Early life factors and the long-term development of asthma

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"Das schönste Glück des denkenden Menschen ist, das Erforschliche erforscht zu haben und das Unerforschliche zu verehren."

*Johann Wolfgang von Goethe (1749-1832)*
ORIGINAL PUBLICATIONS

This thesis is based on the following four papers, which will be referred to in the text by their Roman numerals.

I. Preterm Birth and Inhaled Corticosteroid Use in 6- to 19-Year-Olds: A Swedish National Cohort Study
   Hartmut Vogt, Karolina Lindström, Lennart Bråbäck, Anders Hjern
   Pediatrics 2011;127:1052–1059

II. Asthma heredity, cord blood IgE and asthma-related symptoms and medication in adulthood: a long-term follow-up in a Swedish birth cohort
    Hartmut Vogt, Lennart Bråbäck, Olle Zetterström, Katalin Zara, Karin Fälth-Magnusson, Lennart Nilsson
    Submitted

III. Migration and asthma medication in international adoptees and immigrant families in Sweden
    Lennart Bråbäck, Hartmut Vogt, Anders Hjern
    Clinical & Experimental Allergy, 2011 (41), 1108–1115

IV. Does pertussis vaccination in infancy increase the risk of asthma medication in adolescents?
    Hartmut Vogt, Lennart Bråbäck, Anna-Maria Kling, Maria Grünewald, Lennart Nilsson
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ABSTRACT

Asthma, a huge burden on millions of individuals worldwide, is one of the most important public health issues in many countries. As genetic and environmental factors interact, asthma may be programmed very early in life, perhaps even in utero.

The aim of this thesis was to assess the impact of gestational age, cord blood immunoglobulin E (IgE), a family history of asthma, migration, and pertussis immunization in early life on the development of asthma in child and adult populations.

As a proxy for asthma disease, dispensed asthma medication was used as the main outcome variable based on data from the Swedish Prescribed Drug Register. Data from other national registers were used to control for confounders. Three of our studies were based on national cohorts, and one on a local birth cohort that was initiated in 1974–75.

Gestational age had an inverse dose-response relationship with dispensed asthma medication in 6– to 19-year-olds. Odds ratios for dispensed asthma medication increased with degree of prematurity compared with children born at term. Furthermore, asthma medication was more likely to be dispensed among children and adolescents born early term after 37–38 weeks’ gestation than among those at the same age who were born at term.

Elevated cord blood IgE and a family history of asthma in infancy were associated with a two- to threefold increased likelihood of dispensed asthma medication and self-reported allergen-induced respiratory symptoms at the age of 32–34 years, but the predictive power was poor.

Age at migration had an inverse dose-response relationship with dispensed asthma medication at the age of 6–25 years in adoptees and foreign-born children with foreign-born parents. International adoptees and children born in Sweden to foreign-born parents had three- to fourfold higher rates of dispensed asthma medication compared with foreign-born children who were raised by their foreign-born birth parents.

No association was found between pertussis immunization in early infancy and dispensed asthma medication in 15-year-olds. The type of vaccine or vaccine schedule did not affect the outcome.

Fetal life is a vulnerable period. This thesis strengthens the evidence that every week of gestation is important for lung maturation. Cord blood IgE, however, did not predict the risk of asthma in adults. Furthermore, the study of migrating populations demonstrated that environmental changes at any age during childhood may affect the risk of asthma. Another important public health message from this thesis is that vaccination against pertussis in early childhood can be considered safe with respect to the long-term development of asthma.
SVENSK SAMMANFATTNING

Astma har blivit allt vanligare och är idag ett stort folkhälsoproblem runt om i världen. En samverkan mellan arv och miljö bidrar till utvecklingen av astma och sjukdomen grundläggs ofta mycket tidigt i livet, inte sällan redan under fostertiden.

Syftet med denna avhandling var att undersöka hur graviditetslängd, immunoglobulin E (IgE) i navelsträngsblod, ärftlighet för astma, migration och vaccination mot kikhosta tidigt i livet kan påverka utvecklingen av astma bland barn och vuxna.


Förhöjt navelsträngs-IgE och ärftlighet för astma som barn visade ett samband med uttag av astmamedicin och självrapporterade allergeninducerade luftvägsymptom vid 32-34 års ålder, men varken IgE eller ärftlighet kunde förutsäga risken för astmamedicinering som vuxen på ett tillräckligt bra sätt.

Uttag av astmamedicin jämfördes mellan utlandsfödda adoptivbarn med svenska adoptivföräldrar, svenskfödda barn till invandrade föräldrar, utlandsfödda barn till utlandsfödda föräldrar och svenskfödda barn till svenskfödda föräldrar. Uttaget av astmamedicin minskade med stigande ålder vid invandring till Sverige. Åldern vid invandring spelade större roll än varifrån i världen man kom till Sverige.

Vaccination mot kikhosta i tidig barndom påverkade inte risken för astma vid 15 års ålder. Typ av kikhostevaccin eller tidpunkt för vaccination påverkade inte heller sambandet.

DEUTSCHE ZUSAMMENFASSUNG


 Diese Dissertation beschäftigt sich mit der Bedeutung verschiedener perinataler Faktoren für die Entwicklung von Asthma bei Kindern und Erwachsenen (Schwangerschaftsdauer, Nabelschnur-Immunglobulin E (IgE), positive Asthmafamilienanamnese, Migration, Keuchhustenimpfung im Kleinkindesalter). Die Ergebnisse dieser Untersuchungen basieren hauptsächlich auf Daten aus nationalen Registern. Informationen aus dem Schwedischen Medikamentenregister über eingelöste Rezepte für Asthmamedikamente dienten als Indikator für Asthma.

 Es zeigte sich ein umgekehrter Zusammenhang zwischen Schwangerschaftsdauer und dem Anteil eingelöster Rezepte bei Kindern und jungen Erwachsenen (6-19 Jahre). Das Risiko war für extrem Frühgeborene am größten, jedoch hatten selbst Kinder, die zwei bis drei Wochen vor dem Termin geboren waren, immer noch ein erhöhtes Risiko im Vergleich zu am Termin geborenen Kindern.


 Eine Impfung gegen Keuchhusten hatte keinen Einfluss auf das Asthmarisiko im Alter von 15 Jahren. Dabei spielte weder der verabreichte Impfstofftyp noch der Zeitpunkt der Impfung eine entscheidende Rolle.

ANTI Anti-inflammatory treatment
ANY Any asthma medication
aP Acellular pertussis
ATC Anatomical Therapeutic Chemical
BETA2 Beta₂-agonist
BMI Body Mass Index
BPD Bronchopulmonary dysplasia
CB-IgE Cord blood immunoglobulin E
CHC Child health center
CI Confidence interval
DAG Directed Acyclic Graph
DPT Diphtheria-pertussis-tetanus
ENRIECO Environmental Health Risks in European Birth Cohorts
GA²LEN Global Allergy and Asthma European Network
ICD International Classification of Disease
ICS Inhaled corticosteroids
IL Interleukin
INF Interferon
ISAAC International Study of Asthma and Allergy in Childhood
ITT Intention to treat
LF+ Positive likelihood ratio
LGA Large for gestational age
LTRA Leukotriene antagonist
OR Odds ratio
PIN Personal identification number
PP Per protocol
PPV Positive predictive value
QN Questionnaire
RSV Respiratory syncytial virus
SE Sensitivity
SGA Small for gestational age
SMBR Swedish Medical Birth Register
SP Specificity
SPDR Swedish Prescribed Drug Register
Th T helper lymphocyte
wP Whole cell pertussis
WHO World Health Organization
# TABLE OF CONTENTS

1 Introduction and background

1.1 Development in utero and fetal programming
  1.1.1 Fetal programming
  1.1.2 Lung development

1.2 Asthma
  1.2.1 Historical background
  1.2.2 Asthma today

1.3 Asthma prevalence

1.4 Birth cohorts

1.5 National registers
  1.5.1 Swedish Prescribed Drug Register
  1.5.2 Swedish Medical Birth Register
  1.5.3 Other registers used

1.6 Preterm birth

1.7 Asthma heredity

1.8 Immunoglobulin E

1.9 Migration

1.10 Immunization

2 Aims of the thesis

3 Material and methods

3.1 Study population
  3.1.1 Cohort I (Study I)
  3.1.2 Cohort II (Study II)
  3.1.3 Cohort III (Study III)
  3.1.4 Cohort IV (Study IV)

3.2 Study variables (Studies I-IV)
  3.2.1 Outcome variables
  3.2.2 Questionnaire data
  3.2.3 Confounding factors

3.3 Statistical analyses
  3.3.1 Study I
  3.3.2 Study II
  3.3.3 Study III
  3.3.4 Study IV

4 Ethical statements
1 INTRODUCTION AND BACKGROUND

1.1 Development in utero and fetal programming

1.1.1 Fetal programming
There is increasing evidence that an insult during early life, especially fetal life, contributes to the development of diseases as an adult [1-3]. The hypothesis describing the result of disturbances during fetal life, often referred to as ‘fetal programming’, has attracted growing interest during the past decade. Reports about the association of low birth weight with metabolic and cardiovascular diseases in adulthood have given rise to a discussion about the influence of early life factors on disease later in life [2, 4].

Variations to maternal nutrition have been of special interest in the study of adverse intrauterine environments [5, 6]. There is convincing evidence that an impaired diet during pregnancy can influence fetal growth and subsequent disease development in adulthood [7]. Low birth weight and intrauterine weight gain are associated with impaired postnatal organ function [8]. Low birth weight, however, is not necessarily an accurate reflection of fetal growth, and can be caused by different factors. Insults at different stages of the gestational process and subsequent prenatal growth can, depending on their magnitude, result either in normal or reduced birth weight [9]. Limited access to food and energy during prenatal life might lead to an inappropriate adaptive response by the fetus to a profuse nutritional postnatal environment, which in turn could lead to the development of disease in adulthood [10].

The placenta, as a natural interface between the maternal and fetal organisms, seems to play a key role in fetal programming. It functions as an immunological barrier and as a mediator of nutritional and hormonal factors [11].

Life in utero can already program for health and disease in adulthood [12, 13]. Different factors early in life seem also to influence the development of asthma [14]. There is a strong association between pre- and perinatal exposure and hospitalization for respiratory symptoms, including asthma, during early infancy [15]. An increased risk of asthma can remain all the way into adulthood [16], but few studies have investigated these associations.

1.1.2 Lung development
The development of the lung is a continuous process that starts early after conception and does not end until the age of 2–3 years, after which the lung continues to grow until body growth stops [17]. Disturbance of the developmental programming of the lung at any point during this development may have a crucial impact on its function and its susceptibility to harmful external factors [18, 19].

The embryonic phase of lung development is critical for cell differentiation and branching morphogenesis. The later stages occur during fetal and early postnatal
life when the lung is still growing and maturing structurally and functionally. During this stage of development, the lung is extra susceptible to adverse effects of environmental pollutants [17]. Lung growth and function are negatively influenced by environmental pollutants such as those from tobacco smoke [20]. Yet, fetal or early postnatal life is not the only period during which lung development can be disturbed; even adolescence might be a vulnerable period as the lung is in its final phase of rapid growth and maturation [21]. Starting to smoke during adolescence has been shown to result in impaired functional pulmonary development [22]. The degree of impaired lung function depends on when the harmful impact during lung development occurs [23].

Maternal smoking has been identified as a risk factor for asthma and wheezing in infants and young children. In a recent review, exposure to maternal and passive smoking, both pre- and postnatal, was shown to increase the incidence of wheezing and asthma in small children and teenagers up to 18 years of age by at least 20% [24]. Some studies even describe an effect of prenatal tobacco exposure independent of postnatal exposure [25].

Lung development and its function seems not only to be susceptible to direct adverse effects of environmental pollutants, but is also influenced by impaired fetal and postnatal growth [26]. Restricted fetal growth has been shown to have adverse functional effects on lung development that can persist into postnatal life. There are multiple causes of impaired growth (e.g. maternal malnutrition, placenta insufficiency), which leads to different types of functional and structural pulmonary impairments depending on when during gestation growth retardation occurs [17]. Structural changes include smaller numbers of enlarged alveoli with thicker septal walls and basement membranes. The structural abnormalities and impaired lung function seen soon after birth persist or even progress with age and can influence lung aging [27].

Exposure to intrauterine infections, e.g. chorioamnionitis, can lead to premature labor that results in premature birth. Levels of proinflammatory cytokines in the amniotic environment are elevated, which could be the cause of premature labor [28]. Animal models have demonstrated the influence of an intrauterine proinflammatory environment on lung development [29, 30], and it is reasonable to believe that a similar effect can occur in human beings.

As proinflammatory factors influence lung development in utero, frequent infections of the lower respiratory tract may influence postnatal lung development. Viral infections of the lower respiratory tract in infants show an association with asthma, and various viral agents have been identified [31-33]. The infections occur most frequently during infancy, the postnatal phase of alveolarization [34].
1.2 Asthma

1.2.1 Historical background
Asthma is believed to have been recognized as a specific disease in ancient Egypt, and perhaps even earlier. A German Egyptologist, Georg Ebers, discovered a medical papyrus (ca. 1550 BC) in the 1870s that contained ancient prescriptions. One of the over 700 remedies described was a “mixture of herbs heated on a brick so that the sufferer could inhale their fumes” [35].

