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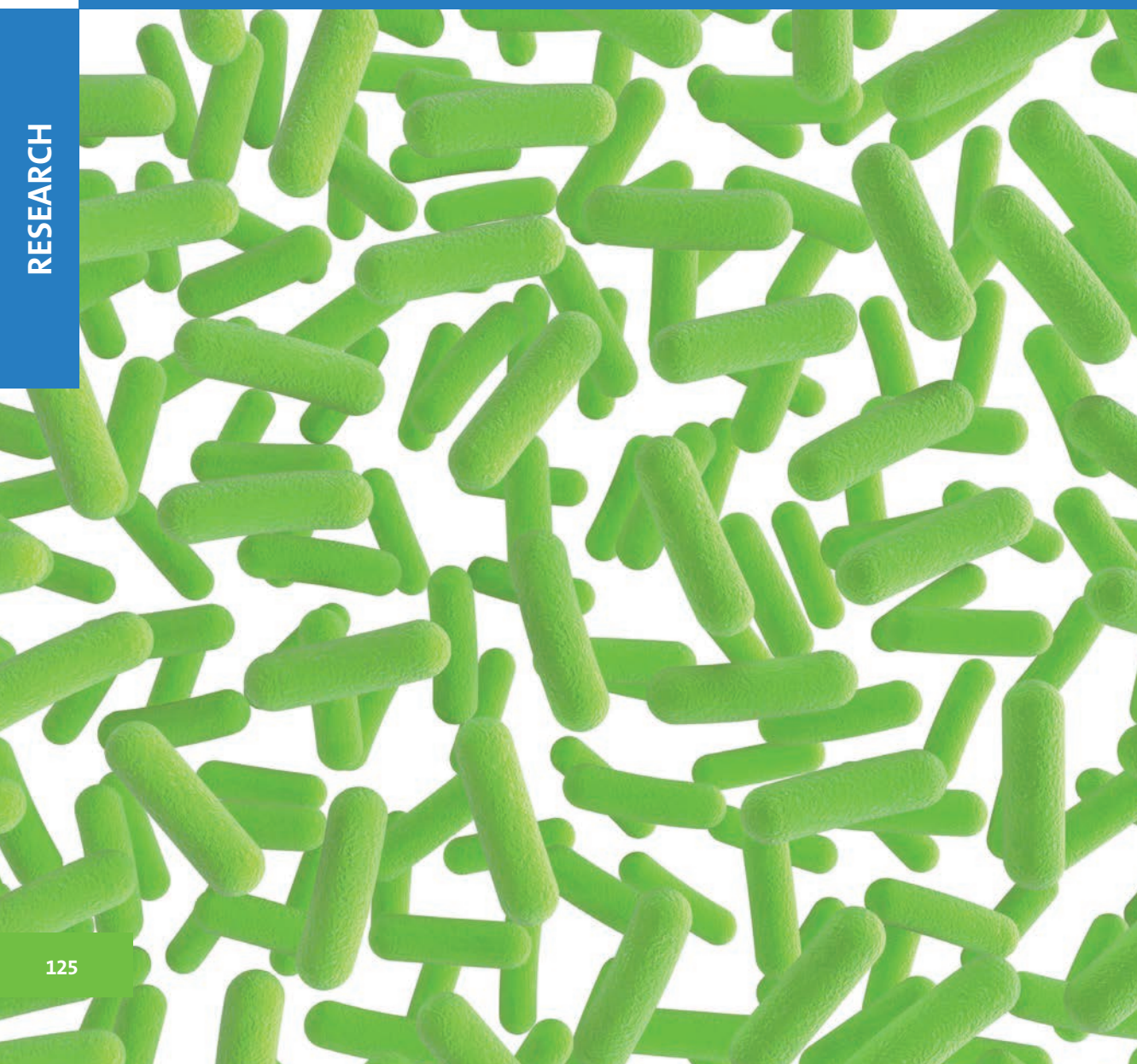
Johanna Metsälä

Maternal and perinatal characteristics, use of antibiotics and the risk of cow's milk allergy and asthma in childhood

RESEARCH



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RESEARCH 125

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ACADEMIC DISSERTATION

To be presented with the permission of the Faculty of Medicine of the University of Tampere, for public examination in the auditorium of Tampere School of Health Sciences, Medisiinarinkatu 3,
on March 21st, 2014, at 12 o'clock noon.

Department of Lifestyle and Participation, Unit of Nutrition, National Institute for Health and Welfare, Finland
and
School of Health Sciences, University of Tampere, Finland

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Supervisors
Professor Suvi M. Virtanen
Department of Lifestyle and Participation
National Institute for Health and Welfare
Helsinki, Finland

School of Health Sciences
University of Tampere
Tampere, Finland

Adjunct Professor Annamari Lundqvist
Department of Health Functional Capacity and Welfare
National Institute for Health and Welfare
Helsinki, Finland

Reviewers
Adjunct Professor Anna Pelkonen
Skin and Allergy Hospital
Helsinki University Central Hospital
Helsinki, Finland

Professor Catarina Almqvist Malmros
Department of Medical Epidemiology and Biostatistics
Karolinska Institutet
Stockholm, Sweden

Opponent
Professor Jouni Jaakkola
Institute of Health Sciences
University of Oulu
Oulu, Finland

To my family

Abstract

Johanna Metsälä. Maternal and perinatal characteristics, use of antibiotics and the risk of cow's milk allergy and asthma in childhood. National Institute for Health and Welfare (THL). Research 125. 87 pages. Helsinki, Finland 2014.
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The prevalence of asthma and allergic diseases in childhood has increased in several industrialized countries since the second half of the twentieth century. In some countries, the prevalence is still rising, although in others it seems to have plateaued or even decreased. It has been suggested that environmental factors operating prenatally and in early life affect the development of asthma and allergic diseases. Particularly changes in microbial exposure are proposed to play an important role in the development and maturation of the immune system. Thus, the factors that affect microbial exposure, such as mode of delivery and the use of antibiotics, may influence the development of asthma and allergic diseases. Several studies have explored the associations between perinatal factors and children's use of antibiotics and the risk of asthma, with inconsistent findings, while less is known about environmental risk factors for cow's milk allergy.

The aim of the present study was to assess whether maternal background factors, perinatal factors and the use of antibiotics are associated with the development of cow's milk allergy and asthma in childhood. These associations were addressed in a register-based case-control study nested in a cohort of all Finnish children born between 1 January 1996 and 30 April 2004. Among these children, all those who were diagnosed with cow's milk allergy (i.e. received a special reimbursement for the cost of special infant formula) or asthma (i.e. received a special reimbursement for the cost of antiasthmatic drugs) by the end of year 2005 and purchased, respectively, special infant formulas or antiasthmatic drugs after diagnosis were identified as cases. For each case, one sex-, birth date- and birth hospital district-matched control child was selected. Altogether, 16,237 cow's milk allergy case-control pairs and 20,272 asthma case-control pairs were included in the study.

Information on received special reimbursements, drug purchases, maternal background and perinatal factors were obtained from, respectively, the Special Reimbursement Register of the Social Insurance Institution, the Drug Prescription Register of the Social Insurance Institution and the Finnish Medical Birth Register of the National Institute for Health and Welfare. Statistical analysis was conducted using conditional logistic regression separately for children diagnosed with asthma before the age of three years and at the age of three years or later, as the asthma diagnosis in young children is challenging. Thus, the results related to cow's milk

allergy and asthma diagnosed at the age of three years or later are considered as the main results of the present study.

The number of maternal previous deliveries was inversely associated with the risk of both cow's milk allergy (OR 0.71, 95% CI 0.59–0.86 for five or more deliveries vs. no previous deliveries) and asthma diagnosed at the age of three years or later (OR 0.36, 95% CI 0.27–0.49 for five or more deliveries vs. no previous deliveries). In addition, maternal age at delivery, socioeconomic status, child's birth weight and delivery by caesarean section were directly associated with the risk of cow's milk allergy. Maternal smoking during pregnancy and multiple pregnancy were associated with a decreased risk of cow's milk allergy. Further, maternal asthma and a short gestational age were associated with an increased risk of asthma diagnosed at the age of three years or later.

Maternal use of antibiotics during pregnancy was associated with an increased risk of cow's milk allergy (OR 1.21, 95% CI 1.14–1.28) and asthma diagnosed at the age of three years or later (OR 1.14, 95% CI 1.04–1.24) in the offspring after considering several maternal sociodemographic background and perinatal factors and the child's use of antibiotics. Maternal use of cephalosporins during pregnancy was associated with the risk of both cow's milk allergy and asthma diagnosed at the age of three years or later. In addition, maternal use of macrolides and penicillins with extended spectrum were associated with the risk of cow's milk allergy.

The children's own use of antibiotics during early childhood was associated with an increased risk of cow's milk allergy (OR 1.71, 95% CI 1.59–1.84) and asthma diagnosed at the age of three years or later (OR 1.55, 95% CI 1.44–1.67). All common specific antibiotics used during early childhood were associated with the risk of both cow's milk allergy and asthma diagnosed at the age of three years or later, except phenoxymethylpenicillin, which was not associated with the risk of asthma.

In summary, the present study found that the use of antibiotics by the mother and the child were associated with an increased risk of both cow's milk allergy and asthma diagnosed at the age of three years or later in childhood. Further, although several maternal sociodemographic background and perinatal factors were found to be associated with either the risk of cow's milk allergy or asthma in childhood, only a high number of maternal previous deliveries were associated with a decreased risk of both cow's milk allergy and asthma diagnosed at the age of three years or later. The antibiotics and asthma findings are in line with results from similar large, register-based studies, but the cow's milk allergy findings will have to be confirmed in future studies. In addition, biological mechanisms underpinning the associations observed in the present study need to be explored further.

The results of the present study provide further evidence that environmental factors affecting microbial exposure during the prenatal period and in early childhood may play a role in the development of cow's milk allergy and asthma in

childhood, although some maternal-related and perinatal risk factors seem to be different for these two diseases.

Keywords: antibiotics, asthma, cow's milk allergy, children, epidemiology, perinatal factors, registries

Tiivistelmä

Johanna Metsälä. Äidin tausta- ja perinataalitekijöiden sekä antibioottien käytön yhteys lapsen maitoallergiaan ja astmaan. Terveyden ja hyvinvoinnin laitos (THL). Tutkimus 125. 87 sivua. Helsinki, Finland 2014.
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Lapsuusiän astman ja allergisten sairauksien esiintyvyys on noussut useissa länsimaissa 1900-luvun puolivälin jälkeen. Esiintyvyys nousee yhä joissakin maissa, vaikka toisissa maissa nousu näyttäisi pysähtyneen tai jopa laskeneen. Varhaislapsuuden ja jopa sikiöaikaisten ympäristötekijöiden arvellaan olevan yhteydessä astman ja allergisten sairauksien syntyyn. Erityisesti muuttuneella mikrobialtistuksella ja suoliston mikrobistolla on mahdollisesti keskeinen vaikutus immunipuolustuksen kehittymiseen ja kypsymiseen. Siten niillä tekijöillä, joilla on vaikutusta mikrobialtistukseen ja suoliston mikrobistoon, kuten synnytystavalla ja antibioottien käytöllä, saattaa olla merkitystä astman ja allergisten sairauksien kehittymisessä. Perinataalitekijöiden ja lapsen antibioottien käytön yhteyttä astman syntyyn on selvitetty useissa tutkimuksissa, kun taas eri ympäristötekijöiden yhteyttä lehmänmaitoallergian syntyyn on tutkittu vain vähän.

Tämän tutkimuksen tavoitteena oli selvittää äidin taustatekijöiden, perinataalitekijöiden ja antibioottien käytön yhteyttä lapsen riskiin sairastua lehmänmaitoallergiaan ja astmaan. Näitä yhteyksiä selvitettiin rekisteritietoihin pohjautuvassa upotetussa tapaus-verrokki asetelmassa. Tutkittavat oli poimittu kaikista Suomessa 1.1.1996–30.4.2004 syntyneistä lapsista. Näistä lapsista kaikki ne, joille oli myönnetty erityisäidinmaidonkorvikkeiden tai lääkkeiden erityiskorvausoikeus lehmänmaitoallergian tai astman johdosta vuoden 2005 loppuun mennessä ja jotka olivat ostaneet erityisäidinmaidonkorvikkeita tai astmalääkkeitä diagnoosin (erityiskorvausoikeuden) jälkeen määriteltiin tapauksiksi. Jokaiselle tapaukselle poimittiin sukupuolen, syntymäajan ja syntymäsairaanhoitopiirin mukaan kaltaistettu verrokki. Tutkimukseen otettiin lopulta mukaan 16237 maitoallergian ja 20272 astman tapaus-verrokkiparia. Erityiskorvausoikeus- ja lääkeostotiedot saatiin Kansaneläkelaitoksen lääketiedostoista ja tiedot äidin taustatekijöistä ja perinataalitekijöistä saatiin Terveyden ja hyvinvoinnin laitoksen ylläpitämästä Syntyneiden lasten rekisteristä. Tilastolliset analyysit toteutettiin käyttäen ehdollista logistista regressiota. Analyysit tehtiin erikseen alle 3-vuotiaina ja 3-vuotiaana tai sitä vanhempana diagnoosin saaneilla lapsilla, sillä astma-diagnoosi on haastellinen pienilä lapsilla. Siten tämän työn päätuloksiksi voidaan katsoa tulokset maitoallergian ja astma-diagnoosin 3-vuotiaana tai sitä vanhempana saaneiden osalta.

Äidin aikaisempien synnytysten määrä oli käänteisessä yhteydessä sekä lehmänmaitoallergian (OR 0.71, 95% CI 0.59–0.86, 5 tai useampi synnytys vs. ei aikaisempia synnytyksiä) että 3-vuotiaana tai sitä vanhempana diagnosoidun astman (OR 0.36, 95% CI 0.27–0.49, 5 tai useampi synnytys vs. ei aikaisempia syn-

nytyksiä) riskiin. Lisäksi, äidin ikä ja sosioekonominen asema synnytyksen hetkellä sekä lapsen syntymäpaino ja syntyminen keisarinleikkauksella olivat suorassa yhteydessä lehmänmaitoallergian riskiin. Äidin tupakointi raskauden aikana ja monisikiöraskaus olivat yhteydessä vähentyneeseen lehmänmaitoallergian riskiin. Lyhyt raskauden kesto oli yhteydessä suurentuneeseen 3-vuotiaana tai sitä vanhempana diagnosoidun astman riskiin.

Äidin raskaudenaikainen antibioottien käyttö oli yhteydessä lapsen suurentuneeseen lehmänmaitomaitoallergian (OR 1.21, 95% CI 1.14–1.28) ja 3-vuotiaana tai sitä vanhempana diagnosoidun astman (OR 1.14, 95% CI 1.04–1.24) riskiin, kun oli huomioitu useita äidin taustatekijöitä, perinataalitekijöitä ja lapsen oma antibioottien käyttö. Äidin raskauden aikana käyttämät kefalosporiinit olivat yhteydessä lapsen suurentuneeseen lehmänmaitoallergian ja astman riskiin. Lisäksi äidin käyttämät makrolidit ja laajakirjoiset penisilliinit olivat yhteydessä suurentuneeseen lehmänmaitoallergian riskiin.

Lapsen oma antibioottien käyttö varhaislapsuudessa oli yhteydessä suurentuneeseen lehmänmaitoallergian (OR 1.71, 95% CI 1.59–1.84) ja 3-vuotiaana tai sitä vanhempana diagnosoidun astman (OR 1.55, 95% CI 1.44–1.67) riskiin. Kaikki yleisesti lapsilla käytetyt antibiootit, paitsi fenoksimetyylipenislliini astman osalta, olivat yhteydessä sekä lehmänmaitoallergian että astman suurentuneeseen riskiin.

Sekä äidin että lapsen antibioottien käyttö oli yhteydessä suurentuneeseen lehmänmaitoallergian ja 3-vuotiaana tai sitä vanhempana diagnosoidun astman riskiin. Lisäksi, vaikka useat äidin taustatekijät ja perinataalitekijät olivat yhteydessä lapsen riskiin sairastua joko lehmänmaitoallergiaan tai astmaan, vain äidin aikaisempien synnytysten lukumäärä oli käänteisessä yhteydessä sekä lehmänmaitoallergian että 3-vuotiaana tai sitä vanhempana diagnosoidun astman riskiin. Antibioottien käyttöön ja astmariskiin liittyvät havainnot ovat samansuuntaisia kuin mitä on aikaisemmin havaittu muissa vastaavissa, isoissa rekisteritietoihin perustuvissa tutkimuksissa, kun taas lehmänmaitoallergiaan liittyvät havainnot tulee vahvistaa lisätutkimuksin. Lisäksi, tässä tutkimuksessa havaittujen yhteyksien taustalla olevista biologisista mekanismeistä tarvitaan lisää tutkimuksia. Tämän tutkimuksen tulokset tukevat käsitystä siitä, että sikiöaikaisilla ja varhaislapsuuden mikrobialtistukseen yhteydessä olevillä ympäristötekijöillä on mahdollisesti vaikutusta lehmänmaitoallergian ja astman kehittymiseen lapsuusiässä, vaikka sairauksien riskitekijät näyttävät osittain eroavan.

Avainsanat: antibiootit, astma, epidemiologia, lapset, lehmänmaitoallergia, perinataalitekijät, rekisterit

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List of original papers

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II Johanna Metsälä, Annamari Lundqvist, Minna Kaila, Mika Gissler, Timo Klaukka, Suvi M. Virtanen. Maternal and perinatal characteristics and the risk of cow's milk allergy in infants up to 2 years of age: A case-control study nested in the Finnish population. Am J Epidemiol 2010; 171:1310–1316.

