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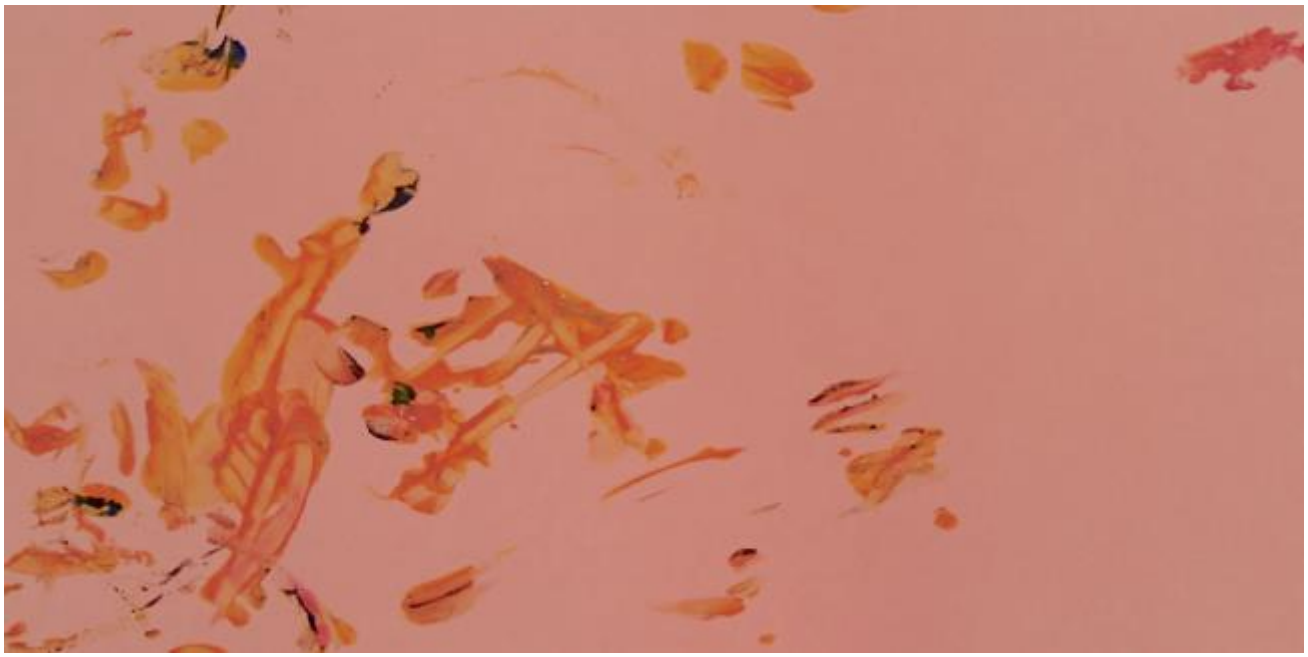

PhD thesis

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Perinatal Risk Factors for Childhood Asthma

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This thesis has been submitted to the Graduate School of Health and Medical Sciences, University of Copenhagen

Herlev og Gentofte Hospital

COPSAC

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II. Stokholm J, Sevelsted A, Anderson UD, Bisgaard H. Preeclampsia Associates with Asthma, Allergy, and Eczema in Childhood. *American Journal of Respiratory Critical Care Medicine*. 2017

III. Stokholm J*, Sevelsted A*, Bønnelykke K, Bisgaard H. Maternal propensity for infections and risk of childhood asthma: a registry-based cohort study. *Lancet Respiratory Medicine*. 2014

IV. Sevelsted A, Pipper CB, Bisgaard H. Stable admission rate for acute asthma in Danish children since 1977. *European Journal of Epidemiology*. 2016

V. Sevelsted A, Stokholm J, Bønnelykke K, Bisgaard H. Cesarean section and chronic immune disorders. *Pediatrics*. 2015

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ABSTRACT

Asthma is a common childhood disease, and the most common cause of medication and hospitalizations of children. The disease is complex and heterogeneous with multiple causes.

The aim of the PhD thesis was to investigate the perinatal programming of childhood asthma based on data from clinical birth cohorts focused on childhood asthma, with replication in the Danish national registries when possible. All studies in the thesis are based on either a combination of COPSAC birth cohort data and National registry data, or entirely on registry.

The Danish population is well registered in our multiple national registries. For the pediatric population of approximately 2 million children there are no alternatives to public hospitals, which means there is complete nationwide coverage. COPSAC2000 is a clinical birth cohort consisting of 411 infants born to mothers with a history of asthma. The major strength of the clinical cohort data is the close clinical follow-up with physician-diagnosed asthma and related diseases.

The thesis consists of the following papers:

I. Risk of Asthma from Cesarean Delivery Depends on Membrane Rupture. Journal of Pediatrics 2016
II. Preeclampsia Associates with Asthma, Allergy, and Eczema in Childhood. American Journal of Respiratory and Critical Care Medicine 2017
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Study I investigates the risk factor cesarean section, where an explorative association in the cohort is further investigated in the registry data where we have power to explore different types of cesarean section, namely sections performed before or after membrane rupture. We find that both types carry a higher risk of offspring asthma compared to vaginally born, but the risk is highest for children born to section before membrane rupture, which could imply microbiome mediated effect on later disease development.

Study II investigates the maternal pregnancy factor, preeclampsia, which we find to be associated to offspring asthma and particularly allergy in the cohorts. The registry data confirm the findings, and shows a higher risk by longer duration of fetal exposure to preeclampsia.

Study III is based on an observation from the birth cohort (although in another manuscript) of an association between maternal use of antibiotics during pregnancy and risk of offspring asthma. Re-investigating this association using the entire Danish population with a new approach to the timing of exposure, we find that the risk factor is unlikely as a causal factor for offspring asthma. Contrary we conclude that maternal use of antibiotics may be seen as a proxy for maternal propensity for infections which appears to be a risk factor for offspring asthma.

Study IV concerns the time trend of childhood asthma. Using the same data source (the Danish National Patient registry) across 35 years we find that the admission rate of school-aged asthma has in fact been stable.

Study V is based on registry data and concerns the comorbidity of asthma and other chronic inflammatory childhood diseases. We investigate the cesarean section and find several of the diseases to share this common early life risk factor. This suggests early life commonality in the origins of these chronic immune disorders.

Together these studies showcase the advantage of using vastly different data sources especially for an outcome as childhood asthma where the clinical cohort have much stronger phenotyping but lack the power compared to registries where outcome uncertainty is leveraged by a large sample size. Overall the studies conclude several perinatal risk factors for childhood asthma, however some risk factors may be interpreted as proxies for maternal factors.

DANSK RESUME

Astma er en almindeligt forekommende inflammatorisk børnesygdom, karakteriseret af tilbagevendende vejrtrækningssymptomer, som debuterer tidligt i livet. Astma er den hyppigste årsag til hospitaliseringer og medicinering af små børn. Der er ingen komplet forståelse af hvorfor astma opstår. Årsagssammenhængene er komplekse og er et samspil mellem gener og miljøfaktorer. Især det tidlige miljø er interessant, da det jo betinger udformningen af immunsystemet.

Formålet med denne PhD var at undersøge forskellige risikofaktorer for astma fra tiden omkring fødslen, baseret på data fra en klinisk fødselskohorte samt registerdata fra hele den danske befolkning. COPSAC2000 er en højriskokohorte af 411 børn født af mødre med astma. Børnene er fulgt tæt siden fødslen med fokus på udviklingen af astma. Danmark er verdenskendt for vores omfattende og veludbyggede registre, som dækker hele befolkningen. Strukturen for flere af studierne er at lave en eksplorativ undersøgelse i fødselskohorten, som derefter replikeres i registerdata.

Afhandlingen består af følgende videnskabelige manuskripter:

I. Risk of Asthma from Cesarean Delivery Depends on Membrane Rupture. Journal of Pediatrics 2016
II. Preeclampsia Associates with Asthma, Allergy, and Eczema in Childhood. American Journal of Respiratory and Critical Care Medicine 2017
III. Maternal propensity for infections and risk of childhood asthma: a registry-based cohort study. Lancet Respiratory Medicine 2014
IV. Stable admission rate for acute asthma in Danish children since 1977. European Journal of Epidemiology 2016
V. Cesarean section and chronic immune disorders. Pediatrics 2015

I det første studie undersøges fødsel ved kejsersnit som en risikofaktor for astma. I kohorten ses en øget risiko for at udvikle astma, hvis man er født ved kejsersnit i forhold til hvis man er født vaginalt. I registerdata udbygges undersøgelsen ved at se på forskellige slags kejsersnit, nemlig kejsersnit udført før eller efter vandet er gået. Vi finder at begge typer øger risikoen for astma, men mest ved kejsersnit før vandafgang. Dette kan tolkes som en effekt gennem barnets første

mikrobielle kolonisering, da kejsersnit før vandafgang implicerer at barnet ikke har haft kontakt med moderens vaginale mikrobiom.

I det andet studie undersøges barnets risiko for astma og andre relaterede allergiske sygdomme efter svangerskabsforgiftning hos moderen i graviditeten. Vi finder at særligt de allergiske sygdomme er associerede med moderens svangerskabsforgiftning. I registerdata ses endvidere at risikoen er større når barnet har været udsat for længere tids eksponering af svangerskabsforgiftning.

Det tredje studie undersøger moderens brug af antibiotika som risikofaktor for udvikling af astma hos barnet. Tidligere er der påvist sammenhænge mellem antibiotika i graviditeten og astma, men i dette studie undersøger vi antibiotikaforbrug ikke kun i graviditeten, men også i perioden før og efter graviditeten. Eksponering specifikt i graviditeten giver ikke højere risiko for astma end hvis moderen bruger antibiotika på andre tidspunkter. Dette fortolker vi således, at moderens generelle forbrug kan ses som et udtryk for moderens infektionstilbøjelighed, og at dette er en risikofaktor for barnet, ikke en specifik antibiotikakur i graviditeten.

Det fjerde studie omhandler ikke en risikofaktor, men ser derimod på hvordan hyppigheden af astma blandt børn i skolealderen har udviklet sig over tid. Ved hjælp af landspatientregisteret undersøges hospitalsindlæggelser for astma blandt alle børn født i Danmark. Vi finder at frekvensen af astma blandt børn i skolealderen faktisk har været stabil over lang tid.

Det sidste studie undersøger samspillet mellem astma og en lang række andre sjældnere inflammatoriske børnesygdomme. Ved at vise at fødsel ved kejsersnit er associeret til ikke bare astma, men også mange andre sygdomme, vises at risikoen for at udvikle immunologiske og inflammatoriske sygdomme grundlægges tidligt i livet. Det er væsentligt for forskningen–og befolkningen, at fastslå at faktorer helt tidligt i livet har betydning for helbredet senere i livet.

ABBREVIATIONS

ATC	Anatomical Therapeutic Chemical classification
CI	Confidence interval
COPSAC	Copenhagen Prospective Studies on Asthma in Childhood
CPR	Personal identification numbers
ICD-8	International Classification of Diseases, 8th edition
ICD-10	International Classification of Diseases, 10th edition
IgE	Immunoglobulin E
WHO	World Health Organization

INTRODUCTION

This section will introduce the topic of the thesis. I will introduce the framework of the perinatal period as a special window of vulnerability which can have a significant impact on health and disease risk in later life. Then I will briefly go through the definitions of asthma, and touch upon childhood asthma as the “canary in the coalmine” for other chronic inflammatory diseases in a population. Finally, the objectives of the thesis are presented.

Perinatal life

Hales and Barker (1992) were the first to suggest that the risk of developing type 2 diabetes may be influenced by factors working in the fetal period. Their “thrifty phenotype hypothesis” is cited almost 3000 times and continues to impact research. Barker's work inspired many, including many epidemiologists and clinicians, and has become the foundation for much research in reproductive epidemiology in the last 10-20 years, not the least in the Nordic Countries.

The perinatal programming suggests that there exists a window in life where the child is specifically vulnerable or susceptible to exposures that may change the health trajectory and risk of developing diseases later in life. The development of the immune system in early life creates a window of heightened susceptibility, where environmental exposures may affect the immature immune system which is prone to alterations (Schaub and Prescott, 2016). Early life exposures to lifestyle factors such as the human microbiome and diet interplay with genetics, epigenetics, and metabolism to influence immune maturation causing a potential trajectory towards diseases.

Changing microbial exposure and diversity remains one of the leading explanations for the increase in rates of many inflammatory diseases (Prescott, 2013). Such phenomena are well documented biologically from animal research (Dostál et al., 1994; Russell et al., 2013), where changing early life microbial exposure and diversity modulates immune programming.

In humans, a tremendous source of the epidemiological evidence on perinatal programming has been from famine studies. Such observational studies include exposures to a famine environment by natural or man-made causes. These natural experiments provide an opportunity to examine long-term outcomes after famine exposures by

comparing exposed and unexposed individuals. One of the most studied famines is the Dutch Hunger Winter caused by the world war II blockade of the Netherlands by the German army (Lumey et al., 2011). The blockade created a short-term extreme famine, which has been extensively used in later research to study the different impacts of famine exposure in either trimester of pregnancy on a wide range of adult outcomes such as obesity, psychiatric diagnoses, and offspring birth weight (Lumey et al., 2011).

Scientific interest for the perinatal period remains high in recent years (Olsen, 2014), and there exist scientific societies dedicated to this sensitive window in development, such as “International Society for Developmental Origins of Health and Disease”. The concept of early programming of disease directs research focus towards the earliest stages of life and the first indication of disease development. Mother-child cohorts contribute to the growing body of literature on perinatal programming, e.g. supplementation of fish oil during pregnancy can help prevent asthma in the offspring (Bisgaard et al., 2016).

So, what are perinatal risk factors? As evident from above any environmental change from the natural life course can constitute as a risk factor, even exposures which are considered normal can be investigated as risk factors. In this thesis, I have dealt with a limited list of perinatal risk factors, namely cesarean section, preeclampsia, and maternal use of antibiotics in the perinatal period.

Cesarean section is the surgical delivery of a child (or multiple children) through incisions in the mother's abdomen and uterus. It is performed when vaginal delivery is deemed unsafe for either child or mother, or it can be performed upon maternal request. As a risk factor, the cesarean section may be interpreted in multiple ways. In itself the section is of course a disruption of the natural life course; the newborn meets the world via a different entrance with a different environment to follow. The procedure could also be interpreted as a surrogate marker for the causes of the section, be they complications during pregnancy or delivery; or the request for the section by the mother as an indicator for a specific personality trait.

Preeclampsia is a potentially dangerous pregnancy complication. It manifests as an aberration of the interaction between placental and maternal tissue with an excessive maternal inflammatory response resulting in higher than usual blood pressure and high levels of protein in the urine. Preeclampsia usually begins after 20 weeks of pregnancy.

The causes of preeclampsia are unknown, but several risk factors are established, including hypertension, diabetes, and family history of preeclampsia (Roberts and Cooper, 2001). For the child, preeclampsia is a risk factor since the treatment of preeclampsia is placental removal, i.e. induced birth and probably lower gestational age than natural life course. Furthermore, the maternal inflammation may have effects in utero.

Antibiotics are used to treat or prevent bacterial infections and work via either killing or inhibiting the growth of bacteria. The general side effect of antibiotics is that some commensal bacteria are killed along with the pathogenic bacteria. Microbes are one of the most relevant and abundant constituents of the human environment, and use of antibiotics causes temporary changes herein. Unlike cesarean section and preeclampsia, antibiotics can be investigated in animal models, and are confirmed here as a risk factor for asthma-related outcomes (Dostál et al., 1994; Russell et al., 2013).

All three factors somehow create a disturbance in the natural life course, and can thereby be classified as potential risk factors. The exact mechanisms these risk factors work through can be difficult to establish and may be multiple. For each, it may be important to spell out the expected mechanisms that are investigated, since different mechanisms may be proven from different models of association.

Canary in the Coalmine

Asthma is defined as a chronic inflammatory disease of the airways with recurrent episodes of lung symptoms, remitting spontaneously or with treatment. Although the clinical presentation has probably changed very little over the centuries (von Mutius and Drazen, 2012), no exact diagnostic criteria for asthma exist. The diagnosis is based on a combination of clinical presentation, anamnesis, measures of lung function, and the exclusion of alternative diagnoses. The measurement of lung function in school-age children and adults is used to support the diagnosis of asthma and to evaluate the need for treatment adjustment (Moth et al., 2010).

Asthma presumably develops from an inappropriate immune response early in life caused by complex interactions between exposures and the host immune system. A large-scale, genome-wide study of asthma showed associations to genes controlling both innate and adaptive immune components (Moffatt et al., 2010), although the genetics as such only explain up to 60% of outcome variation. Alike

other immunological diseases asthma has some interesting features like being more common in western countries. Inflammation is a common feature in asthma and a whole range of other immunological diseases. There is clear indication that the developing immune system is sensitive to modern environmental pressures (Prescott, 2013).

The disease is heterogeneous with varying age at onset and clinical presentation, and many children debut with asthma before the ability to perform standard lung function measurements. Previously pre-school asthma may not have been considered asthma but instead labeled as bronchitis or infections (Whallett and Ayres, 1993), and not until the child reached an age where standard lung function could be measured would the diagnosis asthma come into play. It is useful to divide asthma by age of onset in childhood, i.e. pre-school asthma and school-aged asthma. Importantly the pre-school asthma is common, and since diagnosis cannot be based on standard lung function it must rely on symptoms. Symptoms are coughing, breathlessness and wheezy breathing. Such symptoms are likely initiated by some airway infections, and asthmatic children are more prone to infections and are more impacted by these infections (Bønnelykke et al., 2015; Carlson et al., 2015). The disease is often coined as chronic however many children with pre-school asthma do in fact outgrow symptoms.

Thus, asthma is an early-onset and common immunological disease. It is well researched in both registry studies, cross-sectional studies, and clinical studies including longitudinal mother-child cohorts such as COPSAC's two birth cohorts. However, other chronic inflammatory diseases with later onset and lower prevalence are less investigated and the interplay between risk factors are equally underinvestigated. This is due to the fact that late onset and rare diseases make long-term clinical follow-up most cumbersome and expensive.

The presence of asthma may be considered an early indicator, or "the canary in the coalmine" for an overall increased risk for chronic inflammatory diseases in a population. Asthma may thus serve as a surrogate marker in the path from exposures in pregnancy and early life through childhood to the development of other chronic inflammatory diseases later in life. Understanding the developmental origins of asthma may, therefore, help understanding other chronic inflammatory diseases, and potentially lead to common, far wider reaching, intervention strategies. Even risk factors that may not be preventable are of interest to study since the research outcome may

provide overall disease understanding and point at relevant targets for mitigating mistakes in early life.

In the next section I will present the objectives of the thesis, and thereafter in the methods section present the data used, and the statistical methods.

OBJECTIVES

The objectives of this PhD thesis were to investigate perinatal risk factors for childhood asthma using clinical birth cohort data and national registry data.

The studies fall into two categories. Three studies (I, II and to some extent III) with specific investigations on individual perinatal risk factors, namely cesarean section, maternal preeclampsia, and maternal use of antibiotics, using both cohort and registry data. The other category comprises the remaining studies, which are broader epidemiological studies on overall trends of asthma (IV) and comorbidity between asthma and other chronic inflammatory diseases through a shared early life risk factor (V) using solely registry data.

METHODS AND DATA

This section presents the methods and data used for the five papers included in the thesis. The five papers can be categorized by two overall methods. The first studies share the methodology of combining data from the clinical cohorts with nationwide registry data. The idea is to have discovery and replication. Furthermore, the combination of data from the clinical cohort and nationwide registry data takes advantage of the respective benefits with deep phenotyping of asthma and other outcomes in the cohort data versus the statistical power and nationwide coverage of the pediatric population in the registry data.

In paper I and II an exploratory analysis is conducted in the clinical cohort, and secondly, replication is sought in the national registries. In paper III the major background for the study was another study based on cohort data, but the current study only uses registry data.

The two remaining studies, paper IV, and V, have more broad scopes related to nationwide time trends of school-aged asthma hospitalizations, and to exemplify the comorbidity of several chronic inflammatory diseases via showing a common perinatal risk factor. These studies are based solely on registry data.

The structure of this section is first to present the data sources with a section on the clinical COPSAC cohorts, their aim and relevant measurements. Thereafter a section introducing the Danish national registries; how study populations are defined and importantly how asthma can be defined from registries. After this, a section on the statistical methods describes how the data are analyzed and introduces the statistical methods. In the end, the ethics for the studies are stated before the Results section goes through the findings from the five studies.

COPSAC cohorts

COPSAC is an acronym for Copenhagen Prospective Studies on Asthma in Children. Two birth cohorts exist, COPSAC2000 and COPSAC2010, named for approximate timing of recruitment during 1998-2001 and 2008-2010 respectively.

The main difference between the two cohorts is the inclusion criteria. COPSAC2000 is a high-risk cohort of children born to asthmatic mothers, whereas COPSAC2010 is a population-based cohort.

In this thesis, for paper I and II data from the COPSAC2000 cohort is used. Paper III does not directly include cohort data, but the major background article for the study was an investigation also on the COPSAC2000 cohort. Therefore, I will only go into details regarding this cohort, although there are major overlaps in design and clinical follow-up of the two cohorts.

COPSAC2000 is an ongoing single-center prospective clinical birth cohort study of 411 children born to mothers with a history of asthma (Bisgaard, 2004). It is designed to examine the relation between the genetics, environmental and lifestyle factors and development of asthma, eczema and allergic symptoms in early life. Subjects participating in COPSAC were recruited between August 1998 and December 2001 among pregnant mothers with a history of asthma diagnosed by a doctor and requiring medication. Exclusion criteria were prematurity (less than 36 weeks of gestation) or severe congenital malformations. 411 children of asthmatic mothers were included in a comprehensive program of clinical and objective assessments with prospective data collection at visits to the clinical research unit every 6 months, as well as at acute symptomatic episodes.

At inclusion, the parents conduct a personal interview with the research doctors. Here information regarding maternal antibiotics use in the third trimester, preeclampsia, and delivery mode is collected.

Children are diagnosed and treated for acute respiratory and skin symptoms by the doctors in the research unit following predefined algorithms. Asthma was diagnosed when a child fulfilled all of the following four criteria: (1) recurrent wheeze, defined by troublesome lower lung symptoms with a burden of greater than or equal to five episodes of greater than or equal to 3 consecutive days within 6 months with symptoms captured by daily diary cards filled from birth by the parents; (2) symptoms judged by the clinic pediatricians to be typical of asthma, e.g., exercise-induced symptoms, prolonged nocturnal cough, recurrent cough outside common cold, or symptoms causing wakening at night; (3) in need of intermittent rescue use of inhaled beta-2-agonist; and (4) improvement of symptoms during a 3-month trial of anti-asthmatic inhalation corticosteroids and relapse after end of treatment.

Diagnosis of eczema was based on the criteria of Hanifin and Rajka (1980), requiring the presence of three of four major criteria and at least 3 of 23 minor criteria (Halkjaer et al., 2006).

Allergic sensitization was based on allergen-specific IgE levels measured in blood, and skin prick test. Both measurements were performed at ages 0.5, 1.5, 4, and 6 years for 16 common inhalant and food allergens[1] by ImmunoCAP assay (Pharmacia Diagnostics AB, Uppsala, Sweden) for IgE and ALK-Abello (Copenhagen, Denmark) for skin prick test. For IgE levels, values of specific IgE greater than or equal to 0.35 kU/L were considered indicative of allergic sensitization and was analyzed as dichotomized values for (1) any allergens, (2) food allergens, and (3) inhaled allergens. Total-IgE levels were measured with a detection limit of 2 kU/L (Wickman et al., 2003). For skin prick tests a mean wheal diameter of 2 mm or larger than that elicited by the negative control at 0.5 and 1.5 years, and of 3 mm or larger at 4 and 6 years was considered indicative of sensitization.

Allergic rhinitis was based on relevant aeroallergen sensitization and clinical interviews of the parents on a history of significant nasal congestion, sneezing, and/or a runny nose outside periods with the common cold (Chawes et al., 2009).

The extensive objective assessments and detailed clinical phenotyping of asthma and related diseases with prospective data collection at visits to the clinical research unit every 6 months, as well as at acute symptomatic episodes has proven a unique strength. This minimizes the risk of misclassification of symptoms and diagnostic variation due to local diagnostic tradition. The objective of this approach is to minimize variability in the clinical data and characterize specific clinical features associated with underlying endotypes.

Danish National Registry data

Unique personal identification numbers (CPR) have been assigned to all persons with permanent residence in Denmark since 1968. These identification numbers are used in all contacts with the healthcare system, and in all population and health registers, thus making it possible to link records on individuals electronically across registers using the CPR number.

Several registries are used in this thesis. I will introduce the most important registries for the thesis, that is the Medical Birth Registry,

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[1] cat, dog, horse, birch, timothy grass, mugwort, house dust mites, molds, hen's egg, cow's milk, cod, wheat, peanut, soybean, and shrimp.

the Patient Registry, and the Prescription Registry. I will shortly mention data from other registries and finally, I will detail how asthma can be defined before the section on statistics.

The Danish Medical Birth Registry was an independent register collecting data from midwives from 1973 until 1997 where it became part of the National Patient Registry (described below). The register is often used in research while its primary purpose is to monitor the health of newborns and the quality of antenatal care and delivery care services. The registry contains information on all births in Denmark (at home or at hospitals) by women who at the time of birth are registered in the Danish Civil Registration System and comprise information pertaining to the birth, the birth outcome, parental marital status and complications, and interventions during delivery (Langhoff-Roos et al., 2014; Knudsen and Olsen, 1998).

There are multiple changes over time in how detailed each variable is collected. For instance, is gestational age from 1973 until 1977 registered as term, or number of weeks preterm. There is no specific definition of term, but from the data it appears to be at least 36 full weeks. From 1978 until 1996 the gestational age is registered in weeks, and since 1997 in days.

Cesarean section is also registered in various ways over time. In the earliest time period, there are no distinction between types of section, only an overall registration of any section (1973-1977), and in other periods it is registered as elective versus emergency, or in others as performed during delivery or before delivery, and only since 1997 as defined by operation coding. Investigations of trends over time reveal that some periods may contain faulty or erroneous data when trends are not aligning well with surrounding periods, and therefore the specification of types of cesarean section is difficult to investigate over longer periods.

In this thesis, I have categorized all variables used, in part to account for the variations over time. Still, some variables are only available during certain periods, and therefore the use of these in the studies limits the potential population (data used in this thesis are until 2012). Table 1 indicates categorizations and data limits on data from the Medical Birth Registry.

Table 1. Categorizations and data limits on data from the Medical Birth Registry.

Variable	Categories	Period
Birth weight	below 2.5 kg; 2.5-3.0 kg; 3.0-3.5 kg; 3.5-4.0 kg; and above 4.0 kg.	1973-2012
Gestational age	less than 37 weeks; 37-39 weeks; 40-41 weeks; 42 or more	1978-2012
Maternal age	<20; 20-25; 25-30; 30-35; 35-40; >40 years	1973-2012
Mode of delivery	cesarean section; vaginal	1973-2012
Type of cesarean section	emergency before delivery; emergency during (delivery complications); emergency during (pregnancy complications); planned section	1997-2012
Child gender	male; female	1973-2012
Multiple birth	yes; no	1973-2012
Parity	first child; second child; third or more child	1973-2012
Season of birth	spring; summer; autumn; winter	1973-2012
Maternal smoking during pregnancy	yes; no	1997-2012

The Danish National Patient Registry, a key health register, was established in 1977 as a monitoring instrument for hospital activities, and since 2000 it has also functioned as the basis of government reimbursement. In the first ten years, the register was stable with no changes. Since the 1990es changes have been made yearly. The most important changes are the gradual expansion of the register (Lynge et al., 2011).