The term ‘asthma’ comes from the Greek verb ‘aazein’, meaning to pant or to exhale with open mouth, and is described for the first time in a Greek epic poem, the Iliad. The earliest text containing the word ‘asthma’ as a medical term is believed to be the Corpus Hippocratum (~400 BC). It remains uncertain, however, whether Hippocrates meant asthma as a clinical entity or as merely a symptom. The best clinical description of asthma in later antiquity is offered by the master clinician, Aretaeus of Cappadocia (1st century AD). The numerous mentions of ‘asthma’ in the extensive writings of Galen (130–200 AD) appear to be in general agreement with the Hippocratic texts and to some extent with the statements of Aretaeus [36].

1.2.2 Asthma today
Definitions for asthma are many in the literature, but are nowadays mostly based on the typical symptoms seen and the known pathophysiological changes in the airways.

Similar to the WHO definition [33], the Global Initiative for Asthma (GINA), a collaborating network of health care professionals and public health officials around the world to reduce asthma prevalence, describes asthma as a chronic inflammatory disorder of the airways, in which many cells and cellular elements are involved. Chronic inflammation is associated with airway hyper-responsiveness that leads to recurrent episodes of wheezing, breathlessness, chest tightness and coughing with variable, often reversible, airflow limitation [37].
Asthma is a more heterogeneous than uniform disease, with different phenotypes and probably different causes. They can be defined by their unique interaction between genetic and environmental factors [32]. Asthma phenotypes include allergen-induced asthma as well as non-allergic asthma, of which the latter used to be more common in adults [31]. Non-allergic, also called intrinsic, non-atopic or adult-onset, asthma is characterized by female predominance, increased symptom severity and later debut in life. An association with smoking and chronic rhinosinusitis has been reported [19, 38]. The presence of allergy, or sensitization, is routinely evaluated by skin prick test and/or immunoglobulin E (IgE) immunoassays. Allergic sensitization is rather common and has been demonstrated in about half of the population in the USA, with a positive skin prick test to one or more common allergens [39]. A significant proportion of patients with non-allergic asthma probably have coincidentally positive skin prick tests and allergic rhinitis, which can lead to misclassification of their asthma symptoms. Increased IgE synthesis may be a risk factor for asthma independent of allergen-specific IgE-mediated allergic responses [32]. Non-allergic asthma patients with negative skin prick tests and no heredity of allergy but with elevated levels of total IgE have been found to have more severe asthma and more impaired lung function than those with normal IgE [40]. It has been proposed that total IgE concentration reflects the intensity of a Th2-response (often referred to as an “allergic immune response”) and asthma severity in these patients, but is not an expression of allergic asthma as specific IgE is missing [32].

In children, asthma phenotypes include transient infant wheezing, non-atopic wheezing, wheezing mediated by IgE, and so-called late-onset childhood asthma [31]. Transient infant wheezing occurs mainly during the first years of life and is not associated with atopy or reduced lung function at school age [41]. Non-atopic wheezing is often seen among children who continue to wheeze beyond the first years of life and whose symptoms often started in conjunction with early viral infections of the lower respiratory tract. Viral infections usually continue to trigger wheezing in these children [31]. Another group of children continue to wheeze even when older. This persistent wheezing type is often associated with atopy (IgE-mediated), bronchial hyper-responsiveness and reduced lung function [42]. Late-onset childhood asthma is another subtype of pediatric asthma that has been described as occurring during or after puberty. It affects mainly women and has a low remission rate [43].

1.3 Asthma prevalence

The prevalence of asthma and allergic diseases among children and young adults has steadily increased during the second half of the 19th century [44-46], above all in industrialized countries [47-50]. In countries like the USA or several Western European countries, however, the increase has not continued at the same rate as before [47, 51-53]. Global initiatives like the International Study of Asthma and
Allergies in Childhood (ISAAC) [54] have shown the different prevalence rates from a worldwide perspective [55-57] (Figure 1).

As members of the ISAAC Phase Three Study Group, we have investigated the change in asthma prevalence in the municipality of Linköping, Sweden, in 2002 compared with the identical Phase One investigation in 1994. In contrast to our Nordic neighbors in Finland we found a significant decrease in 12-month wheezing, from 11.2% to 9.7%, between ISAAC Phase One and ISAAC Phase Three among teenagers 13–14 years of age. There was no change in “wheeze ever” between Phases One and Three, with prevalence rates of 18.6% and 18.9%, respectively, but the number of teenagers with a diagnosis of asthma increased from 10.0% to 12.0% during this eight-year period [56]. Equally, a repeated cross-sectional survey in 1985, 1995 and 2005 among school children in Northern Sweden showed that the increase in asthmatic symptoms in school children had peaked. Fewer children had questionnaire-reported wheezing and other severe symptoms in 2005 compared with the previous investigations. On the other hand, physician-diagnosed asthma had increased compared with the same investigations conducted 10 and 20 years before [58]. In contrast, several countries in Eastern Europe where prevalence previously was low showed a dramatic increase in asthma prevalence [57], whereas other investigators in Europe have seen more stable prevalence rates in school children [59]. Similar to the effect we have seen in Swedish children in Linköping and in

▲ Figure 1: Time trends of asthma symptoms in ISAAC Phase Three.
investigations in Northern Sweden [58], studies of asthma in adults in Sweden have also shown that there is no ongoing increase in asthma prevalence. Lötvall and colleagues found a prevalence of physician-diagnosed asthma of 8.3% in adults 16–75 years of age in West Sweden, and the prevalence of respiratory symptoms was found to be lower compared with previous studies [60]. In Northern Sweden, a comparison of two questionnaire-based surveys of respiratory symptoms among young adults from 1990 and 2008 showed a decrease in the 12-month prevalence of wheezing from 20% to 16% and an unchanged rate of other obstructive airway symptoms common in asthma during this period [61]. This stagnation might partly be caused by an increased awareness of asthma among Swedish physicians during recent years, which has led to increased prescription of asthma medication, especially of inhaled corticosteroids (ICS) [62]. With better treatment of asthma symptoms, their prevalence decreases.

1.4 Birth cohorts

Birth cohorts are commonly used in the investigation of causal factors and the development of asthma and allergies over time. Many birth cohorts have been established during recent decades, especially in Europe but also in other regions of the world. One of the first birth cohorts to study allergic disease in Europe was initiated in Denmark in 1985 [63], followed in the late 1980s by the Isle of Wight Birth Cohort in the United Kingdom [64]. Thereafter many other studies started during the 1990s [65-70] and after the turn of the millennium [71].

A prospective birth cohort was initiated as early as 1973 in Linköping, Sweden, with the emphasis on predictors of asthma and allergic disease [72]. Initially, 1,701 children were recruited with a follow-up rate of 97% at the age of 10–11 years [73]. Only a few birth cohorts started before the early 1970s, e.g. the British 1958 Birth Cohort, which has been followed up at different points in time up to the age of 42 years, but which originally had an entirely different focus than the investigation of the development of asthma [74].

Efforts made during recent years to build up networks between different birth cohorts have resulted in the GA²LEN initiative [75] and ENRIECO project [76], for example. Common databases with pooled data were established to allow meta-analysis and develop recommendations for future data collecting.

1.5 National Registers

Most data used in the analyses in this thesis have been retrieved from national registers. We mainly used data from the Swedish Prescribed Drug Register and the Swedish Medical Birth Register.
1.5.1 Swedish Prescribed Drug Register
This register contains data about all prescribed and dispensed drugs for the entire Swedish population from 1999 and onward. Since July 1, 2005 all data in this register are linked on an individual basis by a personal identifier, the personal identification number (PIN), a 10-digit identification code all Swedish residents are assigned at birth. The register also contains the patient’s sex, age and registered residence and is updated monthly [77]. This quite new register offers valuable data on the dispensation of different drugs and provides a useful tool for studying patterns of drug utilization [78].

1.5.2 Swedish Medical Birth Register
This register was established in 1973 and is maintained by the Swedish National Board of Health and Welfare. It contains information on ante- and perinatal factors for almost every newborn child in Sweden. The content and methods of data collection have changed since its establishment in 1973, but the register’s basic structure has remained the same. Between 1973 and 1982 so-called “Medical Birth Reports” were the basis of information for the register, but since 1982 the three records of primary interest—the basic antenatal care record of the mother, the delivery record, and the record for the pediatric examination of the newborn infant—have been sent to the National Board of Health for data registry. Most of the mothers were identified by their unique PIN. Their infants were linked to the Medical Birth Register using the PIN from The Birth Register at Statistics Sweden [79].

The register’s quality has been evaluated at different points in time. Cnattingius et al. studied the register on two different occasions, before and after the change in 1982. Problems with the validity of diagnosis and the risk of misclassification of rare conditions were pointed out. For so-called “hard” data such as perinatal survival or birth weight distribution, however, the register contains data of fairly good quality [80]. The National Board of Health and Welfare gave an overview on the quality of the register in 2003 and found that only an acceptable 1–2% of the records are missing for most of the years [79].

1.5.3 Other registers used
The Swedish Hospital Discharge Register contains data about all hospital discharges in Sweden since 1964. It is mandatory for all public caregivers to report to the register, and since 1987 reporting covers the whole country. For 99% of all registered hospital stays, the register contains one or more diagnoses based on the International Classification of Disease (ICD). Except for the loss of some data, mainly concerning psychiatric diagnoses, the data quality of the register has been assessed as good [81].

The Swedish Register of Education was established in 1985 and contains information on the highest completed education for all Swedish citizens between 16 and 74 years of age. Data are reported continuously to Statistics Sweden, which updates the register annually [82].
The Total Enumeration Survey, maintained by Statistics Sweden, contains information for all Swedish citizens on income, pension, welfare and disability grants, as well as income from sickness assistance and taxes paid [83].

The Total Population Register was established in 1968 as a computerized register containing data which historically had been collected in parish registers and church books. It contains information about name, place of residence, sex, age, marital status, place of birth, citizenship, and immigration and kinship status. Data are updated continuously. This register is maintained by Statistics Sweden [84].

The Swedish Multi-Generation Register, also maintained by Statistics Sweden, is part of the Total Population Register. Data on family relationships for all Swedish citizens are based on information about people registered in Sweden since 1961 and those who were born in 1932 and later [85].

1.6 Preterm birth

Preterm birth is defined as birth before 37 completed weeks of gestation (less than 259 days). Premature birth, especially in infants with very low birth weight (VLBW), accounts for numerous short-term complications such as respiratory distress, retinopathy of prematurity, sepsis, and bronchopulmonary dysplasia (BPD) [86]. The risk of neonatal morbidity decreases with increasing gestational age, but still exists even for infants born not extremely premature after 30–34 weeks of gestation [87]. Premature infants are born with incompletely developed lungs, fewer alveoli and impaired lung function [88]. This contributes to an increased risk of asthma and bronchitis, especially during early infancy [89]. Bronchitis is particularly common among children born prematurely who developed BPD. A study from Alaska showed an inverse association between gestational age and the risk of asthma in children up to 10 years of age. The highest risk was seen in children born prior to week 32 and remained even after controlling for birth weight [90].

The relation between prematurity and respiratory morbidity, including asthma, is rather complex. Many factors can lead to premature birth, which increases the risk of asthma. Apart from influencing the risk of prematurity, prenatal factors can increase the risk of asthma, independent of prematurity itself. Finally, prenatal factors might increase the risk of asthma via prematurity, and remaining exposure post-partum, e.g. tobacco smoke, can further increase the risk of asthma [89].

Chorioamnionitis is a well-known cause of preterm delivery but has also been described as an independent risk factor for wheezing [91] and for physician-diagnosed asthma [92]. The risk of pulmonary diseases, including asthma, is probably higher if prematurity is caused by chorioamnionitis. Many studies that investigated the possible association between prematurity and asthma have not included chorioamnionitis in their analysis, which might explain the different results [93].
Prematurely born children run an increased risk of severe respiratory syncytial virus (RSV) infections. Bronchiolitis caused by RSV infection is a risk factor for asthma. Exposure to tobacco smoke contributes to prematurity and low birth weight, but also increases susceptibility to RSV infection [94]. The relation between RSV infection and an increased risk of asthma attenuates by time. More recently, several studies have also pointed out human rhinovirus as a strong predictor of asthma in schoolchildren [95].

Changes in neonatal care might influence the risk of respiratory morbidity, including asthma. It is plausible that the introduction of surfactant treatment has led to a decreased prevalence of asthma among children born very prematurely [96].

Signs of chronic lung disease among prematurely born children may persist until adulthood and is especially common among children developing BPD [97]. It has been shown that bronchial hyper-reactivity and impaired lung function can persist until the teen years in infants born before gestational week 28 [98, 99]. Many studies that have investigated the possible association between prematurity and respiratory problems have focused on early infancy. Few epidemiological studies have concentrated on the importance of prematurity as a risk factor for asthma and bronchitis among teenagers [99, 100] and young adults [16, 101, 102]—and results have been ambiguous. A lack of control for social status, exposure to tobacco smoke, and respiratory tract infections might have contributed to the different results in these studies.

### 1.7 Asthma heredity

The accumulation of asthma disease in certain families has led to intensive research about the heredity of this disease and its predictive power in identifying individuals at risk. In the late 1970s, Kjellman demonstrated a family history of atopic disease in almost a third of 1,472 school beginners in the municipality of Linköping, Sweden, evaluated by questionnaires [103]. Since then, many studies have investigated the association between family history of asthma or atopy and childhood asthma. Despite methodological differences between studies, family history of asthma has been identified as a strong predictor of asthma risk [104]. Few studies have investigated the potential association of asthma heredity and asthma in adults.

