increased risk of asthma.

In addition, we have assessed the effect of breast-feeding and diet during infancy, suggesting a protective effect by the early introduction of fish on future allergic asthma and by breast-feeding on the risk of wheezing with viral infections in childhood.

Data on exposure during pregnancy and dietary factors in infancy were not available in the follow-up of severe viral wheeze. Information about exposure to tobacco smoke *in utero* and in infancy was, however, available in both studies.

Smoking habits 1983-2003

In the follow-up of viral wheeze in children born in the early 1980s, an association was seen between smoke exposure both *in utero* and in infancy and later asthma. This was not evident in the study of the Children of Western Sweden birth cohort assessing the risk of wheeze at preschool age. One possible explanation for our not finding a clear impact of smoking during pregnancy on wheezing at preschool age is the change in smoking habits in recent decades. There has been a decrease in smoking prevalence among pregnant women in Sweden. In the early 1980s, about 31% of pregnant women smoked, compared with 6.7% in 2009 (Swedish National Board of Health and Welfare 2004, Lafih 2011).

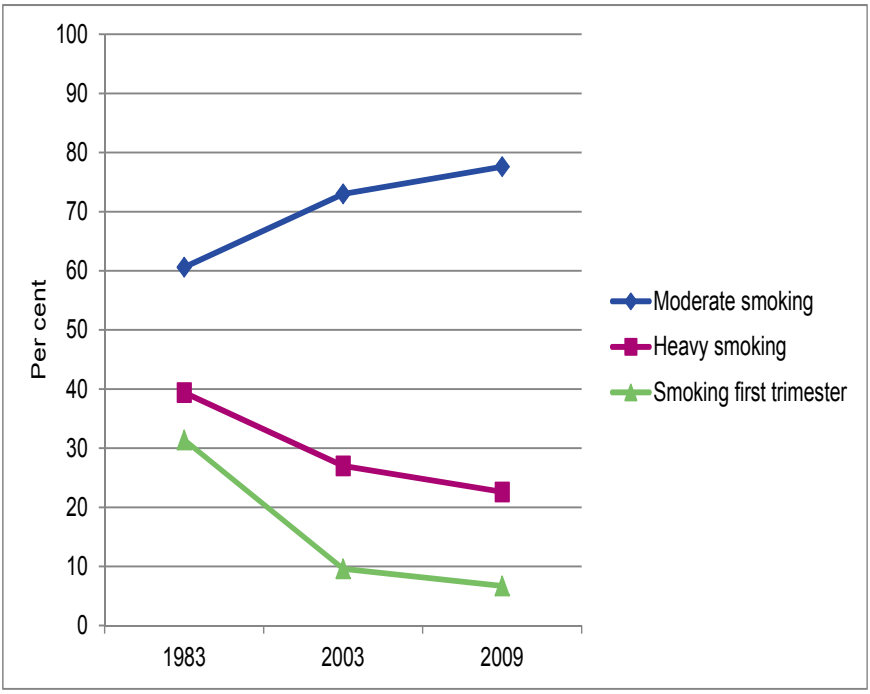
However, it is possible that women who smoke during pregnancy today smoke less than smoking women used to do a few decades ago. When comparing data from 1983 and 2003, it is obvious that not only smoking during pregnancy has decreased but also that women who smoke, smoke less (Swedish National Board of Health and Welfare 2007). The percentage of heavy smokers ($\geq$ 10 cigarettes per day) among women who smoke during early pregnancy has decreased from 39% to 27%, as illustrated in Figure 15 (Lafih 2011). The prevalence of heavy smoking in our studies is 43% and 20%, illustrating a clear difference in smoke exposure during pregnancy between the two cohorts. This difference is clear also when comparing the overall prevalence of smoking during pregnancy in the two cohorts. Fifty-five per cent of the children with severe wheeze in early life were exposed to smoke prenatally, compared with 9.6% in the Children of Western Sweden Study.

In addition, it might be suspected that pregnant women today are not exposed to as much second-hand smoke as they were some decades ago, due to the national legislation in Sweden forbidding smoking in public places. The educational efforts and restrictions that have been made have reduced public acceptance of smoking and possibly also changed smoking habits. This reasoning is also applicable to the second-hand smoke exposure of children postnatally at home and in public.

Summary

To summarise, wheezing with viral infections is common during early childhood and most children who wheeze become symptom free as they grow up. However, children who wheeze run an increased risk of persistent symptoms and the development of asthma. Our studies support the notion that some children are more prone to react by wheezing during viral infections. The interplay of several factors, including genetic inheritance and exposure *in utero* and early life, influences the risk of later asthma development.

Figure 15. *Prevalence of smoking mothers during the first trimester in western Sweden as reported to the MBR from 1983 to 2009 (Sweden National Board of Health). The prevalence of moderate (fewer than 10 cigarettes/day) and heavy (10 or more cigarettes per day) smokers among smoking mothers is shown. The results show a steady decrease in prenatal smoke exposure from 1983 to 2009. In addition, the number of heavy smokers among smoking pregnant women is also reduced.*



Conclusion

The specific aims of the thesis were:

To study factors influencing the risk of wheeze at 4.5 years of age, with special emphasis on the impact of neonatal antibiotics, early fish introduction and paracetamol exposure during pregnancy (Papers I and II)

Treatment with antibiotics during the first week of life increased the risk of recurrent wheeze at age 4.5 years. The effect was more pronounced in children with MTW. In addition, the greatest prevalence of neonatal antibiotic treatment was seen among the children with most frequent wheeze at preschool age.

The early introduction of fish before nine months of age reduced the risk of recurrent wheeze at preschool age. Indications of a combined effect of early fish introduction and neonatal antibiotic treatment were found.

Exposure to paracetamol during pregnancy increased the risk of ICS-treated wheeze at age 4.5 years, with a more pronounced effect on MTW. The attributable fraction for paracetamol exposure was 4.7% in ICS-treated wheeze and 8.4% in ICS-treated MTW.

In addition, the risk of recurrent wheeze at preschool age was increased by male gender, parental atopic disease and doctor-diagnosed food allergy or eczema in infancy. Breast-feeding for more than four months had a risk-reducing effect on recurrent wheeze close to significance.

To study whether the risk factors for multiple-trigger wheeze and episodic viral wheeze differed at preschool age (Paper I)

Our results suggest that the risk factors for MTW and EVW differ. The differences in risk factors between the two phenotypes support the notion of different prognoses and indicate different pathophysiological pathways.

The factors that were most pronounced for MTW at preschool age were neonatal antibiotic treatment, paracetamol exposure during pregnancy, parental atopic disease and doctor-diagnosed food allergy or eczema in infancy. This supports the more allergic nature of children with MTW.

The factors influencing the risk of EVW at preschool age, although only close to significance, were male gender, breast-feeding for four months or more and caesarean section.

To describe the prevalence of asthma in early adulthood following hospitalisation due to virally induced obstructive disease before the age of two years (Paper III)

Children hospitalised due to wheezing bronchitis in early life run an increased risk of asthma in early adulthood. The prevalence of asthma at age 17-20 years was 43% among subjects with early wheezing, compared with 15% in the control group. The risk of asthma in early adulthood was highest among females with current allergic disease.

To study the factors influencing the risk of asthma in early adulthood, following hospitalisation due to virally induced obstructive disease before the age of two years (Paper III)

The factors influencing the risk of asthma in early adulthood are summarised in a model with three pathogenic pathways. The first pathway is the increased risk of asthma seen in females. Secondly, a family history of atopy increases the risk of asthma via the development of current allergic disease. Lastly, exposure to tobacco smoke in *utero* and early life increases the risk of asthma via the development of bronchial hyper-responsiveness and an increased prevalence of active smoking in early adulthood.

To describe the airway function in early adulthood following hospitalisation due to virally induced obstructive disease before the age of two years (Paper IV)

To study the factors influencing the risk of having reduced airway function in early adulthood, following hospitalisation due to virally induced obstructive disease before the age of two (Paper IV)

Children with severe wheezing in early life run an increased risk of reduced airway function in early adulthood. Expiratory flows were most affected. Abnormal airway function was seen in 31% of the cohort compared with 16% in the control group. Affected airway function was seen both pre-BD and post-BD, indicating not only an increase in airway tone but also possible structural changes to the small airways in the cohort.

Asthmatic females in the cohort had the most affected airway function. Female gender, current hyper-responsiveness and prenatal smoke exposure were independent risk factors for abnormal airway function in early adulthood following hospitalisation due to wheezing bronchitis before the age of two years.

To study the impact of prenatal and postnatal smoking on asthma in early adulthood, following hospitalisation due to virally induced obstructive disease before the age of two (Paper V)

Both pre- and postnatal smoke exposure increase the risk of asthma in early adulthood. Prenatal smoke exposure increases the risk of current hyper-responsiveness and affected airway function, which in turn are both associated with a higher prevalence of asthma. Postnatal smoke exposure increases the risk of being an active smoker in early adulthood, which is associated with a higher prevalence of asthma.

Parental smoking cessation during childhood is beneficial and reduces the subsequent risk of asthma compared with continuous smoke exposure throughout childhood.

Populärvetenskaplig sammanfattning

Bakgrund:

Astmabesvär i samband med virusorsakade luftrörsinfektioner är mycket vanliga i de tidiga barndomsåren och har rapporterats vara kopplade till en ökad risk för astma senare i livet. Alla med tidiga astmabesvär utvecklar dock inte astma. Risken för utveckling av efterföljande astma kan påverkas av både genetik och miljöfaktorer.

Målsättning:

Syftet med avhandlingen är att beskriva riskfaktorer för utvecklande av astmabesvär som barn samt långtidsprognosen efter besvär tidigt i livet.