The register contains all hospitalization admission dates, discharge dates, operation codes, and diagnoses on all patients discharged from all hospitals in Denmark. Inpatients in somatic wards have been registered since 1977, and since 1994 outpatient admissions are included. The diagnosis coding is based on the 10th revision of the "International Classification of Diseases and Related Health Problems" (ICD-10) since 1994 and before that on the 8th revision (ICD-8).

Data can be categorized as administrative and clinical. Administrative data include patients' CPR numbers and municipality and region of residence, hospital and department codes, admission type (acute or nonacute), patient contact type (inpatient, outpatient, or emergency department), referral information, contact reason, and dates of admission and discharge. Clinical data comprise diagnosis. Diagnoses associated with each hospital contact are registered as one primary diagnosis and, when relevant, secondary diagnoses. The primary diagnosis is the main reason for the hospital contact. Secondary diagnoses supplement the primary diagnosis by identifying other relevant diseases related to the current hospital contact, e.g. underlying chronic diseases. In addition to primary and secondary diagnoses, the registry records referral, temporary, procedure-related, and supplementary diagnoses. The discharging physician registers all diagnoses at the time of hospital discharge or at the end of an outpatient contact.

The Danish National Patient Registry is considered unique and of very high quality (Andersen et al., 1999). For the pediatric population, there are no alternatives to public hospitals. The registrations are compulsory and necessary for government reimbursement, which results in complete nationwide coverage.

After a description of the Prescription Registry, I will detail how outcomes are defined with data from the Patient Registry.

The Danish National Prescription Registry has since 1994 registered information on all prescription drugs sold in Denmark. The registry is

internationally unique with data on individual-level dispensed prescriptions for an entire nation. The register was established by law in order to track drug price indexes and compile statistics for the authorities (Wallach-Kildemoes et al., 2011).

The register contains information on all prescription drugs dispensed at Danish community pharmacies. There are data on the drug user, the prescriber, the pharmacy, and the date, dose, amount, and anatomical therapeutic chemical classification (ATC) code of the dispensed drug. Before 1996 children under age 16 had drugs dispensed for their mother. Since 1996 drugs for children have been dispensed in the child's own CPR number (Wallach-Kildemoes et al., 2011). The only prescription drugs that are not registered, are drugs given at the hospitals. Such drugs are not registered on an individual level. In this thesis, I have had access to data from the Prescription Registry for the period 1997-2010 and limited to the ATC codes for anti-asthmatic drugs and antibiotics.

Almost all data used in the thesis stem from the Patient Registry, Medical Birth Registry, and the Prescription Registry. But a few other variables from other registries are of importance.

From the Central Person Registry information on vital status – i.e. whether the person is alive and resident in Denmark, disappeared, emigrated or deceased is used along with a date of these events (Pedersen, 2011). From Statistics Denmark, I have data on maternal occupation which is used as a socioeconomic confounder in paper I and III (Petersson et al., 2011).

Definitions of asthma depend on the period of investigation. Paper I and paper III are limited to registry data for 1997-2010 from their respective exposure variables, and asthma is defined by three definitions using either Patient Registry data or Prescription Registry data. Paper II, IV and V use registry data for 1977(8)-2012, and outcomes are defined only from Patient Registry data. For paper I and III the three definitions of asthma for 1997-2010 are as follows:

- Inpatient admissions. At least two separate (separated in time by minimum 2 months) inpatient admissions to hospital with asthma[2] as the primary diagnosis

[2] Asthma codes: ICD-8 439; ICD-10 J45 and J46

- Outpatient admission. Minimum 1 year of outpatient attendance with asthma as the primary diagnosis.
- Anti-asthmatic inhalation corticosteroids[3]. Minimum 200 days of WHO defined daily doses collected at a pharmacy.

From definition, these asthma categories could comprise asthma severity. Figure 1 shows the overlap of the three asthma definitions. Only 5% of children with at least one of the asthma definitions fulfill all three. 19% fulfill at least two criteria, and more than 75% fulfill only one. The lack of a better overlap leads us to investigate the three groups individually as specific asthma phenotypes (Hansen et al., 2012).

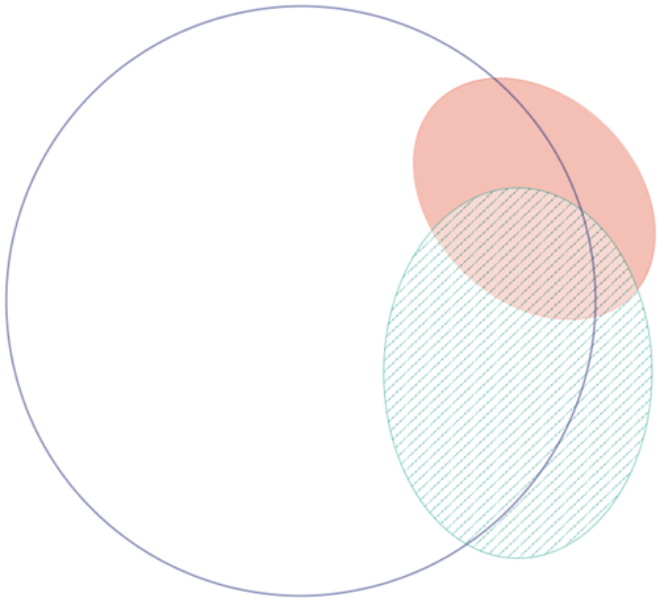

Figure 1. Overlap of asthma definitions. Red fill, minimum 2 in-patient admissions (14% of all); green texture, minimum 1 year outpatient treatment (25% of all); blue outline, minimum 200 defined daily doses anti-asthmatic inhalation corticosteroids collected (89% of all).

[3] ATC codes: R03BA01, R03BA02, R03BA05, and R03BA07

Paper IV on time trends of asthma only concerns in-patient admission with the primary diagnosis of asthma for the full length of the Patient Registry, 1977-2012. In paper II and V, asthma is investigated alongside a range of other diseases. In these papers we use registry data from 1977-2012 and define children as cases at first admission (in- or outpatient) with the defined diagnoses[4] as either primary or secondary. Whenever the maternal disease is adjusted for as a confounder we use a maximum inclusion definition as any hospitalization with the disease in question registered as any type of diagnosis.

Statistical analyses

This section details the statistical methods. I will focus on detailing the methods used on the registry data. Although each study is different, most of the methods are alike. For each study, a definition of population and time frame of observation forms the basis. The population under investigation is always the entire pediatric population consisting of all children born in Denmark limited by birth years. Limitations are based on which data are used[5], and whether these data

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[4] Diagnosis codes in ICD-8 and ICD-10 for the diseases are as follows, where x indicates all subgroups.
Asthma. ICD-8: 439xx; ICD-10: J45.x and J46.x
Eczema. ICD-8: 69100; ICD-10: L20.x
Allergic rhinitis. ICD-8: 50703, 50708, 50709; ICD-10: J30.x
Food allergy. ICD-8: 56102; ICD-10: K52.2
Systemic connective tissue disorder. ICD-8: 714xx, 715xx, 716xx, 734xx, 446xx, 71899, 69549, 73419; ICD-10: M30, M31, M32, M33, M34, M35, M36
Juvenile arthritis. ICD-8: 712xx; ICD-10: M05.x, M08x, M09x, M13.x
Inflammatory bowel diseases. ICD-8: 563xx; ICD-10: K50.x, K51.x
Diabetes type 1. ICD-8: 24906, 24907, 24908, 24909, 25000, 25006, 25007, 25009; ICD-10: E10x, E13x, E14x
Immune deficiencies. ICD-8: 275xx, 288xx; ICD-10: D80–D89
Psoriasis. ICD-8: 696xx; ICD-10: L40.x
Celiac disease. ICD-8: 26900; ICD-10: K90.0
Leukemia. ICD-8: 204xx, 205xx, 206xx; 207xx; ICD-10: C91.x–C96.x
Fractured forearm or elbow. ICD-8: 813xx; ICD-10: S52.x

[5] For administrative reasons, I only have access to data on Danish people born since 1952. This limits the cohort whenever maternal data are used to children born to mothers from 1952 onwards.

are registered only in certain periods. For paper I and paper III where data from the Prescription Registry are included the population is limited to children born 1997-2010. For the remaining studies, we use children born 1977-2012, with minor alterations. For each study, the population may have further inclusion and exclusion criteria, mainly exclusion for missing data or exclusion based on gestational age or birth weight in the studies where prematurity is a principal confounder of the main association. For all studies, there are several sensitivity analyses performed, some of these may include limiting the study population, e.g. to all term children, or to children in specific ages. Children included in the population are then observed for a specified age span, e.g. from birth to a specific age, or (if applicable) the event of death, or the event of outcome, or emigration, or a final follow-up date (either 2010 for studies with prescription data or else 2012).

All studies in this thesis are analyzed with regression models investigating a clinical outcome (mainly asthma) as an effect of chosen predictors.

In the clinical cohort data outcomes such as asthma, asthma exacerbations and eczema are defined by a start date which facilitates survival analyses. Alternatively, other generalized linear regression models are used namely logistic regression and linear regression.

Registry data are summarized with the pyrsstep macro developed by Rostgaard (2008). This macro helps to stratify of individual follow-up time by age, calendar period and other variables, and subsequent aggregation of follow-up time and events over individuals within these strata. The resulting event time tables can be analyzed with Poisson regression of the events offset by the natural logarithm of the person time of observation adjusted for (categorical) confounders and the time-varying factors (calendar year, and child's age). Adjustment for the child's attained age as a categorical factor allows the resulting incidence rate ratios from the offset Poisson regression model to be interpreted as Hazard Ratios from a semi-parametric model (Cox regression) with free baseline hazard. Furthermore, when adjusting the models for the categorical calendar year, the models can be adjusted for underlying time trends in data. This is especially important in analyses over longer time periods like 1977-2012 that include outpatient admissions as cases, since there are huge leaps in numbers of cases.

In all studies, we chose confounders a priori as recommended by Brookhart et al. (2010). In neither of the studies do we impute any data,

instead we exclude observations with missing data. The majority of registry analyses are performed in SAS versions 9.3 and 9.4. Cohort analyses are mainly performed in R versions 3.0.1-3.3.3.

Ethics

The COPSAC2000 study was conducted in accordance with the Declaration of Helsinki and was approved by the Copenhagen Ethics Committee (KF 01-289/96) and the Danish Data Protection Agency (2008-41-1754).

The registry study was based on existing data in national registries and was approved by the Danish Data Protection Agency (J.no. 2012-41-0388). Subjects were not contacted as a part of the study hence the ethics committee did not require written informed consent.

SUMMARY OF RESULTS

In this section, I will go through the results from the five studies. Each paper–including supplemental material–is included at the end of the thesis. In this section I will not detail methods or list all results, but instead introduce the objectives and summarize the main findings and the relation with recent international research when relevant.

Paper I

Risk of Asthma from Cesarean Delivery Depends on Membrane Rupture

Paper I investigates cesarean section as a risk factor for asthma in the offspring and focuses on differences in risk by different types of cesarean section from the perspective of microbiome differences due to lack of vaginal microflora contamination when sections are performed before membrane rupture.

Initially, an overall association between any section and asthma is reported in the high-risk COPSAC2000 cohort. Delivery mode is dichotomized with 22 percent cesarean deliveries (87 of 411 children) and asthma is diagnosed prospectively by the research doctors using strict and standardized criteria of recurrent respiratory symptoms. We found an increased risk of developing asthma within the first seven years of life for children delivered by cesarean section compared to vaginally delivered children. Figure 2 shows the Kaplan-Meier curve for developing asthma in the COPSAC2000 cohort stratified by mode of delivery.

In an adjusted and stratified Cox regression model, cesarean section is confirmed as a risk factor with hazard ratio of 2.18 (95% confidence interval 1.27-3.73). The model was adjusted for the child's birth weight, gestational age, maternal age, smoking and education level, multiple birth; and stratified by parity and the child's gender since these covariates violated the proportional hazards assumption.

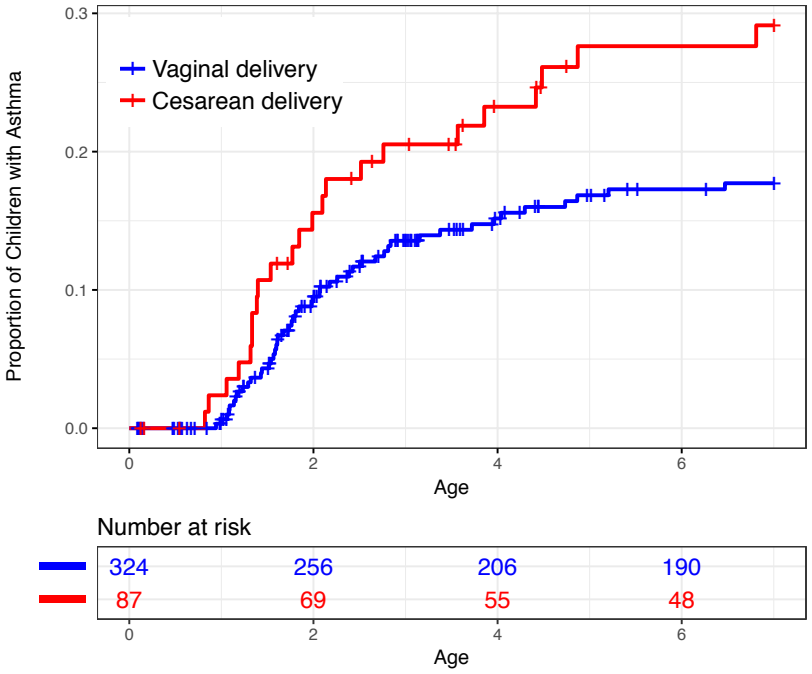

Figure 2. Risk of developing asthma by cesarean delivery in the COPSAC2000 cohort.

For the replication in nationwide registry data we used data from 1997-2010, where we have available data on filled prescription medication as well as hospitalization data (all registered with ICD-10 coding). Furthermore, cesarean section has been reported with the same coding throughout this period. Children are followed in the registry from birth to censoring (death, emigration, or december 31st 2010) or asthma. In the primary analyses, we use the asthma definition of long-term recurrent use of inhaled anti-asthmatic corticosteroids, and the overall association between any cesarean section and asthma is confirmed, although with smaller effect size than in the high-risk cohort, incidence rate ratio 1.16 (95% CI: 1.13-1.19). As described in the methods section the Poisson regression models on asthma events are offset by the natural logarithm of person-years of observation which makes the resulting incidence rate ratios comparable to hazard ratios from Cox regression. From different adjustment models, it is

clear that the most important effect modifiers are birth weight and gestational age.

After confirmation of an overall association, the cesarean section is divided into two subgroups. There are four different types of cesarean section registered; planned cesarean section (B), emergency before delivery (D), emergency section during delivery due to delivery complications (E), and emergency during delivery due to pregnancy complications (A). These four different types of section are grouped by whether or not there has been membrane rupture, ie. planned section is grouped with emergency section performed before delivery; and the two remaining types of emergency sections are grouped together based on being performed after membrane rupture. Figure 3 illustrates mean and standard deviation of birth weight and gestational age by the four different sections.

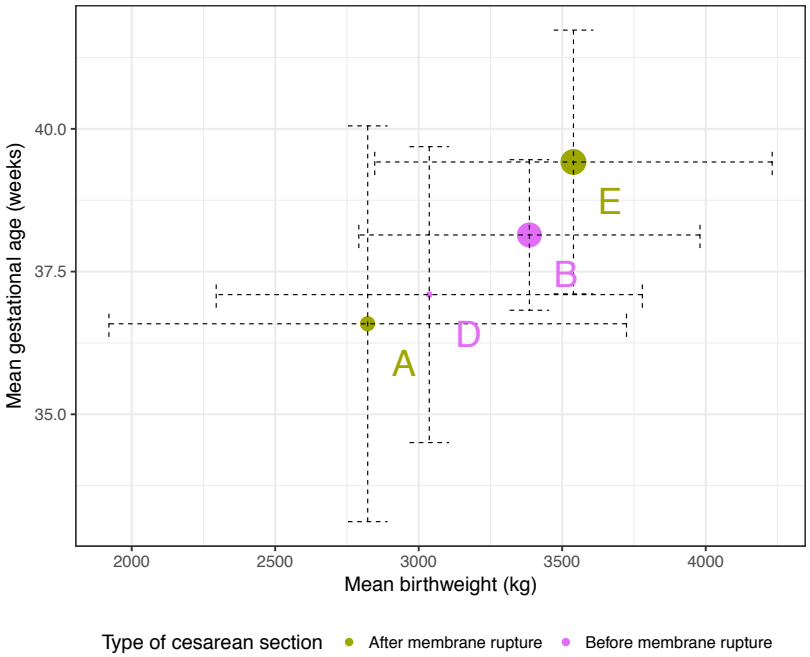

Figure 3. The four operational coding categories of cesarean section are plotted with mean birth weight and mean gestational age. Error bars denote standard deviations, and size of dot denote number of children.

Vaginally delivered children have similar distribution to the common emergency section, i.e. section during delivery due to delivery complications (E). Sections are colored by our defined grouping. It is evident that the grouping merges quite different section types together, especially the two section types performed after membrane rupture differ in respect to birth weight and gestational age distribution. Hence, it is a crude division we have made to investigate the effect of membrane rupture.

In analyses of associations between type of cesarean section and asthma, we used Poisson regression models offset by the log-transformed person years of observation. We did three adjustment models: (1) model adjusted for the timing variables, attained age in one-year groups and calendar year in one-year groups; (2) including sex, parity, multiple birth, maternal factors (age, smoking during pregnancy, usage of antibiotics during pregnancy, employment status, asthma [ever prescription of steroid or ever admission for asthma]); (3) adding birth weight and gestational age. All confounders were chosen a priori. Figure 4 illustrates the incidence rate ratios for childhood asthma by the two types of cesarean section, each compared to vaginal delivery, in the three different adjustment models.

We found that both groups of cesarean sections carry an increased risk of asthma in the offspring, but the sections performed before membrane rupture carry a larger risk in all three adjustment models. As expected in epidemiological studies, the associations are reduced by confounder adjustment, but interestingly the biggest drop from one confounder model to the next in incidence rate ratio happens at inclusion of different confounders for the two categories of section. For cesarean section performed after membrane rupture, the biggest change in estimate occurs after inclusion of parity, sex, multiple birth and a range of maternal factors (age, pregnancy smoking, employment, antibiotics usage, asthma); whereas for cesarean sections performed before membrane rupture the change in estimate occurs after inclusion of birth weight and gestational age. It is un-trivial to determine if gestational age is a true confounder or we observe collider-stratification bias when gestational age is affected by both mode of delivery and other factors (Wilcox et al., 2011). The pattern persisted in the subgroup of children with birth weight over 2.5 kg. We interpret this difference as a validation of the relevance of our cesarean grouping.

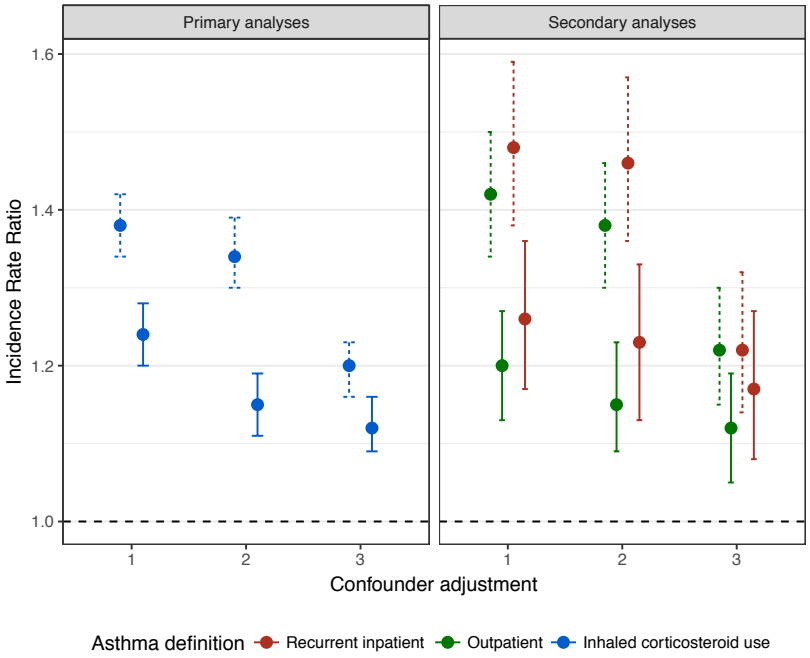

Figure 4. Incidence rate ratios for asthma by type of cesarean section, dotted lines denote section before membrane rupture; solid lines denote section after membrane rupture. Each is compared to vaginal delivery from the three confounder adjustment models. (1) age and calendar year; (2) adding: sex, parity, multiple birth, and maternal factors; (3) adding: birth weight and gestational age. In the primary analyses asthma is defined by long term use of inhaled corticosteroids, in secondary analyses asthma is defined by hospitalizations, red are recurrent inpatient admissions, green are long term outpatient attendance.

The findings were confirmed in several sensitivity analyses, where asthma was defined in alternative ways, namely by long-term outpatient attendance and recurrent inpatient admissions (see second panel in Figure 4). Interestingly, although the other definitions of asthma have far less cases, the associations to cesarean section were in fact stronger. This implies more specific phenotypes based on admissions (Bønnelykke et al., 2014). School-aged asthma was also investigated with only children older than 5 years included, here the

association remained, but somewhat diminished, possibly due to lack of power.

Our findings are comparable to multiple other studies reporting associations between mode of delivery and asthma, both in other clinical cohorts (Magnus et al., 2011; Robson et al., 2015), and registry data (Håkanssons and Källen, 2003), and meta-analyses (Bager et al., 2008).

When it comes to investigating differences between types of cesarean section the general picture is less clear. Mostly because there are multiple ways to define sections and inherent confounders which may be difficult to adjust for. Cesarean section, especially the emergency section happens for a reason, and often this reason could easily be the true cause of increased asthma risk. Lack of agreement between different studies could likely be explained by different setups and different adjustments, e.g. when Almqvist et al. (2012) do not exclude premature children.

A very recent Danish study by Brix et al. (2017), also based on registry data, investigated the effect of emergency section in twins, where the first twin was delivered vaginally and the second twin in emergency section. The situation is rare, hence the study was underpowered and they found no statistically significant differences in risk of asthma. However, the estimates pointed towards a higher risk for the vaginally delivered twin. This study plays well with the findings in our study of the cesarean section after membrane rupture had the biggest drop in estimate after adjustment for maternal factors. The findings in Brix et al. (2017) in fact indicate that the remaining association could be residual confounding.

We interpreted our findings according to the stated hypothesis of microbiome differences in initial colonization of the newborn depending on membrane rupture. When membranes are ruptured the newborn is more likely to be exposed to vaginal microbiota than if membranes are not ruptured. This first encounter could form the basis of the newborn's initial microbiome colonizations, and potential deviations could pose a risk. Apart from the anticipated microbiome differences it could be noted that sections performed before membrane rupture also encaptures births that have been artificially induced. Another explanation could therefore be lack of newborn maturity when the birth is pushed forward. This plays well with the fact that we find the biggest drop in estimate from including birth weight and gestational age. This theory is touched upon by Thysen et al.

(2015) where immune cell maturation is found to be bypassed in prelabor cesarean section.

Paper I including the online supplement can be found at the end of the thesis in the section Papers.

Paper II

Preeclampsia Associates with Asthma, Allergy, and Eczema in Childhood

Preeclampsia is an unusual increase in systemic inflammation during pregnancy. Paper II investigates this maternal pregnancy factor as a risk factor of asthma, allergy, and eczema in the offspring since the fetal immune system is exposed to the excessive maternal inflammation while evolving. This prenatal inflammation exposure in preeclampsia is suspected to be harmful to the developing child and responsible for initiating a skewed trajectory toward disease.

Paper II is primarily based on data from COPSAC2000, where there are multiple clinically diagnosed and objectively measured endpoints. Among 411 children in COPSAC2000 23 (5.6%) were exposed to preeclampsia. We investigated associations between preeclampsia and various outcomes at age 7: asthma, current corticosteroid treatment, eczema, and allergic rhinitis. For allergic sensitization, we used all measures of sensitization collected throughout childhood, and for lung function we investigated both infant lung function and lung function at 7 years of age. Overall, we found a general signal of increased risk of especially the allergic outcomes, diagnosed allergic rhinitis, and objectively measured specific IgE and skin prick tests at the four time points in childhood.

To replicate these findings, we used registry data from 1978-2012. Since there are no registrations of preeclampsia in the birth registry, we extracted preeclampsia from the maternal records of primary or secondary diagnosis of preeclampsia in the Patient Registry during pregnancy week 20 until two weeks after birth. Thereby we only investigate preeclampsia where the mother has been in contact with a hospital. To divide preeclampsia by severity we categorized duration of preeclampsia fetal exposure (date of preeclampsia registration minus date of birth) in less or more than two weeks exposure.

Children followed in ages 0-16 years for any in- or outpatient admission, with a primary or secondary diagnosis of asthma, allergic

rhinitis, eczema or food allergy. These diseases are primarily dealt with in the primary sector, and therefore it is a selected subgroup of cases who are hospitalized. However, this bias is expected to be non-systematic. All analyses are adjusted for timing variables age and calendar year, sex, parity, birth weight, gestational age, season of birth, mode of delivery, maternal age, and maternal disease.

Figure 5 illustrates the confounder adjusted incidence rate ratio for the selected diseases by preeclampsia (any, less than 14 days duration, 14 or more days of duration).

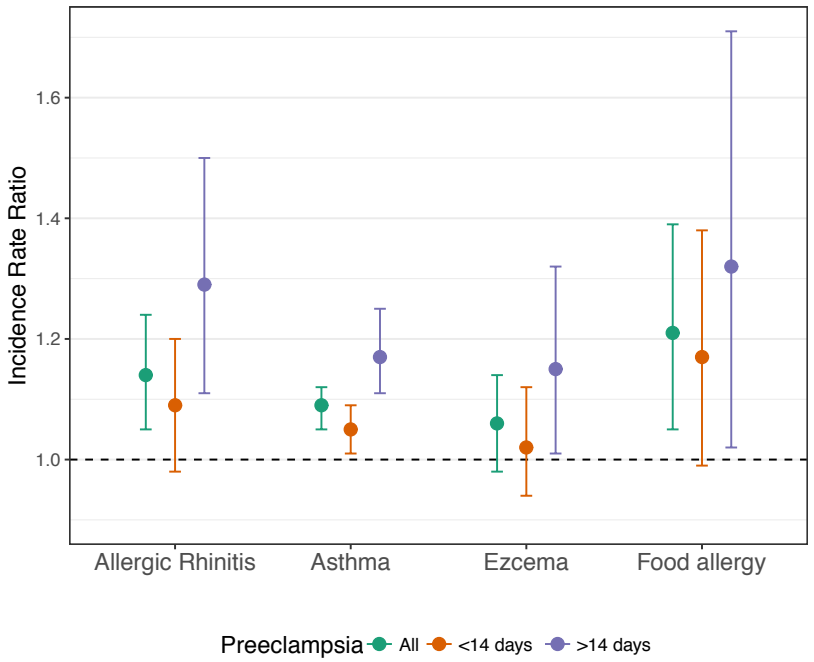

Figure 5. Incidence rate ratios for allergic rhinitis, asthma, eczema, and food allergy by maternal preeclampsia, any and stratified by duration of fetal exposure. All estimates are adjusted for age, calendar year, sex, parity, birth weight, gestational age, season of birth, mode of delivery, maternal age, and maternal disease.

Together with cohort data, we found preeclampsia to be a risk factor for asthma and especially allergic rhinitis and food allergy. From the registry data, we see a clear tendency towards higher risk by longer duration of exposure to preeclampsia. With preeclampsia being

an uncommon condition, and the registry studied outcomes also being uncommon due to the fact that hospitalizations for these conditions are rare, we find that the population attributable risk fractions for the investigated childhood diseases are low, ranging from 0.1 to 0.8% for preeclampsia. Thus, clinically a less important risk factor.