Although it is unclear how asthma is inherited, both maternal and paternal influence seems to be important in the process [105, 106]. However, the fetus shares an environment with the mother. This might explain why maternal asthma tends to be more important than paternal asthma [106]. Imprinting, an epigenetic mechanism, where gene expression is determined by an imbalance between the maternal and the paternal allele, has been discussed as the reason for a maternal dominant inheritance of asthma [107, 108]. Twin studies have shown a possible asthma heritability of up to 60–70%, and heredity seems to be extra important for disease severity [109]. The large variability in the asthma phenotype might be an expression of the variability in genetic factors that determine the development of asthma. Gene-environmental
interaction has been a key concept in the elucidation of genetic susceptibility and environmental influences that finally lead to clinical disease [17, 109, 110].

Although a family history of asthma has been shown to be strongly associated with asthma in childhood, it fails to identify the majority of children at risk [104].

1.8 Immunoglobulin E

Immunoglobulin E (IgE) is one of five isotypes of human immunoglobulins and is produced by plasma cells. Plasma cells normally produce IgM, and it requires different mediators (e.g. interleukin-4 and -13) and cell surface interactions between B and T cells to induce synthesis of another immunoglobulin isotype [111]. IgE is not transferred via the placenta, and cord blood IgE (CB-IgE) levels are believed to represent the infant’s baseline production at birth. Contamination with maternal blood, however, might influence the validity of CB-IgE levels as a measurement of the infant’s atopic predisposition [112]. This has to be taken into account in studies investigating the role of CB-IgE for the development of atopic diseases. Total IgE levels increase from birth and peak in teenage years [113]. The levels of total IgE in human milk are insignificantly low [114], but an association between levels of IgE in mothers and their infants has been reported [115].

CB-IgE has previously been studied as a predictor of asthma and other allergic diseases, with conflicting results [116-121]. A Danish study showed no correlation between high levels of CB-IgE and allergic disease at 18 months of age [118]. In the same birth cohort, a significantly greater number of children with elevated cord blood IgE levels developed allergic disease before 5 years of age [122]. In an American birth cohort, elevated CB-IgE was identified as a risk factor for allergic sensitization at ages 4 and 10 years, and asthma at 10 years of age [120]. Pesonen et al investigated a birth cohort of 200 consecutively born children and concluded that an elevated CB-IgE level predicts subsequent atopy up to the age of 20 years [119]. Few studies have investigated the potential correlation between CB-IgE and atopic diseases until adulthood. Moreover, the number of individuals in the studies that have been conducted was often quite low [119], and follow-up rates were poor [121]. Many studies have concentrated on high-risk infants only [116].

1.9 Migration

The global variation in asthma prevalence has raised many questions about the causes of the differences observed [56, 57, 123, 124]. Rapid changes in asthma prevalence over a short period of time [125] and geographical differences in asthma prevalence within the same ethnic group [126, 127] can hardly be explained by genetics alone. To disentangle the genetic and environmental causes, studies on migrating populations can act as a natural experiment, as these populations
experience faster changes in lifestyle and environment than a more homogenous population does [128]. Studies often deal with population groups emigrating from low-prevalence, poorly developed regions and countries to an affluent region with high disease prevalence [129-131]. An increased prevalence of asthma has been linked to urbanization, affluence and changes in diet and microbial contacts [132]. Farm studies have delivered evidence that protective exposures might already act in utero [133].

Migrants adapt to a different extent to lifestyles in their new environment, and protective factors related to exposures in their birth environment weaken with duration of residence in their new environment. Migrant studies provide an opportunity to investigate the influence of early life conditions such as micro- and macro-environments, as recently pointed out by Kuehni in an editorial [134].

Since 1970 most immigrants in Sweden are refugees or relatives of refugees. Foreign-born adoptees differ in several aspects from other immigrants. Many children are adopted from orphanages into mainly higher social class families, and the children adapt rapidly to the Swedish lifestyle of their host family.

1.10 Immunization

The 'hygiene hypothesis', which was first proposed by Strachan in 1989, suggests that a lack of infections during early infancy might increase the risk of asthma and allergic diseases because of lower exposure to microorganisms [135]. Different causal explanations have been discussed, all leading to a shift in immune regulation and response that results in an increased susceptibility to asthma and allergic diseases [136].

As a consequence of the 'hygiene hypothesis' and the absence of certain infectious diseases, common childhood vaccinations have been suspected as a possible cause of the increase in asthma and allergic diseases in affluent countries [137]. Several investigations have focused on the role of pertussis or combined diphtheria-pertussis-tetanus (DPT) immunization, with contradictory results. Earlier studies have found an increased risk of asthma and atopic disease in certain age groups after pertussis or DTP vaccination [137-140]. Other studies even proposed a protective effect against atopic disease among immunized children [141-144]. More recent studies did not find any association between pertussis vaccination and the risk of asthma [145-147], including the only randomized controlled trial published so far [148]. In most of these previous studies a whole cell pertussis (wP) vaccine was used. Acellular pertussis (aP) toxin has been shown to induce a strong specific IgE response, above all after a booster when originally vaccinated with an aP vaccine [149, 150]. An association between total IgE and specific IgE to pertussis toxin has been described for wP vaccine especially in children with atopy [151], which is why a connection between pertussis vaccine and the development of asthma and allergies might be suspected.
Although there is no convincing evidence for the association between early infancy immunization against pertussis and asthma or atopic disease later in childhood, it seems too early to finally discard the possible causal or contributory role of vaccines in the development of allergic diseases. Due to methodological incongruence between the different studies, further investigations on a larger scale that are well-controlled for possible bias have been requested. This seems particularly important as parental fear concerning vaccine safety and the risk of developing other diseases has been recognized as a major obstacle to the immunization of infants.
2 AIMS OF THE THESIS

The general aim of this study was to investigate different pre- and postnatal factors that might influence the long-term development of asthma. Dispensation of asthma medication was used as a proxy for asthma.

The specific aims of each individual paper were:

1. To examine the potential effect of gestational age in general and the degree of prematurity on dispensation of inhaled corticosteroids as a proxy for asthma in children aged 6–19 years (Paper I).

2. To assess whether CB-IgE levels and a family history of asthma in early childhood were associated with, and could predict, allergy-related respiratory symptoms and dispensation of asthma medication at 32–34 years of age (Paper II).

3. To sort out the independent effects of population of origin and age at immigration/being born in Sweden on dispensation of asthma medication at the age of 6–25 years in international adoptees, raised by Swedish-born parents, and children raised by their foreign-born birth parents (Paper III).

4. To determine whether pertussis immunization in infancy contributes to a higher rate of dispensed asthma medication at the age of 15 (Paper IV).
3 MATERIAL AND METHODS

3.1 Study population

3.1.1 Cohort I (Study I)
The study population was created from the 1,142,806 children born in Sweden during 1987–2000 according to the Swedish Medical Birth Register. All infants fulfilled the criteria of being offspring of two Swedish parents, according to the Swedish Multi-Generation Register, and being residents in Sweden on December 31, 2005 according to the Register of the Total Population. Offspring of foreign-born parents were excluded because of the influence of ethnicity on asthma prevalence in Sweden [128]. From this population we excluded 33,183 children who had at least one malformation (ICD-10 Q00–Q99) reported at birth by the attending pediatrician. However, minor malformations (undescended testicles, pre-auricular appendage and congenital nevus) and hip dislocation were considered insignificant and did not lead to exclusion from the study. Moreover, 8,797 children with a registered birth weight for gestational age above 3 SD or less than -6 SD, according to the growth chart developed by Maršál et al [158], were excluded as probable coding errors [159], leaving 1,100,826 individuals to be included in the study population.

3.1.2 Cohort II (Study II)
The study is based on a follow-up of a Swedish asthma and allergy birth cohort containing all infants consecutively born from December 1, 1974 to December 31, 1975 at Linköping County Hospital. Of 1,884 infants born during that period, 1,701 were able to be enrolled in the original study. Development of asthma and allergic disease in relation to both CB-IgE and family history of asthma or atopy, separately and in combination, was investigated on different occasions [73, 160-162]. Information about the asthma heredity status of the study population, asthma diagnosis at the age of 6–7 years and at 10–11 years was taken from the original paper charts. Information about CB-IgE values was extracted from the original magnetic tape the data were stored on (Figure 2).
In 2007 we conducted a questionnaire-based follow-up of the original study population to investigate the current asthmatic and allergic status of these now adult individuals. Almost all former study participants could be identified by their PIN. Forty-five individuals had to be excluded as either no valid address could be located or their PIN turned out to be incorrect. A total of 1,238 (72.8%) answered the postal questionnaire. An additional 11 individuals had to be excluded for various reasons, leaving 1,227 individuals (72.1%).

In a second phase the same study population was linked to the Swedish Prescribed Drug Register and the Swedish Medical Birth Register. The registers linked to 1,661 (97.6%) individuals at the age of 32–34 years (Figure 3).

3.1.3 Cohort III (Study III)
All individuals born during 1980–2000, who were alive and registered as residents in Sweden on December 31, 2005 were identified in the Register of the Total Population. The biological and/or adoptive parents of these individuals were identified in the Multi-Generation Register.

Information about region of birth, date of immigration, sex and year of birth in the Total Population Register was linked to the study subjects and their parents. Based on this information we identified three categories of residents with a non-Swedish background: (1) international adoptees; (2) residents born outside of Sweden who immigrated to Sweden with their parents; and (3) residents born in Sweden with two foreign-born parents. We selected four regions of origin where there were considerable numbers of children in all three categories: Eastern Europe, East Asia, South Asia and Latin America. Eastern Europe included the former Eastern Bloc countries, excluding Yugoslavia; Latin America included all countries in the Americas south of the USA; South Asia included India, Pakistan, Sri Lanka and Bangladesh. East Asia included all Asian countries east of the Indian peninsula.

This population included 24,252 international adoptees with two Swedish-born adoptive parents, 47,986 foreign-born, and 40,971 Swedish-born with two foreign-born parents. To this population we added 1,770,092 Swedish-born residents with two Swedish-born parents as a comparison group. The age of the study subjects ranged from 6 to 20 years.

3.1.4 Cohort IV (Study IV)
Our study population is based on more than 80,000 former participants in an efficacy trial of aP vaccines that has previously been described in detail [163, 164]. As a control group we included 98,475 children born during a 6-month period before and after the vaccination trial and who were not offered pertussis immunization as there had not been general pertussis vaccination in Sweden for 14 years at that time. Another 21,485 children who were born during the vaccination trial but who were not vaccinated for several reasons were included as a control group in certain analyses (Figure 4).
Figure 3: Flow chart of the study population in Study II. 
CB-IgE = Cord blood immunoglobulin E; PIN = Personal identification number; QN = Questionnaire
In brief, infants born between June 1, 1993 and May 31, 1994 in 22 out of 24 Swedish counties, except the city of Gothenburg and 10 surrounding municipalities were eligible for enrolment in the initial trial, as were infants born between June 1, 1993 and June 30, 1994, in Malmöhus county, Sweden (Appendix 11.2). Infants were enrolled in the study at 1–3 weeks of age if they were residing within the defined study areas, were registered at the child health center (CHC), were examined by a CHC physician/study physician at 6–8 weeks of age, and if parental consent was obtained. Children were excluded because of parental language difficulties or other circumstances that could interfere with communication and follow-up; if they planned to move out of the study area within one year; if they had certain known or suspected chronic diseases according to the following contraindications—serious chronic illness (with signs of cardiac or renal failure or failure to thrive), progressive neurological disease, uncontrolled epilepsy/infantile spasms, treatment with gammaglobulin, immunosuppression due to treatment or disease, HIV, previous culture-confirmed pertussis; or if the first vaccine dose was given later than 92 days post-partum.

Infants were vaccinated with a series of three intramuscular injections with DTP vaccines at ages 3, 5 and 12 months according to the Swedish vaccination schedule.

![Figure 4: Timeline of the study population in Study IV with respect to time of birth, vaccination status and dispensed asthma medication at 15 years of age. 134 individuals were registered as vaccinated outside the vaccination period.](image-url)
for DT at that time. In two counties the trial DTP vaccines were given at the age of 2, 4 and 6 months. As different vaccines were compared in the initial study, infants enrolled in the study were vaccinated with a two-, three-, five-component acellular DTaP vaccine or a whole-cell DTwP vaccine. The four different vaccine groups were roughly the same.

The cohort was linked to the Swedish Medical Birth Registers and the Swedish Prescribed Drug Register using the PIN. Information concerning mother’s country of birth, parity, maternal age at childbirth, maternal body mass index and smoking habits in early pregnancy, mode of delivery, maternal diseases and pregnancy complications, malformations, gestational age and birth weight were obtained from the Swedish Medical Birth Register.

We excluded 1,331 individuals from the analyses who were deceased at the time the register data were retrieved. We also excluded 4,998 children with at least one malformation reported at birth (ICD-9 740–759). However, as minor malformations (undescended testicles, pre-auricular appendage, congenital nevus and hip dislocation) were considered insignificant, children with these conditions were included. Another 1,042 individuals were excluded for different reasons, leaving a total of 199,665 individuals at the age of 15 years for the analyses (Figure 5).

\[\text{Figure 5: Flow chart of the study population in Study IV, children born January 1, 1993 to December 31, 1994 in the study area divided in non-vaccinated and vaccinated. SMBR = Swedish Medical Birth Register}\]
Table 1: List of ATC codes used in Studies I-IV for definition of the outcome variable dispensed asthma medication. ANY = any asthma medication; ICS = inhaled corticosteroids; ANTI = anti-inflammatory treatment.