III Johanna Metsälä, Annamari Lundqvist, Lauri J. Virta, Minna Kaila, Mika Gissler, Suvi M.Virtanen. Mother's and offspring's use of antibiotics and infant allergy to cow's milk. Epidemiology 2013; 24:303–309.

IV Johanna Metsälä, Annamari Lundqvist, Lauri J. Virta, Minna Kaila, Mika Gissler, Suvi M. Virtanen. Prenatal and postnatal exposure to antibiotics and risk of asthma in childhood. (submitted).

Abbreviations

ATC	Anatomical Therapeutic Chemical
CMA	cow's milk allergy
IgE	immunoglobulin E
OR	odds ratio
CI	condifence interval

1 Introduction

Cow's milk allergy (CMA) is a common food allergy in early childhood and asthma one of the most common chronic conditions in childhood. In Finland, a cumulative incidence of 1.9% of challenge-confirmed CMA among infants less than 12 months of age (Saarinen *et al.* 1999) and a prevalence of 7% of asthma among schoolchildren aged 7 to 16 years has been reported (Hugg *et al.* 2008). No reliable data on time trends in the occurrence of CMA have been published, but the prevalence of asthma and allergic diseases like allergic rhinitis in childhood has shown an increasing trend in several industrialized countries since the second half of the twentieth century (Eder *et al.* 2006). In some countries, the prevalence is still rising, although in other countries the prevalence seems to have plateaued or even decreased (Pearce *et al.* 2007).

Although the development of asthma and allergic diseases has a strong genetic component, the rise in the prevalence of these diseases indicates that environmental factors play an important role as well. Increasing evidence suggests that important time periods of these environmental factors to come into play are early life or even in utero (Holt and Jones 2000). The original hygiene hypothesis suggested that modern healthcare and hygiene practices and reduced exposure to infections were linked to the development of allergic diseases (Strachan 1989). Over the years, the hygiene hypothesis has been revised and the focus has shifted from clinical infections to overall microbial exposure. Accumulating evidence suggests that gut microbiota play an important role in the maturation and development of the immune system. The "microflora hypothesis" suggests that perturbations in the gastrointestinal microbiota as a result of reduced microbial exposure result in immature microbiota, which may delay proper maturation of the immune system, and this delay may lead to an increase in asthma and allergic diseases (Noverr and Huffnagle 2005).

The factors that have been suggested to be related to the development of asthma include infections, non-pathogenic microbes, dietary factors and exposure to tobacco smoke, air pollutants and allergens (Eder *et al.* 2006), as well as various factors related to pregnancy, delivery and fetal growth (Bernsen *et al.* 2006). Despite active research, evidence on early life factors and the development of asthma is not consistent. Further, evidence on the role of environmental factors in the development of CMA or any food allergy is limited. Identification of risk and protective factors for CMA, asthma and other allergic diseases is important for prevention strategies. Although not all early life factors are easily modifiable, identification of such factors may help focus preventive strategies on children at high risk. Given the widespread use of antibiotics and their potential as a modifia-

ble exposure, it is important to thoroughly examine the role of antibiotics in the development of asthma and allergic diseases.

The aim of the present study was to assess whether maternal background factors, perinatal factors and the use of antibiotics are associated with the development of cow's milk allergy and asthma in childhood.

2 Review of the literature

2.1 Definitions, mechanisms and occurrence of cow's milk allergy and asthma

Cow's milk allergy

According to a revision of the allergy nomenclature, allergy is defined as a hypersensitivity reaction initiated by specific immunologic mechanisms (Johansson *et al.* 2004). Cow's milk allergy (CMA) is defined as an adverse clinical reaction to ingested cow's milk proteins based on an immunologically mediated adverse reaction to the provoking proteins at doses which are tolerated by healthy persons (World Allergy Organization 2010). Exposure to cow's milk proteins provokes an immune response in all infants, although in healthy infants this response is suppressed and oral tolerance is developed. If oral tolerance is not achieved, processes ending to a release of inflammatory mediators will be activated, and these inflammatory reactions can occur in several organs. Mechanisms of CMA are best understood and described in antibody-mediated, (immunoglobulin E [IgE]-mediated) CMA, while the precise immunologic mechanisms of cell-mediated (non-IgE mediated) CMA remain less clear. In IgE-mediated CMA typical symptoms, which usually develop rapidly within minutes or an hour after ingestion of cow's milk protein, include symptoms in the skin like urticaria and angioedema respiratory symptoms like wheeze, and anaphylaxis. In non-IgE-mediated CMA, symptoms tend to have later onset, from hour to days after ingestion of cow's milk protein, and most commonly involve the gastrointestinal tract, although – as in IgE-mediated CMA – various symptoms are possible. (World Allergy Organization 2010)

There is a consensus that the diagnosis of food allergy has to be based on oral food challenge procedures that establish a causal relation between the ingestion of a particular food and a subsequent clinical reaction (Sampson *et al.* 2012). Of the different challenge procedures, double-blind placebo-controlled food challenge is considered the "golden standard" of diagnosing food allergies. However, only a minority of studies related to CMA have used this procedure and, in epidemiological research, CMA is often defined as either parentally reported or oral food challenge confirmed CMA (World Allergy Organization 2010).

During the last ten years, the occurrence of oral food challenge confirmed CMA has been evaluated in five birth cohort studies: in Finland, a cumulative incidence of 1.9% of challenge confirmed CMA has been reported among infants less than 12 months of age. In other birth cohort studies, CMA was challenge con-

firmed in 2.2% of infants in Denmark, Isle of Wight, and Netherlands, as well as in 4.9% of infants in Norway (World Allergy Organization 2010). Perception of CMA is more common than confirmed CMA; the prevalence of self-reported CMA varies between 1% and 17.5% in preschool-aged children and between 1% and 13.5% in 5–16-year-old children (World Allergy Organization 2010). No reliable data on geographical or time trends on CMA incidence in children have been published.

Asthma

Asthma is defined as a chronic inflammatory disorder associated with variable airflow obstruction and bronchial hyperresponsiveness (Papadopoulos *et al.* 2012). A key component in the pathology is chronic inflammation, and various factors such as viruses, allergens and exercise can trigger inflammation. These factors also induce hyperresponsiveness, and inflammation together with hyperreactivity can lead to airway obstruction. Asthma can present with symptoms such as recurrent episodes of wheezing, coughing, shortness of breath, and chest tightness (Papadopoulos *et al.* 2012). In epidemiological research, asthma is often defined through information on physician-diagnosed asthma, antiasthmatic drug use, hospitalization due to asthma or asthma symptoms and different classification of these symptoms. According to Spycher and co-workers (2010), frequently used asthma or wheeze phenotypes are based on 1) triggers/short-term temporal pattern (including exclusive viral wheeze and multiple-trigger wheeze), 2) long-term temporal pattern (including early transient wheeze, persistent wheeze and late-onset wheeze), 3) presence of allergic sensitization (atopic asthma and non-atopic asthma) or 4) severity (including wheeze and asthma).

In children, the occurence of recurrent wheezing episodes is a universally accepted starting point of asthma diagnosis (Papadopoulos *et al.* 2012). In addition to medical history, methods to establish asthma diagnosis include physical examination, evaluation of lung function, atopy, airway inflammation and bronchial hyperresponsiveness and exclusion of alternative diagnoses (Papadopoulos *et al.* 2012). In young children, the asthma diagnosis is particularly challenging due to difficulties in applying objective lung function measurements. Thus, in infants and preschool-aged children, the diagnosis is often based on medical history and symptoms.

In Finland, a 7–8% prevalence of asthma has been reported among school-aged children (Hugg *et al.* 2008, Lai *et al.* 2009). Further, according to the Finnish Social Insurance Institution's Special Reimbursement Registry, the number of children receiving a special reimbursement for the cost of antiasthmatic medication was 1.9 per 100 children in 1994, 3.1 per 100 children in 2002, and 2.6 per 100 children in 2006 (Mäkelä *et al.* 2008). The prevalence of asthma symptoms in children has been studied at a global level in the International Study on Allergies

and Asthma in Childhood conducted in three phases. In Phase III, wide global variations in the prevalence of asthma symptoms were observed (Lai *et al.* 2009). Among children aged six to seven years, the global total prevalence of asthma at any time was 11.5%, ranging from 3.4% in Africa to 29.2% in Oceania (Lai *et al.* 2009). An increasing trend of asthma symptoms has been observed in several countries, although the rise seems to have achieved a plateau or even decreased in some countries (Pearce *et al.* 2007).

2.2 Development of cow's milk allergy and asthma

For two decades, the hygiene hypothesis has been one of the leading hypotheses in attempts to explain the increasing prevalence of asthma and allergic diseases. The hygiene hypothesis was first developed to explain the inverse association between number of siblings and hay fever (Strachan 1989). This association was interpreted to mean that shared infections among children within large families attenuate the risk of allergy. The hygiene hypothesis stated that modern healthcare and hygiene practices and reduced exposure to pathogens would lead to an imbalance of the immune system predisposing individuals to the development of allergic diseases (Strachan 1989). Since its inception, several revisions of the hygiene hypothesis have been introduced: the focus in research has shifted from clinical infections to overall microbial exposure and the important role of gut microbiota has been recognised (discussed in more detail later). Further, it has been suggested that the hygiene hypothesis may be relevant not only for allergic diseases but for other immune-mediated diseases such as autoimmune diseases (Bach 2002). Recently, the hygiene hypothesis has been expanded to a biodiversity hypothesis: declining biodiversity in the living environment, plants, animals and their habitats, is suggested to increase chronic diseases that are associated with inflammation, such as asthma and allergic diseases (Von Hertzen *et al.* 2011).

According to the Barker hypothesis, restricted fetal growth may reflect insufficient energy supply for organ development and lead to increased susceptibility to coronary heart disease later in life (Barker 1995). This concept of developmental origins of cardiovascular diseases and metabolic syndrome has also been discussed in relation to the development of asthma and allergic diseases (Tedner *et al.* 2012). Asthma has received more research attention than other allergic diseases, and despite active research the mechanisms underpinning the association between fetal growth and asthma and allergic diseases remains unclear. Whether it is the fetal growth restriction, the rapid catch-up growth after birth following restricted fetal growth or some still unknown third factor(s) that predisposes for the development of asthma has yet to be determined (Tedner *et al.* 2012).

The role of gut microbiota in the development of asthma and allergic diseases

The gut microbiota is a major source of microbial exposure, containing an assortment of micro-organisms inhabiting the entire gastrointestinal tract. Colonization of the human gut microbiota begins immediately at birth, as upon passage through the birth canal infants are exposed to a complex microbial population. After the initial establishment of the gut microbiota during the first year of life, the composition of gut microbiota is host-specific, evolving throughout an individual's lifetime, and is susceptible to both exogenous and endogenous modifications (Sekirov *et al.* 2010). Stimuli derived from normal gut microbiota have been demonstrated in experimental studies to be important in facilitating the development of oral tolerance to food allergens (Sudo *et al.* 1997) and tolerance to inhaled aeroallergens (Noverr and Huffnagle 2005).

The "microflora hypothesis", introduced by Noverr and Huffnagle (2005) suggests that perturbations in the gastrointestinal microbiota as a result of reduced microbial exposure result in an underdeveloped microbiota. This immature microbiota delays proper maturation of the immune system, disrupting the development of immunological tolerance and subsequently increasing the development of allergic diseases (Noverr and Huffnagle 2005). The idea of the potential role of gut microbiota in the development of allergic diseases has encouraged several observational studies to assess the association between the gut microbiota composition and allergic diseases. Although an association between the gut microbiota composition and various allergic conditions or symptoms has been reported, no specific harmful or protective microbes have been identified yet (Penders *et al.* 2007). Further, there has been a substantial effort to assess the effects of probiotics, living micro-organisms that exert health benefits, on the prevention and or treatment of allergic diseases in clinical trials. While there is a theoretical basis for probiotics having a positive effect in allergy prevention or treatment and some studies have shown an association between probiotic supplementation and reduced allergic diseases, not all studies have reached the same conclusions (Prescott and Björksten 2007).

2.3 Factors associated with the development of cow's milk allergy and asthma

Despite active research, the causes of asthma and allergic diseases are only partially understood. Heredity is a generally accepted important risk factor, but the rise and international variation in prevalence rates are likely attributable to environmental aspects and gene-environment interactions (Subbarao *et al.* 2009). Whether food allergies and asthma share same genetic and environmental factors or whether there are some unique factors to each disease is unclear.

2.3.1 Genetic factors

Family history and twin studies have indicated that genetics plays an important role in the development of asthma and food allergies (Hong *et al.* 2009, Subbarao *et al.* 2009). Nearly 100 genes have been found to be associated with asthma or asthma-related phenotypes, although not all of these associations could have been replicated in further studies (Subbarao *et al.* 2009). Unlike asthma, only a limited number of genetic studies – namely candidate gene association studies – of food allergies have been reported (Hong *et al.* 2009). Polymorphisms in nine genes have been associated with food allergy or food allergy severity in at least one study, but most of these associations have not been replicated (Hong *et al.* 2009). Some of the genes associated with food allergy are also associated with asthma, although the relevance of these genes to both food allergy and asthma together remains to unexplored.

2.3.2 Environmental factors

The recognition that the priming of the immune system occurs in early childhood or even before birth has prompted the idea of a "critical time-window" for environmental factors to come to play and impact the development of asthma and allergic diseases (Holt and Jones 2000). Knowledge of the role of different environmental factors in the development of CMA or any food allergy remains limited, but more extensive research has been conducted on the associations between different environmental factors and the development of asthma in childhood. Those factors that have been suggested as being related to changes in the environment and thus the development of asthma include infections and other microbes, dietary factors, exposure to tobacco smoke, air pollutants and allergens (Eder *et al.* 2006). As the mother is an exclusive environment for the child for the first nine months and after birth continues as the closest caregiver, she can be considered as a major environmental factor operating in the child's development. Thus, various factors related to prenatal period, i.e. pregnancy, and delivery have received growing attention (Bernsen *et al.* 2006).

Infections and non-pathogenic microbes

Infections in early childhood have been implicated in the development of asthma, although their role is complex. For example, severe lower respiratory tract infections have been associated with an increased risk of asthma (Bartlett *et al.* 2009), while repeated viral infections have been reported to be associated with a decreased risk (Illi *et al.* 2001). Further, indirect measures of infections like the care of a child in a daycare centre and high number of older siblings have been shown to be associated with both a decreased and an increased risk of asthma (Ball *et al.* 2000, McKeever *et al.* 2001, Hagerhed-Engman *et al.* 2006). Despite the fact that severe lower respiratory tract infections have been linked to later development of

asthma, it is still unclear whether these lower respiratory tract infections cause subsequent development of asthma or whether these infections merely mark individuals that have a genetic propensity for developing asthma (Bartlett *et al.* 2009). Further, it has been suggested that qualitative aspects of infections such as timing, type and intensity may be crucial in the development of asthma. There is currently no evidence that infections would play a major role in the development of cow's milk allergy or any food allergy in early childhood.

Not only pathogenic microbes causing symptomatic infection, but also non-pathogenic microbes have been suggested as playing a role in the development of asthma and allergic diseases. Several studies have shown that exposure to environmental microbes is inversely associated with the risk of asthma and allergic diseases; for example, a lower prevalence of asthma and atopy has been observed in a population with higher number of bacterial exposures compared to a population with lower exposure despite these two populations living in geographically adjacent areas (Vartiainen *et al.* 2002). Further, a lower prevalence of asthma and allergic diseases among children living on a farm has been found repeatedly, and the largest reduction in risk has been demonstrated for those exposed to a farming environment both prenatally and postnatally until adulthood (Von Mutius and Vercelli 2011). In addition, an observation that microbial diversity of house dust was inversely related to the prevalence of childhood asthma independently of farming status (Ege *et a.l* 2011) and that a lower environmental biodiversity in the surroundings of study subjects' homes was more common in atopic than in non-atopic adolescents (Hanski *et al.* 2012) further emphasizes the role of overall microbial exposure and environmental biodiversity in the development of asthma and allergic diseases.

Diet

Breast milk, one of the most important early nutritional sources in the postnatal period, contains compounds with immodulatory properties, and it has been hypothesized that it protects children from asthma and allergic diseases (Kneepkens and Brand 2010). Several studies support the protective role of exclusive breastfeeding until at least four months of age, but the discussion on the preventive effects of breastfeeding continues, mainly due to methodological issues (Kneepkens and Brand 2010). The role of complementary feeding in the development of allergic diseases has been revised in recent years. Delaying exposure to solid foods or avoidance of allergenic foods in infancy is no longer recommended for prevention of allergic diseases. In fact, evidence that early introduction of certain foods is beneficial in decreasing the risk of asthma and allergic diseases is emerging (Nwaru *et al.* 2013).