Preeclampsia is a relatively understudied exposure phenomenon, but there are a few studies investigating its association with childhood asthma and allergies. Concurrently with our study, another study based on Danish registry was published. Similar to us they found an increased risk of asthma especially for the longer duration of preeclampsia (Lui et al., 2015). A Norwegian study found similar results (Magnus et al., 2016). Only one study investigated allergy, Byberg et al. (2014) found positive associations to allergy, but not to asthma in a relative small case-control study.

In summary, preeclampsia exemplifies a shared prenatal risk factor for asthma and allergy in childhood. The duration of preeclampsia increases the risk, suggesting a mechanism of in utero inflammation causing a lasting immune deregulation in the fetus.

Paper II including the online supplement can be found at the end of the thesis in the section Papers.

Paper III

Maternal Propensity for Infections and Risk of Childhood Asthma: a Registry-based Cohort Study

Although published in another paper (Stensballe et al., 2012), paper III is also based on an observation from the birth cohort. Stensballe et al. (2012) reported an association between maternal use of antibiotics during pregnancy and a risk of offspring asthma. The effect was speculated to be caused by derangements of the neonatal microbiome, or inflammation due to the pregnancy infection, or an adverse effect of the antibiotic drug.

The aim of paper III was to rethink this association, and via epidemiological methods reconsider the possible causal link between maternal pregnancy antibiotics and offspring asthma. If the association is causal, we would expect to find a strong association between pregnancy antibiotic use and a less strong association to maternal antibiotic use outside pregnancy.

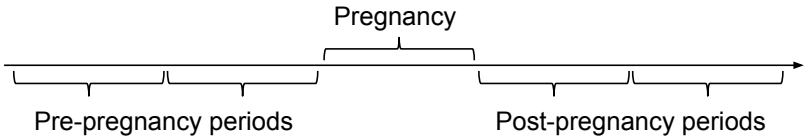

Figure 6. Illustration of the time periods surrounding pregnancy we investigate.

We therefore investigated the timing of maternal antibiotic use during pregnancy or in the time surrounding pregnancy. We constructed "pregnancy" periods of duration 40 weeks prior or post the actual pregnancy. Figure 6 illustrates the timing periods - in total, we investigated almost four years (200 weeks) of the maternal antibiotic prescriptions.

The population used is similar to the population in paper I, namely all Danish children born 1997-2010. Use of antibiotics during pregnancy was similar to the surrounding periods with approximately ⅓ of the women receiving at least one antibiotics treatment during 40 weeks of observation. Over the full 200-week window, 22% had no treatments at all with antibiotics.

We used all three definitions of childhood asthma in the primary analysis and found approximately the same increased risk of offspring asthma regardless of the timing of maternal antibiotics treatment. Figure 7 illustrates the confounder adjusted incidence rate ratios for the three asthma definitions in the five "pregnancy-periods" surrounding and including pregnancy.

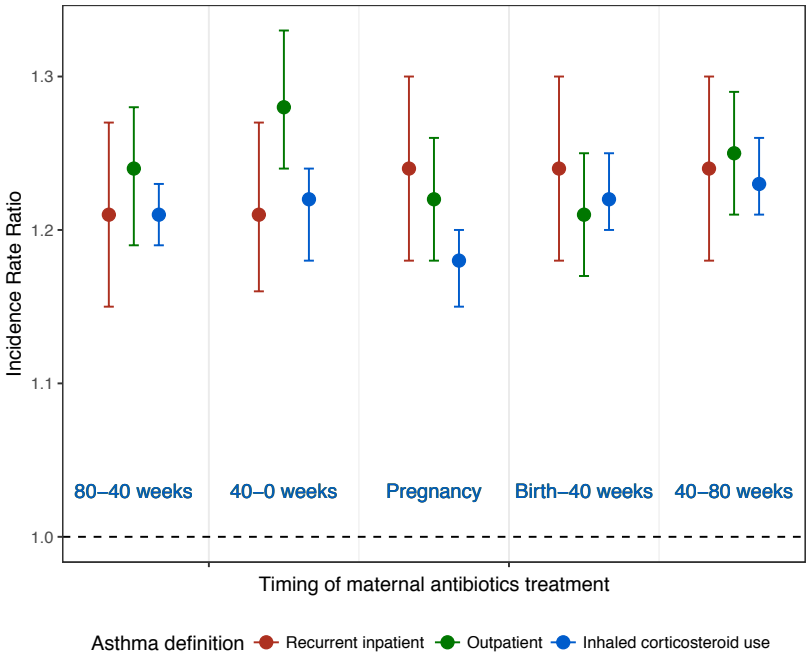

Figure 7. Incidence rate ratios for asthma definitions by maternal use of antibiotics in time periods surrounding pregnancy. All estimates are adjusted for age and calendar year, birth weight, gestational age, sex, mode of delivery, parity, multiple births, season of birth, maternal age, smoking during pregnancy, maternal employment status, and maternal asthma.

Sensitivity analyses confirmed our findings. We excluded women with any antibiotics during pregnancy and found a similar effect for the pre- and post-pregnancy treatments. When stratifying the analysis by maternal asthma we found increased risks in both strata, but with the strongest effects in the non-asthmatic mothers.

Together these results suggest that the association between maternal use of antibiotics in pregnancy and offspring asthma is not of causal nature since then we would have expected stronger association during pregnancy. We even found a linear dose-response relationship between number of maternal antibiotics treatments and rate of each asthma definition with a 5% increased risk of asthma by each additional treatment in the complete 200-week window, illustrated in Figure 8.

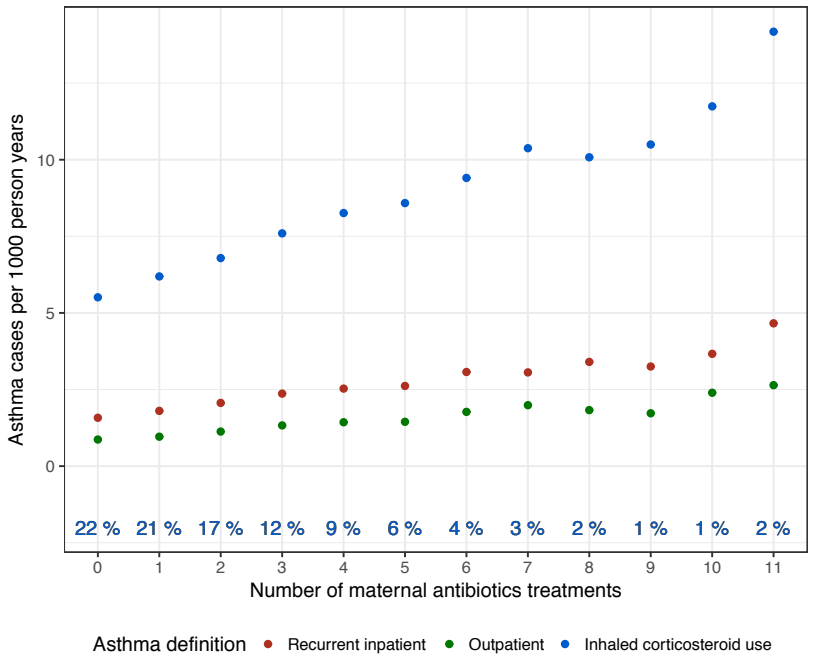

Figure 8. Rates of asthma by cumulative number of maternal antibiotic treatments during the 200-week window surrounding pregnancy. Percentages for antibiotic treatments represent the proportion of the cohort with this exposure.

Altogether this lead us to believe that initial association should be seen as a proxy for other mechanisms rather than a causal factor. Multiple studies have confirmed maternal antibiotics during pregnancy as a risk factor for childhood asthma. However, few dwell on potential mechanisms beyond the causal factors. Even after our study, there are several papers reporting the observational finding with emphasis on the potential causal factors (Lapin et al., 2014; Metsälä et al., 2014; Chu et al., 2015; Mulder et al., 2016).

The paper itself caused two correspondences. Weiss and Litonjua, (2014) suggested the underlying link to be Vitamin D deficiency[6]. Blaser and Bello (2014) interprets the findings still relating to alterations in the child's microbiome via various modes of transfers from the mother.

We suggest that maternal use of antibiotics is a surrogate marker of a general propensity for infections, and this propensity is inherited by the child that increases the risk of infections as the important trigger of asthma.

Paper III including online supplement can be found at the end of the thesis in the section Papers.

Paper IV

Stable Admission Rate for Acute Asthma in Danish Children since 1977

Paper IV concerns the time trend of childhood asthma and does neither include cohort data nor any perinatal risk factor.

As specified in the methods section the national registries undergo many changes over time. However, inpatient admissions are registered across the entire lifespan of the National Patient Registry. We wanted to exploit this aspect to investigate time trends from this unique data source with 35 years of observation.

Since the diagnosis of asthma is less certain in younger ages we investigate children age 5-15 (both years included). We include all children born in Denmark and defines cases as in-patient admission with the primary diagnosis of asthma (1977–1993: ICD-8 493; and 1994–2012: ICD-10 J45, J46). The incidence rate is calculated as number of new cases divided by person-years at-risk. All children contributed person-time from age 5 until age 16 or migration or death or final registry date (december 31st 2011). From 1988-2012 we follow a complete cohort of children without left-truncation of data for 16

[6] Vitamin D has been extensively studied in the later COPSAC cohort, the unselected COPSAC2010, where a randomized trial of Vitamin D supplementation in third trimester of pregnancy has shown Vitamin D to be a protective factor (not statistically significant) against childhood asthma (Chawes et al., 2016)

million person-years[7]. The incidence rate is around 1 cases per 1000 person-years, and the rate is relatively stable from 1988-2012, illustrated in Figure 9.

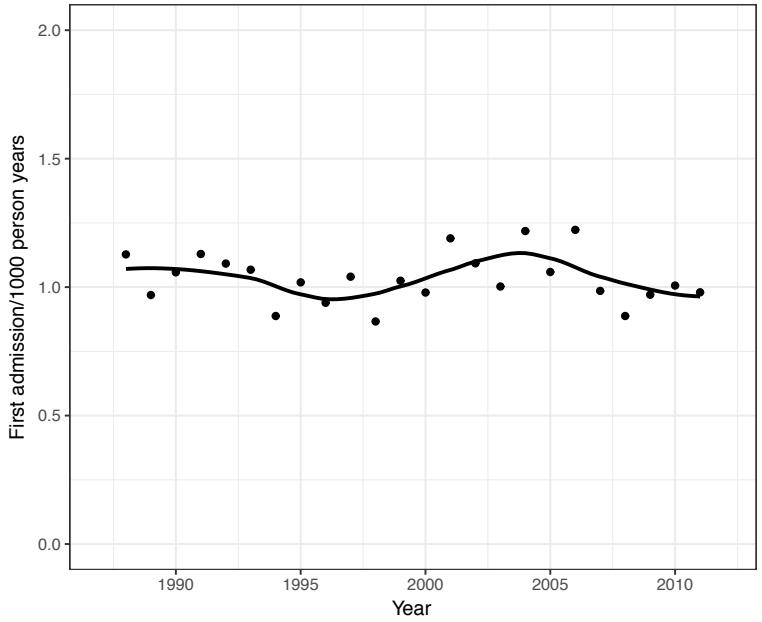

Figure 9. Asthma inpatient admissions in Denmark per 1000 school-aged children at risk 1988–2012.

This finding contrasts the general perception that asthma is on the rise (Pearce et al., 2000; 2007). Hospitalized asthmatics represent the most severe cases that have been filtered through primary care and hospital admission criteria, and although asthma is a complex and heterogeneous disease, we interpret inpatient hospital admission rates as the "tip of the iceberg" of the most severe asthma cases, and thus a surrogate for the underlying disease incidence. We believe hospitalizations may be a less biased marker of disease incidence and disease control in the society than other assessments, such as symptom history and prescribed treatment, which are prone to

[7] In the results section in the paper it erroneously states 1.6 million person-years. That is an unfortunate error missed in proof-readings.

changes in disease perception, management strategy, and definition over time. Other studies who have focused on consistent data sources that are not questionnaire based have likewise found stable incidences of especially hospitalizations: Chawla et al. on Canadian sources (2012); decrease in Portuguese data (Santos et al., 2016). Primary care data from the Netherlands found a rise followed by a fall (Engelkes et al., 2015). A more recent Danish study (Henriksen et al., 2015) focused on recent trends in both Denmark and Sweden in younger children and found stable rates for asthma and related diseases since 1997.

Improved treatments could potentially outbalance underlying increasing disease incidence. However, we find it unlikely that a major asthma epidemic has occurred without leaving an imprint in the admission rates for asthma. This interpretation suggests that the increased asthma prevalence reported by others may be driven by an increase in the milder segment of childhood asthma and pre-school aged children who are not included in our analysis. There may be political or funding related reasons for the constant reporting of increasing incidence when so many sources report stable rates.

Secondary in paper IV we investigate readmission among children with any first admission with asthma. Unlike the incidence analysis, where left truncation may introduce serious bias, the stratified Cox regression analysis can account for the truncation when analyzing readmissions, and therefore we were able to investigate readmission for the full 35 years span. Calendar year at first admission was grouped in seven groups 1977–1981; 1982–1986; 1987–1991; 1992–1996; 1997–2001; 2002–2006; and 2007–2011. We found the risk of readmissions to depend on age at first admission, and time period of first admission. Figure 10 illustrates the decreasing hazard ratios for readmission with calendar year from an age-stratified Cox analysis.

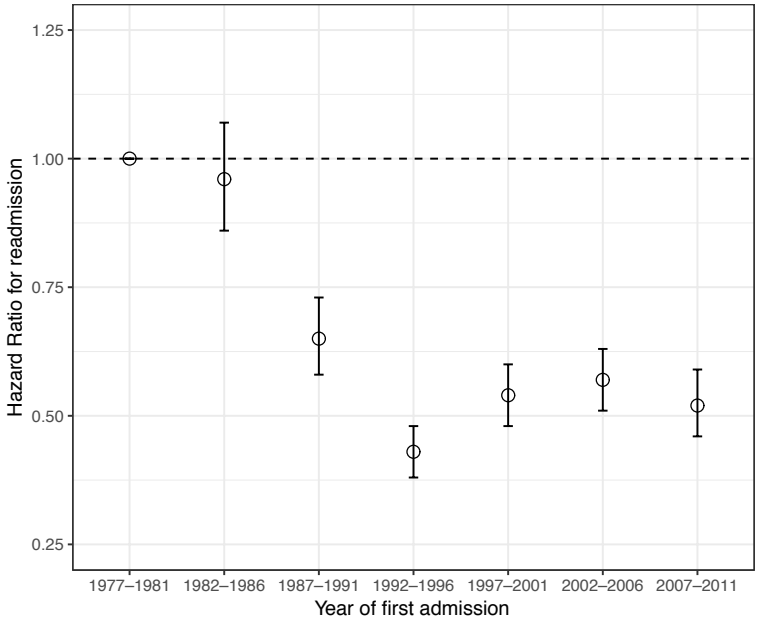

Figure 10. Hazard ratio of readmission by calendar year at first admission from age-stratified Cox regression. 1977–1981 is reference.

We interpreted the drop in readmissions as an indication of improved disease control since the diagnosis was determined at first admission. The fact that the low-point in readmission during 1992–1996 was followed by stabilization is therefore interpreted as no further improvements in asthma management in the recent two decades.

In summary, we report stable rates of first admission and readmissions halved during the 1980es and stabilized in the past twenty years.

Paper IV can be found at the end of the thesis in the section Papers.

Paper V

Cesarean Section and Chronic Immune Disorders

Paper V is also based only on registry data and concerns the comorbidity of asthma and other chronic inflammatory diseases as suggested by e.g. Prescott (2013). Likewise paper IV, this study is aimed at taking advantage of the enormous power in the Patient Registry to investigate the comorbidity of nine chronic inflammatory childhood diseases. Only asthma is common and early enough to be feasible to investigate in a cohort setting. However, as outlined in the Introduction section of this thesis, “Canary in the coalmine”, there are general beliefs that asthma and other chronic inflammatory diseases share etiology. This was the background for paper V, where we investigate the comorbidity through a shared early life risk factor; the cesarean section.

The study cohort was all Danish children born after the initiation of the Medical Birth Registry, i.e. 1973-2012. We excluded children with low birth weight (<2.5 kg). Children were divided by delivery mode (cesarean versus vaginal), and followed in the Patient Registry from 1977-2012 in the ages 0-15 for any hospital in- or outpatient admission with a primary or secondary diagnosis of either:

- Asthma
- Systemic connective tissue disorders
- Juvenile arthritis
- Inflammatory bowel diseases
- Diabetes type 1
- Immune deficiencies
- Psoriasis
- Celiac disease
- Leukemia

As a negative control, we also investigated hospital admissions for fractured forearm or elbow. Asthma was investigated both from age 0-15 and from age 5-15, in accordance with our findings in paper IV that pre-school asthma is a less precise phenotype derived from registry data.

Each disease is investigated separately, and adjusted for the same confounders: the timing variables (attained age and calendar year in varying categories), gender, parity, birth weight categories, season of birth, maternal age and heritability (maternal disease in question). The

forest plot in Figure 11 illustrates the confounder adjusted incidence rate ratios for the diseases by cesarean section versus vaginal delivery.

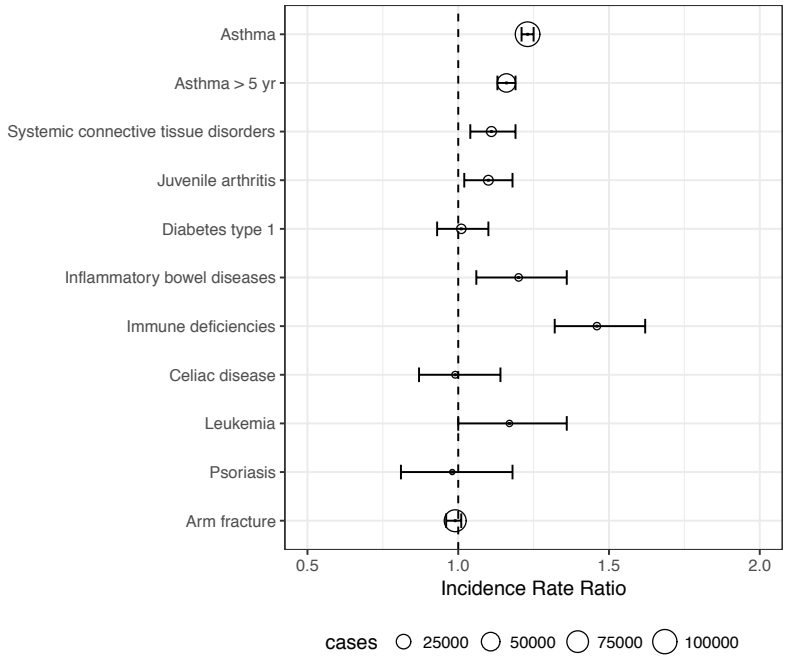

Figure 11. Forest plot of incidence rate ratios for the nine chronic inflammatory diseases (and control condition) by cesarean section. Size of circle denotes number of cases. All estimates are adjusted for age, calendar year, gender, parity, birth weight, season of birth, maternal age, and maternal disease.

We found cesarean section significantly increased the risk for asthma, systemic connective tissue disorders, juvenile arthritis, inflammatory bowel diseases, immune deficiencies, and leukemia. There were no associations to psoriasis and celiac disease. Diabetes type 1 was associated, but the association disappeared after adjustment for maternal diabetes. The negative control of fractured arms was not associated with cesarean section.

We interpreted the finding of a shared early life risk factor, as a sign of communality among the diseases in question. It also suggests that critical events in the perinatal periods may initiate a disease

trajectory. Identifying the commonalities between diseases may help to shed light on the mechanisms of these diseases, and focus research in these diseases on early life.

Many of the diseases in question had already been associated with cesarean section in previous studies - especially asthma had already several studies confirming the association between mode of delivery and asthma (Håkanssons and Källén, 2003; Renz-Polster et al., 2005), but also inflammatory bowel diseases (Bager et al., 2012); and leukemia (Francis et al., 2014). Our main new contribution was the commonality, previously addressed through disease trends, most famously by Bach in 2002 (Bach, 2002).

In line with the findings in paper I, we have interpreted the associations to cesarean section to be related to the altered microbiome met by the newborn which could lead to an immune aberration leading to a variety of chronic immune diseases presenting later in life.

Although this interpretation is pure speculation, it is clear that this particular mechanism strikes a nerve in the scientific as well as the general community. Among the papers included in this thesis, paper V by far has the most citations. A range of studies are citing because they do similar epidemiological studies on cesarean section[8], but the vast majority of citations are done in relation to the microbiome interpretation. This entails studies about the microbiome in general (Moya-Pérez et al., 2017; Thomas et al., 2017; Tamburini et al., 2016); studies investigating the critical window for microbiome development (Stiemsma and Turvey, 2017); investigations of microbiome in fecal samples (Martin et al., 2016); restoration of the microbiota of cesarean-born infants via vaginal microbial transfer (Dominguez-Bello et al., 2016); and investigations of microbes from cesarean section operating rooms (Shin et al., 2015); and a study microbiota samples from different compartments indicating that the gut microbiome initiated in utero (Collado et al., 2016).

Three studies are regarding specific diseases, but interestingly they all regard the microbiome framework; Horton et al. (2016) finds no risk for psoriasis for antibiotic exposure; Stoll and Chron (2016)

[8] Marcotte et al. (2016) do a study confirming cesarean section a risk factor for leukemia; Black et al. (2015) confirms cesarean section a risk factor for asthma but do not find associations to obesity at age 5 years, inflammatory bowel disease, type 1 diabetes, or cancer. The same authors found similar risk for repeat cesarean sections (Black et al. 2016).

reviews microbiome development in relation to juvenile arthritis; and Clausen et al. (2016) finds infancy antibiotics a risk factor for diabetes type 1 in children delivered by cesarean section.

In summary, the finding of commonality between a range of childhood immune disorders via a shared early life risk factors ended up fueling the microbiome hype and perhaps emphasize the risk factor more than necessary for the main point of commonality. The commonality of the chronic inflammatory diseases has since been replicated via shared genetic variants (Kreiner et al., 2017).

Paper V including online supplement can be found at the end of the thesis in the section Papers.

CONCLUSION AND PERSPECTIVES

In this concluding section, I will sum up the main findings and discuss implications for future research, as well as potential public health recommendations. The section is structured to first mention the strengths of the used methods, then the main findings, before a critical stance on the general perinatal risk factor focus, before comments on future research and potential public health strategies.

Reproducibility is a cornerstone in Hill's principles (1965) for establishing causation. For several of the studies in this thesis we have sought replication of the results from the clinical cohort data in the registry data. In paper I and paper II data from COPSAC2000 is used. The strengths of the clinical cohort data are immense for the study of childhood asthma with the close clinical follow-up and meticulous set diagnosis, which are the cornerstones of the COPSAC cohorts.

This cohort strength is also one of the disadvantages in the registry data. There are no registrations of general practitioner diagnosis, and any definition must therefore rely on data from hospital registrations or pharmacy collected prescription medication; and the former only since approximately 1997 for the pediatric population. For the studies in paper I and paper III, where the population is limited to children born 1997-2010 we have investigated three independently defined asthma phenotypes from (1) long term antiasthmatic inhalation corticosteroid filled at a pharmacy, (2) long term outpatient attendance, or (3) recurrent inpatient admissions. As illustrated in the methods section, the overlap between these phenotypes is far from complete, although they theoretically would denote severity of asthma. This clearly proves the muddiness of the field. The fact that we find similar results from each separately investigated asthma definition assures us of the validity of the associations despite the lack of a simple asthma outcome definition.

Across the papers in this thesis, some of the results are in accordance with what other researchers have published, adding to an already existing body of evidence for the particular perinatal risk factors. This is mainly true for paper I on types of cesarean section, IV on time trends (although contrary to common belief), and to some degree paper V on comorbidity of chronic inflammatory diseases.

Paper II on preeclampsia represents a novel finding.

Paper III on maternal antibiotics during pregnancy presents a finding that warrants a new interpretation of previous studies. Previous (and current) interpretations of antibiotics as a causal risk factor cannot be supported in our analysis. Several of Hill's principles are violated for causal inference on maternal pregnancy antibiotics, namely the temporality and the biological gradient. Our findings of similar asthma risk across exposure in several time periods surrounding the pregnancy is novel and needs replication by others.

All perinatal factors in this thesis could be seen as maternal factors, either expressions of maternal genotype or maternal environment. Whether genotype or environment these aspects will follow the newborn through life. All observed associations interpreted as causal could just reflect confounding by family-based socio-demographic or lifestyle-related factors. But since it was measured in perinatal life, this period of life appears especially vulnerable. A reason to find risk factors in perinatal life is that there are many easily done measurements during pregnancy and delivery. The cesarean section is at least a proxy for some maternal disturbances during pregnancy. Preeclampsia is already a maternal immunological disturbance, and we find it a risk factor for offspring immunologic diseases[9]. The antibiotics we indeed interpreted by the maternal propensity for infections. To fully understand the disease mechanisms, and to determine potential prevention strategies it becomes crucial to disentangle maternal factors from intervenable perinatal factors.

Wilcox (2001) put forward similar points of critique of Barker's work on birth weight. Wilcox claimed that birth weight is just an epiphenomenon in another chain of causation and that chain is what we should study rather than the simple birth weight, which is a measure of convenience, used because the data exist.

Perinatal programming is an area of research that provides a good fit for what we can do in the Nordic countries with the national registries. I think future research in the perinatal risk factors should do more to disentangle perinatal risk factors into maternal factors and actually intervenable factors, not least to gain better insight in the disease mechanisms.

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[9] Although we adjust for maternal disease in the analyses, residual confounding could be present, especially in the registries.

Epidemiological associations lack a precise mechanism, and therefore the part is up for speculations in the discussions of the manuscripts. For the findings in paper I and paper V between cesarean section and asthma and other diseases, we have opted for the “microbiome explanation”. Although this has been speculation, is is clear that this particular mechanism strikes a nerve in the scientific as well as the general community. Paper V is by far the manuscript with the most citations, where multiple of these use the microbiome explanation as offset as detailed in the results section.

I believe it is important to avoid tunnel vision on the microbiome. Other potential explanations should be explored. For instance, children delivered via cesarean sections performed before or after membrane rupture do not only differ in relation to microbiome exposure. The different sections also denote how far the natural birth process has come. Sections performed before membrane rupture may indicate a child being delivered earlier than necessary. Induction of the delivery could therefore be an interesting aspect for future research to investigate alternative mechanisms from the microbiome related mechanism.

Perinatal life can be an important window of opportunity for prevention of diseases just as well as a critical period for disease development. Of the risk factors dealt with in this thesis, public health intervention can have several focus points. One could be to change planned cesarean sections to a “planned emergency” section, i.e. when the delivery mode is planned to be a section (for whatever reason), but not performed before the membranes are ruptured and the natural birth process has initiated, since this type of section poses less risk for later disease. Alternatively, the cesarean delivered newborns could be introduced to vaginal microbiota via a smear performed by the midwife immediately after delivery. For the preeclampsia and the propensity for infections, public health recommendations should probably focus on what they already implement: to promote health among potential mothers, screening programmes during pregnancy for preeclampsia, and the general protection of childbearing women from infections.

As a concluding remark, I would like to emphasize that the epidemiological studies, such as the included studies in this thesis based on especially registry data, should be read as proposing questions rather than giving answers. The data are not suited to extract exact mechanisms of disease development, rather they should

help develop frameworks and general theories which could be tested in other settings.