Metod:

I studien Västra Götalands Barn följs en födelsekohort på 5600 barn födda i Västra Götaland år 2003. De följs från spädbarnsåldern och under uppväxten med avseende på förekomst av astma och andra allergiska sjukdomar, samt riskfaktorer för att utveckla dessa. Enkäter har besvarats vid 6 månaders, 12 månaders och 4,5 års ålder. Dessutom har data fåtts från Medicinska födelseregistret. Studien har tidigare redovisat riskfaktorer för astma och eksembesvär vid ett års ålder. I denna avhandling har skyddande faktorer och riskfaktorer för astmabesvär vid 4,5 års ålder undersökts. Effekten av antibiotikabehandling som nyfödd, kostvanor under spädbarnstiden och exponering för paracetamol under graviditeten har varit särkilt intressant att kartlägga.

I en uppföljningstudie av 101 barn som sjukhusvårdats för astmabesvär till följd av virusorsakad luftrörsinfektion har karaktäristika och prognos undersökts vid 17-20 års ålder. Undersökningen bestod i en enkät och testning för allergisk sensibilisering samt testning av lungfunktion och överretbara luftrör. Även effekten av tidig tobaksexponering har undersökts. Data på moderns rökning under graviditeten erhölls från Medicinska födelseregistret. Gruppen med tidiga astmabesvär jämfördes med en kontrollgrupp.

Resultat:

Kända riskfaktorer för att ha upprepade besvär av pipande och väsande andning (astmabesvär) vid 4,5 års ålder, såsom ärftlighet för astma och allergi, eget eksem eller födoämnesallergi under spädbarnsåret samt manligt kön, kunde bekräftas i studien Västra Götalands Barn. Dessutom sågs en ökad risk efter antibiotikabehandling under första levnadsveckan. Tidig introduktion av fisk (före 9 månaders ålder) minskade risken för astmabesvär i förskoleåldern.

Exponering för paracetamol under fostertiden ökade risken för besvär som krävde behandling med inhalationssteroider vid 4,5 års ålder. Risken var tydligast hos de som hade besvär även mellan förkylningsepisoder.

I uppföljningsstudien av individer som sjukhusvårdats för astmabesvär före 2 års ålder, sågs en ökad förekomst av astma i tidig vuxen ålder jämfört med kontrollgruppen. Risken att ha astma i tidig vuxenålder var ökad bland dem med aktuell allergi, vilket i sin tur var kopplat till att ha ärftlighet för astma och allergi. Även att ha överretbara luftrör och egen rökning som ung vuxen ökade risken för samtidig astma. Därtill var kvinnligt kön en oberoende riskfaktor för astma i tidig vuxen ålder. Pojkarna, som dominerar bland dem med astmabesvär i de tidiga barnaåldrarna, tillfrisknar i högre grad än flickorna.

Även lägre lungfunktionsvärden vid 17-20 års ålder sågs hos gruppen med tidiga astmabesvär, vilket var tydligast hos de flickor som utvecklat astma, men även hos de symptomfria individerna sågs en skillnad. Också rökexponering under fostertiden tycktes kopplat till sämre lungfunktion i tidig vuxenålder.

Ett annat intressant fynd i undersökningen är att stor risk för astmautveckling ses både hos de barn där så kallat RS-virus påvisades vid förstagångsinläggningen och hos de barn som insjuknade med andra luftvägsvirus.

Våra data tyder på att rökexponering under fostertiden är en oberoende riskfaktor för att ha överretbara luftrör i ung vuxen ålder. Den passiva rökningen under uppväxten är i stället signifikant kopplad till att barnet själv blir rökare, vilket i sin tur ger ökad risk för astma.

Slutsats:

Exponering för faktorer under fostertiden och under spädbarnstiden påverkar risken för astmabesvär under barndomen. Paracetamol under fostertiden och antibiotikabehandling under första levnadsveckan ökade risken för astmabesvär i förskoleåldern. Tidig introduktion av fisk minskade risken.

Individer som haft astmabesvär som små har en ökad risk för astma i tidig vuxen ålder. Risken är ökad hos dem som utvecklat en allergisk sjukdom eller överretbarhet i luftvägarna samt hos kvinnor. Exponering för tobaksrök under fosterlivet ökar risken för överretbara luftrör och astma, medan passiv rökning under spädbarnsåret och uppväxten ökar risken att själv bli rökare. De tidiga astmabesväreн är också kopplade till långtidseffekter på lungfunktionen.

Acknowledgements

First of all, I would like to express my gratitude to all the participants in the studies. I would like to thank all the parents of the participants in the Children of Western Sweden Study who, as a result of their participation, have created a solid, valuable data source. Special thanks to all the participants and parents in the follow-up study of severe wheezing, who have contributed to research for so many years. I would like to emphasise my gratitude to those participants who were willing to participate in testing, spending hours at the hospital each time.

My particular gratitude goes to:

Göran Wennergren, my supervisor and mentor, for your never-ending encouragement and kindness. For always taking the time to answer my questions and listen to my worries. I am so grateful for the opportunities you have given me and for your support and guidance in research, clinical work and everyday life

Bernt Alm, my co-supervisor, for sharing your vast knowledge of allergy research and statistics. Thank you for your patience and encouragement and for all the fruitful discussions in front of the computer with SPSS and the data file

Both Bernt and Göran – I am grateful for all the historical anecdotes, linguistics and poetry you have shared. You have both been great travelling companions and have taught me wisdom applicable not only in research but also in clinical and everyday life, such as to work a little harder ("lägga manken till"), to keep on trying ("never up, never in") and never to give up ("however, all is not lost"). I am forever grateful for your friendship

Per Gustafsson, my co-supervisor, for your enthusiasm and for sharing your knowledge of paediatric pulmonary physiology

Mainor Åmark, who has performed all the clinical tests and most of the practical work in the follow-up study. Thank you for your friendship and for sharing your knowledge and remarkable clinical experience. You are truly humble and kind and I am grateful for the opportunity to have "seen you in action"

To the rest of the Children of Western Sweden Study group: Rolf Pettersson, Hrefna Thengilsdottir, Per Möllborg, Laslo Erdes, Nils Åberg and Gunnar Norvenius. I am grateful for the opportunity to be a part of the group. Thank you for sharing your knowledge and experience and for your kindness and support. Thanks for letting me bring Peter to our meetings. Special thanks to Nils for reading my thesis and sharing his views. I am also grateful to Gunnar for enlightening me with his vast paediatric knowledge and his kind words

Staffan Nilsson, for excellent statistical advice and valuable discussions

Jeanette Kliger, for correcting all the English texts

Ann Nordström, for helping me with the layout of my thesis and making it printable

Ann-Sofie Peterson, for patiently photographing my two wild kids and making the very nice picture for the cover

Marlene Mussman, for her administrative support

Diana Swolin-Eide, for her support and encouragements. Your never-ending ambition and compassion for others are inspiring!

Cecilia Hallén, for your kindness and administrative support. For solving every problem with a smile

Annika Funnermark-Törnkvist, for your friendship and for letting me know there is a clinical future ahead

Without the support and encouragement of my family and friends, this work would not have been completed

A special thank-you to:

My wonderful parents, Ingela and Lars Åke, for your endless love, encouragement and support. For being the most attentive and loving grandparents to my sons. I am forever grateful for all your help during this summer and autumn. For letting me grow up, knowing I am loved

My sisters and brothers, Clara, Elin, Rasmus, Joakim and Sebastian, and your families – being a part of "villa villervalla" is a blessing and you are always in my heart

My parents-in-law, Mona and Ronny, and my sister-in-law, Sofie, and her family, for your love and support

My special friends, Malin and Sanna

Malin, my BVIV, you are my hero – your strength and endurance during the last years have been extraordinary. Without your friendship, I would be lost

Sanna, my soulmate, I am so grateful that faith made us fall into friendship that day outside Kemi huset so many years ago. You have been a true companion

My grandmother, Liv, who is the most headstrong person I know. You have taught me never to give up

To Bertil, for your kindness and encouragement

For looking down on me from heaven – to Ast, for your love and for daring me to "rubba gubbarna", and to Peter, for his legacy of persistence and ambition

Lastly,

To my children, Erik and Peter, with all my love, you are my greatest joy and comfort

To Peter, for dragging me away from the computer, forcing me to cuddle, read stories and play. For your –"äska däj"!

To Erik, for putting my life into perspective, discussing the meaning of life, why we are here and how everything works. For always keeping me in your heart. You are right! We are always a part of each other

To Mattias, my beloved companion in life – for standing by my side and holding my hand. For your courage and curiosity and for always encouraging me to move forward and for catching me when I fall.

There is nothing greater in life than to love and be truly loved.

The studies were supported by:

The Sahlgrenska Academy at the University of Gothenburg, the Research Foundation of the Swedish Asthma and Allergy Association, the Swedish Foundation for Health Care Sciences and Allergy Research and the Health & Medical Care Committee of the Regional Executive Board, Västra Götaland Region.

References

Abramson JH, Abramson ZH. Making sense of data: a self-instruction manual on the interpretation of epidemiological data. 3rd ed. New York: Oxford University Press. 2001.

Allander T, Jartti T, Gupta S, Niesters HG, Lehtinen P, Osterback R, Vuorinen T, Waris M, Bjerkner A, Tiveljung-Lindell A, van den Hoogen BG, Hyypiä T, Ruuskanen O. Human bocavirus and acute wheezing in children. Clin Infect Dis. 2007; 44: 904-10.