On a nutrient level, it has been hypothesized that changes in antioxidant intake, the ratio of dietary n-6:n-3 polyunsaturated fatty acids, and vitamin D may play a

role in the development of asthma and allergic diseases (West *et al.* 2010). Observational studies have reported inverse associations between asthma and n-3 polyunsaturated fatty acids and dietary antioxidants in the postnatal diet, although supplementation of infants' diet with fish oil or vitamin D have so far failed to show any major benefit (West *et al.* 2010). A maternal intake of antioxidant rich foods, fish oil and vitamin D during pregnancy has been reported to be inversely associated with wheezing, asthma and other allergic diseases in the offspring. In addition, limited intervention data suggest some benefits of fish oil supplementation during pregnancy in reducing the risk of allergic disease in offspring. (West *et al.* 2010)

2.4 Maternal background, perinatal factors and use of antibiotics as risk factors for cow's milk allergy and asthma

For the purposes of this review, a literature search from the PubMed and Web of Science databases was conducted to find studies that had assessed the association between selected maternal background factors and perinatal factors (referring to factors operating during pregnancy, delivery and just after pregnancy), as well as maternal and child's use of antibiotics and CMA or asthma. Medical Subject Heading (MeSH) search terms "Birth Weight", "Apgar Score", "Delivery, Obstetric", "Cesarean Section", "Pre-Eclampsia", "Multiple Birth Offspring", "Gestational Age", "Fetal Development", "Premature Birth", "Birth Order", "Reproductive History", "Anti-Bacterial Agents", "Asthma" and the key words wheeze, wheezing, cow's milk allergy and food allergy were used. Further, reference lists of the obtained articles were reviewed. For this review, those observational studies that included children or adolescents up to the age of 18 years and were published by April 2013 were included (Tables 1–4). Of the studies related to maternal background and perinatal factors and asthma, only cohort studies published since the year 2000 with large sample size (n>1000) were included (Table 2). Of the studies related to children's use of antibiotics and asthma or wheeze, only cohort or nested case-control studies were included (Table 4). Further, if a study reported results for several asthma outcomes, the outcome that was considered most reliable or comparable with the present study was included (Tables 1–4).

2.4.1 Maternal background

Maternal sociodemographic background and perinatal factors and CMA or other food allergies have been explored in 13 studies (Table 1). CMA was used as the outcome in three studies, any food allergy in eight studies and egg allergy in two studies. Studies on CMA had a cohort design, whereas cross-sectional design was the dominant study design in studies on any/other food allergies. Studies were conducted mainly in Europe or North America. The size of the study population varied from 154 to 102,353 subjects, and all studies included both boys and girls.

Two studies were solely register-based, while in other studies the outcome was obtained from medical examination or questionnaires and the exposures from questionnaires or registers/medical records.

Since the year 2000, 46 large cohort studies on maternal background and perinatal factors and asthma have been published (Table 2). The majority of these studies were conducted in Europe or North America. The size of the study population varied from 1,033 to 1,576,700 subjects and all studies included both boys and girls. Data were obtained solely from registers or medical records in 21 studies, of which 18 had a sample size of over 10,000 subjects. In other studies, information on outcome and exposure was obtained either from questionnaires only or from both questionnaires and registers/medical records. Only a minority (ten) of the studies reported associations on several (>4) different maternal and perinatal factors at the same time.

Maternal age

Evidence on the association between maternal age and the risk of food allergies is limited to one case-control study (Dioun *et al.* 2003) reporting an increased risk of any food allergy by age 11 years in those children whose mothers were older than 30 years at the delivery compared with children whose mothers were younger (Table 1). Maternal age at delivery has been observed to be inversely associated with the risk of asthma in two studies (Nafstad *et al.* 2000, Yuan *et al.* 2003), directly associated in one study (Juhn *et al.* 2005), and not associated in two studies (Kiechl-Kohlendorfer *et al.* 2007, Midodzi *et al.* 2010) (Table 2). Studies by McKeever *et al.* (2001) and Bråbäck *et al.* (2003) found age-dependent associations; the inverse association was stronger or present in children diagnosed with asthma at early ages, whereas the association was weaker or not present in children diagnosed at older ages.

Maternal socioeconomic status

In the course of history, asthma and allergic diseases have been considered as conditions of relatively affluent populations, although recent evidence on the association between parental socioeconomic status and food allergies and asthma in children is controversial (Tables 1 and 2). Two recent studies have reported a decreased risk of food allergy in children with low family income (Liem *et al.* 2007, Gupta *et al.* 2011). Victorino and Gauthier (2009) did not observe an association between household income and food allergy, but they observed that children in families with a higher education were more likely to have food allergies. Five large cohort studies on asthma have reported an inverse association (Nafstad *et al.* 2000, Sin *et al.* 2004, Almqvist *et al.* 2005, Juhn *et al.* 2005, Midodzi *et al.* 2010) and three studies a null association (Dik *et al.* 2004, Hancox *et al.* 2004, Davidson

et al. 2010) between maternal socioeconomic status/income/education and the risk of asthma.

Maternal smoking

Evidence on the role of exposure to smoking on the development of verified food allergies is limited (Table 1). A Finnish birth cohort study exploring infant feeding patterns and subsequent immunological patterns of CMA reported no association between maternal smoking during pregnancy and CMA in the infant (Saarinen and Savilahti 2000). On the contrary, maternal smoking during pregnancy is a generally accepted risk factor for respiratory symptoms and reduced lung function, and a 20%–68% increased risk of asthma in children whose mothers had smoked during pregnancy has been reported (Yuan *et al.* 2003, Jaakkola and Gissler 2004, Kiechl-Kohlendorfer *et al.* 2007, Midodzi *et al.* 2010) (Table 2). However, also a null association (Nafstad *et al.* 2000) and children's age-dependent associations (McKeever *et al.* 2001, Bråbäck *et al.* 2003, Davidson *et al.* 2010) between maternal smoking and the risk of asthma have been reported. In the studies by McKeever *et al.* (2001), Bråbäck *et al.* (2003) and Davidson *et al.* (2010), an increased risk of asthma was observed in children diagnosed with asthma at early ages, whereas no association was observed in children diagnosed at older ages.

Maternal pregnancy history

Since Strachan's observation of an inverse association between number of siblings and hay fever (Strachan 1989), the association between asthma and thr number of siblings, parity, birth order or sibship size has been further explored in several studies (Karmaus and Botezan 2002). Saarinen and Savilahti (2000) reported no association between presence of siblings and CMA in infancy (Table 1). Similarly, Eggesbo and co-workers (2003) reported no association between older siblings and egg allergy, although three other studies observed that children with older siblings were less likely to develop egg or any food allergy (Dioun *et al.* 2003, Koplin *et al.* 2012, Kusunoki *et al.* 2012). Four large cohort studies reported a decreased risk of asthma in children having older siblings or high birth order (Ball *et al.* 2000, Nafstad *et al.* 2000, Dik *et al.* 2004, Midodzi *et al.* 2010) (Table 2). For example, Ball and co-workers (2000) observed a 20% decrease in the risk of asthma at ages 6–13 years per each additional sibling. On the contrary, Yuan and co-workers (2003) observed an increased risk of antiasthmatic medication use during the first year of life in children having older siblings. In addition, null (Kiechl-Kohlendorfer *et al.* 2007) and diagnosis-age-dependent (McKeever *et al.* 2001, Bråbäck *et al.* 2003, Davidson *et al.* 2010) associations between older siblings and the risk of asthma have been reported. In the studies observing age-dependent associations, the association was direct in children diagnosed with

asthma at early ages and either inverse or null in children diagnosed at older ages (McKeever *et al.* 2001, Bråbäck *et al.* 2003, Davidson *et al.* 2010).

2.4.2 Perinatal factors

Mode of delivery

The majority of the studies assessing mode of delivery and the subsequent development of CMA, other food allergies or asthma have focused on a comparison between caesarean section and vaginal delivery (Tables 1 and 2). Two Norwegian cohort studies assessed the association between delivery by caesarean section and the risk of CMA, and both studies reported no association (Eggesbo *et. al* 2005, Kvenshagen *et al.* 2009). Although Eggesbo and co-workers (2005) did not find an association in the total population, they observed an increased risk among children whose mothers were allergic. No previous studies related to CMA or any food allergy have assessed planned and emergency caesarean sections separately or assisted and normal vaginal delivery separately.

Two meta-analyses have recently summarized the association between delivery by caesarean section and the risk of asthma (Bager *et al.* 2008, Thavagnanam *et al.*2008). Both meta-analyses concluded that delivery by caesarean section was associated with a roughly 20% increase in the subsequent development of asthma compared with vaginal delivery. These meta-analyses included most studies presented in Table 2, but also studies with smaller sample sizes and various designs. Four more recent cohort studies found a direct association (Tollånes *et al.* 2008, Roduit *et al.* 2009, Magnus *et al.* 2011, Almqvist *et al.* 2012a) and one study a null association (Midodzi *et al.* 2010) between caesarean section and the risk of asthma. The largest of these most recent studies, a register-based Norwegian cohort study (Tollånes *et al.* 2008), reported a 52% increased risk of asthma by the age of 18 in children delivered by caesarean section. This study assessed also emergency and planned caesarean section separately, and both section types were associated with an increased risk of asthma, although the strength of the association was slightly stronger for emergency caesarean. Studies by Smith *et al.* (2004) and Almqvist *et al.* (2012a) observed similar associations for both emergency and planned caesarean section. In addition, some studies have reported an increased risk of asthma in children born by assisted vaginal delivery compared with children born by normal spontaneous vaginal delivery (Xu *et al.* 2000, Tollånes *et al.* 2008, Keski-Nisula *et al.* 2009, Hancox *et al.* 2013), whereas also null associations with assisted vaginal delivery have been reported (McKeever *et al.* 2002a, Bernsen *et al.* 2005).

Twin and other multiple pregnancies

There is currently no evidence related to multiple pregnancy and the development of CMA or other food allergies. Studies on asthma have reported a 15–53% de-

creased risk among twins compared with singletons (Strachan *et al.* 2000, McKeever *et al.* 2001, Dik *et al.* 2004) (Table 2).

Complications during pregnancy

All five cohort studies exploring the association between the presence of various complications and the risk of asthma in the offspring have reported increased risks (Nafstad *et al.* 2000, Annesi-Maesano *et al.* 2001, McKeever *et al.* 2002b, Nafstad *et al.* 2003, Dik *et al.* 2004) (Table 2). There was, however, some variation in the strength of the associations between different complications: ORs varied from 1.43 for hemorrhage to 2.33 for preterm contractions (Nafstad *et al.* 2003). Further, only weak or no association was observed with maternal hypertension or preeclampsia (McKeever *et al.* 2002b, Nafstad *et al.* 2003).

Gestational age and children's anthropometric measures at birth

Evidence on fetal growth and the risk of CMA and other food allergies is limited. Low birth weight has been associated with a decreased risk of food allergy (Hikino *et al.* 2001, Koplin *et al.* 2012), but also no association between low birth weight and/or gestational age has been reported (Hikino *et al.* 2001, Liem *et al.* 2007) (Table 1). Liem and co-workers (2007) assessed also the role of high birth weight on food allergy, but they failed to find an association. On the contrary, several studies have explored associations between gestational age and birth weight and the risk of asthma, with inconsistent findings (Table 1). Nine cohort studies have reported short gestational age or prematurity to be a risk factor for the subsequent development of asthma (McKeever *et al.* 2002a, Yuan *et al.* 2003, Dik *et al.* 2004, Jaakkola and Gissler 2004, Bernsen *et al.* 2005, Gessner and Chimonas 2007, Dombkowski *et al.* 2008, Örtqvist *et al.* 2009, Vogt *et al.* 2011), but no such association was found in four other studies (Annesi-Maesano *et al.* 2001, Yuan *et al.* 2002, Taveras *et al.* 2006, Abe *et al.* 2010) (Table 2). Further, studies by Davidson *et al.* (2010) and Bråbäck *et al.* (2003) found children's age-dependent associations with low gestational age: an increased risk of asthma was observed in children diagnosed with asthma at early ages, whereas no association was observed in children diagnosed at older ages. Previous studies on birth weight and the risk of asthma are even more contradictory than those with gestational age. Ten studies have reported low birth weight to be associated with an increased risk of asthma (Annesi-Maesano *et al.* 2001, McKeever *et al.* 2002a, Dik *et al.* 2004, Jaakkola and Gissler 2004, Juhn *et al.* 2005, Nepomnyaschy and Reichman 2006, Örtqvist *et al.* 2009, Kindlund *et al.* 2010, Midodzi *et al.* 2010, To *et al.* 2012), but five studies failed to find an association (Yuan *et al.* 2002, Yuan *et al.* 2003, Taveras *et al.* 2006, Caudri *et al.* 2007, Kiechl-Kohlendorfer *et al.* 2007). Similar to gestational age, Davidson and co-workers (2010) observed an increased risk with low birth weight only in children diagnosed during the first two years of life,

but not among those diagnosed later. High birth weight has been associated with both an increased risk (Brooks *et al.* 2001, Sin *et al.* 2004, Remes *et al.* 2008) and a decreased risk (To *et al.* 2012) of asthma, but also null associations have been reported (Yuan *et al.* 2002, Yuan *et al.* 2003, Bernsen *et al.* 2005, Taveras *et al.* 2006).

Table 1. Associations between maternal background and perinatal factors and the risk of cow's milk allergy and other food allergies in childhood and adolescence. Summary of results from observational studies

Reference, country	Study design	Year(s) of birth	Subjects *n*	Out-come	Age[1]	Data source[2]	High maternal age	Maternal smoking during pregnancy	High maternal/ household SES/ income	High birth order/ siblings	Caesarean section	Compli-cations during pregnancy	Low gestational age	Low birth weight	High birth weight
Kvenshagen *et al.* 2009, NO	cohort	nr	512	CMA	0–2	E/Q					ns				
Eggesbo *et al.* 2005, NO	cohort	1992–1993	2,656	CMA	2.5	E/R					ns				
Saarinen and Savilahti 2000, FI	cohort	1994–1995	6,209	CMA	0–1	E/Q		ns		ns					
Kusunoki *et al.* 2012, JP	cross-sectional	1991–1997[3]	14,669	FA	7–15	Q/Q				↓					
Koplin *et al.* 2012, AU	cross-sectional	2006–2010[3]	5,276	EA	1	E/Q				↓	ns			↓	
Gupta *et al.* 2011, US	cross-sectional	1991–2010[3]	38,480	FA	0–18	Q/Q			↑						
Victorino and Gauthier 2009, US	cross-sectional	1986–2004[3]	102,353	FA	0–17	Q/Q			ns						
Liem *et al.* 2007, CA	cohort	1995	13,980	FA	6–7	R/R			↑		ns		ns	ns	ns
Salam *et al.* 2006, US	cohort	1993–1996	3,464	FA	8–17	Q/R					ns				
Renz-Polster *et al.* 2005, US	cross-sectional	1990-1992	7,872	FA	3–10	R/R					ns				
Eggesbo *et al.* 2003, NO	cohort	1992–1993	2,803	EA	2.5	E/R				ns	ns				
Dioun *et al.* 2003, US	case-control	nr	58/96[4]	FA	0–11	E/R	↑			↓					
Hikino *et al.* 2001, JP	cross-sectional	nr	21,766	FA	1.5	Q/Q							ns	↓	

Abbreviations: NO= Norway, FI= Finland, JP= Japan, AU= Australia, US= USA, CA= Canada, CMA= cow's milk allergy, FA=food allergy, EA= egg allergy, PA= peanut allergy, SES= socioeconomic status, E= medical examination, Q = questionnaire, R = register or medical record ↑ = associated with increased risk, ↓= associated with decreased risk, nr = not reported, ns = non-significant association

[1] Age (years) at outcome assessment

[2] Outcome/exposure

[3] Years of birth estimated from reported study years and participants ages

[4] Cases/controls

Table 2. Associations between maternal background and perinatal factors and the risk of asthma in childhood and adolescence. Summary of results from large cohort studies published since 2000

Reference, country	Year(s) of birth	Subjects *n*	Age[1]	Data sources[2]	High maternal age	Maternal smoking during pregnancy	High maternal/ household SES/ income	High birth order/ siblings	Caesarean section	Assisted vaginal birth	Twin pregnancy	Complications during pregnancy	Short gestational age	Low birth weight	High birth weight
Hancox *et al.* 2013, NZ	1972–1973	1,037	13	Q/R						↑					
Almqvist *et al.* 2012a, SE	1993–1999	17,5110	9–12	R/R					↑						
To *et al.* 2012, CA	1995–2001	68,7194	6	R/R										↑	↓
Magnus *et al.* 2011, NO	1999–2008	37,171	3	Q/R					↑						
Vogt *et al.* 2011, SE	1987–2000	1,100,826	6–9	R/R									↑		
Midodzi *et al.* 2010, CA	1996–2003	8,499	2–5	Q/Q	ns	↑	↓	↓	ns					↑	
Kindlund *et al.* 2010, DK	1994–2000	9,908	3–9	Q/R										↑	
Abe *et al.* 2010, US	1981–1994	6,187	0–7	Q/R									ns		
Davidson *et al.* 2010, UK	1970–1999	24,8612	0–1 ≥2	R/R		↑ ns	ns ns	↑ ns					↑ ns	↑ ns	
Örtqvist *et al.* 2009, SE	1992–1998	10,918	9 or 12	Q/R									↑	↑	
Roduit *et al.* 2009, NL	1996–2004	2,917	8	Q/Q					↑						
Keski-Nisula *et al.* 2009, FI	1985–2002	5,823	15–16	Q/R						↑					
Tollånes *et al.* 2008, NO	1967–2002	1,576,700	0–18	R/R					↑	↑					
Dombkowski *et al.* 2008, US	1983–1998[3]	150,204	5–18	R/R									↑		
Remes *et al.* 2008, FI	1985–2002	5,500	0–16	Q/nr											↑