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PAPERS

PAPER I:

Sevelsted, A., Stokholm, J., and Bisgaard, H. (2016).

Risk of Asthma from Cesarean Delivery Depends on Membrane Rupture.

J. Pediatr. *171*, 38–42.e4.

PAPER I

PAPER I

Risk of Asthma from Cesarean Delivery Depends on Membrane Rupture

Astrid Sevelsted, MSc[1], Jakob Stokholm, MD, PhD[1,2], and Hans Bisgaard, MD, DMSc[1]

Objective To assess our prospective mother-child cohort and the national registry data to analyze the risk of asthma by delivery mode and whether cesarean delivery before or after membrane rupture affects this risk differently.

Study design The Copenhagen Prospective Studies on Asthma in Childhood$_{2000}$ is a high-risk birth cohort of 411 Danish children. Asthma was diagnosed prospectively by physicians at the research site, and associations with cesarean delivery were investigated using Cox proportional hazard models. From the Danish national prospective registry we included data from 1997-2010. Childhood asthma was defined from recurrent use of inhaled corticosteroids filled at pharmacies. Cesarean delivery was classified as either before or after rupture of membranes, and the risk of asthma was compared with vaginal delivery. Results were adjusted stepwise for age and calendar year, sex, birth weight, gestational age, multiple births, parity, and maternal factors (age, smoking/antibiotics during pregnancy, employment status, and asthma).

Results In the Copenhagen Prospective Studies on Asthma in Childhood$_{2000}$ cohort, the adjusted hazard ratio for asthma was increased by cesarean delivery relative to vaginal birth 2.18 (1.27-3.73). Registry data replicated these findings. Cesarean delivery performed before rupture of membranes carried significantly higher risk of asthma, (incidence rate ratio to vaginal delivery 1.20 [1.16-1.23]) than cesarean delivery after rupture of membranes (incidence rate ratio to vaginal delivery 1.12 [1.09-1.16]).

Conclusions We confirmed cesarean delivery to be a risk factor for childhood asthma. This effect was more pronounced for cesarean delivery performed before rupture of membranes. *(J Pediatr 2016;171:38-42)*.

Birth by cesarean delivery seems to be a risk factor for childhood asthma.[1,2] However, the mechanisms leading to this increased risk remain unknown. Population-based studies have previously investigated the risk for asthma after delivery by emergency or planned cesarean delivery reporting conflicting results.[3-5] Emergency cesarean delivery may be performed for different reasons most commonly during labor because of delivery complications. However, emergency cesarean delivery may also be performed before onset of labor because of pregnancy complications.

We speculate that cesarean delivery could mediate the asthma risk through alterations of the newborn's microbiome.[6] Hence, the rupture of membranes and thereby possible microbial transmission may cause different effects of cesarean delivery. We analyzed the association between cesarean delivery and asthma in our prospective clinical birth cohort Copenhagen Prospective Studies on Asthma in Childhood$_{2000}$ (COPSAC$_{2000}$) with stringent asthma criteria. To further investigate the potential mechanisms leading to an increased asthma risk after cesarean delivery, we used registry data on the entire Danish pediatric population between 1997-2010.

Methods

The COPSAC$_{2000}$ cohort study was conducted in accordance with the guiding principles of the Declaration of Helsinki and was approved by the Local Ethics Committee (KF 01-289/96) and the Danish Data Protection Agency (2008-41-1754). Both parents gave written informed consent before enrollment. The registry study was based on existing data in national registries and was approved by the Danish Data Protection Agency (J.no. 2012-41-0388). Because subjects were not contacted in the registry study, written informed consent was not required.

COPSAC$_{2000}$ Birth Cohort

The COPSAC$_{2000}$ birth cohort consists of 411 children born 1998-2001 to mothers with a history of asthma, excluding children born before 36 weeks of gestation and anyone suspected of chronic diseases or lung symptoms prior to inclusion, as previously described in detail.[7]

COPSAC$_{2000}$	Copenhagen Prospective Studies on Asthma in Childhood$_{2000}$
HR	Hazard ratio
IRR	Incidence rate ratio

From the [1]Copenhagen Prospective Studies on Asthma in Childhood, Herlev and Gentofte Hospital, University of Copenhagen, Copenhagen, Denmark; and [2]Department of Pediatrics, Naestved Hospital, Naestved, Denmark

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Registry Study Population

In the prospective registry based cohort study, we identified a cohort of live born children in Denmark in the period 1997-2010 and used their unique identification number to link information on maternal age, sex, parity, maternal smoking during pregnancy, mode of delivery, and maternal identification number from the Danish Medical Birth Registry; information on prescribed antibiotics and anti-asthmatic steroids from the National Prescription Registry; information on hospital admissions (inpatient and outpatient attendance) with asthma as primary diagnosis from the Danish National Patient Registry; information on maternal employment status from Statistics Denmark; and information on date of migration from the Danish Person Registry. The diagnoses are based on the International Statistical Classification of Diseases and Related Health Problems version 10.[8]

Cesarean Delivery Classification

In the $COPSAC_{2000}$ cohort, we defined delivery by cesarean delivery as a dichotomous variable. The National Registry classified cesarean delivery as: (1) elective cesarean performed before delivery; (2) emergency cesarean because of delivery complications performed during delivery; (3) emergency cesarean because of pregnancy complications performed during delivery; and (4) emergency cesarean performed before onset of labor. Children with no registration of cesarean delivery were coded as vaginal delivery. We combined the different classifications to comprise either cesarean delivery performed before rupture of membranes (1 and 4) or cesarean delivery performed after rupture of membranes (2 and 3).

Asthma Definitions

In the $COPSAC_{2000}$ cohort, asthma was diagnosed by trained physicians at the research unit in accordance with strict, standardized criteria based on daily diary cards since birth. The burden of recurrent symptoms was quantified from an algorithm of 5 episodes of at least 3 consecutive days of troublesome lower lung symptoms within 6 months and need of short-acting ß2-agonists as previously described in detail.[9] Furthermore, the diagnosis required symptom improvement during a 3-month trial of inhaled corticosteroids and relapse when this medication was stopped. Asthma exacerbations were defined by need for oral prednisolone, high-dose inhaled corticosteroids, or acute hospitalization with asthmatic symptoms.

In the registry, we defined asthma as long-term recurrent use of inhaled corticosteroids: at least 200 defined daily doses (World Health Organization index) filled at a pharmacy (R03BA01; R03BA02; R03BA05; R03BA07). The child becomes a case at first collection of medication. In the sensitivity analyses, we investigated 2 alternative definitions of childhood asthma based on asthma hospitalizations as described earlier[10]: (1) recurrent hospital admissions for asthma: at least 2 inpatient admissions (primary diagnosis of asthma ICD10: J45.x; J46.x) separated by at least 1 month (child is considered case at first admission); or (2) long-term outpatient attendance related to asthma: child followed in outpatient care (primary diagnosis of asthma ICD10: J45.x; J46.x) for minimum 1 year (child becomes case at date of outpatient treatment initiation). For each definition, cases were compared with noncases where noncases were all children not fulfilling case definitions.

Confounders

Confounders were chosen a priori as sex, parity, birth weight, gestational age, maternal age, mother smoking during pregnancy, maternal disease, multiple births, mother's use of antibiotics during pregnancy, and maternal employment/education. All confounders were included in the regression models as categorical variables in registry analyses: parity (first child, second child, third child or more), birth weight (2.5-3.0 kg, 3.0-3.5 kg, 3.5-4.0 kg, >4.0 kg), gestational age (<37 weeks, 37-39 weeks, 40-41 weeks, 42 or more weeks), maternal age (4 categories), maternal disease (mothers ever hospital admission for asthma or mothers prescription of inhaled steroid ever), multiple births (singleton, twins, triplets or more), mothers use of antibiotics during pregnancy (ever prescription of antibiotics 14 days before last menstruation until offspring's birthdate), mothers smoking during pregnancy (yes/no), and maternal employment status in the year of child birth or the previous year if child is born during the first 8 months (7 categories).

Statistical Analyses

Time to first asthma diagnosis before the age of 7 years was illustrated with Kaplan Meier plots. For clinical cohort data, confounder adjusted hazard ratios (HRs) were calculated with Cox regression. All confounders were investigated for proportionality, and nonproportional variables (sex and parity) were added as stratifying variables to get adjusted estimates of cesarean delivery.

In the registry analyses, children contributed to person time of observation from date of birth to becoming asthmatic, death, migration, or December 31, 2010. The number of asthma cases by cesarean delivery was investigated with log-linear Poisson regression models offset by the log-transformed person years of observation adjusted for attained age (1-year group) and attained calendar year (1-year group) (Model 1). In 2 additional models, we included stepwise the a priori chosen confounders. Model 2: adding sex, parity, multiple birth, maternal factors (age, smoking during pregnancy, usage of antibiotics during pregnancy, employment status, asthma [ever prescription of steroid or ever admission for asthma]); and model 3: adding birth weight and gestational age. The offset of log-transformed person-years models the rate of diseases, and with the categorical adjustment for timing variable (attained age and calendar year), the resulting incidence rate ratios (IRRs) with 95% CIs can be interpreted as HRs from Cox regression.

Term children (birth weight >2.5 kg) were investigated in a separate sensitivity analysis. In another sensitivity analysis for children above 6 years of age, only children born from 1997-2005 were included and contribute only from the date of the 6-year birthday to case definition, death, migration, or December 31, 2010.

Registry data were summarized with the Pyrsstep macro.[11] A significance level of .05 was used in all analyses. The data processing was performed using R v 3.1.0 (R Foundation for Statistical Computing, Vienna, Austria) and SAS v 9.3 for Windows (SAS Institute Inc, Cary, North Carolina).

Results

During 1998-2001, 411 children from the greater Copenhagen area were included in the $COPSAC_{2000}$ cohort. The cohort has been extensively described earlier.[12-14] In Denmark, 864 049 (95%) of 910 301 children born had data on all confounders. These children were followed in the registries from birth to death/emigration or final follow-up date of December 31, 2010 covering 5 987 664 person years in the age range 0-15 years with declining observation time with child age. Distributions of all confounders in the population are presented in **Table I** (available at www.jpeds.com).

Prevalence of Cesarean Delivery

Eighty-seven of 411 children (22%) in the $COPSAC_{2000}$ cohort were delivered by cesarean delivery. In the registry cohort, 19% (163 462) of the Danish children were born by cesarean delivery during the period 1997-2010. Ten percent (87 559) of the children were born by cesarean delivery performed before rupture of membranes (71% elective and 29% emergency before delivery), 9% (75 863) of the children were born by cesarean delivery performed after rupture of membranes (88% emergency because of delivery complications, 12% emergency because of pregnancy complications).

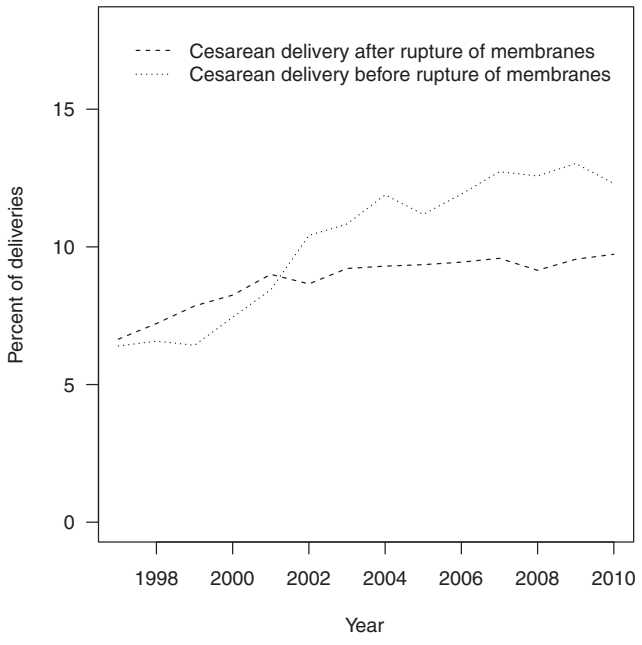

Figure 1. Prevalence of cesarean delivery.

The prevalence of birth by cesarean delivery increased during the study period, primarily because of increasing numbers of cesarean deliveries before rupture of membranes (**Figure 1** and **Table I**). Cesarean deliveries performed before rupture of membranes included more preterm children with a lower birth weight (**Table I**).

Prevalence of Asthma

In the high-risk $COPSAC_{2000}$ birth cohort, 18% (72 children) developed asthma before the age of 7 years. In the registry cohort 4.4% (38 085) had filled at least 200 defined daily doses of anti-asthmatic steroids.

Cesarean Delivery and Asthma

We found significant association between delivery by cesarean delivery and development of asthma in the $COPSAC_{2000}$ cohort with a confounder adjusted HR of 2.18 (1.27-3.73); $P = .005$ (**Figure 2**). Similarly, we found significant association with asthma exacerbations with adjusted HR of 2.48 (1.37-4.49); $P = .003$ (**Figure 3**; available at www.jpeds.com). This overall association between cesarean delivery and increased asthma risk was confirmed in the population cohort, where we found a smaller effect of cesarean delivery with IRR 1.16 (1.13-1.19). The main confounder appeared to be prematurity because the biggest drop in risk occurs from model 2 (all confounders except birth weight and gestational age) to model 3 including those (**Table II**).

When separating cesarean delivery by rupture of membranes in the population cohort, we found differences in the risk of asthma. Cesarean deliveries performed before rupture of membranes carried a significant larger risk of asthma, IRR to vaginal delivery (1.20 [1.16-1.23]) compared with IRR to vaginal delivery (1.12 [1.09-1.16]) after rupture of membranes (**Table II**). For cesarean deliveries performed after rupture of membranes, we found increased risk of asthma in all adjustment models with the biggest decrease in effect size from model 1 to model 2. For cesarean deliveries performed before rupture of membranes, we observed overall larger effect sizes and mainly a decrease when including birth weight and gestational age as confounders in model 3 (**Table II**). When restricting the population cohort to term children only (birth weight >2.5 kg), the results are similar (**Table III**; available at www.jpeds.com). In sensitivity analyses on the population cohort, we investigated asthma among children above 6 years of age. We found similar results with cesarean deliveries performed before rupture of membranes being a stronger risk factor for later asthma (**Table IV**; available at www.jpeds.com).

In another sensitivity analysis on the population cohort, we investigated 2 alternative asthma definitions: recurrent inpatient hospitalizations and long-term outpatient treatment for asthma. The results are shown in **Tables V** and **VI** (available at www.jpeds.com). There were fewer cases by these definitions of asthma, but the associations to cesarean delivery were in fact stronger, indicating more strict asthma

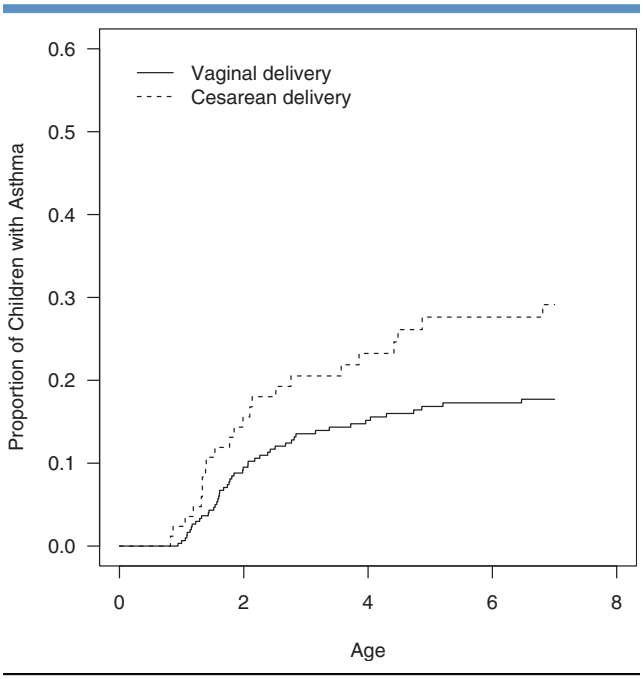

Figure 2. Kaplan Meier curves for asthma by delivery method in the $COPSAC_{2000}$ cohort. Confounder adjusted HR 2.18 (1.27-3.73); $P = .005$.

definitions. Cesarean deliveries performed before rupture of membranes remained a stronger risk factor for asthma regardless of asthma definition.

Discussion

The major strengths of the $COPSAC_{2000}$ clinical cohort is the thorough prospective clinical monitoring and diagnosing based on highly standardized operating procedures. The birth cohort was followed prospectively with diary cards and 6 monthly routine visits, strengthening the asthma diagnosis. However, the size of the cohort does not allow for investigation of different types of cesarean deliveries. For this purpose, we used 14 years of nationwide population follow-up in national registries. The content and validity of the Danish National Registries have been well documented previously.[8,15,16]

The Danish National Prescription Registry has nationwide information on steroids prescribed and filled at the pharmacies. The registry is highly accurate and in Denmark all anti-asthmatic steroid inhalants can be prescribed only by authorized physicians and purchased from authorized pharmacies. Medication was collected on the children's own unique identification number. Unlike the clinical cohort, asthma must be defined based on registered events (there are no registrations of diagnoses from general practitioners). Previous studies have used parental reporting, hospital records, and prescription medication as outcomes. This study is strengthened by defining asthma from both hospitalizations and medication filled. The Danish National Patient Registry covers all hospital admissions nationwide as well as children attending outpatient clinics. Asthma diagnosis in the National Patient Registry has previously been validated,[17] and we recently demonstrated the phenotypic specificity of asthma hospitalization in our discovery of a novel genetics risk variants.[18]

A wide range of potential confounders were included as categorical variables, and no assumptions were made on directions of associations. To ensure that associations were not confounded by prematurity, we excluded children born before week 36 in our mother-child cohort and children with birth weight below 2.5 kg in the registry based cohort, which did not affect the conclusions (**Table III**). However, we cannot exclude residual confounding in our results.

Diagnosis of asthma in a young child may be inaccurate. We, therefore, did a sensitivity analysis in the registry-based cohort excluding children below 6 years of age (**Table IV**). We studied only children born after 1997, and, therefore, the number of observation years was inversely reduced with age in the study-base. This limits our study power for asthmatics with a late onset. Despite this, we still found cesarean delivery to have a significant risk for asthma, and we were still able to differentiate types of cesarean deliveries.

A potential study limitation is that we defined rupture of membranes based solely on the obstetrician's diagnoses of being in active labor or not. The obstetric classification may furthermore be inaccurate and affected by changing medical practice in the long follow-up period. Any misclassification of the types of cesarean delivery could increase noise but would not lead to systematic bias in the results.

Table II. Confounder adjusted IRR for asthma (200 defined daily dose collected anti-asthmatic steroids) by type of cesarean delivery vs vaginal birth in 3 models with increasing confounder adjustments

	N (person y)	Model 1	Model 2	Model 3
Population	864 049 (5 987 664)	N = 41 633	N = 41 633	N = 38 085
Vaginal delivery	700 587 (4 963 792)	Ref	Ref	Ref
Cesarean delivery	163 462 (1 023 872)	1.31 (1.28-1.34); $P < .001$	1.25 (1.22-1.28); $P < .001$	1.16 (1.13-1.19); $P < .001$
Type of cesarean vs vaginal delivery				
Cesarean delivery after rupture of membranes	75 863 (496 772)	1.24 (1.20-1.28); $P < .001$	1.15 (1.11-1.19); $P < .001$	1.12 (1.09-1.16); $P < .001$
Cesarean delivery before rupture of membranes	87 599 (527 100)	1.38 (1.34-1.42); $P < .001$	1.34 (1.30-1.39); $P < .001$	1.20 (1.16-1.23); $P < .001$

Ref, reference group.
Children are investigated from birth to asthma/censoring.

We found an increased risk of asthma and asthma exacerbations in the longitudinally followed $COPSAC_{2000}$ birth cohort among children born by cesarean delivery. The diagnosis and treatment of this cohort are very stringent based on symptom load and clinical appearance ruling out the possibility that treatment-seeking behavior in the mother may lead to both cesarean delivery in pregnancy and asthma diagnosis in the child. This observation is replicated in the national registries by different asthma categories strengthening the confidence in the findings.

The birth setting around cesarean delivery is different from vaginal birth with respect to several factors including anesthetic agents and antibiotics administered during birth, physiological effects of the newborn, and the hospital environment after birth.[19] We speculate that the effect from cesarean delivery may be mediated by changes in the microbiome of the newborn.[6] Cesarean delivery performed before rupture of membranes could exaggerate the differences in microbiota, as the child in this setting is born without vertical microbial transmission from the mother.[20] This could explain the higher risk of asthmatic outcomes following this type of cesarean delivery. Our findings are in line with other studies differentiating between cesarean delivery by rupture of membranes, where the electively born children likewise had increased risk of asthma.[21] One previous study found the opposite, but this could be confounded by not accounting for types of emergency cesarean delivery and no exclusion of premature children.[5]

It cannot be determined whether long-term effects observed after delivery by cesarean delivery is caused by the procedure itself or by the obstetric indication for the procedure. Because a randomized controlled trial on cesarean delivery cannot be performed, we can only attempt to settle this issue by optimizing observational studies such as the present one based on both strong disease phenotypes from clinical cohort data and national registry data with the power for stratification.

Cesarean delivery was a risk factor for childhood asthma in several different disease phenotypes. We found a higher asthma risk among children born by cesarean performed before rupture of membranes compared with cesarean after rupture of membranes, which could imply microbiome mediated effect on later disease development. ■

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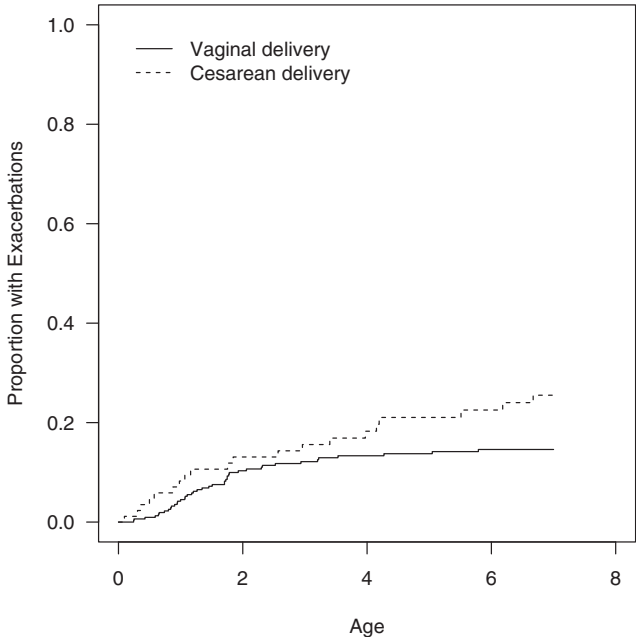

Figure 3. Kaplan Meier curves for asthma exacerbations by delivery method in the $COPSAC_{2000}$ cohort. Confounder adjusted HR 2.48 (1.37-4.49); $P = .003$.

Table I. Baseline characteristics by mode of delivery

	Vaginal delivery	Cesarean delivery after rupture of membranes	Cesarean delivery before rupture of membranes	Total
N (%)	700 587	75 863	87 599	864 049
Birth y				
1997-1999	86.29	7.24	6.47	184 387 (21.34%)
2000-2002	82.61	8.63	8.75	188 567 (21.82%)
2003-2005	79.41	9.29	11.30	187 198 (21.67%)
2006-2008	78.20	9.39	12.41	183 584 (21.25%)
2009-2011	77.70	9.64	12.66	120 313 (13.92%)
Sex				
Male	50.91	55.32	50.58	442 967 (51.27%)
Female	49.09	44.68	49.42	421 082 (48.73%)
Birth weight				
<2.5 kg	2.93	10.42	15.40	41 911 (4.85%)
2.5-3.0 kg	10.49	12.68	16.53	97 619 (11.3%)
3.0-3.5 kg	31.74	24.06	30.69	267 513 (30.96%)
3.5-4.0 kg	35.64	28.70	24.53	292 951 (33.9%)
>4.0 kg	19.20	24.14	12.86	164 055 (18.99%)
Gestational age				
<36 wk	4.16	12.10	17.31	53 476 (6.19%)
37-39 wk	15.33	18.95	47.53	163 391 (18.91%)
40-41 wk	53.25	36.88	29.23	426 623 (49.37%)
>42 wk	27.27	32.06	5.94	220 559 (25.53%)
Parity				
First child	42.45	64.21	36.89	378 445 (43.8%)
Second child	37.74	26.17	40.07	319 335 (36.96%)
Third (or more) child	19.81	9.62	23.05	166 269 (19.24%)
Multiple birth				
Singleton	97.89	89.43	87.09	829 929 (96.05%)
Multiple	2.11	10.57	12.91	34 120 (3.95%)
Maternal age				
..-25	13.83	11.38	7.31	111 918 (12.95%)
26-30	34.95	33.79	26.27	293 494 (33.97%)
31-35	35.47	36.00	39.61	310 492 (35.93%)
36-..	15.75	18.82	26.81	148 145 (17.15%)
Maternal use of antibiotics during pregnancy				
No	67.73	67.14	64.38	581 807 (67.33%)
Yes	32.27	32.86	35.62	282 242 (32.67%)
Maternal asthma (hospitalization or steroid prescription)				
No	88.13	86.54	85.81	758 230 (87.75%)
Yes	11.87	13.46	14.19	105 819 (12.25%)
Smoking during pregnancy				
Yes	18.67	18.38	17.92	160 469 (18.57%)
No	81.33	81.62	82.08	703 580 (81.43%)
Maternal employment status in y or previous y of childbirth				
Unemployed	10.21	9.41	12.14	89 273 (10.33%)
Entrepreneur/leader	1.14	1.31	1.44	10 259 (1.19%)
Employee, unknown status	9.69	10.36	9.92	84 455 (9.77%)
Employee, basic skilled	29.83	30.98	29.65	258 464 (29.91%)
Employee, medium skilled	20.50	21.54	22.10	179 337 (20.76%)
Employee, highly skilled	12.38	12.17	12.92	107 266 (12.41%)
Entrepreneur, no employees	1.89	1.89	2.07	16 484 (1.91%)
Education	4.98	4.56	3.17	41 111 (4.76%)
Other	9.38	7.79	6.59	77 400 (8.96%)

For overall N and birth year, numbers represent row-percentages. For all other confounders, numbers represent column percentages.

Table III. Population: term children only (birth weight >2.5 kg)

	N (person y)	Model 1	Model 2	Model 3
Population	822 138 (5 707 421)	N = 38 085	N = 38 085	N = 38 085
Vaginal delivery	680 066 (4 822 345)	Ref	Ref	Ref
Cesarean delivery	142 072 (885 075)	1.26 (1.23-1.29); $P < .001$	1.21 (1.17-1.24); $P < .001$	1.16 (1.13-1.19); $P < .001$
Type of cesarean vs vaginal delivery				
Cesarean delivery after rupture of membranes	67 960 (443 453)	1.20 (1.16-1.25); $P < .001$	1.13 (1.09-1.17); $P < .001$	1.13 (1.09-1.17); $P < .001$
Cesarean delivery before rupture of membranes	74 112 (441 621)	1.31 (1.27-1.35); $P < .001$	1.29 (1.24-1.33); $P < .001$	1.20 (1.16-1.24); $P < .001$

Ref, reference group.

Table IV. Population: only children above 6 years of age

	N (person y)	Model 1	Model 2	Model 3
Population	498 371 (1 937 919)	N = 12 189	N = 12 189	N = 12 189
Vaginal delivery	414 452 (1 644 591)	Ref	Ref	Ref
Cesarean delivery	83 919 (293 327)	1.26 (1.20-1.31); $P < .001$	1.18 (1.13-1.24); $P < .001$	1.12 (1.06-1.17); $P < .001$
Type of cesarean vs vaginal delivery				
Cesarean delivery after rupture of membranes	41 249 (149 996)	1.21 (1.13-1.28); $P < .001$	1.10 (1.04-1.18); $P = .002$	1.08 (1.01-1.15); $P = .021$
Cesarean delivery before rupture of membranes	42 670 (143 331)	1.31 (1.23-1.39); $P < .001$	1.27 (1.19-1.35); $P < .001$	1.16 (1.09-1.24); $P < .001$

Confounder adjusted IRRs for asthma by type of cesarean vs vaginal delivery birth in term children in 3 models with increasing confounder adjustments. Children are followed from birth to asthma/censoring.