Alm B, Milerad J, Wennergren G, Skjaerven R, Øyen N, Norvenius G, Daltveit AK, Helweg-Larsen K, Markestad T, Irgens LM. A case-control study of smoking and sudden infant death syndrome in the Scandinavian countries, 1992 to 1995. The Nordic Epidemiological SIDS Study. Arch Dis Child. 1998; 78: 329-34.

Alm B, Erdes L, Möllborg P, Pettersson R, Norvenius SG, Åberg N, Wennergren G. Neonatal antibiotic treatment is a risk factor for early wheezing. Pediatrics. 2008; 121: 697-702.

Alm B, Åberg N, Erdes L, Möllborg P, Pettersson R, Norvenius SG, Goksör E, Wennergren G. Early introduction of fish decreases the risk of eczema in infants. Arch Dis Child. 2009; 94: 11-5.

Alm B, Goksör E, Thengilsdottir H, Pettersson R, Möllborg P, Norvenius G, Erdes L, Åberg N, Wennergren G. Early protective and risk factors for allergic rhinitis at age 4½ yr. Pediatr Allergy Immunol. 2011; 22: 398-404.

Almqvist C, Egmar AC, van Hage-Hamsten M, Berglind N, Pershagen G, Nordvall SL, Svartengren M, Hedlin G, Wickman M. Heredity, pet ownership, and confounding control in a population-based birth cohort. J Allergy Clin Immunol. 2003; 111: 800-6.

Almqvist C, Garden F, Xuan W, Mihrshahi S, Leeder SR, Oddy W, Webb K, Marks GB; CAPS team. Omega-3 and omega-6 fatty acid exposure from early life does not affect atopy and asthma at age 5 years. J Allergy Clin Immunol. 2007; 119: 1438-44.

Almqvist C, Garden F, Kemp AS, Li Q, Crisafulli D, Tovey ER, Xuan W, Marks GB; CAPS Investigators. Effects of early cat or dog ownership on sensitisation and asthma in a high-risk cohort without disease-related modification of exposure. Paediatr Perinat Epidemiol. 2010; 24: 171-8.

Almqvist C, Wettermark B, Hedlin G, Ye W, Lundholm C. Antibiotics and asthma medication in a large registerbased cohort study – confounding cause and effect. Clin Exp Allergy. 2011 sept 23. [Epub ahead of print]

Altman DG. Practical statistics for medical research. London: Chapman & Hall/CRC. 1999: 337-49.

American Thoracic Society. Standardization of spirometry, 1994 Update. Am J Respir Crit Care Med. 1995; 152: 1107–36.

Anderson HR, Pottier AC, Strachan DP. Asthma from birth to age 23: incidence and relation to prior and concurrent atopic disease. Thorax. 1992; 47: 537-42.

Anderson HR, Gupta R, Strachan DP, Limb ES. 50 years of asthma: UK trends from 1955 to 2004. Thorax. 2007; 62: 85-90.

Andersson M, Bjerg A, Forsberg B, Lundbäck B, Rönmark E. The clinical expression of asthma in schoolchildren has changed between 1996 and 2006. Pediatr Allergy Immunol. 2010; 21: 859-66.

Asher MI, Montefort S, Björkstén B, Lai CK, Strachan DP, Weiland SK, Williams H; ISAAC Phase Three Study Group. Worldwide time trends in the prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and eczema in childhood: ISAAC Phases One and Three repeat multicountry cross-sectional surveys. Lancet. 2006; 368: 733-43. Erratum in: Lancet. 2007; 370: 1128.

Bakkeheim E, Mowinckel P, Carlsen KH, Håland G, Carlsen KC. Paracetamol in early infancy; the risk of childhood allergy and asthma. Acta Paediatr. 2011; 100: 90–6.

Ball TM, Castro-Rodriguez JA, Griffith KA, Holberg CJ, Martinez FD, Wright AL. Siblings, day-care attendance, and the risk of asthma and wheezing during childhood. N Engl J Med. 2000; 343: 538-43.

Beasley R, Clayton T, Crane J, von Mutius E, Lai CK, Montefort S, Stewart A; ISAAC Phase Three Study Group. Association between paracetamol use in infancy and childhood, and risk of asthma, rhinoconjunctivitis, and eczema in children aged 6–7 years: analysis from Phase Three of the ISAAC programme. Lancet. 2008; 372: 1039–48.

Bibi H, Shoseyov D, Feigenbaum D, Genis M, Friger M, Peled R, Sharff S. The relationship between asthma and obesity in children: is it real or a case of over diagnosis? J Asthma. 2004; 41: 403-10.

Björkstén B. The intrauterine and postnatal environments. J Allergy Clin Immunol. 1999; 104: 1119-27.

Björkstén B, Naaber P, Sepp E, Mikelsaar M. The intestinal microflora in allergic Estonian and Swedish 2-year-old children. Clin Exp Allergy. 1999; 29: 342-6.

Bolte G, Kompauer I, Fobker M, Cullen P, Keil U, Mutius E, Weiland SK. Fatty acids in serum cholesteryl esters in relation to asthma and lung function in children. Clin Exp Allergy. 2006; 36: 293-302.

Bråbäck L, Kjellman NI, Sandin A, Björkstén B. Atopy among schoolchildren in northern and southern Sweden in relation to pet ownership and early life events. Pediatr Allergy Immunol. 2001; 12: 4-10.

Brand PL, Baraldi E, Bisgaard H, Boner AL, Castro-Rodriguez JA, Custovic A, de Blic J, de Jongste JC, Eber E, Everard ML, Frey U, Gappa M, Garcia-Marcos L, Grigg J, Lenney W, Le Souëf P, McKenzie S, Merkus PJ, Midulla F, Paton JY, Piacentini G, Pohunek P, Rossi GA, Seddon P, Silverman M, Sly PD, Stick S, Valiulis A, van Aalderen WM, Wildhaber JH, Wennergren G, Wilson N, Zivkovic Z, Bush A. Definition, assessment and treatment of wheezing disorders in preschool children: an evidence-based approach. Eur Respir J. 2008; 32: 1096-110. Review.

Braun-Fahrländer C, Riedler J, Herz U, Eder W, Waser M, Grize L, Maisch S, Carr D, Gerlach F, Bufe A, Lauener RP, Schierl R, Renz H, Nowak D, von Mutius E; Allergy and Endotoxin Study Team. Environmental exposure to endotoxin and its relation to asthma in school-age children. N Engl J Med. 2002; 347: 869-77.

Breckler LA, Hale J, Jung W, Westcott L, Dunstan JA, Thornton CA, Prescott SL. Modulation of in vivo and in vitro cytokine production over the course of pregnancy in allergic and nonallergic mothers. Pediatr Allergy Immunol. 2010; 21: 14–21.

Busse WW, Lemanske RF Jr, Gern JE. Role of viral respiratory infections in asthma and asthma exacerbations. Lancet. 2010; 376: 826-34. Review.

Byremo G, Rød G, Carlsen KH. Effect of climatic change in children with atopic eczema. Allergy. 2006; 61: 1403-10.

Camargo CA Jr, Rifas-Shiman SL, Litonjua AA, Rich-Edwards JW, Weiss ST, Gold DR, Kleinman K, Gillman MW. Maternal intake of vitamin D during pregnancy and risk of recurrent wheeze in children at 3 y of age. Am J Clin Nutr. 2007; 85: 788-95.

Camargo CA Jr, Ingham T, Wickens K, Thadhani R, Silvers KM, Epton MJ, Town GI, Pattemore PK, Espinola JA, Crane J; New Zealand Asthma and Allergy Cohort Study Group. Cord-blood 25-hydroxyvitamin D levels and risk of respiratory infection, wheezing, and asthma. Pediatrics. 2011; 127: e180-7.

Castro-Rodríguez JA, Holberg CJ, Wright AL, Martinez FD. A clinical index to define risk of asthma in young children with recurrent wheezing. Am J Respir Crit Care Med. 2000; 162: 1403-6.

Castro-Rodríguez JA, Holberg CJ, Morgan WJ, Wright AL, Martinez FD. Increased incidence of asthmalike symptoms in girls who become overweight or obese during the school years. Am J Respir Crit Care Med. 2001; 163: 1344-9.

Castro-Rodriguez JA. The asthma predictive index: early diagnosis of asthma. Curr Opin Allergy Clin Immunol. 2011; 11: 157-61. Review.

Caudri D, Wijga A, Schipper CM, Hoekstra M, Postma DS, Koppelman DH, Brunekreef B, Smit HA, de Jongste JC. Predicting the long-term prognosis of children with symptoms suggestive of asthma at preschool age. J Allergy Clin Immunol. 2009; 124: 903–910; e1–e7.

Corne JM, Marshall C, Smith S, Schreiber J, Sanderson G, Holgate ST, Johnston SL. Frequency, severity, and duration of rhinovirus infections in asthmatic and non-asthmatic individuals: a longitudinal cohort study. Lancet. 2002; 359: 831-4.

Coughlin SS, Benichou J, Weed DL. Attributable risk estimation in case-control studies. Epidemiol Rev. 1994; 16: 51-64.

Court SD. The definition of acute respiratory illnesses in children. Postgrad Med J. 1973; 49: 771-6.

De Vries H, Engels R, Kremers S, Wetzels J, Mudde A. Parents' and friends' smoking status as predictors of smoking onset: findings from six European countries. Health Educ Res. 2003; 18: 627-36.

Douwes J, Cheng S, Travier N, Cohet C, Niesink A, McKenzie J, Cunningham C, Le Gros G, von Mutius E, Pearce N. Farm exposure in utero may protect against asthma, hay fever and eczema. Eur Respir J. 2008; 32: 603-11.

Douwes J, Travier N, Huang K, Cheng S, McKenzie J, Le Gros G, von Mutius E, Pearce N. Lifelong farm exposure may strongly reduce the risk of asthma in adults. Allergy. 2007; 62: 1158-65.