<table>
<thead>
<tr>
<th>ATC code</th>
<th>Substance</th>
<th>Study I</th>
<th>Study II</th>
<th>Study III</th>
<th>Study IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>R03AC02</td>
<td>Salbutamol</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>R03AC03</td>
<td>Terbutaline</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>R03AC12</td>
<td>Salmeterol</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>R03AC13</td>
<td>Formoterol</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>R03AK04</td>
<td>Salbutamol + others</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>R03AK06</td>
<td>Salmeterol + others</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>R03AK07</td>
<td>Formoterol + others</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>R03BA01</td>
<td>Beclometasone</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>R03BA02</td>
<td>Budesonide</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>R03BA05</td>
<td>Fluticasone</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>R03BA07</td>
<td>Mometasone furoate</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>R03BA08</td>
<td>Ciclesonide</td>
<td></td>
<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>R03BC01</td>
<td>Sodium cromoglicate</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R03CC02</td>
<td>Salbutamol</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R03CC03</td>
<td>Terbutaline</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R03DC03</td>
<td>Montelukast</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
</tbody>
</table>

Number of dispensed prescriptions: ≥1/year, ≥2/3 years, ≥1/year, ≥1/year

3.2 Study variables (Studies I–IV)

3.2.1 Outcome variables
Dispensation of asthma medication was used as a proxy for asthma diagnosis in all four studies. The information on asthma medication was based on data from the Swedish Prescribed Drug Register. All study individuals were linked to the register using their PIN.

Data about the dispensation of anti-asthmatic drugs were recorded according to their corresponding Anatomical Therapeutical Chemical (ATC) code. Codes that were used in the different studies included selective β₂-agonists (R03AC), inhaled corticosteroids (ICS; R03BA), combinations of β₂-agonists and other drugs for obstructive airway disease (R03AK04 through R03AK07) and leukotriene antagonists (LTRA; R03DC03).

Different drug variables were created (any asthma medication, inhaled corticosteroids, anti-inflammatory treatment) using different combinations of ATC codes from the register. The combination of variables differed slightly between the four studies and is displayed in Table 1.

3.2.2 Questionnaire data
In the follow-up study (Paper II) former participants in a birth cohort were asked to answer a postal questionnaire with a total of 20 questions regarding different respiratory symptoms as well as nose-eye symptoms and skin symptoms (Appendix 1). Additionally, the questionnaire contained questions about current and former smoking habits, current and former occupation, dietary and physical habits and residential status ("Having lived on a farm") during the first five years of life. A positive answer to the question “Do you become breathless, start to wheeze or cough due to contact with pollen from trees or grass?” or “… due to contact with furred pets?” was used as a marker of respiratory symptoms resulting from contact with pollen or furred pets.

3.2.3 Confounding factors
Data from different national registers were used to control for the potential influence of different confounding variables on our outcome variables, asthma medication (Studies I–IV) and reported respiratory symptoms (Study II). In Study II additional data from the postal questionnaires were available for the cohort (current smoking, residential status during the first five years of life and fish diet), which were used in the analysis. Table 2 displays the different variables used in the corresponding studies and their source.
<table>
<thead>
<tr>
<th>VARIABLES</th>
<th>SOURCE OF DATA</th>
<th>DEFINITIONS/DESCRIPTIONS</th>
<th>STUDY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational Age</td>
<td>Swedish Medical Birth Register</td>
<td>Mainly (70.1%) according to ultrasound measurements in early pregnancy (weeks 10-18); remaining: reported last menstrual period (II = reported last menstrual period)</td>
<td>I, II, IV</td>
</tr>
<tr>
<td>SGA</td>
<td>Swedish Medical Birth Register</td>
<td>Study I: &lt;-2 SD according to scale created by Maršál et al (based on intrauterine ultrasound measurements) [158] Study II, IV: dichotomized; calculated using sex, birth weight, gestational age (weeks, days) similar to Study I</td>
<td>I, II, IV</td>
</tr>
<tr>
<td>LGA</td>
<td>Swedish Medical Birth Register</td>
<td>Dichotomized; calculated using sex, birth weight, gestational age (weeks, days) similar to Study I</td>
<td>II, IV</td>
</tr>
<tr>
<td>Cesarean delivery</td>
<td>Swedish Medical Birth Register</td>
<td>Dichotomized (yes/no)</td>
<td>I, II, IV</td>
</tr>
<tr>
<td>Asphyxia</td>
<td>Swedish Medical Birth Register</td>
<td>Apgar score ≤7 at 5 minutes</td>
<td>I, II, IV</td>
</tr>
<tr>
<td>Multiple birth</td>
<td>Swedish Medical Birth Register</td>
<td>Dichotomized (singleton = yes, no singleton = no)</td>
<td>I</td>
</tr>
<tr>
<td>Chorioamnionitis</td>
<td>Swedish Medical Birth Register</td>
<td>Maternal diagnosis at birth = O41.1 (ICD-10)</td>
<td>I</td>
</tr>
<tr>
<td>Maternal smoking</td>
<td>Swedish Medical Birth Register</td>
<td>Information routinely collected by midwife at the first visit to the maternity health clinic after 8 to 12 weeks’ gestation. Categorized into no, 1–9 cigarettes/d, ≥10 cigarettes/d, and missing</td>
<td>I, IV</td>
</tr>
<tr>
<td>Respiratory Syncytial Virus</td>
<td>Swedish Hospital Discharge Register 1987–2005</td>
<td>≥1 discharge with diagnosis J12.1 or J20.5 (ICD-10) before 12 months of age</td>
<td>I, IV</td>
</tr>
<tr>
<td>Maternal education</td>
<td>Swedish National Education Register</td>
<td>Highest formal education attained by each individual up to 2005. If the mother was no longer a Swedish resident, we reported paternal education if possible. Categorized by years of education into ≤9, 10–12, 13–14, ≥15 and missing</td>
<td>I, III</td>
</tr>
<tr>
<td>Maternal age</td>
<td>Swedish Medical Birth Register</td>
<td>Categorized in years 12–19, 20–24, 25–29, 30–34, 35–39, ≥40</td>
<td>I, II, IV</td>
</tr>
<tr>
<td>Social assistance</td>
<td>Total Enumeration Income survey of 2005</td>
<td>Cash income allowance from local social authorities after a thorough means investigation to guarantee the applicant a minimum standard of living</td>
<td>I, III</td>
</tr>
<tr>
<td>Maternal/paternal asthma medication</td>
<td>Swedish Prescribed Drug Register</td>
<td>Study I: ≥1 dispensed drug with ATC code starting with R03 during 2006 Study IV (maternal asthma only): ≥1 dispensed drug (ANY, ICS, ANTI) with ATC code according to Table 1</td>
<td>I, IV</td>
</tr>
<tr>
<td>Family history of asthma</td>
<td>Original data birth cohort</td>
<td>At least one of the parents or a sibling was reported to have asthma (data collected on enrolment to the birth cohort at 18 months of age)</td>
<td>II</td>
</tr>
</tbody>
</table>
3.3 Statistical analyses

The statistical analyses were performed using PASW statistics 18 for Windows Release 18.0.1 (SPSS Inc, Chicago, IL) or IBM SPSS Statistics for Windows Release 19.0.0, respectively, in Studies I-III, and partly in Study IV. All remaining analyses in Study IV were performed using R statistical software version 2.14.2 (Vienna, Austria) [165].

Logistic regression was used to calculate odds ratios (OR) with 95% confidence intervals (95% CI) as estimates of effect with asthma medication (Studies I–IV) and self-reported respiratory symptoms (Study II) as the outcome variable while controlling for numerous potential confounders.

3.3.1 Study I

In Study I we used four models to investigate the effects of preterm birth on dispensed ICS. Age was entered as a continuous variable in all models according to the age profile of ICS dispensation. As the age profile differed between boys and girls, decreasing with older age in boys and increasing with older age in girls, we included an interaction term of age*sex in all models. Model 1 was adjusted for age and sex only. In Model 2 we added county of residence, maternal education, social assistance, parental asthma medication and maternal smoking during pregnancy as confounders. In Models 3 and 4 we investigated potential mediating variables

**Table 2: continued**

<table>
<thead>
<tr>
<th>VARIABLES</th>
<th>SOURCE OF DATA</th>
<th>DEFINITIONS/DESCRIPTIONS</th>
<th>STUDY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Geographical residency</td>
<td>Register of the Total Population</td>
<td>Study I: Dichotomized (urban/rural) Study III: large city, other city and rural</td>
<td>I, III</td>
</tr>
<tr>
<td>Current smoking</td>
<td>Postal questionnaire</td>
<td>Dichotomized (yes/no)</td>
<td>II</td>
</tr>
<tr>
<td>Residential status</td>
<td>Postal questionnaire</td>
<td>Dichotomized “Having lived on a farm during the first 5 years of life” (yes/no)</td>
<td>II</td>
</tr>
<tr>
<td>Fish diet</td>
<td>Postal questionnaire</td>
<td>Categorized as never; 1–2x/week and &gt;2x/week</td>
<td>II</td>
</tr>
<tr>
<td>Season of birth</td>
<td>Original data birth cohort</td>
<td>Categorized as winter (Dec–Feb), spring (Mar–May), summer (Jun–Aug) and fall (Sep–Nov)</td>
<td>II</td>
</tr>
<tr>
<td>Mother’s country of birth</td>
<td>Swedish Medical Birth Register</td>
<td>Dichotomized: Study II = Scandinavian/Other; Study IV=Swedish/Other</td>
<td>II, IV</td>
</tr>
<tr>
<td>Parity</td>
<td>Swedish Medical Birth Register</td>
<td>Categorized as 1, 2, 3, ≥4 or missing</td>
<td>I, II, IV</td>
</tr>
<tr>
<td>Cord blood IgE</td>
<td>Original data birth cohort</td>
<td>Dichotomized (&lt;0.9 kU/l, ≥0.9 kU/l)</td>
<td>II</td>
</tr>
<tr>
<td>Mother’s body mass index</td>
<td>Swedish Medical Birth Register</td>
<td>Continuous variable</td>
<td>IV</td>
</tr>
<tr>
<td>Neonatal respiratory distress</td>
<td>Swedish Medical Birth Register</td>
<td>Dichotomized (yes/no) according to neonatal diagnosis 768–770 (ICD-9)</td>
<td>IV</td>
</tr>
</tbody>
</table>
by adding the potential perinatal mediators SGA, chorioamnionitis, multiple birth, asphyxia and cesarean delivery in Model 3, and hospital admission due to RSV infection in Model 4.

3.3.2 Study II
Chi-square test was used for bivariate comparisons between CB-IgE level and dispensed self-reported respiratory symptoms and anti-asthma medication, respectively, and to compare basic characteristics of the study population with respect to the two different outcomes measured. Fisher’s exact test was used instead, in case there were cells with expected counts of less than five. Sensitivity, specificity, positive predictive value and positive likelihood ratio were calculated following standard statistical measures [166].

We used two models to investigate the role of elevated CB-IgE and a positive family history of asthma on self-reported respiratory symptoms and the dispensation of anti-asthmatic medication at the age of 32–34 years. Both models were adjusted for sex, season of birth, maternal age, SGA, LGA, gestational age, mother’s country of birth, cesarean section and parity. When analyzing the role of self-reported respiratory symptoms we added current smoking, having lived on a farm during the first five years of life and fish consumption per week as covariates in the model.

The flow of the study population with respect to asthma diagnosis at 6–7 years of age, at 10–11 years of age and asthma medication at 32–34 years of age was evaluated using common contingency tables.

3.3.3 Study III
All models were adjusted for residency (large city, other city and rural) and for sex. Age was entered as a continuous variable with an interaction term age*sex reflecting the linear age pattern when each sex was analyzed separately. The final model also included maternal education and the socio-economic indicator, social assistance.

3.3.4 Study IV
We performed two main types of analyses: an intent to treat analysis (ITT) and a per protocol analysis (PP). In the PP analysis we compared children who received vaccination as registered in the initial trial with non-vaccinated children with no regard to vaccination period. In the ITT analysis all children born in the vaccination period, regardless of whether they were immunized (n=79,705) or non-immunized (n=21,451) children, were compared with non-vaccinated children born during the 6 months before and after the vaccination period.

The influence of confounders was deemed to be different for these two types of analyses. To disentangle potential confounding factors in this study, a Directed Acyclic Graph (DAG) was used (Figure 6). The technique of using DAGs has been described before and will only be outlined here [167]. A crucial assumption in the DAG is that there are no causal relationships in the data other than those drawn
as directed arrows into the graph. An arrow, however, does not imply that a causal relationship is present.

Instead of only investigating the association between vaccination and prescription (PP analysis), we initially studied the relationship between intended vaccination schedule and prescription. This setting allowed us to avoid certain types of confounders, described as Confounders 2 in Figure 6. This method of avoiding confounders is referred to as “intent to treat” in the clinical trials setting. Treatment was allocated deterministically based on time of birth. Controlling for time of birth per se was not possible here, as at every point in time only one of the alternatives (offer vaccination/do not offer vaccination) was available. Instead, we had to try to control for the variables described in Confounders 1 in Figure 6. We also tested whether time period was associated with the potential confounders. Spring 1993 (non-vaccination period) was compared with spring 1994 (vaccination period), and fall 1993 (vaccination period) with fall 1994 (non-vaccination period).

Confounders 1 includes variables that might have changed during the two-year period from which the cohort was collected. The vaccination schedule was performed so that seasons were similar for vaccination and non-vaccination periods; thus seasons could be omitted from the analysis. Other factors, such as pollen counts and
circulation of infections, may have changed during the two years, but have not been measured in this study. For the PP analysis, additional variables such as attitudes toward health care may act as confounders (Confounders 2 in Figure 7). We checked which of the potential confounders measured were associated with non-compliance in the vaccination period. For the ITT analysis, all confounders that were statistically associated with time period were included in the multivariate analysis. For the PP analysis, all confounders statistically significant for one or both of time period and compliance were used. Confounders were added one at a time and all simultaneously for each scenario. Pearson’s chi² test was used to analyze categorical data on the univariate association between vaccination status and potential confounders and asthma medication. For continuous variables, a Wilcoxon test was used. A logistic regression model was used to calculate odds ratios (OR), with 95% confidence intervals (95%CI) as effect size for vaccination on asthma medication while controlling for numerous potential confounders.