Table V. Population: all children. Asthma defined by recurrent inpatient hospitalizations

	N (person y)	Model 1	Model 2	Model 3
Population	864 049 (5 987 664)	N = 7166	N = 7166	N = 6466
Vaginal delivery	700 587 (4 963 792)	Ref	Ref	Ref
Cesarean delivery	163 462 (1 023 872)	1.38 (1.30-1.45); $P < .001$	1.35 (1.28-1.43); $P < .001$	1.20 (1.13-1.27); $P < .001$
Type of cesarean vs vaginal delivery				
Cesarean delivery after rupture of membranes	75 863 (496 772)	1.26 (1.17-1.36); $P < .001$	1.23 (1.13-1.33); $P < .001$	1.17 (1.08-1.27); $P < .001$
Cesarean delivery before rupture of membranes	87 599 (527 100)	1.48 (1.38-1.59); $P < .001$	1.46 (1.36-1.57); $P < .001$	1.22 (1.14-1.32); $P < .001$

Confounder adjusted IRRs for asthma by type of cesarean vs vaginal delivery in 3 models with increasing confounder adjustments. Children are followed from birth to asthma/censoring. Asthma defined by recurrent inpatient hospitalizations.

Table VI. Population: all children. Asthma defined by long term outpatient treatment

	N (person y)	Model 1	Model 2	Model 3
Population	864 049 (5 987 664)	N = 12 711	N = 12 711	N = 11 711
Vaginal delivery	700 587 (4 963 792)	Ref	Ref	Ref
Cesarean delivery	163 462 (1 023 872)	1.31 (1.26-1.37); $P < .001$	1.27 (1.21-1.33); $P < .001$	1.17 (1.12-1.23); $P < .001$
Type of cesarean vs vaginal delivery				
Cesarean delivery after rupture of membranes	75 863 (496 772)	1.20 (1.13-1.27); $P < .001$	1.15 (1.09-1.23); $P < .001$	1.12 (1.05-1.19); $P < .001$
Cesarean delivery before rupture of membranes	87 599 (527 100)	1.42 (1.34-1.50); $P < .001$	1.38 (1.30-1.46); $P < .001$	1.22 (1.15-1.30); $P < .001$

Confounder adjusted IRRs for asthma by type of cesarean vs vaginal delivery birth in 3 models with increasing confounder adjustments. Children are followed from birth to asthma/censoring. Asthma defined by long-term outpatient treatment.

PAPER II:

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Preeclampsia Associates with Asthma, Allergy, and Eczema in Childhood.

Am. J. Respir. Crit. Care Med. *195*, 614–621.

PAPER II

PAPER II

ORIGINAL ARTICLE

Preeclampsia Associates with Asthma, Allergy, and Eczema in Childhood

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Abstract

Rationale: Preeclampsia reflects an unusual increase in systemic inflammation during pregnancy.

Objectives: We studied associations between preeclampsia and asthma, allergy, and eczema in Copenhagen Prospective Studies on Asthma in Childhood$_{2000}$ (COPSAC$_{2000}$) and in national registries.

Methods: COPSAC$_{2000}$ is a high-risk birth cohort of 411 Danish children. Asthma, allergy, and eczema were diagnosed prospectively, and lung function measured at age 1 month and 7 years. Sensitization was evaluated at age 6 months, 18 months, 4 years, and 6 years by skin prick tests and IgE measurements. The register-based cohort included 1.7 million children from Danish national registries in the 35-year period 1977–2012. Children born to mothers with preeclampsia were analyzed regarding risk of asthma, allergy, and eczema.

Measurements and Main Results: In the COPSAC$_{2000}$ cohort, 5.6% (n = 23) were diagnosed with preeclampsia. Preeclampsia was associated with increased risk of treatment with inhaled corticosteroids at age 7 years (adjusted odds ratio, 4.01 [95% confidence interval (CI), 1.11–14.43]; $P = 0.0337$), increased bronchial responsiveness to methacholine (adjusted β-coefficient log-μmol, −0.80 [95% CI, −1.55 to −0.06]; $P = 0.0348$), and allergic rhinitis (adjusted odds ratio, 4.83 [95% CI, 1.58–14.78]; $P = 0.0057$) in the 7-year-old children. Furthermore, the children had an increased risk of sensitization to both aeroallergens and food allergens, and increased amount of total IgE during childhood. In the registry-based cohort, 3.7% (n = 62,728) were born to mothers with preeclampsia. Preeclampsia was associated with increased risk of asthma, eczema, and aeroallergen and food allergy, especially pronounced after a duration of preeclampsia of 14 days or more. Maternal asthma increased the risk of preeclampsia.

Conclusions: Preeclampsia is a shared prenatal risk factor for asthma, eczema, and allergy in childhood pointing toward *in utero* immune programming of the child.

Keywords: preeclampsia; asthma; hypersensitivity; dermatitis, atopic; embryonic and fetal development

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At a Glance Commentary

Scientific Knowledge on the Subject: Preeclampsia is a major cause of pregnancy inflammation due to a disturbed immune tolerance between mother and fetus, a tolerance otherwise characterizing a healthy pregnancy. Pregnancy inflammation is a suspected risk factor for childhood asthma, allergy, and eczema.

What This Study Adds to the Field: We demonstrate that preeclampsia is a shared prenatal risk factor for asthma, eczema, and allergy in childhood. These results point toward pregnancy as a critical period for immune programming of the child. Longer duration of preeclampsia was associated with higher risk, suggesting a mechanism of *in utero* inflammation causing a lasting immune deregulation in the fetus.

Chronic immune-mediated diseases have increased with socioeconomic affluence (1). Childhood asthma, allergy, and eczema likely originate in early life, where environmental exposures may affect the immature immune system, which is prone to alterations (2). Early programming of immune-mediated disease may happen already during pregnancy, where the fetal immune system is evolving and exposed to the maternal immune responses, normally dampened to protect the fetus from rejection (2, 3). Chronic low-grade inflammation in the pregnant woman is suspected to be harmful for the child developing *in utero* (4), and responsible for initiating a skewed trajectory toward disease. The inflammatory status of the pregnant woman is affected by various factors (e.g., a diet low in antiinflammatory n3-long-chain polyunsaturated fatty acid has been linked to increased risk of childhood asthma and related disorders) (5–7).

Preeclampsia is characterized by excessive maternal inflammation in pregnancy with both innate and adaptive immune activation (8, 9), and absence of the T-helper type 2 (Th2) skewness typical for a healthy pregnancy, and thus the predominance of Th1-type immunity (10). It is a condition occurring after pregnancy week 20 determined by new-onset proteinuria and hypertension (11) and is among the main causes of maternal and fetal morbidity and mortality (12). Changes in the earliest immune cell compartments of the newborn child after a pregnancy with preeclampsia have been described previously (13).

We hypothesized that *in utero* exposure to excessive inflammation caused by preeclampsia could mediate immune deregulation in the child leading to a trajectory toward inflammatory disease in childhood. We investigated the association between preeclampsia and development of asthma, eczema, allergic rhinitis, sensitization, and lung function in our prospective clinical birth cohort, the Copenhagen Prospective Studies on Asthma in Childhood$_{2000}$ (COPSAC$_{2000}$). To test whether such associations could be replicated, we used data from the Danish national registries in the 35-year period 1977–2012.

Methods

Ethics

The COPSAC$_{2000}$ cohort study was conducted in accordance with the guiding principles of the Declaration of Helsinki and was approved by the Local Ethics Committee (KF 01-289/96) and the Danish Data Protection Agency (2015-41-3696). Both parents gave written informed consent before enrollment.

The registry study was based on existing data in national registries and approved by the Danish Data Protection Agency (2012-41-0388). Because subjects were not contacted as part of the study, written informed consent was not required.

Study Population

COPSAC$_{2000}$ birth cohort. COPSAC$_{2000}$ is a prospective clinical birth cohort study of 411 children born 1998–2001 to mothers with doctor-diagnosed asthma, excluding children with gestational age less than 36 weeks, severe congenital abnormalities, or any lung symptoms before enrollment, as previously described in detail (14).

Participants were assessed until age 7 years at the research unit with 6-month intervals and additional visits were arranged immediately at onset of lung or skin symptoms. At every visit, a full physical examination was performed, and history was obtained using structured questions and closed response categories focusing on the child's lung and skin symptoms, medication, healthcare use, lifestyle, and home environment. The COPSAC pediatricians were solely responsible for diagnosis and treatment of all respiratory, allergy, and skin-related symptoms according to predefined validated algorithms (15, 16). Data validation and quality control follow the guidelines for good clinical practice.

Clinical investigator-diagnosed endpoints. Asthma was diagnosed when a child fulfilled all of the following criteria: (*1*) recurrent wheeze, defined by troublesome lung symptoms with a burden of greater than or equal to five episodes of greater than or equal to 3 consecutive days within 6 months with symptoms captured by daily diary cards filled from birth by the parents; (*2*) symptoms judged by the COPSAC pediatricians to be typical of asthma (e.g., exercise-induced symptoms, prolonged nocturnal cough, recurrent cough outside common cold, or symptoms causing wakening at night); (*3*) in need of intermittent rescue use of inhaled β_2-agonist; and (*4*) improvement of symptoms during a 3-month trial of inhaled corticosteroids (ICS) and relapse after end treatment (16, 17).

Eczema was based on the criteria of Hanifin and Rajka (18), requiring the presence of three of four major criteria and at least 3 of 23 minor criteria (19, 20).

Allergic rhinitis was based on relevant aeroallergen sensitization and clinical interviews of the parents on history of significant nasal congestion, sneezing, and/or runny nose outside periods with common cold (21–23).

Objective assessments. Allergen-specific IgE levels was measured at age 0.5, 1.5, 4, and 6 years for 16 common inhalant and food allergens (cat, dog, horse, birch, timothy grass, mugwort, house dust mites [*Dermatophagoides pteronyssinus* and *D. farinae*], molds, hen's egg, cow's milk, fish, wheat, peanut, soybean, and shrimp) by ImmunoCAP assay (Pharmacia Diagnostics AB, Uppsala, Sweden). Values of specific IgE greater than or equal to 0.35 kU/L were considered indicative of allergic sensitization (24), and was analyzed as dichotomized values for (*1*) any allergens, (*2*) food allergens, and (*3*) inhaled allergens.

Total-IgE levels were measured at age 0.5, 1.5, 4, and 6 years by ImmunoCAP (Pharmacia Diagnostics AB) with a detection limit of 2 kU/L (25).

Skin prick test was performed at 0.5, 1.5, 4, and 6 years of age for eight inhalant allergens (dog, cat, horse, birch, timothy grass, mugwort, *Dermatophagoides pteronyssinus*, and molds), and six food allergens (milk, egg, wheat flour, soybean, cod, and peanut) (ALK-Abelló, Copenhagen, Denmark), as well as fresh cow's milk and pasteurized hen's egg. A mean wheal diameter of 2 mm or more larger than that elicited by the negative control at 0.5 and 1.5 years, and of 3 mm or larger at 4 and 6 years was considered indicative of sensitization and was analyzed as dichotomized values for (*1*) any allergens, (*2*) food allergens, and (*3*) inhaled allergens.

Infant spirometry was performed during sedation at age 1 month by applying the raised volume rapid thoracoabdominal compression technique as previously detailed (26, 27). The $FEV_{0.5}$ and forced expiratory flow at 50% of the forced vital capacity (FEF_{50}) were used as lung function indices.

Spirometry was performed at age 7 years using a pneumotachograph Masterscope Pneumoscreen spirometer (Erich Jaeger, Wurtzburg, Germany) measuring the FEV_1 and FEF_{50} (28).

Bronchial responsiveness was assessed at age 1 month as previously detailed (26) by continuous measurements of Ptc_{O_2} during quadrupling methacholine dose-steps and the provocative dose was defined as causing a 15% drop in Ptc_{O_2} (PD_{15}). At age 7 years bronchial responsiveness was defined as the provocative dose of methacholine causing a 20% drop in FEV_1 from baseline (PD_{20}) (29).

Preeclampsia. The diagnosis of preeclampsia was collected by personal interview and validated against registry information.

Covariates. Covariates included sex, maternal age at childbirth, maternal smoking and antibiotic use in pregnancy, gestational age, birth weight, delivery by caesarean section, cat and dog exposure, older children, paternal asthma, allergy or eczema, household income, and maternal educational level.

Registry-Based Cohort

The Danish Civil Registration System (CRS) provides a unique 10-digit number for every citizen, encoding date of birth and sex, and is used as the identifying number in all Danish registries. Furthermore, this registry contains date of emigration (30). The Danish Medical Birth Registry contains data on birth weight, parity, mode of delivery, and maternal smoking during pregnancy. The Danish Register of Causes of Death contains data on date of death. The Danish National Patient Registry contains data on all primary and secondary diagnosis on all inpatient discharges since 1977, and on outpatient admissions since 1994. Diagnoses are based on the International Statistical Classification of Diseases and Related Health Problems (ICD-8 until 1994; ICD-10 thereafter) (31).

We identified a cohort of live born children in Denmark in the period January 1, 1978, through December 31, 2011, and used their CRS numbers to link to information on maternal age, sex, parity, maternal smoking during pregnancy, mode of delivery, and maternal identification number from the Danish Medical Birth Registry; information on date of death from the Danish Register of Causes of Death; information on date of first hospital inpatient or outpatient admission for any of the predefined diseases (primary or secondary diagnosis) from the Danish National Patient Registry; and information on date of migration from the Danish CRS. Only children whose mothers were born after 1952 were included in the analyses. The selection process is illustrated in a flow diagram (*see* Figure E1 in the online supplement).

Case definitions. Cases were identified by the ICD-8 and -10 diagnoses. Four diseases were investigated: asthma (ICD-8, 439.x; ICD-10, J45.x and J46.x), eczema (ICD-8, 69100; ICD-10, L20.x), allergic rhinitis (ICD-8, 50703, 50708, and 50709; ICD-10, J30.x), and food allergy (ICD-8, 56102; ICD-10, K52.2).

Preeclampsia definitions. Preeclampsia was identified from maternal hospitalization with diagnoses 637.x (ICD-8) and O14.x (ICD-10) during pregnancy (20 wk before birth to 2 wk after birth). Duration was calculated from the first hospital contact with a preeclampsia diagnosis until date of birth. Preeclampsia was dichotomized as (*1*) ever, (*2*) less than 14 days duration, and (*3*) greater than or equal to 14 days duration.

Maternal disease. We obtained information from the Danish National Patient Registry on maternal hospital admission for the specified diseases to adjust the analyses for maternal disease.

Confounders. Confounders were chosen *a priori* as sex, parity (first child, second child, third child, or more), birth weight ($\geq$2.5–$<$3.0 kg, $\geq$3.0–$<$3.5 kg, $\geq$3.5–4.0 kg, $\geq$4.0 kg), attained age (1-yr intervals), calendar time (3-yr intervals), season of birth (December to February, March to May, June to August, September to November), maternal age ($\leq$25, 26–30, 31–35, $\geq$36 yr), mode of delivery, gestational age ($<$37 wk, $\geq$37 wk), and maternal disease.

An overview of the diagnostic criteria for maternal preeclampsia and clinical outcomes in the two cohorts is shown in Table E1.

Statistics

In the $COPSAC_{2000}$ cohort, chi-square test or Student's *t* test was used for simple associations in the baseline characteristics of pregnancies with preeclampsia. Associations between preeclampsia and the dichotomized variables asthma, ICS treatment, eczema, and allergic rhinitis at age 7 years were assessed using logistic regression. Total IgE levels, specific IgE levels, and skin prick test results measured at four time points were assessed by using a generalized estimating equation analysis (repeated measures). Specific IgE levels and skin prick test results were analyzed as dichotomized variables with a log link. The associations between preeclampsia and lung function indices were analyzed by using generalized linear models. PD_{15}, PD_{20}, and total-IgE were log-transformed and *z* scores were calculated for the height and sex calibrated $FEV_{0.5}$, FEV_1, and FEF_{50} before analyses. Results were adjusted for confounders associated with preeclampsia.

In the registry-based cohort, for each disease category we accumulated person-time from birth to age 15, or death, or emigration (whichever came first) and events (cases) stratified by preeclampsia and chosen confounders (32). Thereby we calculated the specific incidence rates (cases per person- years). The overall effects of preeclampsia on the number of cases in each separate disease category in the complete time period and across the entire age span were estimated with log-linear Poisson regression models offset by the log of person time (yrs of observation), where any underlying age and calendar time variations for the various disease groups can be taken into account by adjusting for the attained age and calendar year as categorical confounders. Furthermore, we added the chosen confounders to obtain the fully adjusted estimates for the effect of

preeclampsia. All confounders were included as categorical variables. The incidence rate ratio (IRR) was calculated to show the effect of preeclampsia. The population attributable risk (PAR) fractions were calculated from the adjusted IRR estimates as follows: PAR = Pe (RRe-1)/[1 + Pe (RRe-1)], where Pe is the prevalence of the exposure and RRe is the relative risk of disease caused by that exposure.

A significance level of 0.05 was used in all types of analyses. All estimates were reported with 95% confidence intervals. Missing data were treated as missing observations. The data processing was conducted using SAS version 9.3 for Windows (SAS Institute Inc., Cary, NC).

Results

COPSAC$_{2000}$

The COPSAC$_{2000}$ cohort included 411 children born to mothers with asthma. A total of 5.6% (n = 23) of the mothers were diagnosed with preeclampsia in pregnancy. Compared with the background population from the register-based cohort with a preeclampsia prevalence of 3.7%, maternal asthma significantly increased the risk of preeclampsia ($P = 0.0408$). There was a higher prevalence of delivery by caesarean section in pregnancies with preeclampsia (39%; n = 9) compared with 20% (n = 77) in pregnancies without preeclampsia. No other significant associations were found between cohort baseline characteristics and preeclampsia. Therefore, all analyses in COPSAC$_{2000}$ were only adjusted for mode of delivery (Table 1).

Preeclampsia and asthma. In COPSAC$_{2000}$ 14% (n = 47) of the 336 children fulfilled the study criteria for asthma at age 7 years. Preeclampsia was not significantly associated with risk of asthma. However, we found an increased risk of ongoing treatment with inhaled corticosteroids at age 7 years (adjusted odds ratio [aOR], 4.01 [95% confidence intervals (CI), 1.11–14.43]; $P = 0.0337$) (Table 2).

Preeclampsia and lung function. Preeclampsia was not associated with any changes in lung function measurements at age 1 month ($FEV_{0.5}$, FEF_{50}, PD_{15}) nor the forced flows assessed at 7 years (FEV_1, FEF_{50}). Preeclampsia was, however, associated with increased bronchial responsiveness to methacholine (PD_{20}) at age 7 years (adjusted β coefficient log-μmol, −0.80 [95% CI, −1.55 to −0.06]; $P = 0.0348$) (Table 2).

Table 1. Baseline Characteristics for COPSAC$_{2000}$ Grouped According to Preeclampsia in Pregnancy

	Preeclampsia		
	Yes	No	*P* Value
All, % (n)	**6 (23)**	94 (388)	—
Male sex, % (n)	57 (13)	49 (190)	0.482
Gestational age, mean (SD), wk	39.6 (2.2)	40.1 (1.4)	0.271
Birth weight, mean (SD), kg	3.5 (0.9)	3.5 (0.5)	0.828
Caesarean section, % (n)	39 (9)	20 (77)	**0.028**
Maternal age at birth, mean (SD), yr	31.6 (4.6)	29.9 (4.5)	0.087
Maternal smoking in pregnancy, % (n)	26 (6)	24 (92)	0.795
Antibiotic use in pregnancy, % (n)	39 (9)	30 (116)	0.350
Paternal asthma, allergy, or eczema, % (n)	55 (12)	46 (173)	0.449
Older children in the home, % (n)	41 (9)	39 (142)	0.859
Cat in the home from birth, % (n)	13 (3)	16 (58)	0.750
Dog in the home from birth, % (n)	14 (3)	14 (52)	0.976
Maternal educational level*, % (n)			0.291
Low	57 (12)	60 (214)	
Medium	38 (8)	26 (92)	
High	5 (1)	14 (51)	
Household income at birth (yearly)†, % (n)			0.077
Low	9 (2)	30 (110)	
Medium	55 (12)	47 (170)	
High	36 (8)	23 (82)	

Definition of abbreviation: COPSAC$_{2000}$ = Copenhagen Prospective Studies on Asthma in Childhood$_{2000}$.
All mothers in the cohort have asthma.
*Low (primary school, secondary school, or college graduate), medium (tradesman or bachelor degree), high (master's degree).
†Low (<53.000 €), medium (53.000–80.000 €), high (>80.000 €).
Bold indicates a statistically significant difference with a *P* value less than 0.05.

Preeclampsia and eczema. Preeclampsia was not significantly associated with risk of eczema (aOR, 1.84 [95% CI, 0.67–5.10]; $P = 0.2399$) (Table 2).

Preeclampsia and allergy. Preeclampsia was associated with an increased risk of allergic rhinitis at age 7 years (aOR, 4.83 [95% CI, 1.58–14.78]; $P = 0.0057$). Using generalized estimating equation models for analysis of repeated assessments from age 0.5 to 6 years demonstrated increased levels of total IgE (adjusted estimate log-kU/m, 0.64 [95% CI, 0.17–1.12]; $P = 0.0081$) and any specific IgE sensitization (aOR, 2.63 [95% CI, 1.18–5.87]; $P = 0.0178$) and any skin prick test positivity (aOR, 4.71 [95% CI, 2.12–10.46]; $P = 0.0001$). Preeclampsia was significantly associated with increased risk of sensitization toward inhalant and food allergens in both specific IgE measurements and skin prick tests (Table 2).

Registry-Based Cohort

The registry-based cohort included 1,698,638 Danish children born between 1978 and 2012 with data on preeclampsia, outcomes, and all confounders. A total of 3.7% (62,728) of the pregnancies were complicated with preeclampsia. Mothers with asthma had preeclampsia significantly more often than the control population (4.6 vs. 3.7%; $P < 0.0001$), but not eczema or allergy (*see* Table E2). Table 3 describes characteristics for pregnancies with preeclampsia. Especially first-born children, lower maternal age, and multiple birth were determinants for preeclampsia, and children were more often delivered by caesarean section at a lower gestational age and with a lower birth weight. In 1.0% (16,146) of the pregnancies, the preeclampsia was present for 14 days or more before delivery (*see* Table E3 for differences by duration of preeclampsia).

Preeclampsia and asthma. The risk of asthma was increased in children born to mothers with preeclampsia (adjusted IRR [aIRR], 1.09 [95% CI, 1.05–1.12]; $P < 0.001$). In the stratified analysis, the risk of asthma increased with increased duration of preeclampsia (⩾14 d, aIRR of 1.17 [95% CI, 1.11–1.25], $P < 0.001$ compared with <14 d, aIRR of 1.05 [95% CI, 1.01–1.09], $P = 0.010$) (Table 4).

Preeclampsia and eczema. Preeclampsia was overall not associated with the risk of eczema, but in the stratified analysis, a

Table 2. $COPSAC_{2000}$: Preeclampsia in Pregnancy and Risk of Disease in the Children

Endpoint	Age		Estimate (95% CI)	*P* Value
Current disease		Cases/N	Odds Ratio	
Asthma	7 yr	3/47	1.13 (0.31 to 4.13)	0.8496
ICS treatment (ongoing)	7 yr	4/24	4.01 (1.11 to 14.43)	**0.0337**
Eczema	7 yr	6/71	1.84 (0.67 to 5.10)	0.2399
Allergic rhinitis	7 yr	6/38	4.83 (1.58 to 14.78)	**0.0057**
Repeated assessments		N	GEE Estimate	
Total IgE, log-kU/ml	0.5, 1.5, 4, and 6 yr	1,336	0.64 (0.17 to 1.12)	**0.0081**
		N	GEE Odds Ratio	
Specific IgE (any positive)	0.5, 1.5, 4, and 6 yr	1,336	2.63 (1.18 to 5.87)	**0.0178**
Inhalants		1,342	3.55 (1.50 to 8.41)	**0.0040**
Food		1,336	2.93 (1.19 to 7.19)	**0.0189**
Skin prick test (any positive)	0.5, 1.5, 4, and 6 yr	1,251	4.71 (2.12 to 10.46)	**0.0001**
Inhalants		1,284	4.76 (2.09 to 10.86)	**0.0002**
Food		1,198	5.22 (1.89 to 14.39)	**0.0014**
Lung function		N	β Coefficient	
z-$FEV_{0.5}$	1 mo	402	−0.22 (−0.65 to 0.20)	0.3052
z-FEF_{50}	1 mo	397	0.11 (−0.33 to 0.54)	0.6221
log-PD_{15}	1 mo	361	0.25 (−0.56 to 1.06)	0.5450
z-FEV_1	7 yr	313	0.17 (−0.32 to 0.67)	0.4874
z-FEF_{50}	7 yr	309	−0.08 (−0.57 to 0.41)	0.7475
log-PD_{20}	7 yr	253	−0.80 (−1.55 to −0.06)	**0.0348**

Definition of abbreviations: CI = confidence interval; $COPSAC_{2000}$ = Copenhagen Prospective Studies on Asthma in $Childhood_{2000}$; FEF_{50} = forced expiratory flow at 50% of the forced vital capacity; GEE = generalized estimating equation; ICS = inhaled corticosteroids; PD_{15} = provocative dose of methacholine causing a 15% drop in transcutaneous oxygen saturation; PD_{20} = provocative dose of methacholine causing a 20% drop in FEV_1. All analyses are adjusted for type of delivery. Bold indicates a statistically significant difference with a *P* value less than 0.05.

duration of preeclampsia of greater than or equal to 14 days was associated with increased risk of eczema (aIRR, 1.15 [95% CI, 1.01–1.32]; $P = 0.042$) (Table 4).

Preeclampsia and allergy. Preeclampsia was significantly associated with increased risk of both allergic rhinitis (aIRR, 1.14 [95% CI, 1.05–1.24]; $P = 0.002$) and food allergy (aIRR, 1.21 [95% CI, 1.05–1.39]; $P = 0.009$). In the stratified analyses the association remained significant only with a duration of preeclampsia of greater than or equal to 14 days for allergic rhinitis (aIRR, 1.29 [95% CI, 1.11–1.50]; $P < 0.001$) and food allergy (aIRR, 1.32 [95% CI, 1.02–1.71]; $P = 0.037$) (Table 4).

Stratified analyses for ICD-8 versus ICD-10 disease coding showed similar results (data not shown).

With preeclampsia being an uncommon condition, the population attributable risk fractions for the investigated childhood diseases range from 0.1 to 0.8% for preeclampsia.

Discussion

Primary Findings

We have demonstrated significant associations between preeclampsia in pregnancy and increased risk of current inhaled corticosteroid treatment, allergic rhinitis, and increased bronchial responsiveness in 7-year-old children from the $COPSAC_{2000}$ cohort. Furthermore, these children had an increased risk of sensitization to both aeroallergens and food allergens. We replicated these findings in the Danish national registries. Preeclampsia was associated with increased risk of both asthma and eczema and allergic rhinitis and food allergy in childhood, especially pronounced after a duration of preeclampsia of greater than or equal to 14 days, although being a minor contributor for the overall risk burden of these diseases. Maternal asthma increased the risk of preeclampsia.