Droste JH, Wieringa MH, Weyler JJ, Nelen VJ, Vermeire PA, Van Bever HP. Does the use of antibiotics in early childhood increase the risk of asthma and allergic disease? Clin Exp Allergy. 2000; 30: 1547–53.

Du Toit G, Katz Y, Sasieni P, Mesher D, Maleki SJ, Fisher HR, Fox AT, Turcanu V, Amir T, Zadik-Mnuhin G, Cohen A, Livne I, Lack G. Early consumption of peanuts in infancy is associated with a low prevalence of peanut allergy. J Allergy Clin Immunol. 2008; 122: 984-91.

Duchén K, Yu G, Björkstén B. Atopic sensitization during the first year of life in relation to long chain polyunsaturated fatty acid levels in human milk. Pediatr Res. 1998; 44: 478-84.

Duijts L, Ramadhani MK, Moll HA. Breastfeeding protects against infectious diseases during infancy in industrialized countries. A systematic review. Matern Child Nutr. 2009; 5: 199-210. Review.

Dunstan JA, Mori TA, Barden A, Beilin LJ, Taylor AL, Holt PG, Prescott SL. Fish oil supplementation in pregnancy modifies neonatal allergen-specific immune responses and clinical outcomes in infants at high risk of atopy: a randomized, controlled trial. J Allergy Clin Immunol. 2003; 112: 1178-84.

Eder W, Klimecki W, Yu L, von Mutius E, Riedler J, Braun-Fahrländer C, Nowak D, Martinez FD; ALEX Study Team. Toll-like receptor 2 as a major gene for asthma in children of European farmers. J Allergy Clin Immunol. 2004; 113: 482-8.

Ege MJ, Bieli C, Frei R, van Strien RT, Riedler J, Ublagger E, Schram-Bijkerk D, Brunekreef B, van Hage M, Scheynius A, Pershagen G, Benz MR, Lauener R, von Mutius E, Braun-Fahrländer C; Parsifal Study team. Prenatal farm exposure is related to the expression of receptors of the innate immunity and to atopic sensitization in school-age children. J Allergy Clin Immunol. 2006: 117: 817-23.

Ege MJ, Frei R, Bieli C, Schram-Bijkerk D, Waser M, Benz MR, Weiss G, Nyberg F, van Hage M, Pershagen G, Brunekreef B, Riedler J, Lauener R, Braun-Fahrländer C, von Mutius E; PARSIFAL Study team. Not all farming environments protect against the development of asthma and wheeze in children. J Allergy Clin Immunol. 2007; 119: 1140-7.

Ege MJ, Mayer M, Normand AC, Genuneit J, Cookson WO, Braun-Fahrländer C, Heederik D, Piarroux R, von Mutius E; GABRIELA Transregio 22 Study Group. Exposure to environmental microorganisms and childhood asthma. N Engl J Med. 2011; 364: 701-9.

Elliott L, Henderson J, Northstone K, Chiu GY, Dunson D, London SJ. Prospective study of breast-feeding in relation to wheeze, atopy, and bronchial hyperresponsiveness in the Avon Longitudinal Study of Parents and Children (ALSPAC). J Allergy Clin Immunol. 2008; 122: 49-54.

Elphick HE, Ritson S, Rigby AS, Everard ML. Phenotype of acute respiratory syncytial virus induced lower respiratory tract illness in infancy and subsequent morbidity. Acta Paediatr. 2007; 96: 307-9.

Eriksson J, Ekerljung L, Lötvall J, Pullerits T, Wennergren G, Rönmark E, Torén K, Lundbäck B. Growing up on a farm leads to lifelong protection against allergic rhinitis. Allergy. 2010; 65: 1397-403.

Erwin EA, Woodfolk JA, Ronmark E, Perzanowski M, Platts-Mills TA. The long-term protective effects of domestic animals in the home. Clin Exp Allergy. 2011; 41: 920-2.

Etminan M, Sadatsafavi M, Jafari S, Doyle-Waters M, Aminzadeh K, Fitzgerald JM. Acetaminophen use and the risk of asthma in children and adults: a systematic review and metaanalysis. Chest. 2009; 136: 1316–23.

Eyers S, Weatherall M, Jefferies S, Beasley R. Paracetamol in pregnancy and the risk of wheezing in offspring: a systematic review and meta-analysis. Clin Exp Allergy. 2011; 41: 482–9.

Farquhar H, Stewart A, Mitchell E, Crane J, Eyers S, Weatherall M, Beasley R. The role of paracetamol in the pathogenesis of asthma. Clin Exp Allergy. 2010; 40: 32-41.

Flaherman V, Rutherford GW. A meta-analysis of the effect of high weight on asthma. Arch Dis Child. 2006; 91: 334-9.

Flohr C, Nagel G, Weinmayr G, Kleiner A, Strachan DP, Williams HC; the ISAAC Phase Two Study Group. Lack of evidence for a protective effect of prolonged breastfeeding on childhood eczema: lessons from the International Study of Asthma and Allergies in Childhood (ISAAC) Phase Two. Br J Dermatol. 2011 Aug 24. [Epub ahead of print].

Fogarty A, Davey G. Paracetamol, antioxidants and asthma. Clin Exp Allergy. 2005; 35: 700-2.

Forrest JA, Clements JA, Prescott LF. Clinical pharmacokinetics of paracetamol. Clin Pharmacokinet. 1982; 7: 93–107.

Furuhjelm C, Warstedt K, Larsson J, Fredriksson M, Böttcher MF, Fälth-Magnusson K, Duchén K. Fish oil supplementation in pregnancy and lactation may decrease the risk of infant allergy. Acta Paediatr. 2009; 98: 1461-7.

Garcia-Marcos L, Sanchez-Solis M, Perez-Fernandez V, Pastor-Vivero MD, Mondejar-Lopez P, Valverde-Molina J. Is the effect of prenatal paracetamol exposure on wheezing in preschool children modified by asthma in the mother? Int Arch Allergy Immunol. 2009; 149: 33–7.

Garcia-Marcos L, Martinez FD. Multi-trigger versus episodic wheeze in toddlers: new phenotypes or severity markers? J Allergy Clin Immunol. 2010; 126: 489-90.

Gern JE, Brooks GD, Meyer P, Chang A, Shen K, Evans MD, Tisler C, Dasilva D, Roberg KA, Mikus LD, Rosenthal LA, Kirk CJ, Shult PA, Bhattacharya A, Li Z, Gangnon R, Lemanske RF Jr. Bidirectional interactions between viral respiratory illnesses and cytokine responses in the first year of life. J Allergy Clin Immunol. 2006; 117: 72-8.

Gilliland FD, Berhane K, McConnell R, Gauderman WJ, Vora H, Rappaport EB, Avol E, Peters JM. Maternal smoking during pregnancy, environmental tobacco smoke exposure and childhood lung function. Thorax. 2000; 55: 271-6.

Gilliland FD, Li YF, Dubeau L, Berhane K, Avol E, McConnell R, Gauderman WJ, Peters JM. Effects of glutathione S-transferase M1, maternal smoking during pregnancy, and environmental tobacco smoke on asthma and wheezing in children. Am J Respir Crit Care Med. 2002; 166: 457-63.

Gilliland FD, Berhane K, Islam T, McConnell R, Gauderman WJ, Gilliland SS, Avol E, Peters JM. Obesity and the risk of newly diagnosed asthma in school-age children. Am J Epidemiol. 2003; 158: 406-15.

Gold DR, Bloomberg GR, Cruikshank WW, Visness CM, Schwarz J, Kattan M, O'Connor GT, Wood RA, Burger MS, Wright RJ, Witter F, Lee-Parritz A, Sperling R, Sadovsky Y, Togias A, Gern JE. Parental characteristics, somatic fetal growth, and season of birth influence innate and adaptive cord blood cytokine responses. J Allergy Clin Immunol. 2009; 124: 1078-87.

Greer FR, Sicherer SH, Burks AW; American Academy of Paediatrics Committee on Nutrition; American Academy of Paediatrics Section on Allergy and Immunology. Effects of early nutritional interventions on the development of atopic disease in infants and children: the role of maternal dietary restriction, breastfeeding, timing of introduction of complementary foods, and hydrolyzed formulas. Pediatrics. 2008; 121: 183-91. Review.

Guerra S, Lohman IC, Halonen M, Martinez FD, Wright AL. Reduced interferon gamma production and soluble CD14 levels in early life predict recurrent wheezing by 1 year of age. Am J Respir Crit Care Med. 2004(a); 169: 70-6.

Guerra S, Wright AL, Morgan WJ, Sherrill DL, Holberg CJ, Martinez FD. Persistence of asthma symptoms during adolescence: role of obesity and age at the onset of puberty. Am J Respir Crit Care Med. 2004(b); 170: 78-85.

Guilbert TW, Morgan WJ, Zeiger RS, Bacharier LB, Boehmer SJ, Krawiec M, Larsen G, Lemanske RF, Liu A, Mauger DT, Sorkness C, Szefler SJ, Strunk RC, Taussig LM, Martinez FD. Atopic characteristics of children with recurrent wheezing at high risk for the development of childhood asthma. J Allergy Clin Immunol. 2004; 114: 1282–1287.

Gustafsson PM, Kjellman B. Asthma from childhood to adulthood: course and outcome of lung function. Respir Med. 2000; 94: 466–4474.

Hancox RJ, Milne BJ, Poulton R, Taylor DR, Greene JM, McLachlan CR, Cowan JO, Flannery EM, Herbison GP, Sears MR. Sex differences in the relation between body mass index and asthma and atopy in a birth cohort. Am J Respir Crit Care Med. 2005; 171: 440-5.