Table 2 *(Continued)*

Reference, country	Year(s) of birth	Subjects *n*	Age[1]	Data sources[2]	High maternal age	Maternal smoking during pregnancy	High maternal/ household SES/ income	High birth order/ siblings	Caesarean section	Assisted vaginal birth	Twin pregnancy	Compli-cations during pregnancy	Short gestational age	Low birth weight	High birth weight
Gessner and Chimonas 2007, US	1999–2002	37,349	0–10	R/R									↑		
Werner *et al.* 2007, DK	1984–2005	7,119	15–18	Q/R					ns						
Kiechl-Kohlendorfer *et al.* 2007, AT	1994–2005	33,808	6–10	R/Q	ns	↑		ns						ns	
Caudri *et al.* 2007, NL	1996–1997	3,628	0–7	Q/R										ns	
Taveras *et al.* 2006, US	1999–2002	1,372	0–2	Q/Q				↑					ns	ns	ns
Salam *et al.* 2006, US	1993–1996	3,464	8–17	Q/R					ns <3y[4] ↑ >3y						
Nepomnyaschy and Reichman 2006, US	1998–2000	1,803	0–3	Q/R										↑	
Almqvist *et al.* 2005, SE	1994–2000	4,089	4	Q/Q			↓								
Renz-Polster *et al.* 2005, US	1990–1992	7,872	6–10	R/R					↑						
Bernsen *et al.* 2005, NL	1988–1990	1,727	6	R/R					ns	ns			↑		ns
Juhn *et al.* 2005, US	1976–1982	7,106	0–7	R/R	↑		↓		ns					↑	
Dik *et al.* 2004, CA	1980–1990	170,960	0–6	R/R			ns	↓			↓	↑	↑	↑	
Maitra *et al.* 2004, UK	1991–1992	7,495	6–7	Q/R					ns						
Smith *et al* 2004, UK	1992–1995	173,319	0–7	R/R					↑						

Table 2 *(Continued)*

Reference, country	Year(s) of birth	Subjects *n*	Age[1]	Data sources[2]	High maternal age	Maternal smoking during pregnancy	High maternal/ household SES/ income	High birth order/ siblings	Caesarean section	Assisted vaginal birth	Twin pregnancy	Complications during pregnancy	Short gestational age	Low birth weight	High birth weight
Hancox *et al.* 2004, NZ	1972–1973	1,033	0–15	Q/Q			ns								
Sin *et al.* 2004, CA	1985–1988	83,595	0–10	R/R			↓								↑
Jaakkola and Gissler 2004, FI	1987	58,841	0–7	R/R		↑							↑	↑	
Bråbäck *et al.* 2003, SE	1987–1995	214,276	<2 2–3 ≥4	R/R	↓ ns ns	↑ ↑ ns		↑ ↓ ns					↑ ↑ ns		
Yuan *et al.* 2003, DK	1996–1997	9,705	0–1	R/R	↓	↑		↑					↑	ns	ns
Nafstad *et al.* 2003, NO	1967–1993	1,548,429	0–7	R/R								↑			
Benn *et al.* 2002, DK	1992–1994	3,003	4–5	R/R					ns						
Kero *et al.* 2002, FI	1987	59,927	0–7	R/R					↑						
Yuan *et al.* 2002, DK	1984–1987	10,440	0–12	R/R									ns	ns	ns
McKeever *et al.* 2001, UK	1988–1999	24,690	0–11	R/R	↓	↑		↑			↓				
McKeever *et al.* 2002a, UK	1988–1999	24,690	0–11	R/R					ns	ns			↑	↑	
McKeever *et al.* 2002b, UK	1988–1999	24,690	0–11	R/R								↑			
Brooks *et al.* 2001, US	1988	8,071	3	Q/Q											↑
Annesi-Maesano *et al.* 2001, UK	1973–1990	4,065	0–18	Q/Q					ns			↑	ns	↑	

Table 2 *(Continued)*

Reference, country	Year(s) of birth	Subjects *n*	Age[1]	Data sources[2]	High maternal age	Maternal smoking during pregnancy	High maternal/ household SES/ income	High birth order/ siblings	Caesarean section	Assisted vaginal birth	Twin pregnancy	Compli-cations during pregnancy	Short gestational age	Low birth weight	High birth weight
Nafstad *et al.* 2000, NO	1992–1993	2,531	0–4	Q/Q	↓	ns	↓	↓				↑			
Ball *et al.* 2000, US	1980–1984	1,035	6–13	Q/Q				↓							
Xu *et al.* 2000, FI	1985–1986	8,088	7	Q/R					↑	↑					
Strachan *et al.* 2000, UK	1981–1984	262,939	0–10	R/R							↓				

Abbreviations: NZ= New Zeeland, SE= Sweden, CA= Canada, NO= Norway, US= USA, UK= United Kingdom, NL= The Netherlands, FI= Finland, DK= Denmark, AT= Austria, SES= socioeconomic status, Q = questionnaire, R = register or medical record ↑ = associated with increased risk, ↓= associated with decreased risk, nr = not reported, ns = non-significant association

[1] Age (years) at outcome assessment

[2] Outcome/exposure

[3] Years of birth estimated from reported study years and participants ages

[4] Age (years) at diagnosis

2.4.3 Use of antibiotics

Antibiotics are natural or synthetic drugs which inhibit microbial growth or kill microbes such as bacteria. In Finland, 2.5 million courses of antibiotics are prescribed annually in the primary healthcare system, and the majority (80%) of these courses are prescribed for respiratory tract infections (Rautakorpi *et al.* 2001). According to a survey assessing current treatment practices for common infections in Finnish primary care, the most common (84%) infections among children less than five years of age were respiratory tract infections with otitis media and upper respiratory tract infections comprising the majority (70%) of these infections (Rautakorpi *et al.* 2001). The corresponding figure for these two respiratory infections among children aged 5–14 years was 52%. The most frequently used antibiotic for otitis media infections was amoxicillin (53% of antibiotics prescribed for otitis media), followed by macrolides (16%) and sulphatrimetoprim (16%). For upper respiratory tract infections the most frequently used antibiotics were macrolides (28%) and amoxicillin (27%). The most commonly prescribed antibiotics were different in adults and in children due to different infection profiles. In children, the most common antibiotic agent was amoxicillin (45% of all antibiotic prescriptions).

Increasing bacterial resistance to antibiotics is a major public health threat worldwide (Hawkey 2008) and a significant contributing factor to this problem is the frequent use of antibiotics. In addition, the adverse effect of antibiotics on human microbiota has received increasing attention as an important threat to individual's short- and long-term health. The association between children's exposure to antibiotics during early childhood and the development of asthma and other allergic diseases such as eczema and hay fever has been studied extensively, whereas food allergies and prenatal exposure to antibiotics has received less attention. The results of the studies exploring the association between exposure to antibiotics and the risk of asthma or wheeze in childhood or adolescence are summarized in Tables 3 and 4.

Maternal use of antibiotics

No studies assessing the association between maternal use of specific or any antibiotics during pregnancy and the risk of CMA have been published previously. A Norwegian cohort study of 2803 children reported no association between the maternal use of antibiotics and the risk of egg allergy in the offspring (OR 1.1, 95% CI 0.3–4.9) (Eggesbo *et al.* 2003).

Maternal use of antibiotics during pregnancy and the risk of asthma or wheeze in the offspring has been explored in nine studies (Table 3). Six studies had a cohort design, one study was a nested case-control study, one a case-control study and one a cross-sectional study. The majority of the studies were conducted in

Europe, and the size of the study population varied from 102 to 571,277 subjects. Information on outcome and exposure was obtained solely from registers or medical records (four studies), solely from questionnaires (four studies) or from both questionnaires and records (one study). The outcome was defined based on diagnosed asthma, antiasthmatic drug use or wheezing symptoms.

An increased risk of asthma or wheeze in the offspring who were exposed to antibiotics prenatally was observed in five studies (Benn *et al.* 2002, McKeever *et al.* 2002b, Jedrychowski *et al.* 2006, Martel *et al.* 2009, Källén *et al.* 2013), but three studies found no association (Calvani *et al.* 2004, Rusconi *et al.* 2007, Dom *et al.* 2010). The largest cohort study reported a 10% increased risk of asthma in children whose mothers were non-asthmatic and had used antibiotics during pregnancy (Källén *et al.* 2013). McKeever and co-workers (2002b) found only minor variation between the strength of the associations with different specific antibiotics, while in the study by Stensballe *et al.* (2013) the strongest association was observed for macrolides. In addition, Stensballe and co-workers (2013) observed that maternal antibiotics for both non-respiratory infections and other indications were associated with an increased risk of asthma.

Children's use of antibiotics

Evidence on the role of children's use of antibiotics in the development of CMA or food allergies is limited. Eggesbo and co-workers (2003) assessed in a cohort of 2803 children whether exposure to antibiotics during the first six months of life was associated with subsequent development of egg allergy among children followed from birth to three years of age, but they failed to find an association (OR 1.5, 95% CI 0.6–3.7). However, children's use of antibiotics and the subsequent development of asthma or wheeze has been explored more extensively, and 22 cohort/nested case-control studies have been published thus far. The majority of the studies were conducted in Europe and North America. The size of the study population varied from 154 to 251,817 subjects and the age from 0 to 10 years. Over half of the studies obtained information on outcome and exposure solely from questionnaires (13 studies). Six studies were register- or record-based, and one study used both questionnaires and records. The outcome was defined based on diagnosed asthma, antiasthmatic drug use, asthma hospitalization, asthma or wheezing symptoms, or various combinations of these. The most commonly used exposure period was the first year of life, but also different periods from the first six months to the first three years were used.

A systematic review and meta-analysis of studies on antibiotics and asthma published by September 2004 concluded that exposure to antibiotics during the first year of life was associated with an increased risk of asthma in childhood, but the association was significantly stronger in retrospective studies than in prospective studies (Marra *et al.* 2006). Since this meta-analysis, several cohort studies on

asthma or wheeze have been published, but the results have been inconsistent (Table 4). Recently, two systematic reviews, including cohort studies published before year 2011, concluded that exposure to antibiotics was associated with a weak increase in risk of asthma or wheeze (Murk *et al.* 2011, Penders *et al.* 2011). The largest studies assessing the dose-response effect of antibiotics reported a 30% to 99% increased risk of asthma in children exposed to antibiotics five, six or more times during early childhood (McKeever *et al.* 2002c, Kozyrskyj *et al.* 2007, Marra *et al.* 2009, Almqvist *et al.* 2012b). Age-dependent associations have been observed in some studies: the association between exposure to antibiotics and the risk of asthma was found to be stronger in children diagnosed at early ages than in later childhood (McKeever *et al.* 2002c, Marra *et al.* 2009). Further, an increased risk of asthma has been observed in children diagnosed at early ages, but not in children diagnosed at older ages (Celedon *et al.* 2004, Mai *et al.* 2010). The child's use of macrolides, cephalosporins and amoxicillin (McKeever *et al.* 2002c, Marra *et al.* 2009) as well as penicillins (Marra *et al.* 2009) and broad-spectrum antibiotics (Kozyrskyj *et al.* 2007, Wickens *et al.* 2008, Almqvist *et al.* 2012) has been associated with an increased risk of asthma, although one study reported that no particular specific antibiotic was associated with the risk of asthma (Celedon *et al.* 2004).

Table 3. Association between maternal antibiotic use (ever exposed) during pregnancy and the risk of asthma or wheeze in the offspring in childhood or adolescence. Summary of results from observational studies

Reference, country	Study design	Year(s) of birth	Subjects *n*	Outcome	Age[1]	Data sources[2]	Results		Adjusted for
							Crude OR (95% CI)	Adjusted OR (95% CI)	
Stensballe *et al.* 2013, DK	cohort	1997–2003	30,675	asthma drug use	0–5	R/R	1.28[3] (1.19–1.37)	1.18[3] (1.10–1.27)	sex, maternal age, allergies, smoking, alcohol, paracetamol use, exercise, parental education, asthma, population density,pets, child's birth weight, gestational age, mode of delivery, breasetfeeding, day care, siblings
Källén *et al.* 2013, SE	cohort	1999–2007	571,277	asthma drug use	2–10	R/R		1.10 (1.02–1.19)	Maternal age, parity, smoking, body mass index, child's year of birth
Dom *et al.* 2010, BE	cohort	1997–2002	773	wheeze	0–4	Q/Q	1.27 (0.86–1.88)	1.29 (0.80–2.09)	sex, maternal age, siblings, smoking, education, day care, pets, allergies, child's antibiotic use
Martel *et al.* 2009, CA	nested case-control	1990–2002	5,226/ 104,520[4]	asthma diagnosis	0–10	R/R	1.25[3] (1.22–1.29)	1.09[3] (1.06–1.12)	sex, maternal age, education, area of residence, mode of delivery, complications, child's antibiotic use, small for gestational age -status
Rusconi *et al.* 2007, IT	cross-sectional	1995–1996	15,609	late-onset wheeze	6–7	Q/Q	nr	1.13 (0.77–1.65)	sex, maternal age, siblings, smoking, parental allergies, low birth weight, study center, socioeconomic status
Jedrychowski *et al.* 2006, PO	cohort	2000–2003	102	wheeze	1	Q/Q	nr	4.42 (1.05–18.5)	sex, maternal education
Calvani *et al.* 2004, IT	case-control	nr	338/467[4]	asthma diagnosis or drug use	>3	Q/Q	1.23 (0.76–1.99)	1.65 (0.92–2.93)	sex, maternal asthma, smoking, allergies
Benn *et al.* 2002, DK	cohort	1992–1994	3,003	asthma drug use	4–5	R/Q	1.7 (1.1–2.6)	1.7 (1.1–2.6)	maternal age, education, allergies prematurity
Mckeever *et al.* 2002b, UK	cohort	1988–1999	24,690	asthma diagnosis	0–9	R/R	2.14[5] (1.85–2.47)	1.36[5] (1.16–1.60)	doctor visits, maternal allergy, child's antibiotic use

Abbreviations: SE= Sweden, BE= Belgium, CA= Canada, IT= Italy, PO=Poland, DK= Denmark, UK= United Kingdom, Q = questionnaire, R = register or medical record, nr = not reported

[1] Age (years) at outcome assessment

[2] Outcome/exposure

[3] Odds ratio per additional antibiotic prescription

[4] Cases/controls

[5] Hazard ratio for 3 or more antibiotic prescriptions

Table 4. Associations between exposure to antibiotics during early childhood and the risk of asthma or wheeze in childhood or adolecence. Summary of results from cohort and nested case-control studies

Reference and country	Year(s) of birth	Subjects *n*	Outcome	Age[1]	Data sources[2]	Exposure period	Results I: ever vs. never exposed		Results II: highest exposure category vs. never exposed		Adjusted for
							Crude OR (95% CI)	AdjustedOR (95% CI)	Crude OR (95% CI)	AdjustedOR (95% CI)	
Goksör *et al.* 2013, SE	2003	4,051	Asthma diagnosis + symptoms/ drug use	8	Q/Q	neonatal ward	1.9 (1.2–3.2)	2.3 (1.2–4.2)			Maternal smoking, medication during pregnancy, parental allergy, parental education, paternal employment, rural living, prematurity, caesarean section, small for gestational age, child's sex, diet, allergies
Almqvist *et al.* 2012b, SE	2005–2009	211,192	Asthma drug use	0–4	R/R	birth-outcome	1.15 (0.73–1.83)[3]		1.77 (1.10–2.86)[4]		
Jedrychowski *et al.* 2011, PO	2001–2004	261	Asthma diagnosis	4–5	Q/Q	4–5 years	2.14 (1.37–3.34)	1.65 (0.93–2.93)			maternal age, education, atopy, parity, child's sex, exposure to smoking, respiratory infections
Risnes *et al.* 2011, US	1997–2000	1,401	Asthma diagnosis + symptoms	6	Q/Q	0–6 months	1.81 (1.31–2.53)	1.52 (1.07–2.16)		1.72 (1.11–2.65)[5]	parental asthma, income, child's respiratory infections
Dom *et al.* 2010, BE	1997–2001	773	Recurrent wheeze	0–4	Q/Q	first year	1.50 (0.90–2.49)	0.91 (0.51–1.63)			maternal age, smoking, parental education, allergies, antibiotic use, child's sex day care, pet ownership, siblings
Mai *et al.* 2010, SE	1994–1996	3,306	Asthma symptoms + drug use	8	Q/Q	first year	1.7 (1.3–2.2)	1.4 (1.1–1.7)	2.4 (1.7–3.2)[5]	1.9 (1.2–3.0)[5]	maternal age, smoking, allergies, breastfeeding, child's sex, respiratory infections
Su *et al.* 2010, US	1997–2003	424	Asthma diagnosis + symptoms	0–5	Q/I	0–9 months		1.2 (0.6–2.3)		1.1 (0.7–1.9)[6]	child's illness visits
Martel *et al.* 2009, CA	1990–2002	5,226/ 104,520	Asthma diagnosis + drug use	0–10	R/R	birth–diagnosis or 0–6 months			1.83 (1.73–1.94)[7]	1.59 (1.50–1.68)[7]	sex, small for gestational age birth, child's allergy, maternal age, education, area of residence, mode of delivery, complications, use of antibiotics during pregnancy
Marra *et al.* 2009, CA	1997–2003	251,817	Asthma diagnosis or drug use	2–9	R/R	first year		1.12 (1.08–1.16)		1.30 (1.20–1.41)[8]	SES, urban/rural residence, birth by caesarean section, gestational age, child's sex, birth weight, doctor visits, respiratory infections
Kusel *et al.* 2008, AU	1996–1998	198	Asthma diagnosis	4–5	Q/Q	first year	2.3 (1.2–4.5)	1.5 (0.7–3.2)			antibiotic predictor score, child's sex, pet ownership, day care, doctor visits