4 ETHICAL STATEMENTS

Study I was approved by the Regional Ethical Review Board in Stockholm. The Regional Ethical Review Board in Linköping, Linköping University, approved all procedures and study protocols for Studies II–IV. Participants in Study II provided written informed consent by answering the postal questionnaires. The linkage of data to the National registers was approved and performed by the National Board of Health and Welfare and did not require any verbal or written consent as data were analyzed anonymously.

5 RESULTS

5.1 Prevalence

The prevalence rates for anti-asthmatic medication varied between the different studies (Table 3). The prevalence of dispensed inhaled corticosteroids in Studies I, II and IV ranged from 3.16% to 4.89% depending on sex and age. In Studies I and III we mainly used ICS (ICS alone or as a combination with other drugs) as the outcome measurement (dispensation period 2006). In Studies II and IV we included all combination medicines (R03AK) as well as leukotriene receptor antagonists (R03DC03) in the variable “anti-inflammatory medication”, above all as montelukast became more frequently used in recent years (dispensation period 2006–2008 and 2008–2010 respectively). The prevalence rates varied between 3.58% and 4.53% depending on sex and age. Self-reported respiratory symptoms associated with contact with pollen and furred pets were found to be 15.0% and 9.7%, respectively. Certain sex-associated variation has been noticed and is displayed in Table 3.
Degree of immaturity showed an inverse dose-response relationship with dispensed prescribed ICS in the model adjusted for sex and age (Model 1). Compared with children born between 39 and 41 weeks of gestation, the OR for dispensed prescribed ICS increased with degree of prematurity, from 1.11 (95% CI 1.08–1.14) for children born moderately premature (weeks 37–38) to 2.23 (95% CI 1.93–2.58) for children born extremely premature (weeks 23–28). Adjusting the model for potential confounding factors such as county of residence, maternal education, social assistance, parental asthma medication and maternal smoking during pregnancy had only marginal effects on the association (Figure 7).

The ORs were slightly attenuated when adjusting the analysis for perinatal mediators such as SGA, chorioamnionitis, multiple birth, asphyxia and cesarean delivery (Figure 7, Model 3). The introduction of cesarean delivery contributed to the attenuation of the ORs in this model with an OR for cesarean delivery of 1.13 (95% CI 1.09–1.16) and 1.20 (95% CI 1.17–1.23) after removal of gestational age from the model. The addition of SGA and chorioamnionitis did not affect the ORs for gestational age. The ORs for SGA in models with and without gestational age were 1.06 (95% CI 1.00–1.12) and 1.16 (95% CI 1.10–1.22), respectively. Corresponding ORs for chorioamnionitis were 1.03 (95% CI 0.83–1.26) and 1.32 (95% CI 1.09–1.61).

### Table 3: Prevalence rates (%) for dispensed asthma medication (Studies I-IV) and self-reported allergen-induced respiratory symptoms (Study II).

<table>
<thead>
<tr>
<th>Study</th>
<th>Age (years)</th>
<th>ANY ICS</th>
<th>ANTI</th>
<th>BETA2</th>
<th>Respiratory symptoms (pollen)</th>
<th>Respiratory symptoms (furred pets)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M</td>
<td>F</td>
<td>M</td>
<td>F</td>
<td>M</td>
<td>F</td>
</tr>
<tr>
<td>I¹</td>
<td>6–19</td>
<td>7.39</td>
<td>6.45</td>
<td>4.89</td>
<td>3.78</td>
<td></td>
</tr>
<tr>
<td>II²</td>
<td>32–34</td>
<td>6.69</td>
<td>6.55</td>
<td>3.58</td>
<td>3.16</td>
<td>3.70</td>
</tr>
<tr>
<td>IV¹</td>
<td>15</td>
<td>6.60</td>
<td>7.35</td>
<td>4.53</td>
<td>4.35</td>
<td>4.68</td>
</tr>
<tr>
<td>III¹</td>
<td>6–25</td>
<td>Prevalence rates of ICS differed between 1.4% and 10.4% dependent on mean age at adoption/immigration and country of birth</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

#### 5.2 Gestational age (premature birth)

Prevalence rates of ICS differed between 1.4% and 10.4% dependent on mean age at adoption/immigration and country of birth.
An increased prevalence of dispensed prescribed ICS was seen among children who had been hospitalized for RSV infection. The addition of hospitalization due to RSV infection in the multivariate analysis, however, did not affect the ORs for gestational age (Model 4).

**5.3 Cord blood immunoglobulin E**

Elevated CB-IgE (≥0.9 kU/l) was found in 153/1,227 (12.5%) of the study participants who answered the postal questionnaires and 205/1,661 (12.3%) of those who could be linked to the prescription register at the age of 32–34 years. Elevated CB-IgE levels ranged from detection level 0.9 to 9.7 kU/l and were slightly more common in males than in females. Respiratory symptoms were twice as common in those with elevated CB-IgE levels with a crude OR 2.01 (95% CI 1.33–3.05) for those reporting respiratory symptoms.
due to contact with pollen and 2.03 (95% CI 1.25–3.31) for those reporting respiratory symptoms due to contact with furred pets, respectively. Adjusting for potential confounding factors (sex, current smoking, having lived in a farm during the first five years of life, fish consumption per week, season of birth, maternal age, SGA, LGA, gestational age, mother’s country of birth, cesarean section, parity and positive family history of asthma) did not alter the associations significantly.

Elevated CB-IgE was associated with a 2–2.5-fold increased likelihood of dispensed asthma medication at 32–34 years of age. The crude OR for any asthma medication was 1.98 (95% CI 1.22–3.21) and for anti-inflammatory treatment 2.70 (95% CI 1.50–4.89) for individuals born with elevated CB-IgE. Adjusting the multivariate analysis for several potential confounding factors (sex, season of birth, maternal age, SGA, LGA, gestational age, mother’s country of birth, cesarean section, parity and positive family history of asthma) changed the association only marginally (Table 4).

**Table 4:** Odds ratios (ORs) with 95% confidence intervals (95% CI) for the risk of respiratory symptoms and the risk of asthma medication (≥2 prescriptions/2006–2008) at the age of 32–34 years.

1Final model adjusted for sex, current smoking, having lived on a farm during the first five years of life, fish consumption per week, season of birth, maternal age, small for gestational age, large for gestational age, gestational age, mother’s country of birth, cesarean section, parity and positive family history of asthma.

2Final model adjusted for sex, season of birth, maternal age, small for gestational age, large for gestational age, gestational age, mother’s country of birth, cesarean section, parity and positive family history of asthma.

<table>
<thead>
<tr>
<th></th>
<th>CB-IgE &lt; 0.9 kU/l</th>
<th>CB-IgE ≥ 0.9 kU/l</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR</td>
<td>Crude OR (95% CI)</td>
</tr>
<tr>
<td>Respiratory symptoms</td>
<td></td>
<td></td>
</tr>
<tr>
<td>due to contact with</td>
<td>1</td>
<td>2.01 (1.33–3.05)</td>
</tr>
<tr>
<td>pollen</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Respiratory symptoms</td>
<td>1</td>
<td>2.03 (1.25–3.31)</td>
</tr>
<tr>
<td>due to contact with</td>
<td></td>
<td></td>
</tr>
<tr>
<td>furred pets</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any asthma medication</td>
<td>1</td>
<td>1.98 (1.22–3.21)</td>
</tr>
<tr>
<td>Anti-inflammatory treatment</td>
<td>1</td>
<td>2.70 (1.50–4.89)</td>
</tr>
</tbody>
</table>
5.4 Family history of asthma

Self-reported respiratory symptoms and the need of dispensed asthma medication were increased among those with a positive family history of asthma. The crude OR for self-reported respiratory symptoms due to contact with pollen and due to contact with furred pets were 2.38 (95% CI 1.38–4.10) and 3.04 (1.68–5.51), respectively. For any asthma medication and anti-inflammatory treatment the corresponding rates were 2.75 (95% CI 1.55–4.87) and 2.72 (95% CI 1.30–5.70), respectively. Adjusting for different confounding variables changed the estimates only marginally (Table 5).

Table 5: Odds ratios (ORs) with 95% confidence intervals (95% CI) for the risk of respiratory symptoms and the risk of asthma medication (≥2 prescriptions/2006–2008) at the age of 32–34 years in relation to family history of asthma (FHA). CB-IgE = Cord blood immunoglobulin E

1Final model adjusted for sex, current smoking, having lived on a farm during the first five years of life, fish consumption per week, season of birth, maternal age, small for gestational age, large for gestational age, gestational age, mother’s country of birth, cesarean section, parity and elevated CB-IgE (≥ 0.9 kU/l).
2Final model adjusted for sex, season of birth, maternal age, small for gestational age, large for gestational age, gestational age, mother’s country of birth, cesarean section, parity and elevated CB-IgE (≥ 0.9 kU/l).

<table>
<thead>
<tr>
<th></th>
<th>Negative FHA</th>
<th>Positive FHA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR</td>
<td>Crude OR (95% CI)</td>
</tr>
<tr>
<td>Respiratory symptoms due to contact with pollen</td>
<td>1</td>
<td>2.38 (1.38–4.10)</td>
</tr>
<tr>
<td>Respiratory symptoms due to contact with furred pets</td>
<td>1</td>
<td>3.04 (1.68–5.51)</td>
</tr>
<tr>
<td>Any asthma medication</td>
<td>1</td>
<td>2.75 (1.55–4.87)</td>
</tr>
<tr>
<td>Anti-inflammatory treatment</td>
<td>1</td>
<td>2.72 (1.30–5.70)</td>
</tr>
</tbody>
</table>
5.5 Prediction of asthma

The predictive values of elevated CB-IgE or a family history of asthma at 18 months were low for allergen-induced self-reported respiratory symptoms and asthma medication in this adult population (Table 6).

The combination (“and/or”) of these two presumptive factors did not increase the predictive values sufficiently. If both factors were combined (“and”) the sensitivity (SE) for anti-inflammatory medication was 2, specificity (SP) 99 and positive predictive value (PPV+) 7. If either of the factors was positive (“or”), the corresponding numbers were SE 40, SP 83 and PPV+ 8.

**Table 6:** Sensitivity, specificity and predictive values of elevated CB-IgE (≥ 0.9 kU/l) or positive family history of asthma (FHA+) for respiratory symptoms due to pollen or furred pets and asthma medication (≥ 2 prescriptions/3 years) at the ages of 32–34 years. CB-IgE = Cord blood immunoglobulin E; CB-IgE+ = Cb-IgE ≥ 0.9 kU/l; SE = sensitivity; SP = specificity; PPV = positive predictive value; LR+ = positive likelihood ratio. SE, SP and PPV given as percentages.

<table>
<thead>
<tr>
<th></th>
<th>CB-IgE+</th>
<th>FHA+</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SE</td>
<td>SP</td>
</tr>
<tr>
<td>Respiratory symptoms due to pollen</td>
<td>20</td>
<td>89</td>
</tr>
<tr>
<td>Respiratory symptoms due to pets</td>
<td>21</td>
<td>89</td>
</tr>
<tr>
<td>Any asthma medication</td>
<td>21</td>
<td>88</td>
</tr>
<tr>
<td>Anti-inflammatory medication</td>
<td>27</td>
<td>88</td>
</tr>
</tbody>
</table>

Figure 8 shows the flow of different individuals with asthma diagnosis at 6–7 and 10–11 years of age as well as those with dispensed anti-inflammatory asthma treatment at 32–34 years of age.

Most of the cases with dispensed anti-inflammatory treatment at 32–34 years of age had not been reported having asthma at the age of 6–7 or 10–11 years (49/60). Only 9 cases had been diagnosed with asthma on both follow-up occasions in childhood and had been dispensed asthma medication at 32–34 years of age. The proportion of individuals with elevated CB-IgE was higher in those who had asthma in childhood but had not been dispensed any anti-inflammatory asthma medication (no longer asthma) at the age of 32–34 years (36.7%) compared with those who had been dispensed asthma medication as adults (26.7%).
Figure 8: Flow of individuals with asthma diagnosis at 6–7 and 10–11 years and anti-inflammatory treatment at 32–34 years of age. Corresponding individuals are marked with blue or red numbers describing individual flows over time.

\(^1\)figures based on the total numbers of individuals linked to the Swedish Medical Birth Register (n=1,661).
5.6 Congruence between respiratory symptoms and asthma medication

Reported respiratory symptoms and dispensation of asthma medication corresponded only partly. In all, 65.3% of those with at least two dispensed prescriptions of any asthma medication and 71.4% of those who purchased at least two prescriptions of anti-inflammatory drugs had reported respiratory symptoms due to pollen. The corresponding rates for respiratory symptoms due to contact with furred pets were 52.1% and 56.1%, respectively. Among those with respiratory symptoms due to pollen, only 27.4% had purchased at least two prescriptions of any asthma medication and 16.8% at least two purchases of an anti-inflammatory drug. For those with reported respiratory symptoms due to contact with pets, the corresponding rates were 33.0% and 20.0%, respectively.

The rates were similar for agreement between self-reported allergen-induced respiratory symptoms and dispensation of beta$_2$-agonists. The agreement between respiratory symptoms and asthma medication of beta$_2$-agonists and anti-inflammatory medication (≥2 dispensations/3-year period) is displayed in Figure 9.