Hanrahan JP, Tager IB, Segal MR, Tosteson TD, Castile RG, Van Vunakis H, Weiss ST, Speizer FE. The effect of maternal smoking during pregnancy on early infant lung function. Am Rev Respir Dis. 1992; 145: 1129-35.

Hedenström H, Malmberg P, Agarwal K. Reference values for lung function tests in females. Regression equations with smoking variables. Bull Eur Physiopathol Respir. 1985; 21: 551–557.

Hedenström H, Malmberg P, Fridriksson HV. Reference values for pulmonary function tests in men: regression equations which include smoking variables. Ups J Med Sci. 1986; 91: 299–310.

Hedman L, Bjerg A, Sundberg S, Forsberg B, Rönmark E. Both environmental tobacco smoke and personal smoking is related to asthma and wheeze in teenagers. Thorax. 2011; 66: 20-5.

Hesselmar B, Aberg N, Aberg B, Eriksson B, Björkstén B. Does early exposure to cat or dog protect against later allergy development? Clin Exp Allergy. 1999; 29: 611-7.

Hesselmar B, Saalman R, Rudin A, Adlerberth I, Wold AE. Early fish introduction is associated with less eczema, but not sensitization, in infants. Acta Paediatr. 2010; 99: 1861–7.

Heymann PW, Carper HT, Murphy DD, Platts-Mills TA, Patrie J, McLaughlin AP, Erwin EA, Shaker MS, Hellems M, Peerzada J, Hayden FG, Hatley TK, Chamberlain R. Viral infections in relation to age, atopy, and season of admission among children hospitalized for wheezing. J Allergy Clin Immunol. 2004; 114: 239-47.

Hill AB. The environment and disease: Association or causation? Proc R Soc Med. 1965; 58: 295-300.

Holick MF. Vitamin D: a D-Lightful health perspective. Nutr Rev. 2008; 66: S182-94. Review.

Hosmer DW, Lemeshow S. Applied logistic regression. 2nd ed. New York: Wiley-Interscience. 2000: 91-6.

Hyppönen E, Sovio U, Wjst M, Patel S, Pekkanen J, Hartikainen AL, Järvelinb MR. Infant vitamin d supplementation and allergic conditions in adulthood: northern Finland birth cohort 1966. Ann N Y Acad Sci. 2004; 1037: 84-95.

Hyvärinen M, Piippo-Savolainen E, Korhonen K, Korppi M. Teenage asthma after severe infantile bronchiolitis or pneumonia. Acta Paediatr. 2005(a); 94: 1378-83.

Hyvärinen MK, Kotaniemi-Syrjänen A, Reijonen TM, Korhonen K, Korppi MO. Teenage asthma after severe early childhood wheezing: an 11-year prospective follow-up. Pediatr Pulmonol. 2005(b); 40: 316-23.

Hyvärinen MK, Kotaniemi-Syrjänen A, Reijonen TM, Korhonen K, Korppi MO. Lung function and bronchial hyper-responsiveness 11 years after hospitalization for bronchiolitis. Acta Paediatr. 2007; 96: 1464-9.

Hyvärinen M. Wheezing in early childhood – Predictive factors for asthma in the 11-year follow-up. Kuopio University Publications D. Medical sciences 464. 2009.

Hyvärinen MK, Kotaniemi-Syrjänen A, Reijonen TM, Piippo-Savolainen E, Korppi M. Eosinophil activity in infants hospitalized for wheezing and risk of persistent childhood asthma. Pediatr Allergy Immunol. 2010; 21: 96-103.

Illi S, von Mutius E, Lau S, Nickel R, Grüber C, Niggemann B, Wahn U; Multicenter Allergy Study Group. The natural course of atopic dermatitis from birth to age 7 years and the association with asthma. J Allergy Clin Immunol. 2004; 113: 925-31.

Illi S, von Mutius E, Lau S, Niggemann B, Grüber C, Wahn U; Multicentre Allergy Study (MAS) group. Perennial allergen sensitisation early in life and chronic asthma in children: a birth cohort study. Lancet. 2006; 368: 763-70.

Jackson DJ, Gangnon RE, Evans MD, Roberg KA, Anderson EL, Pappas TE, Printz MC, Lee WM, Shult PA, Reisdorf E, Carlson-Dakes KT, Salazar LP, DaSilva DF, Tisler CJ, Gern JE, Lemanske RF Jr. Wheezing rhinovirus illnesses in early life predict asthma development in high-risk children. Am J Respir Crit Care Med. 2008; 178: 667-72.

Jartti T, Lehtinen P, Vuorinen T, Osterback R, van den Hoogen B, Osterhaus AD, Ruuskanen O. Respiratory picornaviruses and respiratory syncytial virus as causative agents of acute expiratory wheezing in children. Emerg Infect Dis. 2004; 10: 1095-101.

Jenmalm MC. T-Cell function in atopic children. Int Arch Allergy Immunol. 1999; 118: 395-8.

Kabesch M, Hoefler C, Carr D, Leupold W, Weiland SK, von Mutius E. Glutathione S transferase deficiency and passive smoking increase childhood asthma. Thorax. 2004; 59: 569-73.

Kalliomäki M, Salminen S, Poussa T, Arvilommi H, Isolauri E. Probiotics and prevention of atopic disease: 4-year follow-up of a randomised placebo-controlled trial. Lancet. 2003; 361: 1869-71.

Kang EM, Lundsberg LS, Illuzzi JL, Bracken MB. Prenatal exposure to acetaminophen and asthma in children. Obstet Gynecol. 2009; 114: 1295-306.

Koeppen-Schomerus G, Stevenson J, Plomin R. Genes and environment in asthma: a study of 4 year old twins. Arch Dis Child. 2001; 85: 398-400.

Koniman R, Chan YH, Tan TN, van Bever HP. A matched patient-sibling study on the usage of paracetamol and the subsequent development of allergy and asthma. Pediatr Allergy Immunol. 2007; 18: 128–34.

Korppi M, Halonen P, Kleemola M, Launiala K. Viral findings in children under the age of two years with expiratory difficulties. Acta Paediatr Scand. 1986; 75: 457-64.

Kotaniemi-Syrjänen A, Reijonen TM, Romppanen J, Korhonen K, Savolainen K, Korppi M. Allergen-specific immunoglobulin E antibodies in wheezing infants: the risk for asthma in later childhood. Pediatrics. 2003(a);111: e255-61.

Kotaniemi-Syrjänen A, Vainionpää R, Reijonen TM, Waris M, Korhonen K, Korppi M. Rhinovirus-induced wheezing in infancy-the first sign of childhood asthma? J Allergy Clin Immunol. 2003(b); 111: 66-71.

Kozyrskyj AL, Ernst P, Becker AB. Increased risk of childhood asthma from antibiotic use in early life. Chest. 2007; 131: 1753-9.

Kramer MS, Kakuma R. Optimal duration of exclusive breastfeeding. Cochrane Database Syst Rev. 2002;(1):CD003517. Review.

Kremmyda LS, Vlachava M, Noakes PS, Diaper ND, Miles EA, Calder PC. Atopy risk in infants and children in relation to early exposure to fish, oily fish, or long-chain omega-3 fatty acids: a systematic review. Clin Rev Allergy Immunol. 2011; 41: 36-66.

Kristjansson S, Bjarnarson SP, Wennergren G, Palsdottir AH, Arnadottir T, Haraldsson A, Jonsdottir I. Respiratory syncytial virus and other respiratory viruses during the first 3 months of life promote a local TH_2-like response. J Allergy Clin Immunol. 2005; 116: 805-11.

Kuehni CE, Spycher BD, Silverman M. Causal links between RSV infection and asthma: no clear answers to an old question. Am J Respir Crit Care Med. 2009; 179: 1079-80.

Kulig M, Luck W, Lau S, Niggemann B, Bergmann R, Klettke U, Guggenmoos-Holzmann I, Wahn U. Effect of pre- and postnatal tobacco smoke exposure on specific sensitization to food and inhalant allergens during the first 3 years of life. Multicenter Allergy Study Group, Germany. Allergy. 1999; 54: 220-8.

Kull I, Almqvist C, Lilja G, Pershagen G, Wickman M. Breast-feeding reduces the risk of asthma during the first 4 years of life. J Allergy Clin Immunol. 2004; 114: 755-60.

Kull I, Böhme M, Wahlgren CF, Nordvall L, Pershagen G, Wickman M. Breastfeeding reduces the risk for childhood eczema. J Allergy Clin Immunol. 2005; 116: 657-61.

Kull I, Bergström A, Lilja G, Pershagen G, Wickman M. Fish consumption during the first year of life and development of allergic diseases during childhood. Allergy. 2006; 61: 1009–15.

Kull I, Melen E, Alm J, Hallberg J, Svartengren M, van Hage M, Pershagen G, Wickman M, Bergström A. Breast-feeding in relation to asthma, lung function, and sensitization in young schoolchildren. J Allergy Clin Immunol. 2010; 125: 1013-9.

Kummeling I, Stelma FF, Dagnelie PC, Snijders BE, Penders J, Huber M, van Ree R, van den Brandt PA, Thijs C. Early life exposure to antibiotics and the subsequent development of eczema, wheeze, and allergic sensitization in the first 2 years of life: the KOALA Birth Cohort Study. Pediatrics. 2007; 119: e225-31.

Kurukulaaratchy RJ, Matthews S, Holgate ST, Arshad SH. Predicting persistent disease among children who wheeze during early life. Eur Respir J. 2003; 22: 767-71.

Lafih J. Personal communication, Swedish National Board of Health and Welfare (Socialstyrelsen); 2011.