Table 4 *(Continued)*

Reference and country	Year(s) of birth	Subjects *n*	Outcome	Age[1]	Data source[2]	Exposure period	Results I: ever vs. never exposed		Results II: highest exposure category vs. never exposed		Adjusted for
							Crude OR (95% CI)	Adjusted OR (95% CI)	Crude OR (95% CI)	Adjusted OR (95% CI)	
Wickens *et al.* 2008, NZ	1997–2001	967	Asthma diagnosis + symptoms/ drug use	4	Q/Q	0–15 months	1.35 (0.85–2.14)	0.78 (0.46–1.32)			parental allergies, mother's parity, child's sex, ethnicy, infections, antibiotics used at age 15 moths–4 yrs
Alm *et al.* 2008, SE	2003	4,921	Wheeze	1	Q/Q	neonatal ward	1.8 (1.3–2.4)	1.6 (1.0–2.5)			parental asthma, maternal smoking, breastfeeding, child's sex, prematurity, pacifier use, sleep
Verhulst *et al.* 2008, BE	nr	154	Wheeze	0–1	Q/Q	first year		2.94 (1.59–5.43)			breastfeeding, chid's sex, gut bacteria
Harris *et al.* 2007, UK	1993	642	Wheeze	8	Q/R	first year			1.08 (0.98–1.19)[7]		parental atopy,child's birth order, exposure to tobacco smoke
Kozyrskyj *et al.* 2007, CA	1995	13,116	Asthma diagnosis or hospitalization or drug use	7	R/R	first year			1.74 (1.37–2.22)[8]	1.46 (1.14–1.88)[8]	maternal asthma, urban/rural residence, child's sex, siblings, doctor visits, infections
Kummeling *et al.* 2007, NL	2000–nr	2,764	Wheeze	2	Q/Q	0–6 months	3.14 (2.36–4.18)	2.65 (1.95–3.60)			parental allergy, breast feeding, child's sex, birth by caesarean section, siblings, day care, pet ownership, exposure to smoking, vaccination, fever, subcohort
Thomas *et al.* 2006, UK	1996–1998[9]	74/74	Wheeze+ sensitization	3–5	Q/R	first year	1.32 (0.99–1.78)[10]				
Celedon *et al.* 2004, US	1992–1993	4,408	Asthma hospitalization or drug use	2–5	R/R	first year			1.1 (0.8–1.5)[8]	1.0 (0.7–1.4)[8]	child's sex, respiratory infections, doctor visits
Mckeever *et al.* 2002c, UK	1988–1999	29,238	Asthma diagnosis	1–9	R/R	first year			3.13 (2.75–3.57)[8,11]	1.99 (1.72–2.31)[8,11]	parental atopy, smoking, child's sex doctor visits, older siblings
Celedon *et al.* 2002, US	1994–1996	498	Asthma diagnosis + symptoms	5	Q/Q	first year			0.8 (0.4–1.7)[5]	0.9 (0.4–1.8)[5]	maternal asthma, income, child's sex
Illi *et al.* 2001, DK	1990	1,314	Asthma diagnosis	7	Q/Q	0–3 years				1.08 (0.59–1.99)[5]	maternal education, smoking, parental allergies
Ponsonby *et al.* 1999, AU	1988	863	Asthma ever	7	Q/Q	0–35 days	1.10 (0.80–1.36)	1.04 (0.78–1.37)			parental asthma, breastfeeding, exposure to smoking, child's age, low birth weight, prematurity, gas heater at home, respiratory infections

Abbreviations: OR= odds ratio, CI= confidence interval, SE= Sweden, PO= Poland, US= United States, BE= Belgium, CA= Canada, AU= Australia, NZ= New Zeeland, UK= United Kingdom, US= USA, DK= Denmark, R= register or medical record, Q= questionnaire, I= interview nr= not reported

[1] Age (years) at outcome assessment
[2] Outcome/exposure
[3] Hazard ratio for time varying exposure to antibiotics and asthma drug use (reference is time before first exposure)
[4] Hazard ratio for time varying exposure to antibiotics 6 or more courses and asthma drug use (reference is time before first exposure)
[5] 2 or more courses
[6] Odds ratio (95% CI) per additional antibiotic prescription
[7] Hazard ratio (95% CI) per additional antibiotic prescription
[8] 5 or more courses
[9] Estimated from prenatal recruitment years
[10] Ratio of antibiotic receipt in cases over controls (95%CI)
[11] Hazard Ratio (95%CI)

3 Aims of the study

The aim of this study was to assess whether maternal and perinatal factors and the use of antibiotics are associated with the development of CMA and asthma in childhood. More precisely, the study aimed to answer the following questions:

- Are maternal background factors, including maternal age, socioeconomic status, smoking during pregnancy and pregnancy history, associated with the risk of CMA or asthma in the offspring?

- Are perinatal factors, including mode of delivery, complications during pregnancy and anthropometric measures of the child at birth, associated with the risk of CMA or asthma in childhood?

- Is the use of antibiotics by the mother before and during pregnancy associated with the risk of CMA or asthma in the offspring?

- Is the use of antibiotics by the child during early childhood associated with the risk of CMA or asthma

In addition, this study aimed to examine the determinants of use of antibiotics: i.e. are maternal background factors and/or perinatal factors associated with maternal and child use of antibiotics?

4 Subjects and methods

4.1 Data sources

Data for the present study were obtained from four national registers: the Special Reimbursement Register, the Population Register and the Drug Prescription Register maintained by the Social Insurance Institution and the Medical Birth Register maintained by the National Institute for Health and Welfare (Table 5). All the registers include the mothers' and children's unique personal identity codes, which are assigned to all Finnish citizens shortly after birth. Linkages between the registers were based on these identity codes.

Table 5. Data sources: name of the register, maintaining institution and information obtained from the register

Register	Maintainer	Information on
the Special Reimbursement Register	the Social Insurance Institution	start and end dates of granted special reimbursements, disease code (based on diagnosed cow's milk allergy or asthma), personal identity code
the Population Register	the Social Insurance Institution	date of birth, gender, hospital district were the child was born and personal identity code
the Drug Prescripti-on Register	the Social Insurance Institution	date of dispense of antiasthmatic drugs, special infant formulas and antibiotics, ATC code, personal identity code
the Medical Birth Register	National Institute for Health and Welfare	maternal bakcground and perinatal factors, personal identity code

Abbreviations: ATC= Anatomical Therapeutic Chemical

The Special Reimbursement Register and the Population Register were used to respectively select cases and controls. In Finland, patients with a certificate from a physician stating that the diagnostic criteria of certain diseases (i.e. asthma) are fulfilled are entitled to special reimbursement of the drug costs. In the case of CMA, the special reimbursement is for the cost of special infant formulas, and the special reimbursement is available for children up to two years of age. In the case of asthma, the special reimbursement is available for children of any age.

The Drug Prescription Register was established in 1993 and was almost complete in 1995. This register includes information on all drugs and special infant

formulas prescribed by physicians, reimbursed by the National Sickness Insurance Scheme and dispensed from the pharmacists. The Drug Prescription Register includes information on drug class (Anatomical Therapeutic Chemical [ATC] Classification System) and dispensing date of the prescription. Information on purchased antibiotics, antiasthmatic drugs and special infant formulas was obtained from the Drug Prescription Register. In Finland, outpatient antibiotics are available by prescription only. The Finnish Medical Birth Register includes information on mother's sociodemographic background, maternal health care and interventions during pregnancy and delivery, and the newborn's medical interventions and outcome up to the age of seven days.

The study was approved by the national data protection authority, the institutions keeping the registers and the Institutional Review Board of the National Public Health Institute (National Institute for Health and Welfare since 2009).

4.2 Study design and population

This study was a nested case-control study. The study population was selected from a birth cohort consisting of all children born in Finland between 1 January 1996 and 30 April 2004. From this initial birth cohort, all children with a diagnosed CMA or asthma were identified. For each case, one control child without a diagnosed index disease and matched to the case by age (±28 days), gender and hospital district in which the child was born was randomly selected at each time when the case occurred. Controls were allowed to be selected only once and twins from the same family as case-control pairs were not allowed. The Medical Birth Register data were not available for the children who were not born in Finland, and these children were excluded from the study population.

4.2.1 Cow's milk allergy population

All children who belonged to the initial birth cohort and had received a special reimbursement for the costs of special infant formulas (soy-based, extensively hydrolysed or elemental infant formulas) prescribed for the management of their diagnosed CMA by the end of November 2005 were identified (n=19,111) (Figure 1). The Social Insurance Institution's criteria for the special reimbursement for CMA included a clinical examination with a careful history, symptoms suggestive of CMA and disappearance of the symptoms when cow’s milk was eliminated from the diet. Further, either a positive skin-prick test, an elevated serum specific IgE or an open challenge test performed in the hospital’s in- or out-patient unit were required. In Finland, most cases had a final confirmation of the diagnosis based on an open challenge test (Kaila *et al.* 2000). For a strict definition of CMA case for the present study, infants with a special reimbursement of short duration (< 6 months) or none or only occasional purchases (maximum two purchases) of

special infant formulas or both were excluded. Finally, 16,237 case-control pairs were included in the CMA population.

4.2.2 Asthma population

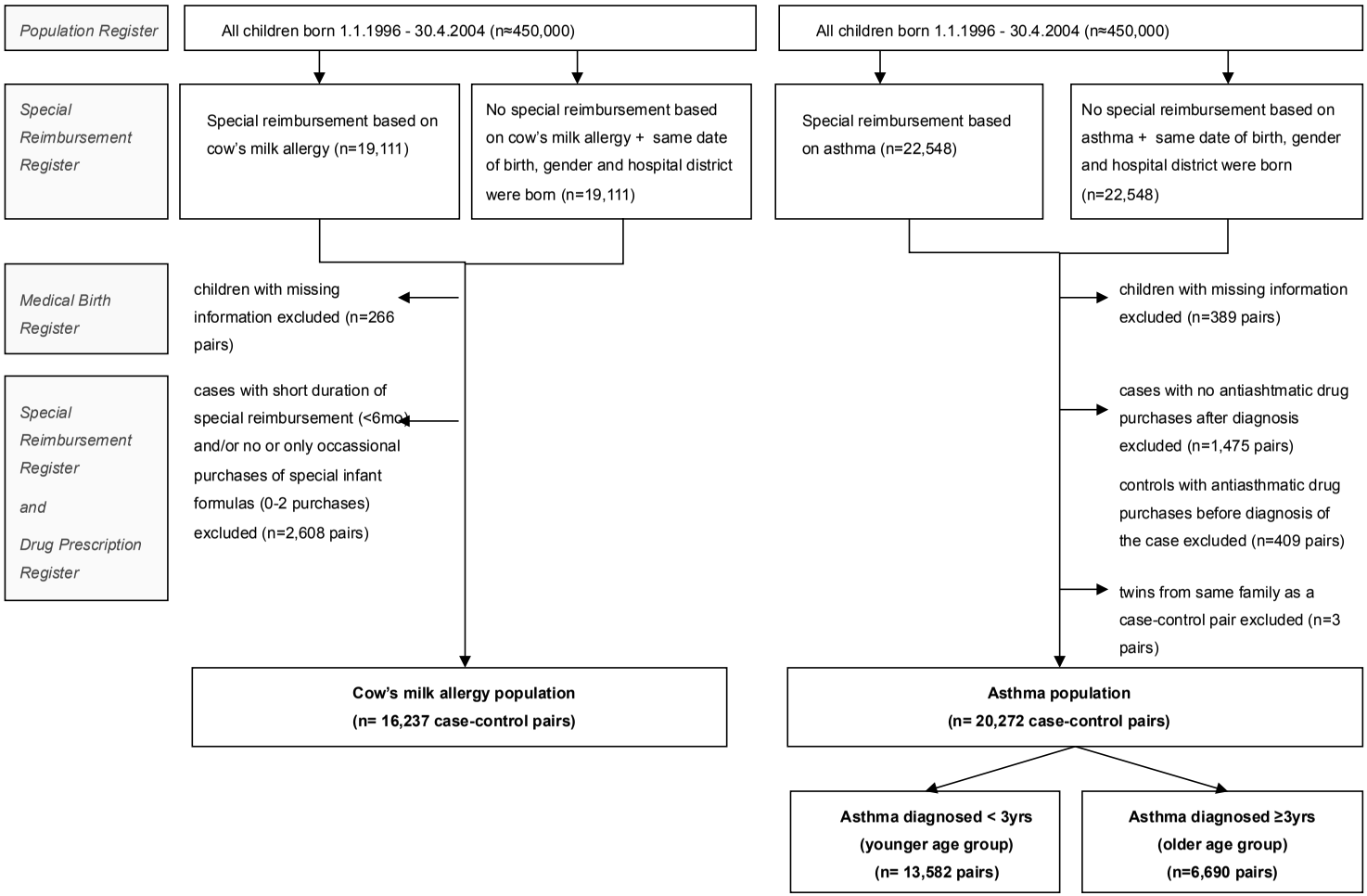
All children who belonged to the initial birth cohort and had received a special reimbursement for the cost of antiasthmatic drugs by the end of 2005 were identified (n=22,548) (Figure 1). The Social Insurance Institution's criteria for the special reimbursement for asthma included a diagnosis made according to explicit predefined diagnostic criteria and a plan of a long-lasting (at least six months) drug therapy. For a strict definition of an asthma case for the present study, children with no antiasthmatic drug purchases after the diagnosis were excluded. Selection of the antiasthmatic drugs was based on the Finnish Current Care guidelines of asthma and the recommendations by the Social Insurance Institution. In addition, those control children who had purchased antiasthmatic drugs from birth until the date of asthma diagnosis of the case without special reimbursement were excluded. Finally, 20,272 case-control pairs were included in the asthma population. As asthma is challenging to diagnose in young children, the asthma population was further divided into children diagnosed before the age of three years (n= 13,582 case-control pairs, the younger age group) and children diagnosed between the age of three and nine years (n= 6,690 case-control pairs, the older age group). The age division was based on national diagnostic criteria (Asthma: Current Care Summary. 2012) and international observations of different wheezing phenotypes (Martinez *et al.* 1995).

4.3 Data collection

4.3.1 Maternal background and perinatal factors

The maternal sociodemographic background variables extracted from the Medical Birth Register were age at delivery, smoking during pregnancy and socioeconomic status. The variables related to maternal pregnancy history and index pregnancy were previous miscarriages, previous deliveries and complications during pregnancy as well as mode of delivery and gestational age. Child-related variables were birth weight, birth length, ponderal index (birth weight/birth length3) and Apgar score at one minute after birth. In addition, the variables gestational age adjusted birth weight, prematurity and low birth weight were calculated. Information on maternal asthma – based on granted special reimbursement for the costs of antiasthmatic drugs – was obtained from the Special Reimbursement Register of the Social Insurance Institution. Detailed information on maternal background and the perinatal factors used in the present study is presented in the Appendix 1.

Figure 1. Study population and data sources: formation fo cow´s milk allergy and asthma populations (case-control pairs)



4.3.2 Antibiotics

To evaluate the maternal use of antibiotics, information on all antibiotics (ATC code J01, antibacterials for systemic use) purchased during pregnancy and one year preceding pregnancy was extracted. The term of pregnancy was calculated from the date of birth of the child and gestational age obtained from the Medical Birth Register. In the absence of information on gestational age (n= 224, 0.6% in the asthma population and n=121, 0.7% in the CMA population) an assumption of 280 days was made. To evaluate the children's use of antibiotics, information on all antibiotics purchased from birth until the date of CMA or asthma diagnosis of the case was extracted. In the CMA population, antibiotics purchased in the month before diagnosis were excluded in order to diminish reverse causation due to antibiotics prescribed during the diagnosis process. In the asthma population, antibiotics purchased in the six months before diagnosis were excluded in order to diminish protopathic bias (i.e. the early symptoms of an undiagnosed asthma could have been the reason for the use of antibiotics). To evaluate the determinants of antibiotic use, only antibiotics used among control mothers and control children were taken into account. Variables used to assess the use of antibiotics were defined and categorized based on exposure period, number of purchases, type of antibiotic and indication (Appendix 2).

4.4 Statistical analysis

To examine the associations between maternal background factors and perinatal factors among control mothers and children, the Pearson chi-squared test for two categorical variables, the Mann-Whitney U test for the combination of a continuous and a dichotomous variable, and the Spearman rank correlation for two continuous variables were used. Logistic regression was used to examine the determinants of the use of antibiotics among control mothers and control children.

The associations between maternal background factors, perinatal factors and the use of antibiotics and the risk of CMA or asthma were analysed by conditional logistic regression. A test for trend was performed by fitting a conditional logistic regression model for the linear effect of maternal age, gestational age, ponderal index, birth weight, birth length and the number of antibiotic purchases using a likelihood ratio test. To examine interactions between selected variables, an interaction term was included in the crude and adjusted models (see below) and a likelihood ratio test was used to compare the models with and without the interaction term. In the asthma population, all analysis was conducted separately for children diagnosed before the age of three years and at the age of three years or later. Statistical significance was set at the five percent level and two-sided P values were used. All analyses were performed using Version 9.1 of STATA software.

Models for maternal background and perinatal factors and the risk of cow's milk allergy and asthma

Firstly, a model with only one explanatory variable at a time was fitted. The ponderal index, the Apgar score at one minute, the mode of delivery, the multiple pregnancy and complications were, however, adjusted for gestational age in the univariate analysis. Secondly, a multivariate model was fitted. The variables included in the multivariate models in the CMA and asthma populations are presented in Table 6.