Strengths and Limitations

The main strength of this study is the prospective design of the $COPSAC_{2000}$ birth cohort. Children from the cohort were subjected to meticulous clinical monitoring, diagnosing of symptoms based on standard operating procedures by the investigators from the clinical research unit through 7 years of life at regular 6-month intervals for acute lung and skin manifestations. The longitudinal assessments from birth ensure robust clinical endpoints and improved statistical power from these longitudinal data. Another significant strength is the range of available objective assessments of lung function and bronchial responsiveness both in the neonate and at age 7 years performed by skilled research staff according to standard operating procedures. In the cohort the allergy endpoints are strengthened by the clinical evaluated allergic rhinitis diagnosis along with repeated measures of both skin prick testing and specific IgE for inhalant and food sensitization.

The main limitation in the $COPSAC_{2000}$ cohort is the few cases of preeclampsia, which limits the study power. This also limits the possibility to analyze possible effects of the duration of preeclampsia.

In the registry-based cohort, we had the possibility to replicate and expand the findings given the great statistical power. The main strength of the registry-based cohort is the vast material from 35 years of data collection in national registries. The cohort covers all hospital admissions and outpatient clinic contacts nationwide. The high numbers allowed for stratification of preeclampsia according to duration of the diagnosis. The associations were mainly found in children exposed to preeclampsia for a longer duration *in utero*, which provides a strong proof concept.

We adjusted the associations for a vast number of potential confounders; especially

Table 3. Baseline Characteristics for the Registry-Based Cohort, Grouped According to Preeclampsia in Pregnancy (N = 1,698,638)

		Preeclampsia (%)	
		Yes (*n = 62,728*)	No (*n = 1,635,910*)
Season of birth	Autumn	24.45	24.62
	Winter	24.18	23.08
	Spring	26.39	25.66
	Summer	24.99	26.64
Child sex	Male	52.07	51.29
	Female	47.93	48.71
Parity (child number)	First	66.72	46.92
	Second	24.48	37.52
	Third or more	8.80	15.56
Maternal age, yr	<25	37.42	27.86
	26–30	36.85	39.40
	31–35	18.98	24.88
	⩾36	6.76	7.87
Singleton/multiple birth	Singleton	92.21	96.83
	Multiple	7.79	3.17
Delivery mode	Vaginal	65.71	84.89
	Caesarean section	34.29	15.11
Gestational age, wk	<37	17.13	5.48
	⩾37	82.87	94.52
Birthweight, kg	<2.5	17.61	4.75
	2.5–3.0	16.68	11.90
	3.0–3.5	26.91	32.20
	3.5–4.0	24.46	33.60
	⩾4.0	14.34	17.56

low birth weight, low gestational age, and delivery by caesarean section are associated with preeclampsia and could also be associated with increased disease risk in the children. These confounders did not alter the results considerably; however, there may still exist residual confounding (e.g., from the categorical definitions used in the confounders). Maternal asthma was associated with preeclampsia, which could lead to an issue of reverse causation. This was, however, not found for eczema or allergy and adjusting for maternal disease did not alter the results.

Most patients in Denmark suffering from asthma, eczema, and allergies attend their general practitioner for diagnosis and treatment. This limits the number of cases in our registry-based study, because we only use diagnoses from inpatient and outpatient hospital contacts. Even though we may miss many (milder) cases, we still provide statistical significant correlations, and the cases we included are probably the most severe cases requiring hospital contact.

The diagnostic classification changed in 1994 from ICD-8 to ICD-10, and registration of outpatient hospitalizations was initiated here. The disease categories used across these different classifications may not be completely congruent. However, stratified analyses showed similar results and the analyses were adjusted for calendar year, which corrected the main association from time-changing effects, limiting the effect of trend bias.

Interpretation

Our study proposes preeclampsia in pregnancy causes an altered fetal immunity. The developing immune system of the child is maturing during pregnancy, and may be susceptible to pregnancy exposures, such as maternal inflammation (2), smoking (33), and diet (7), influencing fetal immune programming through immune regulatory processes at the fetomaternal interface. The maternal inflammation caused by preeclampsia is a process going on before the onset of clinical symptoms in the woman (11, 12), starting already at the end of the first trimester of pregnancy (9). We mainly found significant associations between preeclampsia and asthma, eczema, and allergies with a longer duration of symptoms. This could point toward a disease mechanism going on *in utero*, rather than complications leading to a more acute course of preeclampsia. We speculate that an extended duration of *in utero* exposure to preeclampsia could thereby exacerbate the possible impact on the fetus, which could be facilitated through the fetal immune cell maturation. Immune tolerance is in particular mediated by thymus-derived regulatory T (Treg) cells and it has been described that the fetal thymus is significantly smaller in pregnancies with preeclampsia (34). Alternatively, the observations might originate from different disease entities in early onset and late-onset preeclampsia (35).

In normal pregnancies, increased levels of Th2 and Treg cells adjust the maternal immune responses to avoid rejection of the fetus; however, this balance is skewed in

Table 4. Registry-Based Cohort

	Cases	Preeclampsia		
		All	<14 Days	⩾14 Days
Asthma	90,114	1.09 (1.05–1.12); ***P* < 0.001**	1.05 (1.01–1.09); ***P* = 0.01**	1.17 (1.11–1.25); ***P* < 0.001**
Eczema	19,164	1.06 (0.98–1.14); *P* = 0.14	1.02 (0.94–1.12); *P* = 0.59	1.15 (1.01–1.32); ***P* = 0.042**
Allergic rhinitis	13,167	1.14 (1.05–1.24); ***P* = 0.002**	1.09 (0.98–1.20); *P* = 0.11	1.29 (1.11–1.50); ***P* < 0.001**
Food allergy	4,788	1.21 (1.05–1.39); ***P* = 0.009**	1.17 (0.99–1.38); *P* = 0.06	1.32 (1.02–1.71); ***P* = 0.037**

Preeclampsia in pregnancy and risk of disease in the children. Results are presented as incidence rate ratios with 95% confidence intervals; *P* values correspond to Wald test. N = 1,698,638.
All estimates are adjusted for age and calendar year, birthweight, gestational age, sex, mode of delivery, parity, multiple births, season of birth, maternal age, and mother's disease. Bold indicates a statistically significant difference with a *P* value less than 0.05.

pregnant women, who develop preeclampsia (10). Increasing evidence suggests wide transplacental regulation of cellular immunity between mother and fetus (36), and the levels of both Th2 and Treg cells have been found lowered in blood from women with ongoing preeclampsia (37) and in cord-blood of the newborn after preeclampsia (13). The proliferative responses of T cells derived from cord-blood are reduced in children, who later become allergic (38), and cytokine levels at birth have likewise been associated with a later development of allergy in the child (39). High cord-blood levels of Th2-related chemokines have been associated with increased total-IgE levels (40) and asthma, allergy (41), and eczema (42) in childhood, and thereby skewed levels of immune cells, especially Treg cells after preeclampsia could be a possible link between preeclampsia and childhood disease. We found consistent associations between preeclampsia and allergic sensitization. This may be a contributing factor to diagnosis of both asthma and eczema.

The prevalence of preeclampsia was found higher among women carrying their first child, with lower maternal age, and in women carrying more than one child. When the mother had preeclampsia the children were more often delivered by caesarean section, at a lower gestational age, and with a lower birth weight. Very interestingly, the prevalence was markedly increased if the mother had asthma, but not eczema or allergy. Asthma in pregnancy has previously been associated with preeclampsia (43). These characteristics may help to identify women at risk for developing preeclampsia along with biochemical markers (12) and/or clinical measures, such as uterine artery Doppler ultrasound (12) or ultrasonographic parameters (34) in pregnancy. A pregnant woman with asthma could be subject to evaluation for early signs of preeclampsia. If the systemic effects caused by preeclampsia could be attenuated by early treatment in pregnancy or planned delivery initiated earlier after symptom debut, this would perhaps lead to long-term health benefits for the offspring.

Conclusions

Preeclampsia exemplifies a shared prenatal risk factor for asthma, eczema, and allergy in childhood. These results point toward pregnancy as a critical period for immune programming of the child. The duration of preeclampsia increases the risk, suggesting a mechanism of *in utero* inflammation causing a lasting immune deregulation in the fetus. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

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Preeclampsia associates with Asthma, Allergy and Eczema in Childhood

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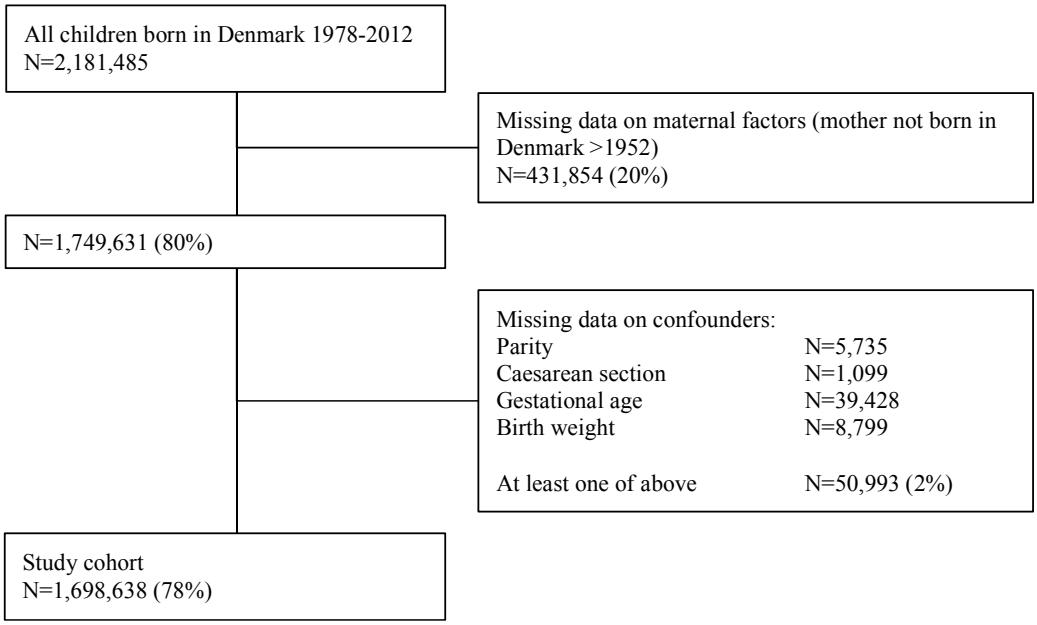
ONLINE DATA SUPPLEMENT

Supplementary Materials

Online Figure E1: Flow diagram describing the selection of the registry based cohort.



Online Table E1: Diagnostic criteria for maternal preeclampsia and clinical outcomes in the two cohorts.

	$COPSAC_{2000}$ (N=411)	**Registry based cohort (N=1,698,638)**
Preeclampsia	Personal interview, validated against registry information (ICD-10 O14.x)	Hospital contact, in- or out-patient diagnosis; ICD-8 (637xx), ICD-10 (O14.x) 20 week before birth to 2 weeks after birth
Clinical end-point		
Asthma	Prospective clinical diagnosis by pediatricians in the research unit based on predefined criteria and diary information.	Hospital contact, in- or out-patient diagnosis (ICD-8: 439xx; ICD-10: J45.x, J46.x)
Eczema	Prospective clinical diagnosis by pediatricians in the research unit based on Hanifin and Rajka's criteria	Hospital contact, in- or out-patient diagnosis (ICD-8: 69100; ICD-10: L20.x)
Allergy	Allergic rhinitis; prospective clinical diagnosis by pediatricians in the research unit, including relevant aeroallergen sensitization and clinical information.	Hospital contact, in- or out-patient diagnosis. Allergic rhinitis (ICD-8: 50703, 50708, 50709; ICD-10: J30.x) Food allergy (ICD-8: 56102; ICD-10: K52.2)

Online Table E2: Association between maternal disease and preeclampsia.

N=1,698,638

	Preeclampsia proportion; Maternal disease/no maternal disease	Chi-square p
Asthma	4.6% / 3.7%	<0.0001
Eczema	3.6% / 3.7%	0.75
Allergic rhinitis	3.8% / 3.7%	0.29
Food Allergy	4.2% / 3.7%	0.27

Table E3: Baseline characteristics for the registry based cohort; grouped according to preeclampsia duration in pregnancy. N=62,728

		Preeclampsia duration	
		<14 days N=46,582	**≥14 days N=16,146**
Season of birth	Autumn	24.73%	23.64%
	Winter	23.96%	24.83%
	Spring	26.25%	26.78%
	Summer	25.07%	24.75%
Child gender	Male	52.27%	51.49%
	Female	47.73%	48.51%
Parity (child number)	First	67.23%	65.25%
	Second	24.31%	24.98%
	Third or more	8.46%	9.76%
Maternal age (years)	-25	37.45%	37.30%
	26 - 30	37.17%	35.93%
	31 - 35	18.81%	19.47%
	36-	6.57%	7.29%
Singleton / multiple birth	Singleton	92.85%	90.38%
	Multiple	7.15%	9.62%
Delivery mode	Vaginal	66.69%	62.89%
	Caesarean section	33.31%	37.11%
Gestational age (weeks)	< 37	16.69%	18.38%
	37+	83.31%	81.62%
Birth weight (kg)	-2.5	16.72%	20.20%
	2.5 - 3.0	16.39%	17.52%
	3.0 - 3.5	27.40%	25.47%
	3.5 - 4.0	25.09%	22.64%
	4.0-	14.40%	14.17%

PAPER III:

Stokholm, J., Sevelsted, A., Bønnelykke, K., and Bisgaard, H. (2014).

Maternal propensity for infections and risk of childhood asthma: a registry-based cohort study.

The Lancet Respiratory Medicine *2*, 631–637.

PAPER III

PAPER III

Maternal propensity for infections and risk of childhood asthma: a registry-based cohort study

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Summary

Background Maternal use of antibiotics during pregnancy has been associated with the development of asthmatic disorders in the offspring. The human microbiome has been suggested to act as an intermediary in this process. To provide clarification on this theory, we studied the temporal relation between maternal use of antibiotics and the risk of childhood asthma.

Methods According to national registries, during the observation period (1997–2010), 910 301 children were born in Denmark and were included in the analysis. From these registries, data for cases of childhood asthma were obtained based on hospital admissions, outpatient attendance at a hospital, or use of inhaled corticosteroids. The effect of timing of maternal antibiotic use on the risk of asthma in the offspring was studied by analysis of maternal antibiotic use in the 80 weeks before pregnancy, during pregnancy, and the 80 weeks after pregnancy. Results were adjusted for age and calendar year, birthweight, gestational age, sex, mode of delivery, parity, multiple births, season of birth, and several maternal factors (age, smoking during pregnancy, employment status, and asthma).

Findings In this study, we replicated our previous finding that maternal use of antibiotics in pregnancy was associated with an increased risk of childhood asthma: the adjusted incidence rate ratio (aIRR) was 1·24 (95% CI 1·18–1·30) for inpatient admission, 1·22 (1·18–1·26) for outpatient attendance, and 1·18 (1·15–1·20) for inhaled corticosteroid use. A similar and independent association was also recorded for maternal antibiotic use in the 80 weeks before and after the pregnancy. A dose-related association occurred between the risk of childhood asthma and the number of maternal antibiotic treatments and was recorded separately for antibiotic treatment for respiratory tract infections and for other types of infections.

Interpretation Maternal use of antibiotics has a dose-related association with the risk of asthma in the offspring, but this association is independent of the temporal relationship with the pregnancy period. This finding suggests that maternal antibiotic use is a surrogate marker of a mother's general propensity for infections as the underlying link between a mother's use of antibiotics and risk of asthma in the offspring.

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Introduction

We have previously reported an association between maternal use of antibiotics during pregnancy and asthmatic disorders in the offspring.[1] These findings were recorded in our clinical birth cohort study[1] and replicated in a national birth cohort study[1] and are consistent with cross-sectional reports.[2,3] This association led us to speculate that the effect might be caused by derangements of neonatal microbial colonisation patterns,[4,5] inflammation caused by an infection during pregnancy,[6] or an adverse effect of the antibiotic drug taken by the mother.

Therefore, in this study, we analysed the association between timing of maternal use of systemic antibiotics during pregnancy, or in the time surrounding pregnancy, and the risk of asthma in the offspring. We postulated that if maternal antibiotic use was causally associated with asthma in children, the recorded association would be strongest for antibiotic use during, or shortly before, the pregnancy period. However, if antibiotic use was merely a surrogate marker of maternal lifestyle or genetic propensity for infections, a similar, independent association would be recorded for maternal antibiotic use outside of the pregnancy period, including the time after birth. We studied this hypothesis by analysing associations between maternal use of antibiotics and the occurrence of asthma in the offspring in a period from 80 weeks before pregnancy, during pregnancy, and up to 80 weeks post partum in a national registry-based dataset, allowing for stratified analyses in relation to timing of antibiotic use, type of antibiotic, and maternal asthma.

Methods

Study design and population

In this prospective registry-based cohort study, we identified a cohort of all liveborn children in Denmark in the period 1997–2010 from the Danish Civil Registration System. The study was based on data from national registries. We used the childrens' unique personal identification number (assigned by the Danish Civil Registration System to all people with permanent residency in Denmark) to link information about

	Any antibiotics	Antibiotics for respiratory tract infections	Antibiotics for urinary tract infections	Antibiotics for other infections
80–40 weeks before pregnancy	278 632 (31%)	217 463 (24%)	69 156 (8%)	31 622 (4%)
40 weeks before start of pregnancy	287 381 (32%)	225 116 (25%)	70 717 (8%)	32 669 (4%)
During pregnancy	289 348 (32%)	185 071 (21%)	142 894 (16%)	8034 (1%)
First trimester	121 377 (14%)	72 945 (8%)	52 061 (6%)	4747 (<1%)
Second trimester	127 776 (14%)	74 559 (8%)	61 339 (7%)	1944 (<1%)
Third trimester	118 388 (13%)	67 744 (8%)	58 488 (7%)	1677 (<1%)
40 weeks post partum	281 416 (32%)	237 596 (27%)	41 333 (5%)	38 548 (4%)
40–80 weeks post partum	311 703 (35%)	264 446 (30%)	54 629 (6%)	30 776 (3%)
Entire period: 80 weeks before pregnancy to 80 weeks post partum	691 850 (78%)	610 351 (68%)	271 181 (30%)	120 396 (14%)

Data are n (%).

Table 1: **Prevalence of antibiotics collected from pharmacies in 891 555 pregnancies**

maternal age, the sex of the child, date of birth, start of pregnancy (1997–2004 from last menstrual period, since 2004 from 12-week routine ultrasound scan), birthweight, parity, maternal smoking during pregnancy, mode of delivery, and maternal personal identification number from the Danish Medical Birth Registry; prescription of antibiotics and inhaled corticosteroids from the National Prescription Registry; hospital admissions in which asthma was the primary diagnosis from the Danish National Patient Registry; maternal employment status from Statistics Denmark; and date of migration away from Denmark from the Danish Civil Registration System.

See Online for appendix

The study was approved by the Danish Data Protection Agency (number 2012-41-0388). The National Committee on Health Research Ethics did not require written informed consent from participants because personal identifiers were stored and encrypted by a trusted third party (the National Registry of Medicinal Product Statistics); therefore, we did not contact the participants as part of this study.

Definitions

We used three independent markers of childhood asthma from the Danish national registries in the 14-year-period 1997–2010. The first of these markers was recurrent hospital admissions for asthma (primary diagnosis of asthma according to the International Classification of Diseases 10th revision [ICD-10]: codes J45.x–J46.x), defined as at least two inpatient hospital admissions separated by at least 1 month, in which a child was judged to be an asthma case at their first admission. The second marker was long-term outpatient attendance related to asthma (primary diagnosis of asthma according to the ICD-10: codes J45.x–J46.x), in which the child was followed in outpatient care for at least 1 year, and was defined as an asthma case at the date of outpatient treatment initiation. The third marker of childhood asthma was recurrent use of inhaled corticosteroids—at least 200 defined daily doses (WHO index) of anti-asthmatic corticosteroid inhalants filled and collected at a pharmacy (classification codes in WHO's anatomical therapeutic chemical [ATC] system: R03BA01, R03BA02, R03BA05, and R03BA07). This information could be tracked because in Denmark, children collect medication under their own Civil Registration System number.[7]

For each case definition, asthma cases were compared with non-cases; the latter group comprised children who did not fulfil the case definition. For example, for inpatient analysis, we compared children with two or more admissions to hospital for asthma versus a group who had one or no admissions to hospital for asthma.

The National Prescription Registry contains individual information about all prescriptions collected in Danish pharmacies since 1994. Antibiotics were registered according to WHO's ATC classification system and grouped by treatment indication in antibiotics for respiratory tract infections, urinary tract infections, and other infections (see appendix for ATC codes).

We studied the administration of antibiotics to women during pregnancy and also in two pre-pregnancy periods (40 weeks before pregnancy, and the 40 weeks before that [ie, up to 80 weeks pre-pregnancy]) and two post-pregnancy periods (40 weeks post partum, and 40 weeks after that [ie, up to 80 weeks post partum]). For each period, we used a dichotomised (yes/no) score for any oral antibiotic use and for the antibiotics grouped by treatment indication. We calculated the total number of antibiotic prescriptions from 80 weeks before pregnancy to 80 weeks post partum.

We investigated the associations between childhood asthma and maternal antibiotic use in pregnancy and in the four different periods surrounding pregnancy versus non-treated mothers in the same periods for all children born in Denmark in 1997–2010.

To investigate the dose–response association, we analysed the number of maternal antibiotic treatments prescribed from 80 weeks before pregnancy to 80 weeks post partum and the association with asthma in the offspring. We plotted incidence rates for each asthma indicator against the number of antibiotics treatments used by the mother.

In a sensitivity analysis, we studied the effect of antibiotic use in the pre-pregnancy and post-pregnancy periods on asthma in children who were not exposed to antibiotics during pregnancy. In another sensitivity analysis, we investigated the effect of maternal antibiotic use on asthma in children older than 6 years, for which we included only children born before 2005.

We chose the confounders to adjust for a priori. We adjusted all analyses for calendar year (year of observation), age (at year of observation), birthweight (five categories; see appendix), gestational age (four

	Cases of asthma, n (person-years of observation, n)	80–40 weeks before pregnancy	40 weeks before pregnancy to pregnancy	Pregnancy	Birth to 40 weeks post partum	40–80 weeks post partum
Inpatient admission	7166 (5 943 271)	1·21 (1·15–1·27)*	1·21 (1·16–1·27)*	1·24 (1·18–1·30)*	1·24 (1·18– 1·30)*	1·24 (1·18–1·30)*
Outpatient attendance	12 711 (5 921 159)	1·24 (1·19–1·28)*	1·28 (1·24–1·33)*	1·22 (1·18– 1·26)*	1·21 (1·17–1·25)*	1·25 (1·21–1·29)*
Inhaled corticosteroid use (200 defined daily doses)	41 163 (5 733 209)	1·21 (1·18–1·23)*	1·22 (1·19–1·24)*	1·18 (1·15–1·20)*	1·22 (1·20–1·25)*	1·23 (1·21–1·26)*

Data are adjusted incident rate ratios (95% CI), unless otherwise indicated. *Denotes significant p values (p<0·0001). p values were calculated by the Wald test. All estimates are adjusted for age and calendar year, birthweight, gestational age, sex, mode of delivery, parity, multiple births, season of birth, maternal age, smoking during pregnancy, maternal employment status, and maternal asthma. The analyses were done in the population of 864 689 children with complete data for all covariates.

Table 2: **Risk (adjusted incident rate ratios) of childhood asthma by maternal use of antibiotics during pregnancy, and in the 80 weeks before and after pregnancy**

categories; see appendix), parity (three categories; see appendix), mode of delivery, sex, multiple births (yes/no), season of birth, maternal age, maternal employment, maternal smoking during pregnancy, and maternal asthma, defined as any admission to hospital for asthma or any collection of anti-asthmatic corticosteroids. We treated all confounders as categorical variables. The appendix provides additional information about the adjusted confounders.

Statistical analysis

We followed children for hospital admissions or medicine collection from their date of birth until Dec 31, 2010, or migration out of Denmark, whichever occurred first. We accumulated person-time (in years) and events (asthma cases) stratified by maternal antibiotic use and our pre-chosen confounders.[8] Thereby, we calculated the specific incidence rates (cases per 1000 person-years).

To estimate the overall association between maternal antibiotic treatment and the number of offspring asthma cases in each disease category (inpatient admission, outpatient attendance, and use of inhaled corticosteroids) in the complete time period and across the entire age range, we used log-linear Poisson regression models offset by the log of person-time (years of observation), in which we took into account any potential underlying age and calendar time variations for the different asthma outcomes (eg, hospitalisations being more frequent in younger children) by adjusting for the age and calendar year as confounders (for which we used categorical coding with 1-year intervals). Furthermore, we included the chosen confounders to obtain the fully adjusted estimates for the effect of maternal antibiotic use. We calculated the adjusted incidence rate ratio (aIRR) and 95% CI to show the effect of maternal antibiotic use on childhood asthma.

We used a significance cutoff level of p<0·05 in all analyses. SAS version 9.3 was used for all data analyses.

Role of the funding source

The funders had no role in design and conduct of the study; collection, management, and interpretation of the data; preparation, review, or approval of the report; or the decision to submit to publication. The corresponding author is the proprietary owner of, and had full access to, all the data in the study, and had final responsibility for the decision to submit for publication.

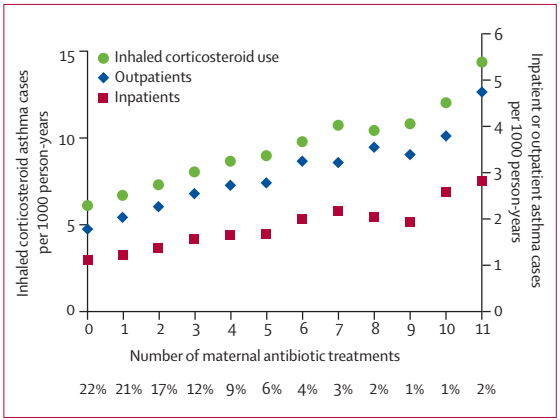

Figure: **Risk of childhood asthma by cumulative number of antibiotics collected from 80 weeks before pregnancy to 80 weeks post partum**
Percentages for antibiotic treatments represent the proportion of the cohort with this exposure.

Results

During the observation period (1997–2010), 910 301 children were born in Denmark in 891 555 unique pregnancies by 545 394 women. In 289 348/891 555 (32%) of all unique pregnancies, the mother had at least one antibiotic prescription filled at a pharmacy. The frequency of maternal antibiotic use was similar in the 40 weeks before pregnancy and in the 40 weeks preceding this period, and in the 40 weeks post partum and in the 40 weeks following this period (table 1). During pregnancy, the frequency of antibiotic treatments prescribed for urinary tract infections was higher than that in the 40 weeks before and after pregnancy (table 1). Antibiotics typically used to treat respiratory tract infections were used most frequently in all time periods (table 1). In 22% of the children (203 725/910 301 children from 199 705 births), the mother had no antibiotic prescriptions filled from 80 weeks before pregnancy up until 80 weeks post partum.

	Cases of asthma, n (person-years of observation, n)	Any antibiotics	Antibiotics for respiratory tract infections	Antibiotics for urinary tract infections	Antibiotics for other infections
Inpatient admission	7166 (5 943 271)	1·30 (1·22–1·39)*	1·30 (1·23–1·37)*	1·21 (1·15–1·27)*	1·22 (1·14–1·30)*
Outpatient attendance	12 711 (5 921 159)	1·36 (1·30–1·43)*	1·37 (1·31–1·42)*	1·18 (1·14–1·23)*	1·20 (1·14–1·26)*
Inhaled corticosteroid use (200 defined daily doses)	41 163 (5 733 209)	1·29 (1·26–1·33)*	1·30 (1·27–1·33)*	1·11 (1·09–1·13)*	1·21 (1·18–1·24)*

Data are adjusted incident rate ratios (95% CI), unless otherwise indicated. *Denotes significant p values (p<0·0001). p values were calculated by the Wald test. All estimates are adjusted for age and calendar year, birthweight, gestational age, sex, mode of delivery, parity, multiple births, season of birth, maternal age, smoking during pregnancy, maternal employment status, and maternal asthma. The analyses were done in the population of 864 689 children with complete data for all covariates.