Lannerö E, Wickman M, Pershagen G, Nordvall L. Maternal smoking during pregnancy increases the risk of recurrent wheezing during the first years of life (BAMSE). Respir Res. 2006; 7: 3.

Lannerö E, Wickman M, van Hage M, Bergström A, Pershagen G, Nordvall L. Exposure to environmental tobacco smoke and sensitisation in children. Thorax. 2008; 63: 172-6.

Le Souëf P. Prediction of asthma in children at 6 and 12 years of age: Perth infant asthma follow-up study. Pediatr Allergy Immunol. 2002; 13 Suppl 15: 44-6.

Lemanske RF Jr, Jackson DJ, Gangnon RE, Evans MD, Li Z, Shult PA, Kirk CJ, Reisdorf E, Roberg KA, Anderson EL, Carlson-Dakes KT, Adler KJ, Gilbertson-White S, Pappas TE, Dasilva DF, Tisler CJ, Gern JE. Rhinovirus illnesses during infancy predict subsequent childhood wheezing. J Allergy Clin Immunol. 2005; 116: 571-7.

Lim RH, Kobzik L, Dahl M. Risk for asthma in offspring of asthmatic mothers versus fathers: a meta-analysis. PLoS One. 2010; 5: e10134.

Lødrup Carlsen KC, Jaakkola JJ, Nafstad P, Carlsen KH. In utero exposure to cigarette smoking influences lung function at birth. Eur Respir J. 1997; 10: 1774-9.

Lødrup Carlsen KC, Carlsen KH, Nafstad P, Bakketeig L. Perinatal risk factors for recurrent wheeze in early life. Pediatr Allergy Immunol. 1999; 10: 89-95.

Lowe AJ, Carlin JB, Bennett CM, Hosking CS, Allen KJ, Robertson CF, Axelrad C, Abramson MJ, Hill DJ, Dharmage SC. Paracetamol use in early life and asthma: prospective birth cohort study. BMJ. 2010; 341: c4616.

Lux AL, Henderson AJ, Pocock SJ. Wheeze associated with prenatal tobacco smoke exposure: a prospective, longitudinal study. ALSPAC Study Team. Arch Dis Child. 2000; 83: 307-12.

Lötvall J, Akdis CA, Bacharier LB Bjermer L, Casale TB, Custovic A, Lemanske RF Jr, Wardlaw AJ, Wenzel SE, Greenberger PA. Asthma endotypes: a new approach to classification of disease entities within the asthma syndrome. J Allergy Clin Immunol. 2011; 127: 355-60.

Mannino DM, Homa DM, Redd SC. Involuntary smoking and asthma severity in children: data from the Third National Health and Nutrition Examination Survey. Chest. 2002; 122: 409-15.

Marks GB, Mihrshahi S, Kemp AS, Tovey ER, Webb K, Almqvist C, Ampon RD, Crisafulli D, Belousova EG, Mellis CM, Peat JK, Leeder SR. Prevention of asthma during the first 5 years of life: a randomized controlled trial. J Allergy Clin Immunol. 2006; 118: 53-61.

Marra F, Marra CA, Richardson K, Lynd LD, Kozyrskyj A, Patrick DM, Bowie WR, Fitzgerald JM. Antibiotic use in children is associated with increased risk of asthma. Pediatrics. 2009; 123: 1003-10.

Martinez FD, Wright AL, Taussig LM, Holberg CJ, Halonen M, Morgan WJ. Asthma and wheezing in the first six years of life. The Group Health Medical Associates. N Engl J Med. 1995; 332:133-8.

Martinez FD, Stern DA, Wright AL, Taussig LM, Halonen M. Differential immune responses to acute lower respiratory illness in early life and subsequent development of persistent wheezing and asthma. J Allergy Clin Immunol. 1998; 102: 915-20.

Matricardi PM, Grüber C, Wahn U, Lau S. The asthma-obesity link in childhood: open questions, complex evidence, a few answers only. Clin Exp Allergy. 2007; 37: 476-84. Review.

Melén E, Wickman M, Nordvall SL, van Hage-Hamsten M, Lindfors A. Influence of early and current environmental exposure factors on sensitisation and outcome of asthma in pre-school children. Allergy. 2001; 56: 646-52.

Melén E, Kere J, Pershagen G, Svartengren M, Wickman M. Influence of male sex and parental allergic disease on childhood wheezing: role of interactions. Clin Exp Allergy. 2004; 34: 839-44.

Melgert BN, Ray A, Hylkema MN, Timens W, Postma DS. Are there reasons why adult asthma is more common in females? Curr Allergy Asthma Rep. 2007; 7: 143-50. Review.

Merkus PJ, Borsboom GJ, Van Pelt W, Schrader PC, Van Houwelingen HC, Kerrebijn KF, Quanjer PH. Growth of airways and air spaces in teenagers is related to sex but not to symptoms. J Appl Physiol. 1993; 75: 2045-53.

Mihrshahi S, Ampon R, Webb K, Almqvist C, Kemp AS, Hector D, Marks GB; CAPS team. The association between infant feeding practices and subsequent atopy among children with a family history of asthma. Clin Exp Allergy. 2007; 37: 671-9.

Mora JR, Iwata M, von Andrian UH. Vitamin effects on the immune system: vitamins A and D take centre stage. Nat Rev Immunol. 2008; 8: 685-98.

Mullins RJ, Camargo CA Jr. Shining a light on vitamin D and its impact on the developing immune system. Clin Exp Allergy. 2011; 41: 766-8.

Mullins RJ, Clark S, Katelaris C, Smith V, Solley G, Camargo Jr CA. Season of birth and childhood food allergy in Australia. Pediatr Allergy Immunol. 2011; 22: 583-89.

Murray CS, Woodcock A, Smillie FI, Cain G, Kissen P, Custovic A; NACMAAS Study Group. Tobacco smoke exposure, wheeze, and atopy. Pediatr Pulmonol. 2004; 37: 492-8.

Nafstad P, Nystad W, Magnus P, Jaakkola JJ. Asthma and allergic rhinitis at 4 years of age in relation to fish consumption in infancy. J Asthma. 2003; 40: 343–8.

Nagakura T, Matsuda S, Shichijyo K, Sugimoto H, Hata K. Dietary supplementation with fish oil rich in omega-3 polyunsaturated fatty acids in children with bronchial asthma. Eur Respir J. 2000; 16: 861-5.

Nagel G, Büchele G, Weinmayr G, Björkstén B, Chen YZ, Wang H, Nystad W, Saraclar Y, Bråbäck L, Batlles-Garrido J, Garcia-Hernandez G, Weiland SK; ISAAC Phase II Study Group. Effect of breastfeeding on asthma, lung function and bronchial hyperreactivity in ISAAC Phase II. Eur Respir J. 2009; 33: 993-1002.

Noakes PS, Holt PG, Prescott SL. Maternal smoking in pregnancy alters neonatal cytokine responses. Allergy. 2003; 58: 1053-8.

Notenboom ML, Mommers M, Jansen EH, Penders J, Thijs C. Maternal fatty acid status in pregnancy and childhood atopic manifestations: KOALA Birth Cohort Study. Clin Exp Allergy. 2011; 41: 407-16.

Noverr MC, Huffnagle GB. The ‘‘microflora hypothesis’’ of allergic diseases. Clin Exp Allergy. 2005; 35: 1511–20. Review.

Nuttall SL, Williams J, Kendall MJ. Does paracetamol cause asthma? J Clin Pharm Ther. 2003; 28: 251–7.

Ober C, Yao TC. The genetics of asthma and allergic disease: a 21st century perspective. Immunol Rev. 2011; 242: 10-30.

Ogra PL. Respiratory syncytial virus: the virus, the disease and the immune response. Paediatr Respir Rev. 2004; 5: S119-26. Review.

Olenec JP, Kim WK, Lee WM, Vang F, Pappas TE, Salazar LE, Evans MD, Bork J, Roberg K, Lemanske RF Jr, Gern JE. Weekly monitoring of children with asthma for infections and illness during common cold seasons. J Allergy Clin Immunol. 2010; 125: 1001-1006.e1.

Ownby DR, Johnson CC, Peterson EL. Exposure to dogs and cats in the first year of life and risk of allergic sensitization at 6 to 7 years of age. JAMA. 2002; 288: 963-72.

Oyama N, Sudo N, Sogawa H, Kubo C. Antibiotic use during infancy promotes a shift in the T(H)1 ⁄ T(H)2 balance toward T(H)2-dominant immunity in mice. J Allergy Clin Immunol. 2001; 107: 153–9.

Perzanowski MS, Rönmark E, Platts-Mills TA, Lundbäck B. Effect of cat and dog ownership on sensitization and development of asthma among preteenage children. Am J Respir Crit Care Med. 2002; 166: 696-702.

Perzanowski MS, Miller RL, Tang D, Ali D, Garfinkel RS, Chew GL, Goldstein IF, Perera FP, Barr RG. Prenatal acetaminophen exposure and risk of wheeze at age 5 years in an urban low-income cohort. Thorax. 2010; 65: 118–23.

Piippo-Savolainen E, Remes S, Kannisto S, Korhonen K, Korppi M. Asthma and lung function 20 years after wheezing in infancy: results from a prospective follow-up study. Arch Pediatr Adolesc Med. 2004; 158: 1070-6.

Piippo-Savolainen E, Remes S, Kannisto S, Korhonen K, Korppi M. Early predictors for adult asthma and lung function abnormalities in infants hospitalized for bronchiolitis. A prospective 18-20 years follow-up. Allergy Asthma Proc. 2006; 27: 341-49.

Piippo-Savolainen E. Wheezy babies – Wheezy adults? Asthma, bronchial reactivity and lung function in adulthood after hospitalization for bronchiolitis in early life. Kuopio University Publications D. Medical sciences 398. 2006.