Table 6. Variables included in the multivariate models estimating the associations between maternal background factors, perinatal factors and the risk of cow's milk allergy and asthma

Variable	Outcome		
	Cow's milk allergy	Asthma (diagnosis <3 yr)	Asthma (diagnosis ≥3yr)
Maternal age	x	x	x
Maternal asthma		x	x
Maternal smoking during pregnancy	x	x	x
Maternal socioeconomic status	x		
Maternal previous deliveries	x	x	x
Maternal previous miscarriages		x	x
Multiple pregnancy	x		
Mode of delivery	x	x	x
Gestational age	x	x	x
Ponderal index	x	x	x

x = included in the model

Models for use of antibiotics and the risk of cow's milk allergy and asthma

Both unadjusted and adjusted models were fitted, and the maternal background and perinatal factors associated with both CMA or asthma and the use of antibiotics were included as confounders in the adjusted models. The variables included in the main adjusted models for maternal and child use of antibiotics in the CMA and asthma populations are presented in Table 7.

Table 7. Variables included as confounders in the main adjusted models estimating the associations between maternal and child use of antibiotics and the risk of cow's milk allergy and asthma

Variable	Outcome					
	Cow's milk allergy		Asthma (diagnosis <3 yr)		Asthma (diagnosis ≥3yr)	
	Mother	Child	Mother	Child	Mother	Child
Maternal age	x	x	x	x		
Maternal asthma			x	x	x	x
Maternal smoking during pregnancy	x	x	x	x		
Maternal socioeconomic status	x					
Maternal previous deliveries	x	x	x	x	x	x
Maternal previous miscarriages			x	x		
Multiple pregnancy	x					
Mode of delivery		x		x		
Gestational age				x		x
Birth weight		x				
Child use of antibiotics	x		x		x	

x = included in the model

The models for child's use of antibiotics were adjusted further with the maternal use of antibiotics preceding and during pregnancy. In addition, the models for the use of specific antibiotics were adjusted further for the total number of antibiotic purchases from which the number of corresponding specific antibiotic purchases was excluded.

As a result of excluding antibiotics purchased one month and six months before diagnosis in the CMA and asthma populations, analysis was restricted to children diagnosed after the age of one month in the CMA population (n=15,672 case-control pairs) and to children diagnosed after the age of six months but before the age of three years (n=12,427 case-control pairs) in the younger asthma age group.

The results of this study are presented as adjusted odds ratios (ORs) and 95 percent confidence intervals (CIs) obtained from the multivariate models (maternal background and perinatal factors) and the main adjusted models (antibiotics).

4.5 Ethical issues

This study was based on information originally collected for administrative purposes. The present study did not require any contact with the participants. Under Finnish law no written consent is needed from the participant or their caretakers in register-based studies. To use administratively collected information for scientific study purposes from those registers that allow it, permission from the institutions keeping the registers and an ethics approval is required. The study protocol of the present study was approved by the institutions keeping the registers used in this study and the Institutional Review Board of the former National Health Institute (now the National Institute for Health and Welfare).

5 Results

5.1 Characteristics of the study population

The mean age at receiving a special reimbursement for CMA was 6.0 months (SD 3.7 months, range 0.0 to 22.9 months) and 1.6 years (SD 0.7 years, range 0.0 to 2.9 years) for asthma in the younger age group and 4.8 years (SD 1.4 years, range 3.0 to 9.7 years) for asthma in the older age group. The majority (91%) of the CMA cases were diagnosed before the age of 12 months and two-thirds (67%) of all asthma cases before the age of three years. Both CMA and asthma diagnosed at any age were more common in boys than in girls (59% vs. 41% and 64% vs. 36%, respectively) (Table 8).

One-third of the mothers in the CMA and asthma populations belonged to the age groups 25–29 years or 30–34 years and less than 20 percent to the youngest age group (<25 years at the time of delivery)(Table 8). A large proportion of the mothers were lower white-collar workers and had previously delivered one or two children (Table 8).

5.2 Association between maternal background factors and the risk of cow's milk allergy and asthma

Several maternal background factors were associated with the risk of CMA and asthma (Table 9). While maternal age was directly associated with CMA, it was inversely associated with asthma diagnosed before the age of three years and not associated with asthma diagnosed at the age of three years or later. Maternal high socioeconomic status was associated with an increased risk of CMA, while the socioeconomic status was not associated with asthma. Maternal smoking during pregnancy was associated with a decreased risk of CMA and an increased risk of asthma diagnosed in early childhood, but not associated with asthma diagnosed at the age of three years or later. Maternal asthma was associated with an increased risk of asthma in both age groups. No information on maternal allergic background was available in the CMA population.

The number of maternal previous deliveries was inversely associated with CMA and asthma diagnosed at the age of three years or later, but directly with asthma diagnosed before the age of three years. Maternal previous miscarriages were associated only with asthma in the younger age group.

Table 8. Characteristics of the cow's milk allergy and asthma study populations

Factors	Cow's milk allergy		Asthma (diagnosis <3 yr)		Asthma (diagnosis ≥3yr)	
	Cases[1]	Controls	Cases[1]	Controls	Cases[1]	Controls
	%	%	%	%	%	%
Maternal age (years)						
<25	15	19	20	9	21	19
25–29	33	33	34	31	35	33
30–34	34	30	31	31	29	31
≥35	18	18	16	19	15	18
Maternal socioeconomic status						
Upper white-collar workers	23	18	16	19	16	17
Lower white-collar workers	47	44	47	45	49	45
Blue-collar worker	13	18	16	17	17	18
Others	17	20	21	20	18	20
Maternal smoking during pregnancy						
No	90	85	81	86	85	86
Yes	10	15	19	15	15	15
Number of maternal previous deliveries						
0	41	42	33	42	47	40
1–2	51	48	54	49	46	50
≥3	8	10	13	10	7	10
Child's sex						
Male	59	59	66	66	60	60
Female	41	41	34	34	40	40

[1] Cases and controls were matched on sex, date of birth (±28 days) and the hospital district in which the child was born

Table 9. Summary of the associations between maternal background and perinatal factors and the risk of cow's milk allergy and asthma

Factor	Outcome		
	Cow's milk allergy	Asthma (diagnosis <3 yr)	Asthma (diagnosis ≥3yr)
Maternal background factors			
Age	↑	↓	ns
Socioeconomic status	↑	ns	ns
Smoking during pregnancy	↓	↑	ns
Previous deliveries	↓	↑	↓
Previous miscarriages	ns	↑	ns
Perinatal factors			
Caesarean section	↑	↑	ns
Multiple pregnancy	↓	ns	ns
High blood pressure	ns	ns	ns
Placental complications	ns	↑	ns
Fetal abnormal presentation	ns	ns	ns
Gestational age	ns	↓	↓
Prematurity (<37 gestational weeks)	ns	↑	↑
Low Apgar score	ns	↑	↑
Ponderal index	ns	↓[1]	↓[1]
Birth weight	↑	ns	ns
Birth length	ns	ns	ns
Low birth weight (birth weight <2500g)	ns	↑	ns
Small-for-gestational-age	ns	↑	ns
Large-for-gestational-age	ns	ns	ns

Abbreviations: ↑ direct association with the outcome, ↓ inverse association with the outcome, ns not associated with the outcome

[1] not associated with asthma when prematurely born children were excluded

5.3 Association between perinatal factors and the risk of cow's milk allergy and asthma

Delivery by both planned and unplanned caesarean section was associated with an increased risk of CMA and asthma diagnosed in early childhood (both section types combined, OR 1.18, 95% CI 1.10–1.27 for CMA and OR 1.27, 95% CI 1.19–1.37 for asthma diagnosed before the age of three years)(Table 9). Multiple pregnancy was associated with a decreased risk of CMA. Most maternal complications during index pregnancy were not associated with CMA or asthma except placental complications, which were associated with an increased risk of asthma diagnosed before three years of age.

None of the variables indicating restricted fetal growth (short gestational age, low birth weight, small-for-gestational age or low ponderal index) were associated with CMA. Short gestational age was associated with asthma diagnosed at any age, but low birth weight, small-for-gestational age and low Apgar score were only associated with asthma diagnosed in early childhood. High birth weight was associated with an increased risk of CMA, but none of the other factors indicating enhanced fetal growth (large-for-gestational age or high ponderal index) were associated with either CMA or asthma.

5.4 Use of antibiotics

In the year preceding pregnancy, 44% of the case mothers in the asthma population, 41% of the case mothers in the CMA population and 35% of all control mothers had used antibiotics. During pregnancy, 32% of case mothers in the asthma population, 28% of case mothers in the CMA population and 24% of all control mothers had used antibiotics. In addition, 19% of case mothers in the asthma population, 16% of case mothers in the CMA population and 12% of all control mothers and had used antibiotics both before and during pregnancy.

Among the children, 21% of the CMA cases and 15% of the CMA controls had used antibiotics at least once during the follow-up period from birth to the diagnosis date of the case (Table 10). In the asthma population, 56% of asthma cases diagnosed before the age of three years and 35% of control children had used antibiotics at least once during the first year of life. The corresponding figures for asthma cases diagnosed at the age of 3 years or later and their controls were 58% and 49% respectively. Further, the prevalence of frequent use (six or more purchases) of antibiotics during the first three years of life was 45% in cases and 26% in controls followed for at least three years. The most commonly used specific antibiotic among all children during the first year of life was amoxicillin, followed by macrolides and cephalosporins (Table 10).

Table 10. Prevalence of antibiotic users* and the mean number of antibiotic purchases per child among children with and without cow's milk allergy and with and without asthma

Type of antibiotic	Cow's milk allergy				Asthma (diagnosis <3yr)				Asthma (diagnosis ≥3yr)			
	Cases (n=15,672)		Controls (n=15,672)		Cases (n=12,427)		Controls (n=12,427)		Cases (n=6,690)		Controls (n=6,690)	
	users %	mean number of purchases	users %	mean number of purchases	users %	mean number of purchases	users %	mean number of purchases	users %	mean number of purchases	users %	mean number of purchases
Any antibiotic	20	1.9	15	1.7	56	2.5	35	2.0	58	2.3	49	2.1
Amoxicillin	14	1.4	11	1.3	43	1.6	27	1.4	45	1.5	38	1.5
Macrolides	8	1.3	6	1.2	29	1.4	14	1.3	26	1.3	19	1.3
Cephalosporins	6	1.3	3	1.2	14	1.3	7	1.3	15	1.3	10	1.4
Sulfonamides and trimethoprim	1	1.2	1	1.1	5	1.2	2	1.2	5	1.2	3	1.2
Phenoxymethyl-penicillin	1	1.0	1	1.1	3	1.1	2	1.1	3	1.1	3	1.0

* use of antibiotics from birth to diagnosis in children with cow's milk allergy (mean age of diagnosis 6 months) and from birth to 1 year of age in children with asthma (from birth diagnosis among if asthma diagnosed before the age of 1 year, n=2443 cases)

5.4.1 Determinants of antibiotic use

Maternal asthma, smoking during pregnancy and previous deliveries were directly associated with the use of antibiotics during pregnancy by the control mothers in both the CMA and asthma populations. Similar associations were observed between maternal background factors and the use of antibiotics before pregnancy.

Maternal age, previous deliveries, previous miscarriages, child ponderal index or birth weight, male sex and maternal use of antibiotics were directly associated with the use of antibiotics by the control children in both the CMA and asthma populations (Table 11).

Table 11. Summary of the determinants of antibiotic use among control children in the cow's milk allergy and asthma populations

Factor	Population	
	Cow's milk allergy[1]	Asthma[2]
Maternal age	↑	↑
Maternal socioeconomic status	ns	ns
Maternal smoking during pregnancy	ns	ns
Maternal previous deliveries	↑	↑
Maternal previous miscarriages	↑	↑
Caesarean section	ns	↑
Gestational age	ns	ns
Ponderal index/birth weight	↑	↑
Male sex	↑	↑
Maternal use of antibiotics	↑	↑

Abbreviations: ↑ direct association with the use of antibiotics, ↓ inverse association with the use of antibiotics, ns not associated with the use of antibiotics

[1] use of antibiotics defined as overall use from birth to diagnosis date of the case

[2] use of antibiotics defined as frequent use from birth to three years of age

5.4.2 Association between maternal use of antibiotics and the risk of cow´s milk allergy and asthma in offspring

Before pregnancy, maternal overall use of antibiotics was associated with an increased risk of CMA (OR 1.26, 95% CI 1.20–1.33) and asthma in the offspring

(OR 1.28, 95% CI 1.21–1.37 for asthma diagnosed before the age of three years and OR 1.20, 95% CI 1.10–1.30 for asthma diagnosed at the age of three years or later) when adjusting for several maternal background factors, perinatal factors and the children's use of antibiotics. In addition, the risk of CMA and asthma increased with an increasing number of antibiotic purchases (P for trend <0.001) (Figure 2).

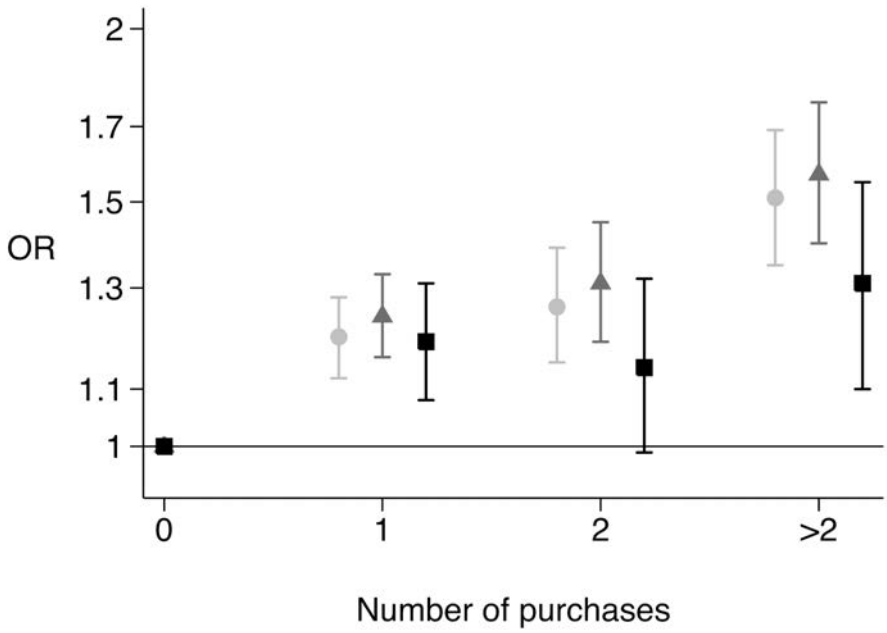


Figure 2. Associations between number of maternal antibiotic purchases before pregnancy and the risk of cow s milk allergy and asthma in the offspring. Data points are adjusted odds ratios (light gray circle, cow's milk allergy; grey triangle, asthma diagnosed before the age of 3 years; black square, asthma diagnosed at the age of 3 years or later) with 95% confidence intervals (bars), 0 purchases as reference.

During pregnancy, maternal overall use of antibiotics was associated with an increased risk of CMA (OR 1.21, 95% CI 1.14–1.28) and asthma (OR 1.28, 95% CI 1.20–1.37 for asthma diagnosed before the age of three years and OR 1.14, 95% CI 1.04–1.24 for asthma diagnosed at the age of three years or later) in the offspring. In addition, the risk of CMA and asthma increased with an increasing number of antibiotic purchases (P for trend <0.001) (Figure 3).

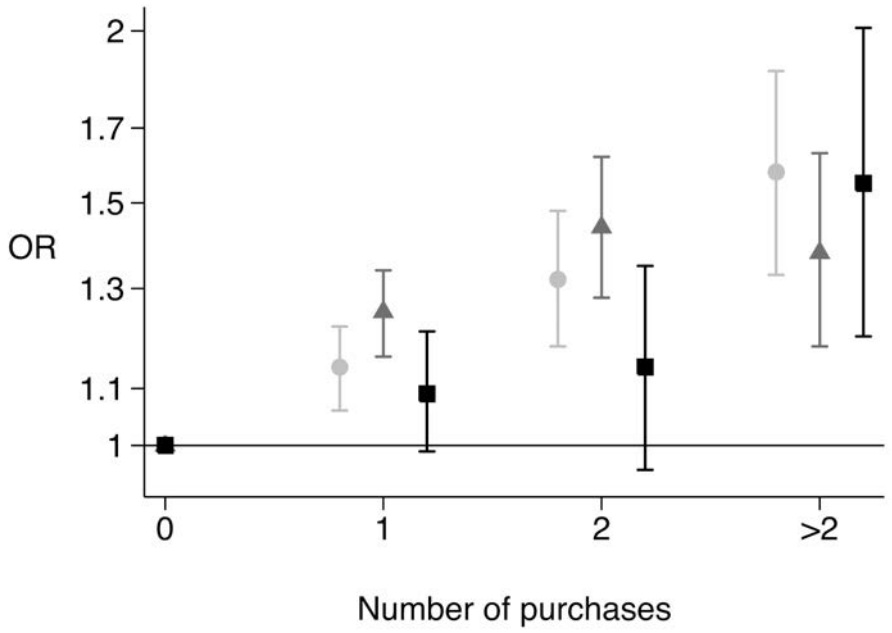


Figure 3. Associations between number of maternal antibiotic purchases during pregnancy and the risk of cow s milk allergy and asthma in the offspring. Data points are adjusted odds ratios (light gray circle, cow's milk allergy; grey triangle, asthma diagnosed before the age of 3 years; black square, asthma diagnosed at the age of 3 years or later) with 95% confidence intervals (bars), 0 purchases as reference.