Table 3: **Risk of childhood asthma by treatment indication of maternal antibiotics prescribed from 80 weeks before pregnancy to 80 weeks after birth**

	Cases of asthma, n (person-years of observation, n)	80–40 weeks before pregnancy	40 weeks before pregnancy to pregnancy	Pregnancy	Birth to 40 weeks post partum	40–80 weeks post partum
Inpatient admission	534 (1 935 979)	1·21 (1·01–1·45); p=0·035	1·20 (1·01–1·44); p=0·039	1·29 (1·08–1·55); p=0·005	1·42 (1·19–1·69); p<0·0001	1·35 (1·13–1·60); p<0·0001
Outpatient attendance	3817 (1 926 443)	1·24 (1·16–1·33); p<0·0001	1·21 (1·13–1·29); p<0·0001	1·09 (1·02–1·17); p=0·015	1·10 (1·03–1·18); p=0·004	1·17 (1·10–1·25); p<0·0001
Inhaled corticosteroid use (200 defined daily doses)	12 189 (1 891 277)	1·15 (1·11–1·20); p<0·0001	1·15 (1·11– 1·19); p<0·0001	1·06 (1·02– 1·11); p=0·002	1·14 (1·09–1·18); p<0·0001	1·15 (1·11–1·19); p<0·0001

Data are adjusted incidence rate ratios (95% CI) and p values, unless otherwise indicated. p values were calculated by the Wald test. All estimates are adjusted for age and calendar year, birthweight, gestational age, sex, mode of delivery, parity, multiple births, season of birth, maternal age, smoking during pregnancy, maternal employment status, and maternal asthma.

Table 4: **Risk of childhood asthma in Danish-born children at least 6 years of age by maternal use of antibiotics**

The prevalence of asthma according to our three different definitions among all 910 301 children were as follows: 0·85% (n=7733) fulfilled the criteria for recurrent inpatient hospital admissions for asthma; 1·50% (n=13 619) had at least 1 year of outpatient hospital attendance for asthma; and 4·80% (n=43 722) had collected at least 200 prescribed defined daily doses of anti-asthmatic inhaled corticosteroids (appendix).

Associations between maternal use of antibiotics and childhood asthma were investigated in the population of 846 689 children with complete data for all potential confounders. Maternal use of antibiotics in pregnancy was associated with an increased risk of childhood asthma according to all three asthma case definitions: aIRR for inpatient hospital admission 1·24 (95% CI 1·18–1·30), aIRR for outpatient attendance 1·22 (1·18–1·26), and aIRR for inhaled corticosteroid use 1·18 (1·15–1·20) (table 2). The association between asthma in the offspring and maternal use of antibiotics during pregnancy was similar to the association with maternal use of antibiotics at any point in the period 80 weeks before pregnancy up until 80 weeks post partum (aIRR range for inpatient admission 1·21–1·24, outpatient attendance 1·21–1·28, and inhaled corticosteroid use 1·18–1·23) (table 2) and with maternal use of antibiotics before or after but not during pregnancy (aIRR range for inpatient admission 1·15–1·22, outpatient treatment 1·19–1·23, and inhaled corticosteroid use 1·16–1·20; appendix). The figure shows the incident rates of asthma in the children by the number of filled maternal antibiotic prescriptions in the whole 200-week study period, from 80 weeks before pregnancy to 80 weeks post partum. The rate of childhood asthma increased by about 5% for every additional maternal antibiotic treatment: inpatient admission aIRR 1·05 (95% CI 1·04–1·05), outpatient attendance aIRR 1·05 (1·05–1·06), and inhaled corticosteroid use aIRR 1·05 (1·05–1·05). This association persisted when we excluded the 203 725 (22%) children whose mothers never collected antibiotics during the entire 200-week study period (data not shown).

The associations between maternal antibiotic use during the whole 200-week period around pregnancy and asthma in the offspring were generally stronger for antibiotics prescribed for respiratory tract infections, but were also significant for antibiotics that treat urinary tract infections and for the small group of other antibiotics. This pattern was similar across asthma case definitions (table 3).

In a sensitivity analysis in which we studied only children older than 6 years of age, the recorded associations between maternal use of antibiotics and asthma in the offspring remained significant at all time points, although the risk decreased slightly (table 4).

Stratification of the analyses by maternal asthma showed statistically significant associations between any maternal antibiotic use during the 80 weeks before to 80 weeks after pregnancy and asthma in both asthmatic and non-asthmatic mothers, with higher effect estimates in the larger group of children of non-asthmatic mothers (appendix).

Discussion

Our main finding in this study was that childhood asthma was associated with maternal use of antibiotics during pregnancy and also with antibiotic use in the 80 weeks before pregnancy and post partum. We recorded this association for antibiotics typically used for respiratory tract infections and for those used to treat urinary tract infections and those prescribed for other infections, and in both mothers with and without asthma. These findings suggest that the association between maternal antibiotic use and asthma in the child does not represent a direct causal effect of antibiotic use during pregnancy but rather is caused by other mechanisms, possibly a heritable predisposition to infections.

Our study was hypothesis driven, based on our previous report of an association between maternal use of antibiotics during pregnancy and risk of asthma in the offspring, shown in our longitudinal clinical birth cohort study, the Copenhagen Prospective Study on Asthma in Childhood (COPSAC), and replicated in The Danish National Birth Cohort.[1] The current study has several strengths. The statistical power of the study allowed for analyses of the maternal antibiotic use in pregnancy and in the periods before pregnancy and after birth, stratification by treatment indication (antibiotics for respiratory tract infection, urinary tract infection, or other antibiotics), and stratification by children with and without mothers with asthma. Finally, we were able to show a dose–response association between maternal antibiotic use and the incidence of childhood asthma.

The data cover 14 years of follow-up in national registries and asthma definitions from different registries. Loss to follow-up is unlikely to bias the recorded association, since emigration is also registered in a central database in Denmark. The content and validity of the Danish national registries has previously been well documented.[9,10] The Danish National Prescription Registry, which contains nationwide information about medication prescribed and filled at pharmacies, is highly accurate, since both antibiotics and anti-asthmatic corticosteroid inhalants can be prescribed only by authorised physicians and can be purchased only from authorised pharmacies. The Danish National Patient Registry covers all hospital admissions nationwide and records data about children attending outpatient clinics. Paediatric asthma diagnoses in this registry have previously been validated[11–13] and we recently demonstrated the phenotypic specificity of admission to hospital for asthma by strong associations in a genetic study.[14] The case definitions in our study mainly represent children with moderate-to-severe asthma in need of long-term treatment since we prioritised specificity over sensitivity.

All our analyses were adjusted a priori for a wide range of potential confounders: birthweight, gestational age, sex, mode of delivery, parity, multiple births, season of birth, maternal age, maternal asthma, smoking in pregnancy, and maternal employment status. Therefore, we reduced the risk of confounding by these factors.

The time trends in both asthma prevalence and prescription practices of antibiotics were accounted for in the analyses by adjustment of all results for calendar year and age of the child as categorical variables.

One limitation of our study is that we studied only children born after 1997, and therefore the number of observation years was inversely reduced with the children's age. This situation limits our study's power for children with late onset of asthma. Childhood asthma had a high prevalence and most asthmatics tend to have an early onset of their symptoms, but diagnosis of asthma in a young child can sometimes be inaccurate. We therefore did a sensitivity test of children excluding those younger than 6 years of age (table 4), which did not change our conclusions, despite limited power.

Another limitation of our study is that we derive the type of infection in the mother from the antibiotic drug prescribed. We hoped that grouping of the antibiotics by suspected indication would enable us to better understand our observations. Furthermore, we cannot exclude the possibility that drugs were filled at the pharmacy but not ingested. However, the large dataset accounts for such possible errors. Another limitation is that we did not gather any data about childhood infections (via child antibiotic prescriptions), which would be interesting to analyse in further study of the mechanisms leading to asthma.

The associations between antibiotic treatment and childhood asthma were adjusted for maternal asthma status, and a significant association remained even when we restricted our analyses to antibiotics for non-respiratory infections. Furthermore, when we stratified the children by whether or not their mothers had asthma, we recorded similar associations in both groups (appendix). This finding suggests that the results were not caused or confounded by the maternal asthma phenotype, and might indicate that the asthma risk is associated with susceptibility to infections, even in non-asthmatic mothers.

Treatment-seeking behaviour of the mother could increase the risk of an asthma diagnosis in the child. However, the definition of asthma in our study requires asthma leading to either long-term outpatient attendance or long-term treatment with inhaled corticosteroids, which makes the case diagnoses robust. A similar strong association was recorded for the asthma definition based upon severe, acute exacerbations leading to hospital admission, which is expected to be subject to less bias from maternal treatment-seeking behaviour.

Our results show a robust association between maternal antibiotic use in pregnancy and increased risk of childhood asthma in the offspring in the Danish population. This finding is consistent with our longitudinal studies from our birth cohorts[1] and cross-sectional studies from others.[2,3] However, we now show that this association is equally strong for maternal use of

Panel: Research in context

Systematic review

We searched PubMed between June 15 and Nov 15, 2013, with no language or date restrictions, for various combinations of the search terms "childhood", "asthma", "maternal antibiotics", and "pregnancy". We focused mainly on more recent articles published within the past 20 years but also included other older relevant publications as background material. The focus of interest was established in our previous publication from 2012, in which we reported an association between maternal pregnancy antibiotic use and childhood asthma in a longitudinal birth cohort. We also searched the reference lists of original research articles to find relevant publications in this subject area.

Interpretation

Use of antibiotics during pregnancy has been associated with the development of asthmatic disorders in the offspring. This association has been suggested to be caused by derangements of the neonatal microbial colonisation patterns, an adverse effect of the drug, or inflammation caused by infection during pregnancy. Our findings show that the same association between maternal use of antibiotics and childhood asthma exists 80 weeks before and 80 weeks after pregnancy in a dose-related manner and independent of type of antibiotics. This outcome suggests that maternal antibiotic use is merely a surrogate marker of a general propensity for infections as the underlying link between maternal use of antibiotics and risk of asthma in the child.

antibiotics any time from 80 weeks before pregnancy until 80 weeks post partum, and even in mothers who use antibiotics before or after, but not during, pregnancy. Maternal use of antibiotics in the whole 200-week period analysed exhibited a dose-related association with the child's asthma risk (panel).

Our initial finding of this association between mother's use of antibiotics during pregnancy and risk of asthma in the child[1] led us to speculate that the effect could be mediated through the human microbiome. The composition of the microbiome is believed to affect early disease trajectory in prenatal and perinatal life, through inflammation in pregnancy[6] and vertical transmission of bacteria during birth.[4,15,16] Perturbation of the symbiotic host–microbial homoeostasis could trigger inflammatory processes in the host[17] and has previously been shown to be associated with the risk of asthma in the child.[4] The maternal microbiome is affected to a much greater extent by a current antibiotic treatment than by previous treatments,[18] but changes in microbial ecology caused by antibiotics could persist for a long time. Similarly, maternal antibiotics after birth can affect the child's microbiome through breastfeeding or bacterial transmission from other maternal compartments that are affected by the antibiotics. However, our present results, which show a similarly strong association for maternal use of antibiotics a long time after delivery (up to 1·5 years later), seem to be incompatible with vertical transmission of an affected microbial community after antibiotics in pregnancy as the causal link. We cannot exclude that several courses of antibiotic treatments are associated with more permanent changes in the microbiome that could then be passed on to the child. However, our data suggest that it is not specifically the antibiotic treatment given during pregnancy that is important. Furthermore, our findings suggest that alternative hypotheses of intrauterine effects from the antibiotic or from the inflammatory processes caused by the infection in pregnancy[2] are unlikely.

Maternal antibiotic use could be indicative of a high pathogen load in the home environment, which can similarly be responsible for pathogenic colonisation or infections in the offspring.[4] In susceptible people, recurrent airway infections and subsequent inflammation in early life can lead to a cycle of tissue damage, tissue repair, and tissue remodelling, which over time can develop into persistent pathological changes of the epithelia and eventually asthma.[19,20] Maternal antibiotic use could also be a marker of some other lifestyle factor that causes an increased risk of infections in both mother and child and thereby an increased risk of childhood asthma. We propose that maternal use of antibiotics is a marker of susceptibility to infections that is inherited by the child and increases the risk of childhood asthma.[4,19] An example of such an inherited susceptibility is represented by genetic variations in the chromosome 17q21 locus, which is linked to both an increased risk of respiratory infections and the development of childhood asthma.[21,22]

Maternal use of respiratory and non-respiratory antibiotics in the 200-week window surrounding pregnancy had a dose-related association with an increased risk of childhood asthma in the offspring, independently of maternal asthma. This result argues against our previous interpretation that the association between antibiotic use in pregnancy and asthma in the offspring is mediated by the human microbiome. Instead, we propose that the mother's general use of antibiotics can be interpreted as a proxy for maternal propensity to infections, which could be the underlying risk factor inherited by the child that increases the risk of infections as the important trigger of asthma.

Contributors

HB is the guarantor of the study and has been responsible for the integrity of the work as a whole, from conception and design to conduct of the study and acquisition of data, analysis and interpretation of data, and writing of the report. JS and AS were responsible for acquisition, analysis, and interpretation of data and writing of the report. KB contributed to data interpretation and writing of the report and approved the final version.

Declaration of interests

We declare no competing interests.

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Web appendix

Methods

Antibiotics grouping

- UTI: antibiotics for urinary tract infections (J01CA08, J01EA**, J01EB**, J01EE**, J01XE**, J01XX05);
- RTI: antibiotics for respiratory tract infections (J01CA** excl. J01CA08, J01CE**, J01CR**, J01DB**, J01DC**, J01DD**, J01FA**, J01XX08, J04AB**, J04AC**, J04AK**);
- OTHER: antibiotics for other infections (J01AA**, J01BA**, J01CF**, J01CR**, J01DF**, J01DH**, J01FF**, J01GB**, J01MA**, J01MB**, J01XA**, J01XB**, J01XC**, J04BA**).

Confounders

We adjusted for calendar year (1997-2010; 1 year categories); age (0-15 years, 1 year categories); parity (first child, second child, third or more child); multiple births (yes/no); season of birth (four categories); birth weight (<2·5; 2·5-3·0; 3·0-3·5; 3·5-4·0; >4·0); gestational age (-36; 37-39; 40-41; 42-); mode of delivery (cesarean section yes/no); child gender (male/female); maternal age (six categories: <20; 20-25; 25-30; 30-35; 35-40; >40 years); maternal smoking during pregnancy (yes/no); maternal employment status in year before child birth or year of child birth for children born September – December (transferred payments/unemployed, top manager/entrepreneur with employees, upper level skilled employee, medium level skilled employee, basic level skilled employee, unknown level skilled employee, entrepreneur without employees/co-working spouse, undergoing education, or other); and maternal asthma (defined by any hospital contact with asthma as principal diagnosis or any collection of anti-asthmatic steroid inhalants in the study period 1997-2012).

Results

Table E1 online: Asthma cases by each case definition

	All cases N = 910,301	Cases minimum 6 years of age N = 526,567
Recurrent inpatient admissions	7,733 (0·85%)	599 (0·11%)
At least one year outpatient attendance	13,619 (1·50%)	4,153 (0·79%)
At least 200 DDD prescriptions steroid collected	43,722 (4·80%)	13,305 (2·53%)

Table E2 online: Effects of pre- and post-pregnancy antibiotics for children whose mothers did not receive any antibiotics during pregnancy. Results are presented as Incidence Rate Ratios with 95% confidence interval, p-values correspond Wald test.

	Cases (person-years)	**80 weeks before gestation to 40 weeks before gestation**	**40 weeks before gestation to gestation**	**Birth to 40 weeks after birth**	**40 weeks after birth to 80 weeks after birth**
Inpatient admission	4,346 (4,114,014)	1·18 [1·11 - 1·26]; p=3·6E-07	1·15 [1·08 - 1·22]; p=2·3E-05	1·18 [1·11 - 1·26]; p=3·0E-07	1·22 [1·14 - 1·29]; p=3·8E-10
Outpatient attendance	8,001 (4,099,262)	1·23 [1·18 - 1·29]; p=1·4E-18	1·22 [1·17 - 1·28]; p=3·0E-17	1·19 [1·13 - 1·25]; p=7·6E-13	1·23 [1·17 - 1·28]; p=1·9E-18
Steroid use (200 DDD)	26,454 (3,976,139)	1·18 [1·15 - 1·21]; p=1·2E-34	1·16 [1·13 - 1·19]; p=1·2E-28	1·20 [1·17 - 1·23]; p=2·3E-41	1·20 [1·17 - 1·23]; p=1·0E-46

Table E3 online: Effects of any antibiotics stratified for maternal asthma. Risk of childhood asthma after maternal antibiotics prescribed from 80 weeks before gestation to 80 weeks after birth. Results are presented as Incidence Rate Ratios with 95% confidence interval, p-values correspond Wald test.

	Asthmatic Mother		**Non-Asthmatic Mother**	
	Cases (person-years)	**Any antibiotics**	**Cases (person-years)**	**Any antibiotics**
Inpatient admission	1,821 (730,332)	1·20 [1·03 - 1·39]; p=0·018	5,345 (5,212,940)	1·32 [1·23 - 1·42]; p=8·3E-15
Outpatient attendance	3,162 (724,800)	1·32 [1·17 - 1·46]; p=2·7E-06	9,549 (5,196,360)	1·37 [1·30 - 1·45]; p=1·0E-31
Steroid use (200 DDD)	9,825 (679,667)	1·24 [1·17 - 1·32]; p=4·1E-12	31,338 (5,053,542)	1·30 [1·27 - 1·34]; p=4·1E-75

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RESPIRATORY EPIDEMIOLOGY

Stable admission rate for acute asthma in Danish children since 1977

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Abstract Childhood asthma is consistently reported to have increased in recent decades in most westernized countries, but it is unknown if this increase is similar across severities. We aimed to study the time-trend of acute hospital admission and readmission for asthma of school-aged children in the recent 35 years in Denmark. We analyzed time-trends in the national incidence rate of hospitalization for acute severe asthma in children aged 5–15 in Denmark during the 35-year period 1977–2012 in the Danish national registry. Only in-patient admissions with a principal diagnosis of asthma (ICD-8: 493** or ICD-10: J45** or J46**) were included. Among children with asthma hospitalizations, we investigated the risk of readmission beyond 1 month of first admission. Admissions were summarized as rates per thousand person years at risk. The overall time-trend is stable with a rate of one admission per year per thousand children at risk and a per-year incidence rate ratio 0.999 [95 % CI 0.997–1.001]. The rate of any readmission decreased from approximately 20 per thousand children in the eighties to less than 10 in the early nineties before stabilizing at around 10 per thousand children from mid-nineties and onwards. During 35 years of nation-wide follow-up, we find a highly stable incidence rate of first hospital admission for acute severe asthma in children. Moreover, rates of readmission halved during the seventies and stabilized in the last twenty years. In conclusion, our data suggest that the reported increase in childhood asthma is mainly due to less severe cases.

Keywords Asthma · Child · Denmark · Hospitalization · Incidence

Abbreviations
ICD International classification of diseases

Introduction

Acute hospitalization for asthma is traumatic to the child and her relatives, and it is a major part of the total direct cost of asthma management in the United States and Europe [1]. It is a main goal of asthma management to avoid asthma-related hospital admissions. Furthermore it is presumably "the tip of the iceberg" reflecting the general asthma control in the population.

The prevalence of childhood asthma has consistently been reported to have increased in recent decades in most westernized countries. In Denmark, one study found the prevalence has increased from 5.3 % in 1986 to 11.7 % in 2001 [2]. Likewise, the latest report from ISAAC on worldwide trends from 2007 [3] reported an increase.

Much of the research behind these claims relies on questionnaire data. Especially questions regarding "wheeze" could be biased, since much higher prevalence has been found in English speaking nations where such terms seem familiar. Therefore, it remains unclear whether these trends represent a consistent increase across severities of asthma.

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In this study, we used the unique National Danish hospital registry data; consistent for more than 35 years of complete national coverage of pediatric population in-patient hospital admissions and analyzed the time-trend of acute hospital admission and readmission for asthma of school-aged children in Denmark.

We analyzed time-trends in the national incidence of hospitalization for acute severe asthma in children aged 5–15 (both included) in Denmark during the 35-year period 1977–2012 in the Danish national registry.

Methods

Study population

The Danish National Patient Registry was established in 1977, and includes information on all in-patient hospitalization in Danish public hospitals comprising a personal identification number, dates of admission and discharge, and diagnoses at discharge classified according to the international classification of diseases versions 8 and 10 [4].

We identified all children born in Denmark 1972–2010 in the Danish Civil Registration System (CRS). From the CRS, information was retrieved regarding date of birth, and possible date of death or emigration, and children were followed in registries from age 5 to 15 for all asthma hospitalizations during 1977–2012.

Case definition

First admission

Children aged 5–15 years (both years included) with in-patient admissions with a principal diagnosis of asthma (1977–1993: ICD-8 493**; and 1994–2012: ICD-10 J45*, J46*) were considered as cases at their first in-patient admission.

Readmission

Inclusion criteria for analyses of readmission selected all children with any first admission in the age span 5–15 years. Children were considered as cases if they had any subsequent admission for asthma (same definition as above) more than 1 month after the first admission and before age 15.

Statistics

The incidence rate is calculated as number of new cases divided by person years at-risk. All children contributed person-time from age 5 until age 16 or migration or death or final registry date (Dec 31st 2011), or first asthma admission, if present. Trend is presented in the window 1988–2012 during which we follow a complete cohort of school-aged children without left-truncation of data. In Fig. 2, the time trend is visualized with a non-parametric LOWESS smoother using a bandwidth of 0.5 years. The linear trend across 1988–2012 is calculated as the effect of calendar year on the number of admissions in a Poisson regression with log link and log to person-years of observation as offset.

Readmissions are investigated among children with any first admission. In Cox regression analysis children are followed from first admission till readmission. They are censored at age 16, migration or death whichever comes first. Rates are compared by year at first admission (5 year intervals 1977–2012) and are stratified by age (1 year intervals) at first admission in a Cox regression to adjust for non-complete cohort until 1988. Pairwise comparisons of the rate of readmission between categories of calendar time are summarized by a letter-based representation [5] and are adjusted for multiple testing using the single step procedure [6].

Admissions and person years are summarized using the SAS macro developed by Rostgaard [7].

Analyses were performed in SAS 9.3 (SAS Institute Inc., Cary, NC, USA) and R version 3.1.2.

Ethics

The study was based on data from national registries and was approved by the Danish Data Protection Agency (J.no. 2012-41-0388). Subjects were not contacted as a part of the study; hence the ethics committee did not require written informed consent.

Results

2.3 million children (51 % male) born in Denmark 1972–2006 are followed during 20.6 million person-years in the ages 5–15 through 1977–2012. Due to changing birth rates over the study period, the number of person years varies slightly over time. Figure 1 depicts the number of person years in the entire period. From 1988 onwards a complete cohort of Danish born children aged 5–15 are investigated for 1.6 million person years.

Excluding 3339 same person admission within a month (31 days), we find 33,219 unique in-patient childhood asthma hospitalizations in 22,023 children (14,143 boys, and 7880 girls) during 1977–2012.

Incidence

Figure 2 shows the national yearly incidence of first asthma admissions of school-aged children. The incidence

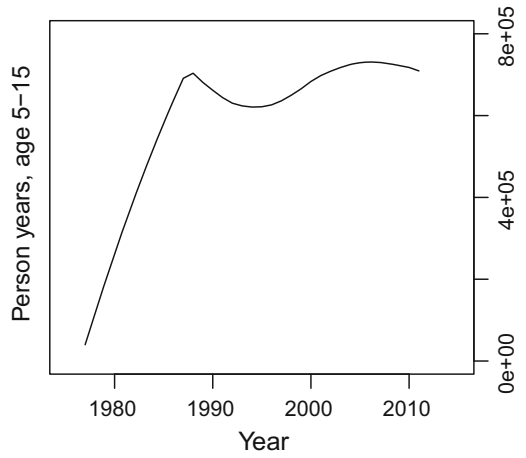

Fig. 1 Person years of observation in children aged 5–15 in Denmark

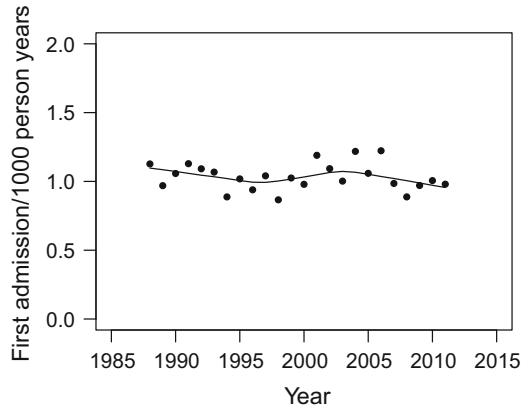

Fig. 2 Asthma admissions in Denmark per 1000 school-aged children at risk 1988–2012

of childhood asthma in Denmark during 1988–2012 is stable with an overall rate of 1.04 [1.02–1.05] first admission in 1000 children per year. More boys than girls have asthma—overall the rate for boys is 1.258 [1.234–1.282] in 1000 boys per year, whereas the rate for girls is 0.743 [0.724–0.762] in 1000 girls per year.

The overall time trend is stable with a per-year incidence rate ratio (IRR) of 0.999 [0.997–1.001]. The overall trend is the same adjusted for gender. The minor fluctuations in trend are mainly due to fluctuations in incidence amongst boys, however this follows the same pattern as the person years of observations, reflecting the changing age distribution over time.

Readmissions

5282 children (64 % boys) have at least one readmission more than 1 month after the first asthma for asthma at age 5–15. The majority (72 %) of readmissions occur within 2 years after the first admission.

Table 1 Risk of readmission by calendar year at first admission (Cox regression stratified by age at first admission)

Year	Cox regression	
	HR [95 % CI]	Letter-based-comparison of rates[a]
1977–1981	Ref	A
1982–1986	0.96 [0.86–1.07]	A
1987–1991	0.65 [0.58–0.73]	B
1992–1996	0.43 [0.38–0.48]	C
1997–2001	0.54 [0.48–0.60]	D
2002–2006	0.57 [0.51–0.63]	BD
2007–2011	0.52 [0.46–0.59]	D

[a] Calendar year groups with the same letter are not significantly different

Risk of readmission differs by age at first admission and calendar year at first admission. Table 1 shows the Hazard Ratios for readmission by year (5-year intervals) at first admission from an age-stratified Cox regression. The risk of readmissions has decreased since 1977–81, to a low-point during 1992–1996, but stabilized from mid 1990s onwards where the trend in readmission rates is similar, but inflated, to the minor fluctuations in overall trend and observation years.

Discussion

Primary findings

The stable incidence of 1 child per thousand per person year through our 35 years of observation extends our report 2 decades ago on a stable incidence of acute asthma hospitalizations of school children during 1978–1993 [8]. Likewise the decreasing readmission rates were reported during 1978–1993, and now we show that these have since stabilized.

Strengths and limitations

The Danish National Patient Registry is considered unique and of very high quality [4]. The registrations are compulsory and necessary for government reimbursement, which results in complete coverage—and for the pediatric population there are no alternatives to public hospitals. This gives us the exclusive opportunity for a complete nation-wide population cohort of standardized observation followed over 35 years.