![Figure 9: Proportional Venn diagrams illustrating the agreement between allergen-induced self-reported respiratory symptoms (pollen- and pet-induced respiratory symptoms) and asthma medication (beta2-agonists and anti-inflammatory medication). Venn diagrams generated with the help of Venn diagram online tool at http://venndiagram.tk/ provided by Dr Tim Hulsen (Radboud University Nijmegen, The Netherlands).]
5.7 Migration

Dispensation of prescribed ICS was less prevalent in foreign-born individuals with foreign-born parents compared with adoptees and children born in Sweden. The ORs for dispensed prescribed ICS by region of origin after logistic regression with adjustment for sex and age were significantly lower in foreign-born children with foreign-born parents in all regions: Eastern Europe 0.34 (95% CI 0.25–0.57), East Asia 0.19 (95% CI 0.16–0.22), South Asia 0.31 (95% CI 0.27–0.36) and Latin America 0.48 (95% CI 0.43–0.54) in comparison with Swedish-born children with Swedish-born parents. In contrast, the prevalence of dispensed prescribed asthma medication varied in Swedish-born individuals with foreign-born parents compared with the Swedish majority population. Swedish-born individuals with parents born in Eastern Europe had a decreased risk (OR 0.54) of dispensed ICS whereas those born in Sweden with parents born in South Asia had a slightly higher risk (OR 1.26).

Dispensation of prescribed ICS was less likely in adoptees from Eastern Europe than in Swedish-born individuals with Swedish-born parents (OR 0.27), but was higher in adoptees from all other continents (Figure 10).

▲ Figure 10: Own and parental region of birth as risk factors for dispensed asthma medication. Odds ratios after adjustment also for age, sex and rural/urban residency (reference group = Swedish-born children with Swedish-born parents).

- □ Foreign-born with Swedish-born adoptive parents,
- ▲ Swedish-born with foreign-born parents
- △ Foreign-born with foreign-born parents
Among adopted individuals we investigated the relation between age at adoption and the dispensation of prescribed ICS and found an inverse dose-response association in the unadjusted model as well as in the models adjusted for region of birth, maternal education and received social assistance (Figure 11).

Region of birth had an effect on the likelihood of ICS being dispensed, with adoptees from South Asia having a higher risk (OR 1.26) compared with adoptees from Latin America. The association became somewhat weaker after adjustment for age at adoption (aOR 1.21). Adjustment for maternal education and receiving social assistance had only marginal effects on the association.

Among non-adopted foreign-born children, age at immigration showed an inverse dose-response relationship with dispensed prescribed ICS similar to the association between adopted children and the age of adoption. Adjustment for region of birth had only a marginal effect on this association (Figure 12).

▲ **Figure 11**: Risk of dispensed asthma medication by age at adoption.
**Blue squares**: unadjusted ORs with 95% CIs.
**Red circles**: ORs after adjustment for sex, geographical residency (urban/rural) and region of birth.
Region of birth influenced the likelihood of ICS being dispensed differently for the different regions. In comparison with immigrants from Latin America, the likelihood of ICS being dispensed was lower in immigrants from all other regions.

The association between the different immigrant categories and dispensed prescribed ICS after logistic regression showed a higher risk for adoptees (aOR 3.94) and Swedish-born individuals with foreign-born parents (aOR 3.36) compared with foreign-born individuals with foreign-born parents in the model adjusted for sex, age and parental region.

5.8 Immunization

The ITT analysis demonstrated a slightly reduced likelihood of dispensed asthma medication in all children born during the vaccination period compared with children born during the 6 months before and during the 6 months after the vaccination period in the univariate analysis. The association disappeared, however, after adjustment for confounding factors (Table 7). The type of vaccination schedule (2, 4 and 6 months or 3, 5 and 12 months) did not affect the association.
When we compared all vaccinated children born in the vaccination period with all non-vaccinated children born both outside and in the vaccination period in the PP analysis, there was no significant association between pertussis vaccination and dispensed asthma medication (Table 8).

<table>
<thead>
<tr>
<th></th>
<th>Vaccination period</th>
<th>aOR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any asthma medication</td>
<td>0.97 (0.93 – 1.00)</td>
<td>0.99 (0.95 – 1.03)</td>
</tr>
<tr>
<td>Anti-inflammatory treatment</td>
<td>0.94 (0.90 – 0.98)</td>
<td>0.97 (0.92 – 1.01)</td>
</tr>
<tr>
<td>β₂-agonists</td>
<td>0.97 (0.94 – 1.01)</td>
<td>0.99 (0.95 – 1.03)</td>
</tr>
<tr>
<td>Inhaled corticosteroids</td>
<td>0.97 (0.91 – 0.99)</td>
<td>0.97 (0.92 – 1.01)</td>
</tr>
</tbody>
</table>

Asthma medication was less likely to be dispensed in non-vaccinated children who were born during the vaccination period than in non-vaccinated children born outside the vaccination period. The crude OR for dispensation of any asthma medication was 0.89 (95% CI 0.84–0.94); the corresponding OR for anti-inflammatory treatment was 0.87 (95% CI 0.81–0.94). These differences disappeared after adjustment for confounding factors.
Additional analyses using selective $\beta_2$-agonists and ICS alone showed similar results with a crude OR for dispensation of $\beta_2$-agonists of 0.88 (95% CI 0.83–0.94) and ICS of 0.87 (95% CI 0.81–0.94) respectively. After adjustment for confounding factors, these associations also became insignificant.

The type of pertussis vaccine and the vaccination schedule did not affect the risk that asthma medication would be dispensed.
6 DISCUSSION

6.1 General methodological considerations

Studies I, III and IV are based entirely on population-based register data except the data about immunization in Study IV. In these data sets we managed to investigate practically the entire Swedish population in the particular age groups, which presumably makes these samples representative of these age groups in Sweden. Indeed, our findings might even be generalized to other similar populations in other countries.

Data from national registers enable us to investigate large sample sizes with high response rates. Other type of studies, e.g. birth cohort or case-control studies, often struggle with these very parameters, resulting in small sample sizes and low response rates. Although these kinds of studies often provide an opportunity to collect extensive data with good quality about exposure and outcome as well as confounding factors, they tend to be vulnerable to selection bias.

In contrast, register-based studies are a useful tool for investigating selection bias related to non-response as data can be analyzed anonymously. As registers in the Nordic countries also contain socioeconomic data on areas like occupations, income and tax returns, it can be an advantage to use these data as they contain other information than self-reported data. The use of data from a smaller study together with data from national registers can sometimes solve the problem of missing data or decrease the loss of study participants, especially in long-term follow-ups like that of our birth cohort in Study II [168].

As register data are collected prospectively and independently from both the study individuals and the research question, it is possible to avoid several types of bias such as recall and selection bias. It also allows the misclassification of outcome and exposure to be minimized. The enormous data sets we retrieved from the registers made it possible to adjust our analyses for various confounding factors that directly or indirectly might have influenced our outcome variable, asthma medication. The “instrumental” use of register data, however, often creates other problems. As we have to accept “proxy” data as a measure of exposure or outcome, we will often end up with attenuated effect measures [168]. It is seldom easy to measure the extent of bias in the data. Moreover, when using proxy data for real confounders in a study, the risk of residual confounding increases [168, 169]. Additionally, certain lifestyle factors, such as breastfeeding and current smoking, normally cannot be controlled for using register data.

Asthma medication as the primary outcome variable has its limitations [170]. Prescription data lack information about diagnosis or disease severity. Besides, the register contains only information about dispensed asthma medication, and it is impossible to know exactly whether the patient really consumed the drug [171]. However, when a drug has been prescribed by a physician and purchased by the
patient, this could be interpreted as a reasonable marker of asthma diagnosis. We have concentrated our investigations on the groups between 6 and 34 years of age due to diagnostic problems and the risk of overtreatment in very young individuals [172].

Poor economic resources on the part of parents could influence the purchase of asthma medication [173] and contribute to an under-diagnosis of asthma related to socioeconomic status. In Sweden there is a reimbursement system for medicines that require a prescription, however, which means that every patient pays only a rather low maximum amount per year for his or her medicines. We also adjusted for various socioeconomic variables in our analyses, but this did not affect the association between measured exposure and asthma medication.

As dispensation of any asthma medication is not entirely equal to asthma diagnosis or symptoms, we mainly investigated the dispensation of ICS and LTRA as these drugs, especially ICS, are used by a considerable number of individuals with active disease [174] and are more likely to present clinically significant disease [62]. Fairly good agreement between register-based prescription data on asthma medication, actual drug use and physician-diagnosed asthma has been demonstrated in the Scandinavian countries [175, 176]. A comparison of prescription databases in the Nordic countries has shown that register data provide valid and reliable data for the study of drug use and that the proportion of coding and data errors is very low [171]. The rates of dispensed asthma medication in our investigations correlate fairly well with other studies about asthma prevalence in Sweden using other outcome variables [60-62, 177].

The birth cohort in Study II has been followed during childhood and investigated on different occasions [73, 161, 162]. Response rates differed slightly between the previous follow-up and our follow-up at 32–34 years of age (≤ 10 individuals between follow up at 6–7, 10–11 and 32–34 years). All subjects in the follow-up in adulthood, however, could be anonymously linked to the Swedish Medical Birth Register, even if they might not have been included in all follow-ups during childhood. There might be individual subjects with an asthma diagnosis that we have missed when investigating asthma prevalence over time in the birth cohort and the flow of individuals with respect to both asthma diagnosis and medication, and elevated CB-IgE (Figure 8). However, the number of these presumably missing subjects would be very small in relation to the whole study population, and the likelihood of participation is probably higher in subjects with asthma than in those without asthma. Therefore, we consider this a minor problem that should not interfere significantly with our results.
6.2 Premature birth

This study showed an increased risk of ICS dispensation depending on the degree of immaturity at birth in 6- to 19-year-olds. The multivariate analysis indicates that immaturity per se seems to be the most important factor for the effect of preterm birth on ICS dispensation in children and teenagers.

Lung development is a continuous process that does not end with birth, but continues into childhood. Every week of maturation is important for the development of the lung, and curtailed pulmonary development as a result of preterm delivery leads to a deficit in the structure and function of the lung [178]. Lung maturation is greatest during the last trimester of pregnancy in particular, [179] making it plausible that damage due to unfinished lung development, possibly combined with the influence of external harmful factors during this vulnerable period, plays an important role in the increased risk of asthma medication in childhood and adolescence.

The association found between the degree of prematurity and the dispensation of asthma medication was attenuated by increasing age. This is in line with findings by Jaakola et al that showed stronger effects of prematurity in younger populations [89]. Another Swedish study similar to ours failed to show an effect of prematurity on asthma medication in infants born after 28 weeks’ gestation [180], perhaps due to the older age group (25.5–35 years) that was investigated.

The population in our investigation was sufficiently large to detect an increased risk even in individuals born early term after 37–38 weeks’ gestation (Figure 7). The risk increase for this group was only 10% but accounted for almost 2% of all cases on a population level as 20% of all children were born early term. The group of infants born extremely preterm, however, accounted only for 0.2% of all cases.

Only a few studies have investigated the long-term outcome of asthma and late preterm birth. A retrospective study from the USA found a significant increase in persistent asthma diagnosis, use of ICS and number of acute respiratory visits related to late preterm birth in a cohort of almost 8,000 children 18 months of age [181]. Another retrospective cohort study of about 71,000 children in the USA found an association between late premature birth and recurrent wheezing at three years of age [182]. Investigations of long-term outcome, however, are rare [180].

Cesarean delivery has been described as a risk factor for asthma [183]. In our study it was associated with a 20% increased risk of asthma medication. Some of the effect, however, was related to immaturity at birth because the OR was reduced after adjustment for gestational age. Unnecessary early deliveries should therefore be avoided to prevent the increased risk of asthma later in life. Studies have shown that the increase in rate of late preterm births has been influenced by the growing use of labor induction and performing cesarean sections at 34–36 weeks’ gestation [184, 185].
Chorioamnionitis is a common complication in pregnancy and contributes to preterm labor [28], but has also been described as an independent risk factor for wheezing [14] and physician-diagnosed asthma in early childhood [92]. We did not find that chorioamnionitis contributed to an increased risk of asthma medication after adjustment for gestational age. The focus on school-aged children in our study, however, does not exclude potential effects on the need for asthma medication in younger children. Furthermore, the setting of our study design probably detects only severe cases of clinically significant chorioamnionitis, which has to be taken into account.

An association between socioeconomic disadvantage and both asthma and preterm birth was demonstrated in our study. Adjustment in our model for our two socioeconomic indicators (maternal education and social assistance), however, resulted only in marginal effects, indicating that socioeconomic confounding was no major problem in this study. A more serious methodological concern in this study is probably the regional variation in our outcome variable. There are differences in access to the health care system, with better access in urban areas than in rural areas. There might be a bias through regional patterns of access to care that overlap with regional rates of preterm birth and asthma prevalence. Adjusting for county variation failed to demonstrate any major effects. Therefore we believe that such bias was minor.

6.3 Cord blood immunoglobulin E and family history of asthma

We were able to follow up 72% of the former infants of the birth cohort by questionnaire at the age of 32–33 years, which is comparable to, or even better than, response rates in cohorts with a similar long-term follow-up [38, 186, 187]. Some other birth cohorts have shown lower response rates also in follow-ups at lower ages [75]. Moreover, almost 98% of the original cohort could be linked to the Swedish Medical Birth Register with information on different confounding factors and information about asthma medication from the Swedish Prescribed Drug Register from 2006–2008.