When taking potential confounders and the total number of antibiotic purchases into account, maternal use of cephalosporins, macrolides and penicillins with extended spectrum both before and during pregnancy was associated with an increased risk of CMA and asthma in the younger age group, while only cephalosporins used both before and during pregnancy were associated with an increased risk of asthma in children diagnosed at the age of three years or later (Table 12). In addition, use of tetracyclines before pregnancy, but not during pregnancy was associated with an increased risk of CMA and asthma diagnosed before the age of three years. When grouping antibiotics used during pregnancy by indication, antibiotics for respiratory tract infections were associated with an increased risk of asthma diagnosed at the age of three years or later in the offspring.

Table 12. Summary of the associations between maternal specific antibiotics used before and during pregnancy and the risk of cow's milk allergy and asthma in the offspring

Type of antibiotic	Outcome		
	Cow's milk allergy	Asthma (diagnosis <3 yr)	Asthma (diagnosis ≥3yr)
Cephalosporins	↑	↑	↑
Penicillins with extended spectrum	↑	↑	ns
Macrolides	↑	↑	ns
Tetracyclines	↑ (before pregnancy) ns (during pregnancy)	↑ (before pregnancy) ns (during pregnancy)	ns
Phenoxymethylpenicillin	ns	ns	ns
Sulfonamides and trimethoprim	ns	ns	ns
Fluoroquinolones	ns	ns	ns

Abbreviations: ↑ direct association with the outcome, ns not associated with the outcome

5.4.3 Association between child use of antibiotics and the risk of cow´s milk allergy and asthma

Both overall use and an increasing number of antibiotics purchased during the first year of life were associated with an increased risk of CMA and asthma (Figure 4). When extending the exposure period from birth to diagnosis in the asthma population, the associations were even stronger (adjusted OR for ⩾10 purchases 19.73, 95% CI 15.30–25.43 in the younger age group and adjusted OR 8.92, 95% CI 7.41–10.73 in the older age group).

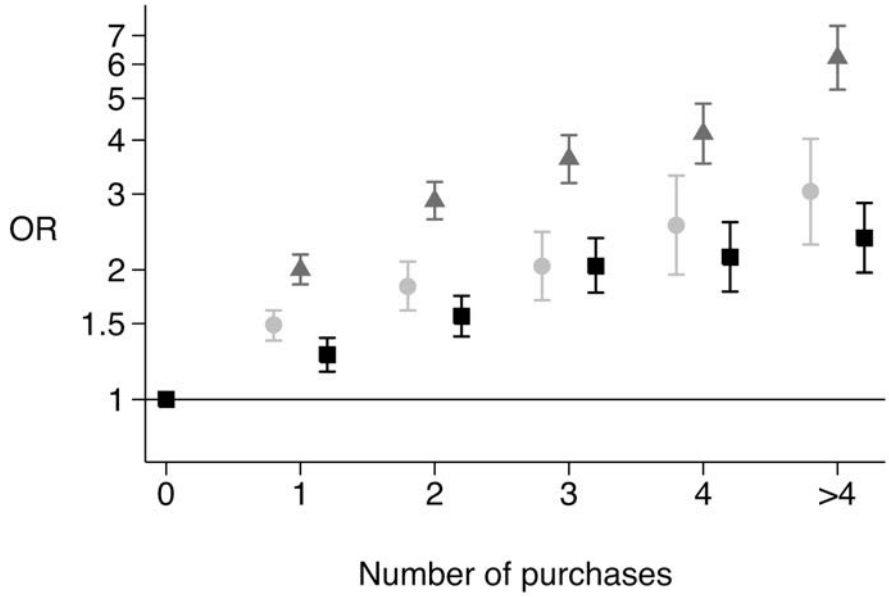


Figure 4. Association between number of children's antibiotic purchases and the risk of cow s milk allergy and asthma. Data points are adjusted odds ratios (light gray circle, cow's milk allergy; grey triangle, asthma diagnosed before the age of 3 years; black square, asthma diagnosed at the age of 3 years or later) with 95% confidence intervals (bars), 0 purchases as reference.

All commonly used specific antibiotics used by the child from birth to diagnosis were associated with CMA and asthma, although the use of phenoxymethylpenicillin during the first year of life was not associated with asthma in the older group (Table 13). Further grouping of specific antibiotics used during the first year of life according to indication did not show clear differences between the effect estimates in children with asthma diagnosed at the age of three years or later (OR 1.51, 95% CI 1.41–1.63 for antibiotics against respiratory tract infections and OR 1.54, 95% CI 0.92–2.59 for antibiotics against urinary tract infections). In fact, the strongest association was observed when antibiotics belonging to both indication groups were used (OR 2.16, 95% CI 1.76–2.66).

Table 13. Summary of the associations[1] between child's use of specific antibiotics and the risk of cow's milk allergy and asthma

Type of antibiotic	Outcome				
	Cow's milk allergy	Asthma (diagnosis <3 yr)		Asthma (diagnosis ≥3yr)	
	Exposure period				
	birth to diagnosis[2]	first year	birth to diagnosis[3]	first year	birth to diagnosis[3]
Amoxicillin	↑	↑	↑	↑	↑
Macrolides	↑	↑	↑	↑	↑
Cephalosporins	↑	↑	↑	↑	↑
Sulfonamides and trimethoprim	↑	↑	↑	↑	↑
Phenoxymethylpenicillin	↑	↑	↑	ns	↑

Abbreviations: ↑ direct association with the outcome, ns not associated with the outcome

[1] adjusted for potential confounders and the total number of antibiotic purchases

[2] those antibiotics purchased one month preceding the diagnosis date of the case were excluded

[3] those antibiotics purchased six months preceding the diagnosis date of the case were excluded

6 Discussion

6.1 Methodological considerations

Data sources

By using the Finnish unique personal identity codes, we were able to establish a large, population-based database and link comprehensive information from several nationwide registers. Use of personal identity codes as a key in register linkage improves the completeness of information in the study (Gissler and Haukka 2004).

The coverage of Finnish health registers is generally high (Gissler and Haukka 2004). The Medical Birth Register covers all births in Finland and several variables included in the register have shown high validity and accuracy (Gissler *et al.* 1995). Completeness and accuracy of the Drug Prescription Register is also considered high (Furu *et al.* 2010), although not optimal. In 1997–1999 and 2000–2005 the register respectively covered 70% and 75% of total antibiotic consumption in Finnish outpatients. The most frequent reason for not being covered by the database was the low price of medication (a threshold of 10 euros).

A major strength in using registers is the avoidance of participation, reporting and recall bias, which are more an issue in studies based on information obtained from questionnaires. Thus, detailed information on both the mother's and the child's lifetime antibiotic exposure and several maternal background and perinatal factors is one of the unique features of the present study.

One of the limitations of using register-based data is that information is usually collected for administrative purposes and not all information needed for research purposes is available. Hence, despite being able to include several putative confounders in the present study, information on certain important confounders – such as indication of antibiotic prescription, antibiotic exposure at hospitals and certain factors presumably affecting the development of diseases like infant feeding and parental allergy status – were missed. Thus, residual confounding, referring to the situation where an observed association between exposure and outcome is produced by an unmeasured third factor, can not be ruled out as an explanation for the results of the present study.

Study design and population

This study was a historical cohort study utilizing a nested case-control approach in the selection of controls for the cases (incidence density sampling). All original information in the registers was collected prospectively and independently of the disease status of the child. The prospective nature of the present study diminishes bias caused by reverse causation, which could occur when the presumed outcome

is responsible for the occurrence of the exposure of interest. Case-control and cross-sectional studies are more amenable to this bias, although it may occur in cohort studies as well.

The study population of the present study was large enough to evaluate the associations between various maternal background factors, perinatal factors and antibiotic exposure in detail and the risk of CMA and asthma. However, due to the large sample size, the possibility of obtaining statistically significant associations even when the differences between the cases and controls were small must be taken into account when interpreting the results. On the other hand, some of the confidence intervals were rather wide due to the use of wide categories with some exposures and the use of several potential confounding factors. Further, the statistical approach needed with match data decreased the number of case-control pairs in each category of the exposure.

Our results on the associations between the use of antibiotics and the risk of CMA and asthma may be generalized to other pediatric populations, providing that potential differences related to, for example, antibiotic prescription practices among other pediatric population are considered.

Outcome and exposure assessment

The identification of CMA and asthma cases through the Special Reimbursement Register can be considered reliable, as the requirement for each special reimbursement is based on a clinical diagnosis made by a paediatrician or physician and, furthermore, the application is further reviewed against criteria by another clinician. In addition, it is worth noting that eligibility for the special reimbursement does not depend on a family's socioeconomic situation or area of residence. However, due to the size of the study population, we were unable to verify individually all the diagnoses and thus can not exclude the possibility that some heterogeneity in the diagnostic procedures among our cohort children exists. For example, some of the CMA cases with severe reactions to cow's milk or a very young age could have received the special reimbursement without undergoing a challenge procedure. The number of these CMA cases is, however, likely to be small. Further, as wheezing is common in early childhood and objective diagnostic measurements for asthma diagnosis are difficult to apply for young children, early transient wheezing may be what some of the youngest children in our asthma cohort have. However, to at least partly disentangle the fact that we were not able to distinguish between different wheezing or asthma phenotypes, all analysis in the asthma population was conducted separately for children diagnosed before the age of three years and at the age of three years or later. Furthermore, in both populations we applied a strict case definition which included specific criteria for the number of purchased antiasthmatic drugs in asthma cases and the duration of the special reimbursement and the number of special infant formula purchases in

CMA cases. This further strengthens the reliability of the case definition used in the present study. However, despite the strict criteria of asthma cases, some uncertainty regarding the diagnosis in the younger age group is likely, and the results of the older age group are considered to be the main findings in the asthma population.

Exposures of the present study – maternal background factors, perinatal factors and the use of antibiotics – were obtained from nationwide registers with high population coverage and accuracy. However, relying on pharmacy records to assess drug use, the information on drug purchases includes only a rough estimation of the actual use. However, data on dispensed drugs suggest a better correspondence with actual drug use than data retrieved from registries based solely on prescriptions would. Further, a misclassification of subjects who failed to take their antibiotics as users only dilutes the associations and biases the relative risks toward unity.

6.2 Maternal background factors as risk factors for cow's milk allergy and asthma

Maternal age, socioeconomic status and smoking

Limited information on the role of maternal background factors – other than maternal allergies – in the development of CMA exists. We observed a direct association between maternal age and socioeconomic status and CMA. Similarly, a higher maternal age and a high socioeconomic status/income have been associated with any food allergy in two previous studies (Dioun *et al.* 2003, Liem *et al.* 2007). We also observed an inverse association between maternal smoking during pregnancy and CMA, but another Finnish cohort study failed to find an association between maternal smoking and CMA (Saarinen *et al.* 1999).

The finding of an increased risk of asthma with low maternal age and smoking during pregnancy in the present study is in line with some previous cohort studies (Nafstad *et al.* 2000, McKeever *et al.* 2001, Yuan *et al.* 2003), although not all (Juhn *et al.* 2005, Kiechl-Kohlendorfer *et al.* 2007, Midodzi *et al.* 2010). Age-dependent associations similar to those in the present study were found in two other studies; low maternal age (Bråbäck *et al.* 2003) and smoking (Bråbäck *et al.* 2003, Davidson *et al.* 2010) were associated with asthma diagnosed at early ages, but not with asthma diagnosed at older ages.

The associations with maternal age, socioeconomic status and smoking and CMA could at least partly support the notion that mothers with a higher socioeconomic status tend to seek consultation for their child's symptoms more often than mothers with a lower socioeconomic status. Smoking during pregnancy has been related to a younger age and lower socioeconomic status of the mother (Jaakkola *et al.* 2001), and thus the association between maternal age and asthma could reflect the social environment and maternal health behaviour. On the other hand,

maternal age could be related to the biology of fetal development as well. The observation of no association between socioeconomic status and asthma in this study may reflect the heterogenic nature of asthma and, on the other hand, the fact that socioeconomic status was based on occupation at the time of delivery. This may have misclassified some women, as some of the women with an occupation that belongs to the highest socioeconomic status group may have reported being housewives at the time of delivery. This may explain the proportion of women with an unidentified socioeconomic position. Thus, even though the occupational information in the Medical Birth Register has been shown to be of high quality (Gissler *et al.* 1995), some uncertainty attributed to the large proportion of women with unidentified socioeconomic status remains.

Previous deliveries

In the present study, the number of maternal previous deliveries was inversely associated with the risk of CMA and asthma in the older age group and directly with asthma in the younger age group. Evidence related to older siblings or birth order and food allergies is limited: three studies have reported an inverse association with egg allergy (Koplin *et al.* 2012) and any food allergy (Dioun *et al.* 2003, Kusunoki *et al.* 2012) whereas two studies have reported a null association with CMA (Saarinen and Savilahti 2000) or egg allergy (Eggesbo *et al.* 2003). Three very large, register-based cohort studies have reported almost similar age-dependent findings with asthma as in the present study: the risk of asthma was increased among the youngest children and decreased or not associated with the older children (McKeever *et al.* 2001, Bråbäck *et al.* 2003, Davidson *et al.* 2010). The differences in these age-dependent associations are likely due to differences in the cut-off points of the diagnosis age or in the outcome definition. Studies by Bråbäck *et al.* (2003) and Davidson *et al.* (2010) used hospitalization due to asthma as the outcome, whereas McKeever and co-workers (2001) relied on information on recoded asthma diagnosis.

The associations between older siblings or birth order and CMA and asthma may have their origin in both the prenatal and postnatal periods. Firstly, it has been suggested that the protective association is due to an increased exposure to infections in early childhood from close contact with siblings. Furthermore, it has been suggested that the more diverse gut microbiota observed in children living in large families (Sjögren *et al.* 2009) could be related to the inverse association between number of siblings and development of asthma and allergic diseases. Secondly, there is growing evidence to support the prenatal origin of "the birth order or sibling effect". As hormonal and immunologic conditions in the uterus in subsequent pregnancies are different from those in first pregnancies, this may affect the health outcomes of the children later in life.

The somewhat divergent associations between maternal sociodemographic background factors, pregnancy history and the outcomes, CMA and asthma, observed in the present study may be due to etiological differences between these diseases. In addition, the age-dependent findings among the asthma population may also be due to etiological differences between asthma starting in earlier rather than later childhood, but also due to the fact that some of the children in our younger asthma cohort may have had only transient early wheezing rather than true asthma. Further, inconsistencies between previous studies exploring the associations between maternal factors and the development of CMA, other food allergies and asthma may be due to etiological differences between the diseases or methodological differences between the studies, or the inconsistencies may indicate that the associations are only weak, due to change or confounding.

6.3 Perinatal factors as risk factors for cow's milk allergy and asthma

Mode of delivery

The finding from the present study that delivery by caesarean section was associated with and increased risk of CMA has some support from one previously published study (Eggesbo *et al.* 2005). Although this cohort study did not find a statistically significant association, a tendency towards an increased risk of CMA was observed (OR 2.5, 95% CI 0.8–7.5) (Eggesbo *et al.* 2005). Another cohort study also failed to find an association between delivery by caesarean section and the development of CMA (Kvenshagen *et al.* 2009), which, however, may have due to small sample size and thus lack of power.

Caesarean section was associated with an increased risk of asthma in the present study, which is in line with several register-based asthma studies (Kero *et al.* 2002, Smith *et al.* 2004, Renz-Polster *et al.* 2005, Tollånes *et al.* 2008, Almqvist *et al.* 2012a), but not them all (Benn *et al.* 2002, McKeever *et al.* 2002a Bernsen *et al.* 2005, Juhn *et al.* 2005). However, the increased risk in the present study was observed only among children diagnosed before the age of three years, but not at later age, which is in contrast with findings from the study by Salam *et al.* (2006). This study reported an increased risk of asthma among those children diagnosed after the age of 3 years but not earlier. Age-dependent associations have not been reported in other studies. The finding that both planned and other caesarean sections were associated with the risk of asthma in the present study is in line with those studies that assessed different caesarean section types (Smith *et al.* 2004, Tollånes *et al.* 2008, Almqvist *et al.* 2012a). Further, there is some evidence – although it is not consistent – supporting the observation of an increased risk of asthma diagnosed in early childhood in children born by assisted delivery (Xu *et al.* 2000, McKeever *et al.* 2002a, Bernsen *et al.* 2005, Tollånes et al. 2008, Keski-Nisula *et al.* 2009, Hancox *et al.* 2013).

It has been hypothesized that the increased risk of asthma in children born by caesarean section may be due to differences in microbial exposures related to caesarean section and vaginal delivery. The gut microbiota in children born by caesarean section has been observed to differ from those of children born vaginally (Grönlund *et al.* 1999), and thus caesarean section may play a role in the development of asthma and allergic diseases by affecting the establishment of a child's gut microbiota. Other explanations suggest that the association may be confounded by the underlying indication for caesarean section or by some other factor or condition already present at or before birth (Bernsen *et al.* 2006, Almqvist and Rejnö 2013). Further, asthma in particular may be predisposed by respiratory problems identified in some newborns born by section (Levine *et al.* 2001). Although children born by assisted vaginal delivery are exposed to maternal vaginal microbes, they may face respiratory problems due to asphyxia and its treatment and thus be at increased risk for developing asthma (Bernsen *et al.* 2006). In addition, the underlying indication for assisted vaginal delivery may also be the explanation for this association (Almqvist and Rejnö 2013). Further, the age-dependent associations with the risk of asthma observed in the present study and the differences between previously published results are likely due to methodological and/or etiological factors.