Piippo-Savolainen E, Remes S, Korppi M. Does early exposure or sensitization to inhalant allergens predict asthma in wheezing infants? A 20-year follow-up. Allergy Asthma Proc. 2007; 28: 454-61.

Piippo-Savolainen E, Korppi M. Wheezy babies--wheezy adults? Review on long-term outcome until adulthood after early childhood wheezing. Acta Paediatr. 2008; 97: 5-11. Review.

Platts-Mills T, Vaughan J, Squillace S, Woodfolk J, Sporik R. Sensitisation, asthma, and a modified Th_2 response in children exposed to cat allergen: a population-based cross-sectional study. Lancet. 2001; 357: 752-6.

Prescott SL, Smith P, Tang M, Palmer DJ, Sinn J, Huntley SJ, Cormack B, Heine RG, Gibson RA, Makrides M. The importance of early complementary feeding in the development of oral tolerance: concerns and controversies. Pediatr Allergy Immunol. 2008; 19: 375-80.

Prescott SL. Allergic disease: understanding how in utero events set the scene. Proc Nutr Soc. 2010; 69: 366-72. Review.

Prymula R, Siegrist CA, Chlibek R, Zemlickova H, Vackova M, Smetana J, Lommel P, Kaliskova E, Borys D, Schuerman L. Effect of prophylactic paracetamol administration at time of vaccination on febrile reactions and antibody responses in children: two open-label, randomized controlled trials. Lancet. 2009; 374: 1339–50.

Rebordosa C, Kogevinas M, Sørensen HT, Olsen J. Pre-natal exposure to paracetamol and risk of wheezing and asthma in children: a birth cohort study. Int J Epidemiol. 2008; 37: 583–90.

Rehan VK, Asotra K, Torday JS. The effects of smoking on the developing lung: insights from a biologic model for lung development, homeostasis, and repair. Lung. 2009; 187: 281-9.

Reijonen TM, Kotaniemi-Syrjänen A, Korhonen K, Korppi M. Predictors of asthma three years after hospital admission for wheezing in infancy. Pediatrics. 2000; 106: 1406-12.

Roduit C, Wohlgensinger J, Frei R, Bitter S, Bieli C, Loeliger S, Büchele G, Riedler J, Dalphin JC, Remes S, Roponen M, Pekkanen J, Kabesch M, Schaub B, von Mutius E, Braun-Fahrländer C, Lauener R; PASTURE Study Group. Prenatal animal contact and gene expression of innate immunity receptors at birth are associated with atopic dermatitis. J Allergy Clin Immunol. 2011; 127: 179-85.

Rothman KJ. Epidemiology: An introduction. New York: Oxford University Press. 2002. 108-89.

Ruotsalainen M, Piippo-Savolainen E, Hyvärinen MK, Korppi M. Adulthood asthma after wheezing in infancy: a questionnaire study at 27 years of age. Allergy. 2010; 65: 503-9.

Rylander E, Pershagen G, Eriksson M, Nordvall L. Parental smoking and other risk factors for wheezing bronchitis in children. Eur J Epidemiol. 1993; 9: 517-26.

Rönmark E, Bjerg A, Hedman L, Perzanowski M, Sundberg S, Lundbäck B. The Obstructive Lung Disease in Northern Sweden (OLIN) longitudinal paediatric study I--the first 10 years. Clin Respir J. 2008; 2 Suppl 1: 26-33.

Salam MT, Li YF, Langholz B, Gilliland FD. Maternal fish consumption during pregnancy and risk of early childhood asthma. J Asthma. 2005; 42: 513-8.

Salam MT, Wenten M, Gilliland FD. Endogenous and exogenous sex steroid hormones and asthma and wheeze in young women. J Allergy Clin Immunol. 2006; 117: 1001-7.

Schatz M, Clark S, Emond JA, Schreiber D, Camargo CA Jr. Sex differences among children 2-13 years of age presenting at the emergency department with acute asthma. Pediatr Pulmonol. 2004; 37: 523-9.

Schaub B, Lauener R, von Mutius E. The many faces of the hygiene hypothesis. J Allergy Clin Immunol. 2006; 117: 969-77. Review.

Schaub B, Liu J, Schleich I, Höppler S, Sattler C, von Mutius E. Impairment of T helper and T regulatory cell responses at birth. Allergy. 2008; 63: 1438-47.

Schauer U, Hoffjan S, Bittscheidt J, Köchling A, Hemmis S, Bongartz S, Stephan V. RSV bronchiolitis and risk of wheeze and allergic sensitisation in the first year of life. Eur Respir J. 2002; 20: 1277-83.

Scholtens S, Wijga AH, Brunekreef B, Kerkhof M, Hoekstra MO, Gerritsen J, Aalberse R, de Jongste JC, Smit HA. Breast feeding, parental allergy and asthma in children followed for 8 years. The PIAMA birth cohort study. Thorax. 2009; 64: 604-9.

Schultz A, Devadason SG, Savenije OE, Sly PD, Le Souëf PN, Brand PL. The transient value of classifying preschool wheeze into episodic viral wheeze and multiple trigger wheeze. Acta Paediatr. 2010; 99: 56-60.

Schumann A, Nutten S, Donnicola D, Comelli EM, Mansourian R, Cherbut C, Corthesy-Theulaz I, Garcia-Rodenas C. Neonatal antibiotic treatment alters gastrointestinal tract developmental gene expression and intestinal barrier transcriptome. Physiol Genomics. 2005; 23: 235–45.

Scialli AR, Ang R, Breitmeyer J, Royal MA. Childhood asthma and use during pregnancy of acetaminophen. A critical review. Reprod Toxicol. 2010; 30: 508–19.

Sears MR, Greene JM, Willan AR, Wiecek EM, Taylor DR, Flannery EM, Cowan JO, Herbison GP, Silva PA, Poulton R. A longitudinal, population-based, cohort study of childhood asthma followed to adulthood. N Engl J Med. 2003; 349: 1414-22.

Sepp E, Julge K, Vasar M, Naaber P, Björkstén B, Mikelsaar M. Intestinal microflora of Estonian and Swedish infants. Acta Paediatr. 1997; 86: 956-61.

Sepp E, Naaber P, Voor T, Mikelsaar M, Björkstén B. Development of intestinal micro-flora during the first month of life in Estonian and Swedish children. Microbial Ecol Health. 2000; 12: 22–26.

Shaheen SO, Newson RB, Henderson AJ, Headley JE, Stratton FD, Jones RW, Strachan DP; ALSPAC Study Team. Prenatal paracetamol exposure and risk of asthma and elevated immunoglobulin E in childhood. Clin Exp Allergy. 2005; 35: 18-25.

Shaheen SO, Newson RB, Ring SM, Rose-Zerilli MJ, Holloway JW, Henderson AJ. Prenatal and infant acetaminophen exposure, antioxidant gene polymorphisms, and childhood asthma. J Allergy Clin Immunol. 2010; 126: 1141–8.

Sidbury R, Sullivan AF, Thadhani RI, Camargo CA Jr. Randomized controlled trial of vitamin D supplementation for winter-related atopic dermatitis in Boston: a pilot study. Br J Dermatol. 2008; 159: 245-7.

Sidoroff V, Hyvärinen MK, Piippo-Savolainen E, Korppi M. Overweight does not increase asthma risk but may decrease allergy risk at school age after infantile bronchiolitis. Acta Paediatr. 2011 Aug 8. [Epub ahead of print]

Sigurs N, Bjarnason R, Sigurbergsson F, Kjellman B, Björkstén B. Asthma and immunoglobulin E antibodies after respiratory syncytial virus bronchiolitis: a prospective cohort study with matched controls. Pediatrics. 1995; 95: 500-5.

Sigurs N, Bjarnason R, Sigurbergsson F, Kjellman B. Respiratory syncytial virus bronchiolitis in infancy is an important risk factor for asthma and allergy at age 7. Am J Respir Crit Care Med. 2000; 161: 1501-7.

Sigurs N, Gustafsson PM, Bjarnason R, Lundberg F, Schmidt S, Sigurbergsson F, Kjellman B. Severe respiratory syncytial virus bronchiolitis in infancy and asthma and allergy at age 13. Am J Respir Crit Care Med. 2005; 171: 137-41.

Sigurs N, Aljassim F, Kjellman B, Robinson PD, Sigurbergsson F, Bjarnason R, Gustafsson PM. Asthma and allergy patterns over 18 years after severe RSV bronchiolitis in the first year of life. Thorax. 2010; 65: 1045-52.

Simoes EA, Groothuis JR, Carbonell-Estrany X, Rieger CH, Mitchell I, Fredrick LM, Kimpen JL; Palivizumab Long-Term Respiratory Outcomes Study Group. Palivizumab prophylaxis, respiratory syncytial virus, and subsequent recurrent wheezing. J Pediatr. 2007; 151: 34-42

Skobeloff EM, Spivey WH, St Clair SS, Schoffstall JM. The influence of age and sex on asthma admissions. JAMA. 1992; 268: 3437-40.

Sly PD, Holt PG. Role of innate immunity in the development of allergy and asthma. Curr Opin Allergy Clin Immunol. 2011; 11: 127–31.

Sly PD. The early origins of asthma: who is really at risk? Curr Opin Allergy Clin Immunol. 2011; 11: 24–8.

Snijders BE, Thijs C, Dagnelie PC, Stelma FF, Mommers M, Kummeling I, Penders J, van Ree R, van den Brandt PA. Breast-feeding duration and infant atopic manifestations, by maternal allergic status, in the first 2 years of life (KOALA study). J Pediatr. 2007; 151: 347-51.