The two- to threefold higher risk of asthma medication being dispensed and allergen-induced respiratory symptoms among individuals with elevated CB-IgE or a family history of asthma which we found in our analyses are consistent with the results of similar investigations in pediatric populations [116, 117, 120]. Only two previous studies have investigated the association between CB-IgE and asthma and allergic disease in adults, with conflicting results [119, 187]. In the Finnish study by Pesonen et al. of 200 unselected full-term newborns, elevated CB-IgE above 0.5 kU/L was associated with a two- to threefold increased risk of allergic symptoms at the age of 20 years [119]. In contrast, Shah et al., who followed up 670 subjects in the Detroit Childhood Allergy Study, was unable to find any consistent association
between elevated CB-IgE and asthma or other atopic diseases at 18–21 years [187]. Methodological variances may explain some of the differences. Continuous cut-off levels for CB-IgE were used in the Detroit study in contrast to the fixed cut-off levels in the two Scandinavian studies, including ours. Besides, the Finnish population is probably genetically more similar to ours, although there are significant differences between Scandinavian populations depending on the geographical distribution [188]. Thus the results of the Finnish study might be more comparable to ours. As we no longer have access to the blood samples from our study population, we were unable to assess how analyses based on IgE measured as a continuous variable or lower cut-off levels for CB-IgE than 0.9kU/l would have affected the outcome.

High CB-IgE values could be the result of maternofetal contamination. A Danish cohort study of 200 children with maternal asthma suggested that maternal IgE antibodies contributed to high CB-IgE values in about 50% of all individuals with CB-IgE values over 0.5 kU/l. The suspected contamination was based on the detection of IgA and allergen-induced specific IgE in the sampled cord blood [112]. In contrary, other studies have suggested that maternofetal contamination of cord blood is uncommon. IgA antibodies were detected in less than 5%, even in mother-child pairs with high IgE levels against the same allergen. Low concordance between specific IgE in cord blood and maternal blood for inhalant allergens was demonstrated as an indicator of no significant maternofetal contamination. The agreement between specific IgE for food allergens in cord blood and maternal blood was much better. The authors interpreted their findings as an indication of sensitization already in utero, especially against food allergens. The high CB-IgE values were considered to be chiefly the result of synthesization by the fetus and not due to maternofetal contamination [189, 190]. It is not easy to elucidate why these different studies have shown such opposing results. However, they differ in several parameters, such as laboratory methods for detecting the different immunoglobulins, the method of securing adequate cord blood samples from the neonates, and the characteristics of the cohorts, just to mention some of the differences between the studies that might have influenced the different results.

As we no longer have access to the original blood samples, we were unable to control for the proportion of IgA in cord blood, which is more or less standard procedure nowadays when investigating the role of CB-IgE. To account for the risk of maternofetal contamination in our study, we excluded subjects with rather high CB-IgE levels (≥ 10.0 kU/l) as suggested in a previous study [116]. Furthermore, good agreement between high CB-IgE levels and high total IgE levels at the age of 18–24 months was reported in our birth cohort [160], indicating that the majority of high IgE levels in cord blood are of fetal origin. This is similar to the findings in the Danish study. Bønnelykke et al found that children with elevated CB-IgE levels and indications of maternofetal contamination had significantly lower IgE levels at 6 months of age compared with children with elevated CB-IgE levels without indications of maternofetal contamination. Furthermore, elevated CB-IgE levels were not significantly associated with IgE levels at 6 months of age in samples with indications of maternofetal transfer of IgE after adjustment for maternal IgE levels.
This was in contrast to a highly significant independent association for samples in which transfer was not indicated [112].

We used information from the Swedish Medical Birth Register and the postal questionnaires to adjust our analysis for different potential confounding factors including season of birth, cesarean section, and gestational age. Controlling for these different factors, however, changed the associations we found only marginally. Elevated CB-IgE and a family history of asthma based on information collected in early life remained as highly significant and independent risk factors for asthma medication and respiratory symptoms in adulthood in the analysis.

The use of questionnaires to investigate the prevalence of asthma is rather common in epidemiological studies, but might not be optimal [191]. Questionnaires are an inexpensive, simple tool which can easily be used in large surveys [192], but their use is not unquestioned, mainly due to the risk of recall bias and other possible subjective errors [193].

To increase the accuracy of our study, we also investigated the dispensation of asthma medication beyond the survey using postal questionnaires. Dispensed asthma medication was less common than self-reported allergen-induced respiratory symptoms, and the two outcomes only partly overlapped (figure 9). Only about 20–30% of those who reported symptoms had purchased any asthma medication. Our definition of allergy-related respiratory symptoms included wheezing, breathlessness or coughing. Some subjects might have reported non-asthmatic symptoms from the airways, and others probably had mild symptoms without need of medication.

In contrast, allergy-related respiratory symptoms were reported by 50–70% of those who had purchased any asthma medication, which is in line with reviews indicating that only approximately 50% of all asthma is associated with allergy [194]. As mentioned above, the prevalence of our variable any asthma medication was fairly similar to prevalence rates of physician-diagnosed asthma in recent studies among young Swedish adults [60, 61]. The odds ratios for both outcomes were fairly comparable even though they were markers of partly different entities. It is important to bear in mind that asthma is a disease with different phenotypes but also different etiologies. We saw that most individuals who had been dispensed anti-inflammatory medication as adults had not had an asthma diagnosis in childhood (Figure 8), which underlines the assumption that we are measuring different phenotypes of asthma. It is not surprising that the use of different methods to measure asthma reveals different prevalence rates [195].

As there is currently no ‘golden standard’ for the definition of asthma in epidemiological studies, different measurements can, and should, be used as long as the investigator is aware of each measurement’s particular advantages and disadvantages.
The predictive capability of our two investigated variables, CB-IgE and family history of asthma, was poor. In line with other previous studies in younger adults [119, 187], neither CB-IgE nor family history of asthma was a sufficient screening parameter for identifying individuals at risk, and the predictive ability did not improve even when both parameters were combined. CB-IgE might be a sensor for sensitization in utero, which is underlined by the higher proportions of individuals with elevated IgE at birth among asthmatics in childhood compared with adult asthmatics in this birth cohort (Figure 8). Bearing in mind that different phenotypes of asthma with allergic asthma are more dominant in childhood years [31], these could have been expected but have not been demonstrated so far in adults below 30 years of age.

6.4 Migration

Migration studies may allow us to disentangle the effects of early environmental exposures from later environmental and socioeconomic influences after migration [134]. In this study we have compared the risk of asthma medication in three well-defined categories of migrants with Swedish-born children of Swedish-born parents.

Previous studies have already shown an association between the risk of asthma and age at immigration [129, 196-198] or duration of residence in the new country [196, 199-201]. In our investigation we also demonstrated a negative relationship between age at immigration and the risk of asthma medication in international adoptees raised by Swedish parents, as well as foreign-born children raised by their birth parents.

An increased risk of asthma is usually a consequence for children of all ages moving from less developed regions with low asthma prevalence to a more developed region with higher asthma prevalence. In a British study, the risk of asthma among South-Asian women was the same regardless of whether they were born in the United Kingdom or had immigrated before the age of five years, whereas South-Asian women migrating after five years of age had a considerably lower risk of asthma, which did not change with increasing age [130]. In our much larger study population we also saw a gradually declining likelihood of dispensed asthma medication in individuals migrating after the age of five years. This was in line with an Israeli study that showed an inverse relation between age at immigration to Israel and the risk of asthma among immigrants from the former Soviet Union and Ethiopia, but not from Western Europe [198].

Adoptees had an almost fourfold increased likelihood of dispensed asthma medication compared with immigrants from the same regions of birth; the exceptions were adoptees from Eastern Europe (Figure 10). Most of the adoptees were adopted before two years of age whereas the mean age at migration among immigrants was around ten years in this study. The highest likelihood of dispensed asthma medication in young adults was seen in subjects who had immigrated in early infancy. Equally,
the protective effect on asthma medication of being born in low- or median-income regions was closely related to the duration of residency in the native country. A low level of cultural adaptation among immigrants may further counteract the effects of asthma-promoting exposures in the new society [202, 203], in contrast to adoptees who are rather instantly integrated into the Swedish lifestyle. It was less likely that asthma medication would be dispensed to adoptees from Eastern Europe than to adoptees from other continents and the Swedish majority population. This could be explained to a large extent by the higher age at migration in adoptees from Eastern Europe.

Dispensed asthma medication was more common among adoptees from Asia and Latin America than in Swedish-born children with Swedish-born parents (Figure 10). This might partly be an effect of differences in behavior related to seeking medical attendance [204]. Adoptive parents might be more eager to seek medical care for their children, after a usually long and time-consuming adoption process. We found the highest rate of dispensed asthma medication in adoptees from South Asia. Similarly, children born in Sweden with parents born in South Asia had slightly increased odds for dispensed asthma medication compared with children born in Sweden to Swedish-born parents. This might suggest a genetic predisposition for asthma in the South-Asian population.

Studies on gene-environment interaction have demonstrated that the expression of specific genes is determined by their setting. A genetic variant might either lead to, or protect from, asthma depending on the environmental influence [205]. Recent multi-center studies have suggested very low asthma prevalence in children in some centers in India [55, 206], leading to the assumption that particular environments in certain regions in India protect from asthma. It has been proposed that certain populations in tropical areas have developed a strong pro-inflammatory immune response that might be important in an environment rich in worms and other parasites. This immune response might deviate into an allergic response when migrating into an environment with less microbial exposure [207]. Parallels can be drawn with the development of metabolic diseases such as diabetes mellitus. An adaptation in utero to an environment determined by an impaired energy supply might lead to an ‘inadequate metabolic response’ when born into an environment with an excess of food [208].

A register-based study in Sweden showed an increased risk of type 1 diabetes mellitus in children born in Sweden with family origins in low incidence countries. In this study the risk of developing diabetes was not influenced by migration after birth, regardless of whether the children grew up with their biological or Swedish-born parents. This led to the conclusion that the effect observed might be the consequence of changes in environmental exposures (changes in nutrition or the exposure to infections) either during pregnancy or early infancy [209]. In contrast, our study showed a clear association between age at migration to Sweden and the development of asthma, indicating the importance of both the pre- and postnatal environment, even several years after birth.
Another, possibly more ‘prosaic’, explanation for the higher prevalence of asthma among South Asians might be diagnostic bias, as suggested by Kuehni, who proposed that South Asians might be diagnosed with, and treated for, asthma more often due to false-positive low lung function as spirometers lack “ethnic-specific norm values” [134]. Similarly, physicians might be influenced by the higher prevalence of allergic diseases in South Asians and therefore tend to prescribe ICS to patients with asthma [134], although only a proportion of asthma is attributable to allergy [210].

Low prevalence of asthma medication could be an expression of under-diagnosed asthma or underuse of prophylactic medication due to economic reasons, which has been reported from different developed countries [211-213]. A similar pattern might also exist in Sweden. In 2001, almost 50% of the immigrant children aged less than 17 years were living in relative poverty in Sweden compared with 8% of Swedish-born children with Swedish-born parents. The proportion of immigrant children living in economic vulnerability decreases with duration of residency in Sweden [214]. Factors such as language barriers, health-seeking behavior, low health literacy and inadequate access to health care contribute to a reduced utilization of asthma medication in immigrants [215]. There is a significant risk that dispensed asthma medication as a proxy for asthma underestimates the true prevalence of asthma in immigrants, especially among those who have recently arrived. This might contribute to the lower rates of dispensation of asthma medication in foreign-born children with foreign-born parents than among adoptees and Swedish-born children with Swedish-born parents seen in our study. This, however, can hardly explain the similar and inverse association between age at migration and dispensed asthma medication both in adoptees and foreign-born children with foreign-born parents despite the socioeconomic disparities and potential differences in health-seeking behavior between the two groups. Adopted children are overrepresented in upper social classes whereas non-adopted immigrants are more common in the lower social classes. Furthermore, the inverse relationship of age at migration with dispensed asthma medication persisted even after adjustment for socioeconomic indicators.

6.5 Immunization

Our study revealed a weak negative association between pertussis vaccination as an infant and asthma medication in some analyses. When adjusting for several sociodemographic and perinatal factors, there was no difference in the prevalence of dispensed asthma medication between unvaccinated and vaccinated children in this cohort. Previous studies investigating the impact of immunization on asthma have shown contradictory results [137-141, 146-148]. In 1994, Odent et al were the first to report a possible increased risk of asthma after infant immunization for pertussis. The authors stated that there was no other explanatory factor except pertussis
vaccination for the tremendous increase in asthma prevalence in this group. However, as this rather short report lacked detailed information, the results remained difficult to assess [137]. Other studies that showed an increased risk of asthma after infant immunization have struggled with certain methodological problems such as recall bias on both exposure and outcome, as well as the problem of very small control groups [139, 140]. McKeever et al also found an association between vaccination and the development of allergic disease in an American birth cohort. He stated, however, that the association that was found could be explained by sampling bias rather than a biological effect, as children with a higher prevalence of allergic disease had fewer physician visits than the group of children with fewer asthma cases [216]. Our findings are in line with a number of more recent studies that do not report any association between pertussis immunization and asthma [145-148]. Yet, these studies have also been confronted with some significant methodological problems, above all very small control groups and/or low response rates [145-147], which is why further large-scale, well controlled investigations have been requested [155]. There have also been discussions about a potential protective effect of infant immunization on the development of asthma and other allergic diseases. But again, studies suggesting such a protective effect have failed to include a sufficient number of non-vaccinated children in their analyses [141-144]. Furthermore, in most of these studies wP vaccines were used, which might induce a more prominent immunological reaction than aP vaccines, which typically show lower reactogenicity regarding adverse events [217]. We have been able to investigate the effect of acellular vaccine on asthma medication on a large scale. This is important as most of the current pertussis vaccines contain aP vaccine, which has been reported to induce a more Th₂-interleukin-dominated profile than wP vaccines, although no increased risk of developing allergy was shown [218].