Twin or other multiple pregnancies

In the present study, multiple pregnancy was associated with a decreased risk of CMA. This association has not been explored previously, and even the evidence regarding the risk of asthma in childhood among members of a multiple pregnancy is limited (Strachan et al. 2000, McKeever *et al.* 2001, Dik *et al.* 2004). Previous studies have observed a decreased risk of asthma among twins (Strachan *et al.* 2000, McKeever *et al.* 2001, Dik *et al.* 2004), but the present study failed to find an association between multiple pregnancy and asthma.

Complications during pregnancy

Complications during pregnancy, neither in general nor any specific complication, were not associated with CMA or asthma in the present study. Previous studies on asthma have found an association between some specific complications or complications in general and an increased risk of asthma (Nafstad *et al.* 2000, Annesi-Maesano *et al.* 2001, McKeever *et al.* 2002b, Nafstad *et al.* 2003, Dik *et al.* 2004), but no specific biological mechanism by which complications would affect the development of asthma has been suggested.

Gestational age and child anthropometric measures

None of the factors indicating restricted fetal growth or low gestational age were associated with CMA in the present study. However, an increased risk of CMA

was observed in children born with a high birth weight. No studies have previously reported an association between gestational age, a child's birth weight or birth length and the risk of CMA. A decreased risk of egg and any food allergy in low birth weight children has previously been reported (Hikino et al. 2001, Koplin *et al.* 2012). Liem and co-workers (2007) assessed the role of both low and high birth weight on the development of food allergy in childhood, but they did not observe any associations (Liem *et al.* 2007). Low birth weight and preterm birth have been associated with a decreased risk of atopy among young adults (Siltanen *et al.* 2011).

Short gestational age was associated with an increased risk of asthma diagnosed at any age in the present study, which is in line the with majority of large, register-based cohort studies published previously (McKeever *et al.* 2002a, Yuan *et al.* 2003, Dik *et al.* 2004, Jaakkola and Gissler 2004, Bernsen *et al.* 2005, Gessner and Chimonas 2007, Dombkowski *et al.* 2008, Vogt *et al.* 2011), but not all of them (Yuan *et al.* 2002). The finding from the present study that low birth weight was associated with asthma diagnosed before the age of three years but not later is in line with results from studies by Davidson *et al.* (2010) and Bråbäck *et al.* (2003).

The association between prematurity and an increased risk of asthma has been explained by the fact that prematurity causes reduced lung growth and reduced airway caliber, which may increase wheezing symptoms during respiratory infections which, in turn, may increase asthma diagnoses (Jaakkola *et al.* 2006). On the other hand, prematurity may predispose the child to respiratory tract infections, which could give a rise to wheezing symptoms often misleadingly diagnosed as asthma in early childhood. Low birth weight often results from low gestational age, but low birth weight can also indicate growth restriction in utero independently from gestational age. The exact mechanism behind fetal growth restriction and subsequent asthma development is not clear, and it has been suggested that, instead of fetal growth restriction per se, the rapid catch-up growth following restricted fetal growth would predispose for the development of asthma (Tedner *et al.* 2012).

6.4 Use of antibiotics as risk factors for cow's milk allergy and asthma

Determinants of antibiotic use

Maternal asthma, smoking during pregnancy and a high number of previous deliveries were associated with the maternal use of antibiotics during pregnancy in the present study. Further, high maternal age, a high number of maternal previous deliveries, maternal use of antibiotics and a child's male gender were associated with the child's use of antibiotics during early childhood. These associations and those identified in previous studies – such as parental educational level (Ciofli

Degli Atti *et al.* 2006, Mangrio *et al.* 2009), parental perceptions (Ciofli Degli Atti *et al.* 2006) and long-term illnesses (Louhi-Pirkanniemi *et al.* 2003, Harris *et al.* 2007) – may reflect parental healthcare-seeking behaviour and thus affect the child's possibility of being taken to a physician and receiving a prescription forantibiotics. Further, a high number of maternal previous deliveries, reflecting a high number of older siblings, may affect a child's use of antibiotics by increasing the risk for respiratory infections from siblings (Louhi-Pirkanniemi *et al.* 2003, Simoes 2003, Harris *et al.* 2007) and thus the child's chance of receiving an antibiotic prescription.

Maternal use of antibiotics

An increased risk of CMA in children whose mothers had used antibiotics before and during pregnancy as observed in the present study has not been reported previously. On the other hand, the evidence on prenatal antibiotics and the risk of asthma is accumulating. Our observation that maternal use of antibiotics during pregnancy was associated with an increased risk of asthma is in line with results from other large cohort studies (McKeever *et al.* 2002b, Martel *et al.* 2009, Källén *et al.* 2013), but not with all previous studies (Calvani *et al.* 2004, Rusconi *et al.* 2007, Dom *et al.* 2010). The inconsistency between results from previously published studies is likely due to methodological issues such as differences in the asthma definition, the study designs and the study populations. Further, as in the present study, several putative confounding factors were considered in the previous studies, but maternal asthma, which may be associated with excess use of antibiotics, was considered only in half of the previous studies.

Exposure to prenatal antibiotics has been suggested to affect the development of asthma in childhood via their adverse effect on human microbiota (Sullivan *et al.* 2001), namely maternal vaginal and gut microbiota. These disturbances in maternal microbiota may, in turn, influence the early colonization of child's gut microbiota and further disturb the maturation of the child's immune system. As the adverse effects of antibiotics on human microbiota may last several months or even years (Sjölund *et al.* 2005, Jakobsson *et al.* 2007, Jakobsson *et al.* 2010), maternal antibiotics used before pregnancy may also influence the process of early colonization of child's gut microbiota. Evidence that different environmental exposures during the prenatal period can modify gene expression and susceptibility to allergic diseases through epigenetic modification is accumulating, although the capacity of antibiotics to induce epigenetic changes in gene expression has not been demonstrated (Prescott and Clifton 2009). On the other hand, exposure to antibiotics could serve as a marker for infections, but the role of infections for which the antibiotics are prescribed in confounding the association between prenatal antibiotics and asthma in childhood has not received attention in previous studies. We investigated this issue of "confounding by indication" by grouping the

antibiotics by their indicated use, but we were not able to draw conclusions from this analysis due to lack of power. On the contrary, the issue of "confounding by indication" has been much debated regarding children's own use of antibiotics and the development of asthma (see discussion later).

Children's use of antibiotics

Associations between children's use of antibiotics and the risk of CMA have not been published previously and thus further studies are needed on this topic.

The finding of an increased risk of asthma in children who had used antibiotics during their first year of life is in line with results from several other large register-based cohort studies (McKeever *et al.* 2002c, Kozyrskyj *et al.* 2007, Marra *et al.* 2009, Martel *et al.* 2009), but not with all results reported previously (Table 4). The majority of previous studies on asthma had explored children's use of antibiotics only during the first year of life (Table 4). When extending the children's exposure period beyond the first year of life, we observed even stronger associations than previously reported. Harris and co-workers (2007) reported associations between total antibiotic counts yearly up to the age of five years and wheeze at eight years of age, but the associations were not substantially different between exposure periods in the first year of life or the first five years of life. In accordance with previous studies, we observed stronger associations between children's use of antibiotics and asthma among children diagnosed in early rather than later childhood (McKeever *et al.* 2002c, Marra *et al.* 2009, Martel et al. 2009). Further, three studies observed an increased risk of asthma at an earlier age, but not at a later age (Celedon *et al.* 2004, Mai *et al.* 2010, Almqvist *et al.* 2012b). We observed some variation in the strength of the associations between different specific antibiotics, with the strongest association observed for cephalosporins in the CMA population and for macrolides in the asthma population. Similarly, some variation in the associations between specific antibiotics and asthma was reported in two other studies (McKeever *et al.* 2002c, Marra *et al.* 2009).

Although results from cohort studies exploring the association between children's use of antibiotics and the risk of asthma are inconsistent, the findings from the present study are in line with those observed in studies most comparable to the present study in methodology. Thus, inconsistencies between results from other studies are likely due to differences in methodology: the null associations were reported mainly by studies that were retrospective in design, had rather small sample size and were relying on reported antibiotic use or asthma symptoms or drug use rather than confirmed asthma. Further, differences in practices for prescribing specific antibiotics between countries may explain inconsistent findings.

As with prenatal antibiotics, exposure to antibiotics in early childhood may affect the development of asthma and allergic diseases via their adverse effect on gut microbiota. Antibiotic-induced disturbances in a child's gut microbiota may inter-

fere the early colonization of gut microbiota and further disturb the maturation of the immune system and development of tolerance towards oral and inhaled antigens (Noverr and Huffnagle 2005). On the other hand, some authors have argued that much if not all of the reported associations between postnatal antibiotics and asthma, in particular, are accounted for respiratory infections for which the antibiotics have been prescribed for or reverse causality (Penders *et al.* 2011, Almqvist *et al.* 2012). However, the relationship between respiratory infections and the development of asthma remains controversial (Bartlett *et al.* 2009), and results pointing towards an independent association of antibiotics with the risk of asthma have been reported (McKeever *et al.* 2002c, Kozyrskyj *et al.* 2007, Marra *et al.* 2009, Martel *et al.* 2009, Risnes *et al.* 2011). Moreover, a variety of social and clinical factors influence the prescribing of antibiotics, and the use of antibiotics is determined both by the infection and its symptoms and by the complex variations in healthcare-seeking behaviour (see discussion earlier).

7 Conclusions

Based on the main findings of the present study, the following conclusions were drawn:

- A high number of maternal previous deliveries were associated with a decreased risk of both CMA and asthma in the offspring. In addition, high maternal socioeconomic status, high maternal age and a child's delivery by caesarean section, as well as a child's high birth weight were associated with an increased risk of CMA, while maternal smoking during pregnancy and multiple pregnancy were associated with a decreased risk of CMA. In addition, maternal asthma and low gestational age were associated with an increased risk of asthma.

- The maternal use of antibiotics both before and during pregnancy as well as the child use of antibiotics during early childhood were dose-dependently associated with an increased risk of both CMA and asthma.

- A high number of maternal previous deliveries were directly associated with use of antibiotics among both control mothers and control children in the CMA and asthma populations. In addition, maternal asthma and smoking during pregnancy were directly associated with the maternal use of antibiotics. Further, maternal age, previous miscarriages, child ponderal index or birth weight, male sex, and maternal use of antibiotics were directly associated with child use of antibiotics.

These findings emphasise the important and complex role of prenatal and early childhood exposures in the development of CMA and asthma in childhood. Although most maternal sociodemographic and perinatal factors included in the present study are not easily modifiable and thus not practical to use in the prevention of asthma and other allergic diseases, identification of such factors may help focus preventive strategies on those children who are at high risk.

8 Future directions

The results of the present study emphasise the need to fill the gap in the literature on the role of different environmental factors such as perinatal factors and the use of antibiotics in the development of CMA. Future research topics raised from the present study include

- assessment of the role of different environmental factors, operating both pre- and postnatally, in the development of CMA in large cohort settings with several possible confounding factors, such as parental allergies and infant feeding taken into account.

- examination of the role of antibiotics in the development of CMA in different populations and study settings and with several possible confounding factors taken into account.

- examination of the causality of the association between maternal and childs use of antibiotics and the risk of asthma in large, prospective settings with follow-up at least to school age and detailed information on antibiotic use and potential confounding factors, e.g. indication for antibiotics, diet and healthcare-seeking behaviour.

- examination of the biological mechanisms behind the associations between maternal background, perinatal factors and the use of antibiotics and the development of CMA and asthma.

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APPENDIX 1 List of maternal background and perinatal factors and their categorization used in the present study

Variable name	Categorization	Details of categorization
Maternal age at delivery	<25, 25–29, 30–34 and ≥35 years	
Maternal smoking during pregnancy	no/yes	yes category included those who quit smoking during the first trimester
Maternal socioeconomic status	upper white-collar workers lower white-collar workers blue-collar workers others	information on occupation or education was converted into socioeconomic status and further aggregated into four groups "others" included students, housewives, farmers, entrepreneurs, unemployed and retired
Previous miscarriages	no/yes	
Previous deliveries	0, 1, 2, 3, 4, ≥5	
Multiple pregnancy	no/yes	based on information on number of fetuses
Mode of delivery	normal vaginal delivery assisted vaginal delivery planned caesarean section unplanned caesarean section	
Gestational age	quartiles[1] quintiles[2]	based on distribution of the controls
Birth weight	quartiles[1] quintiles[2]	based on distribution of the controls
Birth height	quartiles[1] quintiles[2]	based on distribution of the controls
Ponderal index	quartiles[1] quintiles[2]	based on distribution of the controls
Gestational age adjusted birth weight	small-for-gestational age (SGA) average-for-gestational age (AGA) large-for gestational age (LGA)	defined according to Finnish sex-specific population-based growth curves
Prematurity	no/yes	yes = gestational age<37 weeks
Low birth weight	no/yes	yes = birth weight <2,500 g
Apgar score at 1 min	0–6, 7–8, 9–10	
High blood pressure	no/yes	yes = high blood pressure during pregnancy that had to be treated in hospital
Abnormal presentation	no/yes	yes = breech and other abnormal presentation
Placental complications	no/yes	yes = placenta previa and placenta avulsion
Fetal asphyxia	no/yes	

[1] variable was used only in the asthma population

[2] variable was used only in the cow's milk allergy population

APPENDIX 2 List of variables and their categorization used to assess maternal and child use of antibiotics in the present study

Exposure period	Variable name	Categorization (number of purchases)
Before pregnancy	**Maternal use**	
	Overall use of any antibiotic	0 vs ≥1
	Overall use of cephalosporins	0 vs ≥1
	Overall use of penicillins with extended spectrum	0 vs ≥1
	Overall use of macrolides	0 vs ≥1
	Overall use of tetracyclines	0 vs ≥1
	Overall use of phenoxymethyl penicillin	0 vs ≥1
	Overall use of sulphonamides and trimetoprim	0 vs ≥1
	Overall use of fluoroquinolones	0 vs ≥1
	Number of purchases or any antibiotics	0 vs. 1, 2, ≥3
During pregnancy	Overall use of any antibiotic	0 vs ≥1
	Overall use of cephalosporins	0 vs ≥1
	Overall use of penicillins with extended spectrum	0 vs ≥1
	Overall use of macrolides	0 vs ≥1
	Overall use of tetracyclines	0 vs ≥1
	Overall use of phenoxymethyl penicillin	0 vs ≥1
	Overall use of sulphonamides and trimetoprim	0 vs ≥1
	Overall use of fluoroquinolones	0 vs ≥1
	Number of purchases of any antibiotics	0 vs. 1, 2, ≥3
	Overall use of only those antibiotics commonly used to treat respiratory tract infections (penicillins with extended spectrum, macrolides, cephalosporins and phenoxymethylpenicillin)[2]	0 vs ≥1
	Overall use of only those antibiotics commonly used to treat urinary tract infections (trimethoprim, its combinations with sulphonamides and fluoroquinolones)[2]	0 vs ≥1
	Overall use of both of those antibiotics commonly used to treat respiratory tract infections and urinary tract infections[2]	0 vs ≥1

Appendix 2 *(Continued)*

	Child's use	
	Overall use of any antibiotics[1]	0 vs ≥1
	Overall use of amoxicillin[1]	0 vs ≥1
	Overall use of macrolides[1]	0 vs ≥1
	Overall use of cephalosporins[1]	0 vs ≥1
	Overall use of sulphonamides and trimetoprim[1]	0 vs ≥1
During the first year of life	Overall use of phenoxymethyl penicillin[1]	0 vs ≥1
	Overall use of only those antibiotics commonly used to treat respiratory tract infections (amoxicillin, macrolides, cephalosporins and phenoxymethylpenicillin)[2]	0 vs ≥1
	Overall use of only those antibiotics commonly used to treat urinary tract infections (trimethoprim and its combinations with sulphonamides)[2]	0 vs ≥1
	Overall use of both of those antibiotics commonly used to treat respiratory tract infections and urinary tract infections[2]	0 vs ≥1
	Number of purchases of any antibiotics[1]	0 vs. 1, 2, 3, 4, ≥5
	Overall use of any antibiotics	0 vs ≥1
	Overall use of amoxicillin	0 vs ≥1
	Overall use of macrolides	0 vs ≥1
	Overall use of cephalosporins	0 vs ≥1
	Overall use of sulphonamides and trimetoprim	0 vs ≥1
	Overall use of phenoxymethyl penicillin	0 vs ≥1
From birth to diagnosis	Overall use of only those antibiotics commonly used to treat respiratory tract infections (amoxicillin, macrolides, cephalosporins and phenoxymethylpenicillin)[2]	0 vs ≥1
	Overall use of only those antibiotics commonly used to treat urinary tract infections (trimethoprim and its combinations with sulphonamides)[2]	0 vs ≥1
	Overall use of both of those antibiotics commonly used to treat respiratory tract infections and urinary tract infections[2]	0 vs ≥1
	Number of purchases of any antibiotics	0 vs. 1, 2, 3, 4, ≥5
	Number of purchases of any antibiotics[1]	0 vs. 1, 2, 3, 4, 5, 6-7, 8-9, ≥10
During the first 3 years of life	Frequent use of any antibiotics among controls[1]	0-5 vs. ≥6

[1] variable was used only in the asthma population

[2] variable used only among children with asthma diagnosed at the age of 3 years or later

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