Solymar L, Aronsson P-H, Bake B, Bjure J. Nitrogen single breath test, flow volume curve and spirometry in healthy children, 7-18 years of age. Eur J Resp Dis. 1980; 61: 275–86.

Sonnappa S, Bastardo CM, Wade A, Saglani S, McKenzie SA, Bush A, Aurora P. Symptom-pattern phenotype and pulmonary function in preschool wheezers. J Allergy Clin Immunol. 2010; 126 :519-26.

Spergel JM. From atopic dermatitis to asthma: the atopic march. Ann Allergy Asthma Immunol. 2010; 105: 99-106.

Stein RT, Sherrill D, Morgan WJ, Holberg CJ, Halonen M, Taussig LM, Wright AL, Martinez FD. Respiratory syncytial virus in early life and risk of wheeze and allergy by age 13 years. Lancet. 1999(a); 354: 541-5.

Stein RT, Holberg CJ, Sherrill D, Wright AL, Morgan WJ, Taussig L, Martinez FD. Influence of parental smoking on respiratory symptoms during the first decade of life: the Tucson Children's Respiratory Study. Am J Epidemiol. 1999(b); 149: 1030-7.

Stern DA, Riedler J, Nowak D, Braun-Fahrlander C, Swoboda I, Balic N, Chen KW, Vrtala S, Grönlund H, van Hage M, Valenta R, Spitzauer S, Von Mutius E, Vercelli D. Exposure to a farming environment has allergen-specific protective effects on TH_2-dependent isotype switching in response to common inhalants. J Allergy Clin Immunol. 2007; 119: 351-8.

Strachan D, Butland B, Anderson R. Incidence and prognosis of asthma and wheezing illness from early childhood to age 33 in a national British cohort. BMJ. 1996; 312: 1195–9.

Subrata LS, Bizzintino J, Mamessier E, Bosco A, McKenna KL, Wikström ME, Goldblatt J, Sly PD, Hales BJ, Thomas WR, Laing IA, LeSouëf PN, Holt PG. Interactions between innate antiviral and atopic immunoinflammatory pathways precipitate and sustain asthma exacerbations in children. J Immunol. 2009; 183: 2793-800.

Swedish National Board of Health and Welfare (Socialstyrelsen). Smoking habits among pregnant women and parents to infants 2002. (Tobaksvanor bland gravida och spädbarnsföräldrar 2002): 2004.
Available from: URL: http://www.socialstyrelsen.se

Swedish National Board of Health and Welfare (Socialstyrelsen). Smoking habits among pregnant women and parents to infants 2005. (Tobaksvanor bland gravida och spädbarnsföräldrar 2005): 2007.
Available from: URL: http://www.socialstyrelsen.se

Tabak C, Wijga AH, de Meer G, Janssen NA, Brunekreef B, Smit HA. Diet and asthma in Dutch school children (ISAAC-2). Thorax. 2006; 61: 1048-53.

Tang ML, Kemp AS, Thorburn J, Hill DJ. Reduced interferon-gamma secretion in neonates and subsequent atopy. Lancet. 1994; 344: 983-5.

Tapiainen T, Dunder T, Möttönen M, Pokka T, Uhari M. Adolescents with asthma or atopic eczema have more febrile days in early childhood: a possible explanation for the connection between paracetamol and asthma? J Allergy Clin Immunol. 2010; 125: 751–2.

Taussig LM, Wright AL, Holberg CJ, Halonen M, Morgan WJ, Martinez FD. Tucson Children's Respiratory Study: 1980 to present. J Allergy Clin Immunol. 2003; 111: 661-75.

Thavagnanam S, Fleming J, Bromley A, Shields MD, Cardwell CR. A meta-analysis of the association between Caesarean section and childhood asthma. Clin Exp Allergy. 2008; 38: 629-33.

Thomas M, Custovic A, Woodcock A, Morris J, Simpson A, Murray CS. Atopic wheezing and early life antibiotic exposure: a nested case-control study. Pediatr Allergy Immunol. 2006; 17: 184-8.

Thomsen SF, Ulrik CS, Porsbjerg C, Backer V. Early life exposures and risk of atopy among Danish children. Allergy Asthma Proc. 2006; 27: 110-4.

Thomsen SF, van der Sluis S, Stensballe LG, Posthuma D, Skytthe A, Kyvik KO, Duffy DL, Backer V, Bisgaard H. Exploring the association between severe respiratory syncytial virus infection and asthma: a registry-based twin study. Am J Respir Crit Care Med. 2009; 179: 1091-7.

Toelle BG, Ng KK, Crisafulli D, Belousova EG, Almqvist C, Webb K, Tovey ER, Kemp AS, Mellis CM, Leeder SR, Marks GB; Childhood Asthma Prevention Team. Eight-year outcomes of the Childhood Asthma Prevention Study. J Allergy Clin Immunol. 2010; 126: 388-9.

Varner AE, Busse WW, Lemanske RF Jr. Hypothesis: decreased use of paediatric aspirin has contributed to the increasing prevalence of childhood asthma. Ann Allergy Asthma Immunol. 1998; 81: 347-51.

Vassallo MF, Camargo CA Jr. Potential mechanisms for the hypothesized link between sunshine, vitamin D, and food allergy in children. J Allergy Clin Immunol. 2010; 126: 217-22.

Vink JM, Willemsen G, Boomsma DI. Heritability of smoking initiation and nicotine dependence. Behav Genet. 2005; 35: 397-406.

Von Ehrenstein OS, Von Mutius E, Illi S, Baumann L, Böhm O, von Kries R. Reduced risk of hay fever and asthma among children of farmers. Clin Exp Allergy. 2000; 30: 187-93.

Von Mutius E, Schwartz J, Neas LM, Dockery D, Weiss ST. Relation of body mass index to asthma and atopy in children: the National Health and Nutrition Examination Study III. Thorax. 2001; 56: 835-8.

Watts KD, Goodman DM. Wheezing, bronchiolitis and bronchitis. In: Kliegman RM, Behrman RE, Jenson HB, Stanton BF, eds. Nelson Textbook of Pediatrics. 18th ed. Philadelphia: Saunders Elsevier. 2007: 1773-78.

Wegienka G, Johnson CC, Havstad S, Ownby DR, Nicholas C, Zoratti EM. Lifetime dog and cat exposure and dog- and cat-specific sensitization at age 18 years. Clin Exp Allergy. 2011; 41: 979-86.

Wennergren G, Hansson S, Engström I, Jodal U, Åmark M, Brolin I, Juto P. Characteristics and prognosis of hospital-treated obstructive bronchitis in children aged less than two years. Acta Paediatr. 1992; 81: 40-5.

Wennergren G, Åmark M, Åmark K, Oskarsdóttir S, Sten G, Redfors S. Wheezing bronchitis reinvestigated at the age of 10 years. Acta Paediatr. 1997; 86: 351-5.

Wennergren G, Ekerljung L, Alm B, Eriksson J, Lötvall J, Lundbäck B. Asthma in late adolescence--farm childhood is protective and the prevalence increase has levelled off. Pediatr Allergy Immunol. 2010; 21: 806-13.

West CE, Hammarström ML, Hernell O. Probiotics during weaning reduce the incidence of eczema. Pediatr Allergy Immunol. 2009; 20: 430-7.

Willers SM, Devereux G, Craig LC, McNeill G, Wijga AH, Abou El-Magd W, Turner SW, Helms PJ, Seaton A. Maternal food consumption during pregnancy and asthma, respiratory and atopic symptoms in 5-year-old children. Thorax. 2007; 62: 773-9.

Woods RK, Thien FC, Abramson MJ. Dietary marine fatty acids (fish oil) for asthma in adults and children. Cochrane Database Syst Rev. 2002;(3): CD001283. Review.

Wright AL, Holberg CJ, Taussig LM, Martinez FD. Factors influencing the relation of infant feeding to asthma and recurrent wheeze in childhood. Thorax. 2001; 56: 192-7.

Wu P, Dupont WD, Griffin MR, Carroll KN, Mitchel EF, Gebretsadik T, Hartert TV. Evidence of a causal role of winter virus infection during infancy in early childhood asthma. Am J Respir Crit Care Med. 2008; 178: 1123-9.

Young S, Sherrill DL, Arnott J, Diepeveen D, LeSouëf PN, Landau LI. Parental factors affecting respiratory function during the first year of life. Pediatr Pulmonol. 2000; 29: 331-40.

Zhang G, Rowe J, Kusel M, Bosco A, McKenna K, de Klerk N, Sly PD, Holt PG. Interleukin-10⁄interleukin-5 responses at birth predict risk for respiratory infections in children with atopic family history. Am J Respir Crit Care Med. 2009; 179: 205–11.

Zhang Z, Lai HJ, Roberg KA, Gangnon RE, Evans MD, Anderson EL, Pappas TE, Dasilva DF, Tisler CJ, Salazar LP, Gern JE, Lemanske RF Jr. Early childhood weight status in relation to asthma development in high-risk children. J Allergy Clin Immunol. 2010; 126: 1157-62.

Zutavern A, Brockow I, Schaaf B, von Berg A, Diez U, Borte M, Kraemer U, Herbarth O, Behrendt H, Wichmann HE, LISA Study Group. Timing of solid food introduction in relation to eczema, asthma, allergic rhinitis, and food and inhalant sensitization at the age of 6 years: results from the prospective birth cohort study LISA. Pediatrics. 2008; 121: e44–52.

Øien T, Storrø O, Johnsen R. Do early intake of fish and fish oil protect against eczema and doctor-diagnosed asthma at 2 years of age? A cohort study. J Epidemiol Community Health. 2010; 64: 124-9.