The role of infections and immunizations on the immune system is complex and not fully understood. Diseases such as pertussis but also measles, influenza and especially tuberculosis are known to induce a Th₁-deviated immune response [219, 220], which leads to an increased synthesis of interferon γ (INF-γ). INF-γ has been shown to take part in the suppression of a Th₂ immune response [219]. Szabo et al have presented a model in which naive Th cells, via interleukin-12 (IL-12), can develop into either Th₁ or Th₂ cells depending on the presence of interleukin-4 (IL-4). The Th₁-dominated inflammatory environment after certain infections might prevent the development of allergen-specific Th₂ cells and the development of atopic disorders. Moreover, a Th₁-induced cytokine environment could lead naive Th cells toward a Th₁ development by expressing certain transcription factors needed for the differentiation of Th₂ cells or inducing transcription factors for the synthesis of Th₁ cells [221]. However, there are signs that Th₁ and Th₂ responses might be able to coexist simultaneously at different places in the same organism as Mycobacterium bovis, the causative agent of tuberculosis in cattle, strongly inhibits a local Th₂ response in the lung, but not the systemic allergic response [222, 223]. Even the timing of the infection in relation to the infected subject’s age has been discussed as crucial to the suppression of the atopic phenotype. As the neonatal immune system has a
Th\(_2\) overweight which shifts to Th\(_1\) during the infant’s first year of life, it seems that children who develop atopy do not lose their Th\(_2\) response [219]. The inability to produce sufficient amounts of INF-γ as neonates has been suggested as the reason why atopic children do not shift toward a Th\(_1\)-dominated immune response [224]. Following that concept, infectious diseases that induce the synthesis of INF-γ should have the greatest impact on the inhibition of atopic disorders during the immediate postnatal period or early childhood, with infections later in life having no suppressive effect, as suggested by Erb [219]. If the timing of the infection influences the capability to suppress the Th\(_2\) immune response, the timing of the vaccination might also be crucial with regard to its effect on the development of asthma and atopic diseases. A Canadian study of about 12,000 children suggested that a vaccination delay of more than two months reduced the risk of asthma. We were able to compare children vaccinated at 2, 4 and 6 months with children vaccinated at 3, 5 and 12 months, and found that the timing of the vaccination did not affect the outcome. The Canadian study, however, used retrospective data, which could have led to selection bias [225].

The design of our study, with data about exposure and outcome, vaccination status and asthma medication, collected independently of each other and based on registers, minimized recall bias. Access to numerous potential confounding factors from the registers made it possible to adjust for other potential causes of asthma in this age group. To further overcome certain types of confounders, such as parental attitudes to vaccination and health care in general, we performed an ITT analysis. Most previous studies were unable to account for this type of bias as control groups mainly contained children whose parents had decided not to vaccinate their children; and these control groups were very small. This might have influenced the outcome of the studies, as there is reason to believe that this group of individuals differs in more than just attitude to vaccination. This became obvious when we focused on the non-vaccinated children in our study: we saw that dispensed asthma medication was less likely among those born during the vaccination period than among those born outside the vaccination period in the univariate analysis. This difference disappeared after adjusting for multiple confounding factors.

Since misclassification of exposure may reduce the estimated effects in an ITT analysis, we also performed a PP analysis. A true effect of vaccination on asthma medication could otherwise have been somewhat diluted. However, there might still be other factors that may have changed during our study period and that we were unable to measure, e.g. pollen count. However, vaccination with an aP vaccine during the pollen season has been demonstrated not to influence specific IgE for certain outdoor allergens that are prevalent during the immunization season [226]. As the non-vaccinated children born during the vaccination period form a heterogeneous group, the complete impact on our analysis cannot be easily clarified. It is known that the group mainly includes children of parents who chose not to participate and who lived in larger urban areas where the participation rates had been lower than elsewhere [164]. The fact that the dispensation of asthma medication was less likely among children in this group than among non-vaccinated
children born outside the vaccination period, as seen in our unadjusted analysis, emphasizes the importance of the ITT analysis in this population. It underlines the assumption that parental attitudes to vaccination and medication might influence the result. Our inability to control for the different types of non-participation in the trial due to the lack of detailed information is a weakness in our analysis for which we cannot fully compensate. None the less, the unique setting of our study with a large population of vaccinated and non-vaccinated individuals enables us to assess a possible association between immunization and asthma medication in teenagers in such an extensive manner.
7 CONCLUDING REMARKS

We showed an association between degree of immaturity, expressed by gestational age, and dispensed asthma medication as a proxy for asthma in 6–19-year-olds in Sweden. This association was independent of socioeconomic confounders and perinatal mediators. Furthermore, we showed that it was more likely that asthma medication would be dispensed even to children born early term after 37–38 weeks' gestation compared with children born at term.

The clear inverse dose-response relationship of gestational age with dispensed asthma medication shows that every week in the womb is important for the development of the child and its risk of developing diseases such as asthma. Most studies so far have highlighted the part insults play during early fetal development and their causal role in future disease development. As lung development is a continuous process until several years after birth, it is plausible that even infants born almost at term have an increased susceptibility to harmful effects on their lungs, although this had not been shown in such a large population sample earlier.

The follow-up of our birth cohort that was started in 1974 showed a two- to threefold increased likelihood for dispensed asthma medication at the age of 32–34 years for individuals born with an elevated CB-IgE or a positive family history of asthma. CB-IgE has been proposed as a marker for sensitization in utero. As a risk factor it seems to lose its importance with increasing age. Most of the individuals in our birth cohort who had been dispensed asthma medication during 2006–2008 had not been diagnosed as having asthma as a child. Moreover, the proportion of individuals with elevated CB-IgE was higher among children with asthma at 6–7 and 10–11 years of age than among adults with asthma medication. The predictive value of elevated cord blood IgE was poor, even when combined with a family history of asthma. It is not a useful screening marker for adult asthma.

The influence of pre- and postnatal environmental factors is under debate. The genetic susceptibility to disease development probably varies during life in utero and postnatally, as does the impact of different external stimuli. The study of migrating populations can help to differentiate between genetic and various environmental influences on disease development.

We demonstrated the importance of age at migration to Sweden for the need of asthma medication at 6–25 years of age. We were able to assess three different migrating groups originating from regions in which asthma prevalence is usually low. We were further able to show that age at migration to Sweden was a more important factor for the dispensation of asthma medication than population of origin, which further underlines the assumption that environmental factors play an important role in the development of asthma not only during fetal life but also later in childhood.
The importance of infections during early infancy for the development of the immune system has been discussed for a long time. The ‘hygiene hypothesis’ postulated that the lack of infections could lead to an unfavorable development of the immune system, which in turn leads to a higher risk of asthma and allergies.

We studied the potential impact of pertussis vaccination in infancy on dispensed asthma medication at the age of 15 years. In contrast to most previous studies we were able to investigate a large population from a former vaccine trial with about 80,000 vaccinated children and a sufficiently large control group of about 100,000 unvaccinated children.

The unique setting of our investigation was that we were able to compare vaccinated infants with non-vaccinated infants born outside the vaccination trial during a period when general pertussis vaccination was not available in Sweden. We found no statistically significant effect of immunization on dispensed asthma medication in teenagers, regardless of vaccination schedule or vaccine type.

Our study presents evidence that pertussis immunization in early childhood has no significant impact on the development of asthma.

In conclusion, genetic and environmental factors influence the development of asthma disease in the long term. The susceptibility to different insults seems to vary depending on which stage during pre- or postnatal life they occur. Our studies underline above all the importance of different environmental factors and show that the risk of developing asthma seems to be determined by many different factors from life in utero to childhood.
WHAT THIS THESIS SHOWS:

- Every week of gestation is important for lung development and the need of asthma medication at 6–19 years.
- Even infants born early term after 37–38 weeks’ gestation showed a higher rate of dispensed asthma medication compared with children born at term.
- Elevated cord blood immunoglobulin E and a family history of asthma in infancy were associated with a two- to threefold increased likelihood that asthma medication would be dispensed and of allergen-induced self-reported respiratory symptoms at 32–34 years. Their predictive power, however, was poor.
- Age at migration is a more important factor than population of origin for the need of dispensed asthma medication at 6–25 years. The likelihood that asthma medication will be dispensed decreases with increasing age at migration.
- Pertussis immunization in early infancy has no effect on dispensed asthma medication at 15 years of age, regardless of vaccination schedule or vaccine type.
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11 APPENDIX

11.1 Questionnaire Study II

<table>
<thead>
<tr>
<th>Allergi i Östergötland</th>
<th>Kodnr: …..</th>
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</tr>
<tr>
<td>Prövare Olle Zetterström Universitetssjukhuset i Linköping</td>
<td></td>
</tr>
<tr>
<td>Bilaga 2</td>
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</tbody>
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Frågeformulär
Besvara frågorna genom att kryssa i lämpligt alternativ och genom att skriva på den prickade linjen.

Ja  Nej

1 Brukar Du bli andfådd, få pip i bröstet eller hostattacker:
   a) vid ansträngning (  ) (  )
   b) i kyla (  ) (  )
   c) vid ansträngning utomhus i kallt väder (  ) (  )
   d) i dammiga miljöer (  ) (  )
   e) av cigaretter- eller tobaksrök (  ) (  )
   f) av bilavgaser (  ) (  )
   g) av starka dofter (parfym, kryddoft, trycksvärta, rengöringsmedel, etc) (  ) (  )
   h) av pollen från växter som gräs och eller träd (  ) (  )
   i) vid kontakt med pälsdjur (  ) (  )

2 Har Du någon gång reagerat med andningssvårigheter inom 3 timmar efter att Du tagit en värktablett?
   a) Om Ja på fråga 2: Kommer Du ihåg namnet på medicinen? ……………………

3 Har Du nästäppa nästan alltid?

4 Får du nästäppa vid tillfällig exponering för pollen eller pälsdjur?

5 Har du snuva nästa alltid?

6 Får du snuva vid tillfällig exponering för pollen eller pälsdjur?

7 Har du daglig kontakt med något pälsdjur?
   Om Ja på fråga 7: Vilket/vlika djur? ……………………

8 Är Du rökare (inklusive feströkning)?
   a) Om Ja på fråga 8: Ungefär hur många cigaretter röker Du per dag?
      Mindre än 5 (  ) 5 – 14 (  ) 15 – 24 (  ) 25 eller mer (  )
   b) Om Ja på fråga 8: Vid vilken ålder började Du att röka? ……………………

9 Är Du före detta rökare?
   a) Om Ja på fråga 9: Hur många år rökte du? ………………
   b) Om Ja på fråga 9: Hur många cigaretter per dag rökte du? ………………
   c) Om Ja på fråga 9: Vid vilken ålder började Du att röka? ………………

10 Är Du yrkesverksam?
   Om Ja på fråga 10: a) Vilket är ditt nuvarande arbete? ……………………
   b) Hur många år har Du arbetat inom detta yrke? ……………………
11 Har du varit verksam inom något/några andra yrken?

Ja  Nej

11 a) Om Ja på fråga 11 a) Specifiera vilket arbete och antal år i yrket:
Yrke 1………………… Antal år…………
Yrke 2………………… Antal år…………

12 Har Du varit mycket utsatt för damm, gaser eller rök i arbetet?

13 Bodde Du på landsbygden under Dina fem första levnadsår?

Om Ja på fråga 13: Ingick djurhållning i din familj?

14 Hur många gånger/vecka äter Du fisk?   Aldrig (  )    1-2 ggr (  )   Mer än 2ggr (  )

15 Hur många gånger/vecka motionerar Du mer än 30 minuter?
(motionera = springa, cykla, gå snabbt, simma t ex)
   Aldrig (  )    1-2 ggr (  )   Mer än 2ggr (  )

16 Har Du eksem som barn?

17 Har Du någon gång haft ett kliande utslag som kommit och gått i minst 6 månader?

18 Har Du under de senaste 12 månaderna haft handeksem?

19 Är Du allergisk eller överkänslig för Nickel?

20 Har Du haft någon period med nässelutslag (upphöjda, hudfärgade eller blekröda utslag, ofta med röda kanter)?

Jag är  Man (  )   Kvinna (  )

Tack för Din medverkan!
11.2 Map Study IV

Geographic areas of enrolment in Trial II
Study sites Trial I = dots

Participating counties:
23, 5, 12 months schedule;
AB = 01 Stockholm county
C = 03 Uppsala county
D = 04 Södermanland county
E = 05 Östergötland county
F = 06 Jönköping county
G = 07 Kronoberg county
H = 08 Kalmar county
I = 09 Gotland county
K = 10 Blekinge county
L C, N = 11 Kristianstad county C, N
2, 4, 6 months schedule;
L W = 11 Kristianstad county W
M = 12 Malmö community &
Malmöhus county
3, 5, 12 months schedule;
N = 13 Halland county S
O = 14 Göteborg & Bohus county N
R = 16 Skaraborg county
S = 17 Värmland county
T = 18 Örebro county
U = 19 Västmanland county
W = 20 Kopparberg county
X = 21 Gävleborg county
Y = 22 Västernorrland county
Z = 23 Jämtland county
AC = 24 Västerbotten county
BD = 25 Norrbotten county

Non participating counties:
N = 13 Halland county N
O = 14 Göteborg & Bohus county S
P = 15 Älvsborg county