To avoid left-truncation of the data, we only investigated the incidence trends from 1988 onwards where a complete cohort can be followed. We consider this data

restriction compulsory to obtain un-biased estimates of the actual incidence. When investigating the readmissions the stratified Cox regression analysis can account for the truncation, and therefore we were able to investigate readmission for the full 35 years span.

We excluded same-month admissions in same child, since these could be the result of moving a child from one department to another, or simply reflect the same asthmatic period where the child was discharged too soon. This ensures that changing discharge procedures do not bias our analyses of readmissions.

Various political reforms of the Danish health care system have taken place during recent decades. We only investigate the in-patient admission category which has existed in the entire span of registry, and leave out out-patient admissions which were introduced in Denmark in 1994. Most importantly for the hospital admission was the shift from ICD-8 to ICD-10 in 1994. The fact that we do not observe any shift during this change reassures us that asthma diagnosis is stable and in-patient acute hospital admissions for asthma is robust against such changes.

Still, we cannot rule out changes in admission practice over time, and in fact others have dismissed 5 year stable hospitalization rates by indications of changing admission criteria [9]. However, we find that hospitalization rates may be a less biased marker of disease incidence and disease control in the society than other assessments, such as symptom history and prescribed treatment, which are prone to changes in disease perception, management strategy and definition over time.

Interpretation

Time trends

Asthma is a complex and heterogeneous disease. Hospitalized asthmatics represent the most severe cases that have been filtered through primary care and hospital admission criteria. Uncontrolled milder asthmatics may also contribute, but we interpret the incidence of first hospitalization for acute severe asthma as a surrogate marker of the incidence of moderate to severe asthma disease [10], and thereby as the "tip of the iceberg" of the general asthma incidence.

Our findings of a long-term stable incidence of childhood asthma hospitalizations seems to contrast the consistent reports of increased asthma prevalence in industrialized countries through the recent half century [11]. In the same time period there has been a general increase in the awareness and a labeling shift towards asthma. We cannot know if improved treatments outbalance underlying increasing disease incidence, such that our overall observation of a stable admission incidence reflects better control in an increased number of asthmatic children. However, we find it unlikely that a major asthma epidemic has occurred without leaving an imprint in the admission rates for asthma, suggesting that the increased asthma prevalence reported may be driven by increase in the milder segment of childhood asthma and pre-school aged children who are not included in our analysis.

In fact, others who have emphasized consistent data sources have also concluded relative stable rates of childhood asthma [12–14]. This makes it even more intriguing what reports of increasing incidence in fact are reporting? Self-reported asthma symptoms and allergies have definitely been rising [15]. This would fit the labelling shift in childhood asthma and not the actual disease incidence.

We have purposely downplayed the smaller disease incidence fluctuations that are visible from Fig. 2 as these may be chance findings. Alternatively, they may reflect real changes from environmental exposures. For instance smoking bans have been introduced in many countries with variations in stringency during the past decade. Several studies have reported drops in childhood asthma admissions in the period after the legislations. Specifically, a 2010 Scottish study reported a drop in childhood asthma hospitalizations after a peak in 2006, and this was interpreted as a result of the ban of tobacco in restaurants [16]. A more recent and larger study from England, where similar legislation was introduced in 2007, also reported a significant albeit smaller effect of the smoke ban on pediatric asthma admission rates [17].

The Danish population is comparable to the Scottish and English in many respects, including culture and access to public free healthcare. Legislation against smoking was initiated in 1995 in Denmark. In August 2007 the law was extended, now including public restaurants much alike the Scottish. Interestingly we see a drop in the Danish asthma hospitalizations around 2006 i.e. more than a year before the introduction of the smoking ban. Also evident from Fig. 2 is that the local peak around 2004 was preceded by a rise starting around the mid-nineties, actually when the first restrictions were introduced. Both the English and the Scottish study covers a much shorter period of time (1997–2010 and 2001–2009) compared with our 35 years of observation. Indeed, this will affect the baseline trend of hospitalizations and thereby the prediction of a counterfactual post legislation trend.

Smoking and secondhand smoke exposure are well-known risk factors for asthma and asthma exacerbations. But environmental short-term changes should be interpreted with great caution, and inferring causal relations from isolated time trends could be biased by random longer-term fluctuations. In conclusion, our long-term analysis argues no specific effect from tobacco ban on acute asthma hospitalization.

Readmissions

We have reported a drop in readmissions for asthma 2 decades ago [8] where we interpreted this as an indication of improved disease control inasmuch as first-admissions where frequent before regular treatment was initiated while readmission was a better reflection of control after the diagnosis was made. The fact that the low-point in readmission during 1992–1996 was followed by stabilization from mid 1990s onwards where the trend in readmission rates is similar, but inflated, to the minor fluctuations in overall trend could therefore be interpreted as no further improvements in asthma management in the recent two decades.

Conclusion

In summary, this nationwide analysis of asthma hospitalization of children in the recent 35 years shows a stable incidence of first-admissions, suggesting a stable incidence of this particular asthma phenotype among school-aged children.

Milder cases may therefore be contributing to alleged increase in asthma incidence.

The stable rate of readmissions for childhood asthma suggests that there has been no or little improvement in asthma management in the recent 2 decades.

Authors contributions The guarantor of the study is HB, from conception and design to conduct of the study and acquisition of data, data analysis, and interpretation of data. AS was responsible for data acquisition and analysis, interpretation and writing the manuscript. CBP contributed substantially to the analyses and interpretation of the data, and provided important intellectual input. All authors have agreed that the accuracy and integrity of any part of the work has been appropriately investigated and resolved and all have approved the final version of the manuscript. The corresponding author had full access to the data and had final responsibility for the decision to submit for publication. No honorarium, grant, or other form of payment was given to anyone to produce the manuscript.

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Compliance with ethical standards

Conflict of interest None.

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PAPER V:

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Cesarean Section and Chronic Immune Disorders.

Pediatrics *135*.

PAPER V

PAPER V

Cesarean Section and Chronic Immune Disorders

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abstract

OBJECTIVES: Immune diseases such as asthma, allergy, inflammatory bowel disease, and type 1 diabetes have shown a parallel increase in prevalence during recent decades in westernized countries. The rate of cesarean delivery has also increased in this period and has been associated with the development of some of these diseases.

METHODS: Mature children born by cesarean delivery were analyzed for risk of hospital contact for chronic immune diseases recorded in the Danish national registries in the 35-year period 1977–2012. Two million term children participated in the primary analysis. We studied childhood diseases with a suspected relation to a deviant immune-maturation and a debut at young age. The effect of cesarean delivery on childhood disease incidences were estimated by means of confounder-adjusted incidence rate ratios with 95% confidence intervals obtained in Poisson regression analyses.

RESULTS: Children delivered by cesarean delivery had significantly increased risk of asthma, systemic connective tissue disorders, juvenile arthritis, inflammatory bowel disease, immune deficiencies, and leukemia. No associations were found between cesarean delivery and type 1 diabetes, psoriasis, or celiac disease.

CONCLUSIONS: Cesarean delivery exemplifies a shared environmental risk factor in early life associating with several chronic immune diseases. Understanding commonalities in the underlying mechanisms behind chronic diseases may give novel insight into their origin and allow prevention.

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Dr Bisgaard, the guarantor of the study, has been responsible for the integrity of the work as a whole, from conception and design to conduct of the study and acquisition of data, analysis, and interpretation of data and writing of the manuscript; Ms Sevelsted was responsible for acquisition, analysis, and interpretation of data and critically reviewed the manuscript; Drs Stokholm and Bønnelykke contributed to data interpretation and critically reviewed the manuscript; and all authors approved the final manuscript as submitted.

The funding agencies have no role in design and conduct of the study; collection, management, and interpretation of the data; or preparation, review, approval of the manuscript, or decision to publish. The corresponding author is the proprietary owner of and had full access to all the data, and had final responsibility for the decision to submit for publication.

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WHAT'S KNOWN ON THIS SUBJECT: Cesarean delivery has previously been associated with increased risk of specific immune diseases in children. The mechanism remains unknown.

WHAT THIS STUDY ADDS: In 1 large population-based cohort, we demonstrate cesarean delivery as a shared risk factor for several immune-related diseases. Such common risk factor suggests early life commonality in the origins of these chronic immune disorders.

Chronic diseases such as asthma, allergy, inflammatory bowel disease, and type 1 diabetes have shown a parallel increase in prevalence during the last decades in westernized countries.[1] Evidence suggests that early life events may be programming these diseases, as demonstrated by changes in disease prevalence in populations immigrating to westernized countries[1] and by identification of perinatal risk factors.[2] This suggests that the parallel increase in prevalence of immune diseases may be due to shared environmental risk factors and disease mechanisms in early life.

The rate of cesarean delivery has also increased in recent decades.[3] Even though the World Health Organization recommends cesarean delivery as indicated choice of delivery mode in less than 15% of births,[4] many developed countries have a much higher prevalence, pointing toward a less strict medical indication for the procedure.[5,6] Cesarean delivery has been linked to the development of asthma and allergic rhinitis,[7–10] as well as other immune disorders[11–16] in the offspring.

We hypothesized cesarean delivery as an example of a shared environmental risk factor for immune-associated diseases. We analyzed children born by cesarean delivery for risk of hospital contact for diseases suspected to be related to a deviant immune maturation and with a debut at young age, including asthma, systemic connective tissue disorders, juvenile arthritis, inflammatory bowel disease, type 1 diabetes, immune deficiencies, psoriasis, celiac disease, and leukemia in the Danish national registries in the 35-year period 1977–2012.

METHODS

The study was based on data from national registries and was approved by the Danish Data Protection Agency (J.no. 2012-41-0388). Subjects were not contacted as a part of the study; hence the ethics committee did not require written informed consent.

Study Cohort

The Danish Civil Registration System (CRS) has registered every person with a citizenship in Denmark since 1968. This provides a unique 10-digit number, encoding date of birth and gender, which is assigned at birth and is used as the identifying number in all Danish registries. Further, the register contains date of emigration.[17]

The Danish Medical Birth Registry has existed since 1973 and contains data on birth weight, parity, mode of delivery, and maternal smoking during pregnancy. The Danish Register of Causes of Death was computerized in 1970 and contains data on date of death.

The Danish National Patient Registry was established in 1977 and contains data on all primary and secondary diagnosis on all in-patient discharges since 1977, and on outpatient admissions since 1994. The diagnoses are based on the *International Classification of Diseases, 8th Revision* (ICD-8 until 1994; *International Classification of Diseases, 10th Revision* [ICD-10] thereafter).[18]

We identified a cohort of all live born children in Denmark in the period January 1, 1973, through January 1, 2012, and used their unique CRS number to link information on gender, parity, birth weight, mode of delivery, and maternal CRS from the Danish Medical Birth Registry; information on date of death from the National Death Registry, information on date of first hospital in- or outpatient admission for any of the predefined diseases (primary or secondary diagnosis) from the Danish National Patient Registry and information on date of migration from the Danish CRS.

Maternal Disease

We obtained information from the Danish National Patient Registry on mother's hospital admission for the specified diseases to adjust the analyses for maternal disease. Only children whose mothers were born after 1952 were included in the analyses.

Exclusion Criteria

Premature children (defined as birth weight below 2500 g) and children missing information on birth weight were excluded from all analyses.

Case Definitions

Cases were identified by the ICD-8 and ICD-10 diagnoses. Nine groups of diseases were investigated. Asthma (ICD-10 J45.x; J46.x), systemic connective tissue disorders (ICD-10 M3x.x), juvenile arthritis (ICD-10 M05.x; M08.x; M09.x; M13.x), inflammatory bowel diseases (ICD-10 K50.x; K51.x), diabetes type 1 (ICD-10 E10.x; E13.x; E14.x), immune deficiencies (ICD-10 D80.x–D89.x), psoriasis (ICD-10 L40.x), celiac disease (ICD-10 K90.0), and leukemia (ICD-10 C91.x–C96.x). Negative control: fractured forearm or elbow (ICD-10 S52.x). A list of the corresponding ICD-8 diagnosis codes is provided in Supplemental Table 2.

Confounders

Confounders were chosen a priori as gender, parity (first child, second child, third child, or more), birth weight ($\geq$2.5 to $<$3.0 kg; $\geq$3.0 to $<$3.5 kg; $\geq$3.5 to 4.0 kg; 4.0 kg or more), attained age (1-year groups), calendar time (3-year groups), season of birth (December to February; March to May; June to August; September to November), maternal age ($\leq$25; 26–30; 31–35; $\geq$36 years), and maternal illness (maternal diagnosis of the disease in question, see above).

Statistical Methods

We investigated the effect of cesarean delivery versus vaginal birth in all children born 1973–2012. Children were followed for hospitalizations from January 1, 1977, where the registry started, or from date of birth if this occurred after January 1, 1977. Children were followed until January 1,

2012, or until age 16 years, migration, or death, whichever came first.

For each disease category, we accumulated person-time and -events (cases) stratified by cesarean delivery and chosen confounders.[19] Thereby we calculated the specific incidence rates (cases per person years).

The overall effects of cesarean delivery on the number of cases in each separate disease category in the complete time period and across the entire age span were estimated with log-linear Poisson regression models offset by the log of person time (years of observation), where any underlying age and calendar time variations for the various disease groups can be taken into account by adjusting for the attained age and calendar year as confounders (3-year intervals were chosen). Further, we add the chosen confounders to obtain the fully adjusted estimates for the effect of cesarean delivery. All confounders were included as categorical variables. The incidence rate ratio (IRR) with 95% confidence interval was calculated to show the effect of cesarean delivery. The population attributable risk fraction (PARF) was calculated from the adjusted IRR (aIRR) estimates as follows: PAR = Pe (RRe−1)/[1 + Pe (RRe−1)], where Pe is the prevalence of the exposure and RRe is the relative risk of disease because of that exposure. Potential changes in the effect were investigated as interaction terms between cesarean delivery and blocks of calendar years (eg, for changes in registration practice).

A significance level of 0.05 is used in all analyses. The data processing was computed with SAS version 9.3 for Windows (SAS Institute, Inc, Cary, NC).

RESULTS

Two and a half million children were born in Denmark in the period 1973–2012. Five percent had low birth weight (birth weight below 2500 g); 15% had missing data on birth weight or other confounders and were excluded from analysis, leaving 1.9 million (80%) children for the primary analysis. The study population was followed from 1977 to 2012 for a total of 23 million person years in the age range 0 to 15 years. Approximately 14% of the study population was delivered by cesarean delivery with a marked increase in the proportion of cesarean deliveries during the observation time (Fig 1). Disease prevalence is presented in Table 1.

The prevalence of several diseases among the mothers was significantly higher if they had ever delivered by cesarean delivery when compared with vaginal delivery. Especially mothers with type 1 diabetes (27%) had an increased risk of cesarean delivery compared with mothers without diabetes type 1 (14%; Supplemental Table 3).

Children delivered by cesarean section had significantly increased aIRR for asthma, systemic connective tissue disorders, juvenile arthritis, inflammatory bowel diseases, immune deficiencies, and leukemia (Table 1). Type 1 diabetes, psoriasis, and celiac disease were not associated with cesarean delivery. Arm fracture (included as "control condition") was not associated with cesarean delivery.

Limiting the children with asthma to those older than 5 years at first contact did not change the conclusion: aIRR 1.16 (1.13–1.19); $P < .0001$.

The effects of cesarean delivery were not impacted by the registry changes in terms of disease classification systems and outpatient registrations (no significant effect modification considering the number of interaction test; data not shown).

The PARF ranges up to 6% (Table 1).

DISCUSSION

Principal Findings of the Study

Cesarean delivery was a shared risk factor for several immune-related

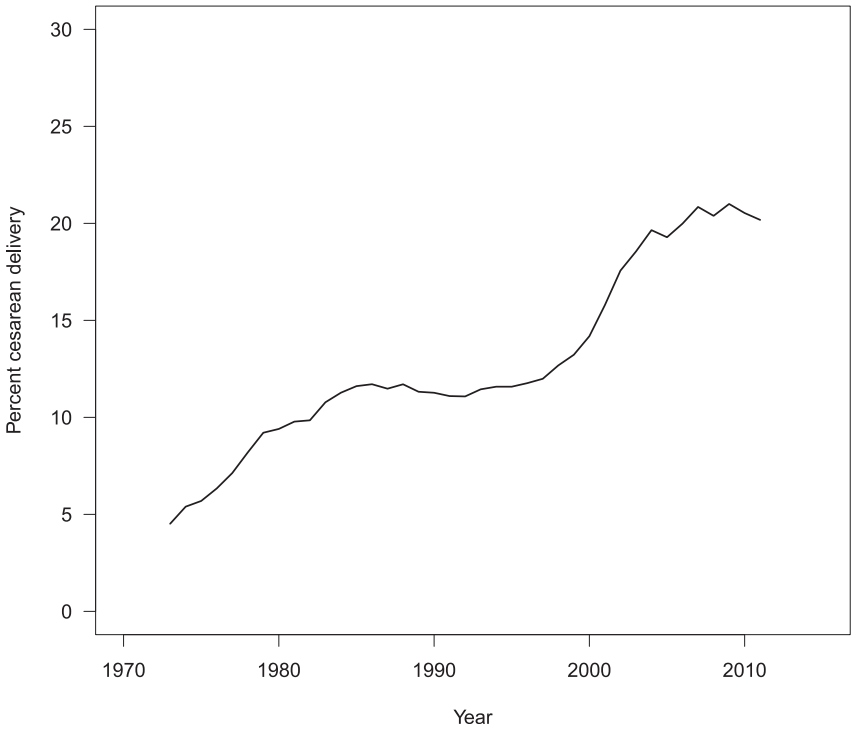

FIGURE 1
Yearly proportion of deliveries by cesarean delivery in relation to birth year. Number of cesarean deliveries has increased over the study period.

TABLE 1 IRRs by Cesarean Delivery in the 35-Year Period 1977–2011 Following 1.9 Million Term Children in the Age Span 0 to 15 Years

	Cases	aIRR (95% Confidence Interval); *P*	PARF (Cases)
Asthma[a]	103 822	1.23 (1.21–1.25); $P < .0001$	3.07 (3187)
Asthma >5 y[b]	48 858	1.16 (1.13–1.19); $P < .0001$	2.19 (1070)
Systemic connective tissue disorders	7498	1.11 (1.04–1.19); $P = .0021$	1.53 (115)
Juvenile arthritis	6946	1.10 (1.02–1.18); $P = .0117$	1.34 (93)
Diabetes type 1	6136	1.01 (0.93–1.10); $P = .82$	—[d]
Inflammatory bowel diseases	2697	1.20 (1.06–1.36); $P = .004$	2.70 (73)
Immune deficiencies	2589	1.46 (1.32–1.62); $P < .0001$	6.09 (158)
Celiac disease	1944	0.99 (0.87–1.14); $P = .89$	—[d]
Leukemia	1631	1.17 (1.00–1.36); $P = .048$	2.31 (38)
Psoriasis	1306	0.98 (0.81–1.18); $P = .81$	—[d]
Arm fracture[a,c]	77 490	0.99 (0.96–1.01); $P = .19$	—[d]

Cases are defined by first in- or outpatient admission to a hospital in Denmark. IRRs are adjusted for age, calendar year, birth weight, parity, gender, season of birth, maternal age, and maternal illness. The PARF is calculated based on an overall prevalence of 14% cesarean deliveries for diseases with significant association to cesarean delivery.

[a] Attained age and calendar year included in 1-y categories.

[b] Attained calendar year included in 3-y and age in 6-y categories.

[c] Not adjusted for maternal disease.

[d] PARF is not calculated for insignificant associations.

diseases including asthma, systemic connective tissue disorders, juvenile arthritis, inflammatory bowel diseases, immune deficiencies, and leukemia. There was no significant association with diabetes type 1, psoriasis, or celiac disease.

Strength and Weaknesses of the Study

Our study was hypothesis driven, searching for a shared risk factor between immune diseases with a debut in children. We did not search all other diagnoses systematically for similar associations because our aim was to exemplify such communality rather than explain individual disorders.

The study base covers 35 years of diagnoses from national registries. The registry on hospitalization covers all admissions and outpatient hospital contacts nationwide.

A priori children with a birth weight below 2500 g were excluded, and all analyses were adjusted for age, calendar year, gender, parity, birth weight, and maternal heredity. By analyzing only children above 2500 g, we hope to get a more comparable group of children, with only the delivery method to differ between them and not an underlying pathology leading to preterm birth.

Particularly, we adjusted for maternal disease of these immune-related diseases, some of which are associated with cesarean delivery (Supplemental Table 3) and could thereby confound an association. After adjustment, the results for type 1 diabetes was no longer statistically significant, but otherwise maternal disease did not materially alter the results.

We included a "negative control" in terms of an analysis of association between cesarean delivery and hospital contact for arm fracture. This demonstrates that the associations seen for other diseases are not caused by a methodological problem of "overregistration" of disease in the children delivered by cesarean delivery.

It may be a limitation to our study that we excluded mild diseases managed in the primary health care sector only. Yet this ascertainment bias is unlikely to influence the conclusion of a common risk factor for the severe chronic diseases.

Diagnostic classification was changed in 1994 from ICD-8 to ICD-10, and registration of outpatient hospitalizations was initiated. The categories used across these different classifications may not be completely congruent. However, the analyses are adjusted for calendar year as a categorical variable, which corrects the main association from time changing effects, and sensitivity analyses did not suggest any effect of this on the results.

Diagnosing asthma in a young child may be inaccurate. We therefore did a sensitivity test of children with a first hospital contact after age 5, where the diagnosis is more robust. The association remained significant.

Interpretation of the Study

Cesarean delivery was a shared risk factor for different immune-related diseases including asthma, systemic connective tissue disorders, juvenile arthritis, inflammatory bowel diseases, immune deficiencies, and leukemia. This suggests that critical events around time of birth initiate a disease trajectory. Such early events may cause an immune aberration leading to a variety of chronic immune diseases presenting later in life. By identifying commonalities between diseases, we may understand mechanisms of the shared "epidemic" seen for these diseases.

Previous studies have revealed individual disorders generally showing associations between cesarean delivery and asthma and allergy,[7–10] inflammatory bowel disease,[11] celiac disease,[12,13] type 1 diabetes,[14] and cancers[15,16] in the offspring. Another register-based study has associated cesarean delivery with a wide range of diagnoses.[20] Our study is the first to reveal cesarean delivery as a common risk factor across several specific immune-related disease categories in the same study and in a nationwide database of children. This reduces the risk of publication bias.

There is increasing evidence that immune-related diseases are programmed in early life as a result of complex gene environment interactions. The prevalence of immune-related diseases is higher in

westernized countries,[21] and immigrant studies reveal that the children of immigrants moving to more westernized countries will acquire the disease risk of westernized countries,[22] indicating the importance of early risk factors associated with western life style. Hygiene, in terms of disturbed early microbial exposure, is hypothesized to be responsible for the increasing prevalence of allergy-related diseases in westernized countries.[23] A similar etiology is suspected behind the increasing prevalence of childhood leukemia,[24] and a recent study revealed an association between allergy and later development of leukemia supporting a potential shared etiology in early life.[25] Reduced cytokine levels at birth have been associated with later development of allergy[26] and atopic predisposition,[27] and children who later developed childhood leukemia also had markedly reduced interleukin-10 levels at birth.[28]

The birth setting around cesarean delivery is different from vaginal birth with respect to several factors including anesthetic agents and antibiotics during birth, physiologic effects on the newborn, and the hospital environment after birth.[29] It may be speculated that the effect from cesarean delivery is mediated by changes in the microbiome of the newborn. The normal delivery canal exposes the child to a composite microbiome different from the one encountered during a cesarean delivery in an operation theater resulting in differences in microbiome of the newborn.[30,31] Furthermore, it has become standard procedure to give prophylactic antibiotics to all women delivering by cesarean delivery to reduce postpartum infections in the mother,[32] which is also likely to affect the microbiome of the newborn child. There are several indications that the diversity and composition of the human microbiome is associated with a variety of diseases such as asthma,[33] allergy,[34] inflammatory bowel diseases,[35] and type 1 diabetes[36] and type 2.[37] However, cesarean delivery entails several other components, which may potentially affect the early environment of the newborn. Biomarkers in the blood in children born by cesarean delivery differ from children born vaginally including lower number of leukocytes, neutrophils, monocytes, and natural killer cells in cord blood,[38,39] and likewise differences in leukocyte composition during the first year of life.[40] Also, stress hormone induction in the fetus is dependent on mode of delivery with a lower production in children born by cesarean delivery,[41] which may affect the immune maturation. Furthermore, pregnancy factors leading to cesarean delivery may already in utero affect the fetus and trigger the progression toward disease.

Because this is an observational study, we cannot rule out that the true casual factor underlying these associations could be a confounding factor related to cesarean delivery. Potential confounders not accounted for in the current study are lifestyle factors associated with maternal request for cesarean delivery or factors increasing the risk of cesarean delivery. Nevertheless, identification of cesarean delivery, or an associated factor, as a potential shared environmental risk factor behind several immune-related diseases is still important. It may give us lead to understand the mechanism and increasing prevalence of these diseases and eventually point to prevention. It is of interest that not all immune-related diseases were affected equally by delivery method, which might suggest different disease pathways. Opposite to some other studies, we did not find association to type 1 diabetes after adjustment for maternal disease. We demonstrated a high prevalence of cesarean delivery among women with type 1 diabetes, which may confound unadjusted results.

Our findings also support the hypotheses that perinatal life is important for later development of chronic diseases, which encourages a research strategy focusing on early life in the origins of chronic diseases.

Furthermore, research targeting the more common immune-related diseases like asthma and allergy may be a relevant approach for understanding the etiology of rare diseases where prospective studies are not feasible. Future research focusing on commonalities in mechanisms behind different disease entities may provide new mechanistic insights and understanding of lifestyle related changes in disease patterns of chronic immune-diseases.

CONCLUSIONS

Cesarean delivery is associated with increased risk of several chronic immune diseases suggesting a shared environmental risk factor in early life. Understanding the underlying disease mechanism may be a key to understanding the origin and increased prevalence of these diseases and promise a perspective for prevention.

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Supplemental Information

SUPPLEMENTAL TABLE 2 Case Definitions

Disease Group	ICD-8	ICD-10
Asthma	439xx	J45x, J46x
Systemic connective tissue disorder	714xx, 715xx, 716xx, 734xx, 446xx, 71899, 69549, 73419	M30, M31, M32, M33, M34, M35, M36
Juvenile arthritis	712xx	M05.x, M08x, M09x, M13.x
Inflammatory bowel diseases	563xx	K50.x, K51.x
Diabetes type 1	24906, 24907, 24908, 24909, 25000, 25006, 25007, 25009	E10x, E13x, E14x
Immune deficiencies	275xx, 288xx	D80–D89
Psoriasis	696xx	L40.x
Celiac disease	26900	K90.0
Leukemia	204xx, 205xx, 206xx; 207xx	C91.x–C96.x
Negative control disease		
Fractured forearm or elbow	813xx	S52.x

SUPPLEMENTAL TABLE 3 Association Between Maternal Disease and Cesarean Delivery

	Cesarean Delivery Proportion; Maternal Illness, %/No Maternal Illness, %	χ^2 *P*
Asthma	16/14	<.0001
Systemic connective tissue disorders	15/14	<.0001
Juvenile arthritis	15/14	<.0001
Inflammatory bowel diseases	17/14	<.0001
Diabetes type 1	27/14	<.0001
Immune deficiencies	15/14	.0037
Psoriasis	15/14	.0005
Celiac disease	16/14	.0031
Leukemia	12/14	.0106

In the $N = 1\,943\,920$ births where mothers' illnesses can be determined, the overall proportion of cesarean deliveries was 14%. The proportion was significantly higher (χ^2) for mothers with all illnesses except leukemia.