Parental gastrointestinal diseases and risk of asthma in the offspring

A review of the specific impact of acid-suppressive drugs, inflammatory bowel disease, and celiac disease

PhD Dissertation

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Preface

The work presented in this thesis was carried out at Aarhus University/Aarhus University Hospital, Department of Clinical Epidemiology. Part of study III was conducted during my stay at the Division of Gastroenterology and Hepatology, University of North Carolina (UNC), NC, USA.

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List of abbreviations

ATC	Anatomical Therapeutic Chemical (code)
AUPD	Aarhus University Prescription Database
BMI	Body mass index
CD	Crohn's disease
CI	Confidence interval
CRS	Civil Registration System
DMBR	Danish National Birth Registry
DNRP	Danish National Registry of Patients
DPR	Danish Pathology Registry
GERD	Gastroesophageal reflux disease
H2RA	Histamine 2 receptor antagonist
IBD	Inflammatory bowel disease
ICD	International Classification of Diseases
IR	Incidence rate
IRR	Incidence rate ratio
NPV	Negative predictive value
OR	Odds ratio
PPI	Proton pump inhibitor
PPV	Positive predictive value
PY	Person years
SNOMED	Systematized Nomenclature of Medicine
Th	T helper (cells)
UC	Ulcerative colitis

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1 Introduction

The prevalence and incidence of asthma are increasing worldwide, and this increase is expected to continue in the future.[1,2] In many parts of the world, asthma is one of the most common reasons for emergency department visits and a leading cause of hospitalization in children.[3,4] Furthermore, the disease may inflict a substantial burden on affected individuals and their families and may have extensive health care expenditures.[5] Thus, asthma constitutes a significant global public health problem, though little is still known about its causes.

Emerging evidence proposes that several prenatal exposures, such as maternal disease and use of medications, may be involved in the development of childhood asthma.[6-8] In individuals, the use of proton pump inhibitors (PPIs) may cause disturbances in the immune system, leading to asthma development.[9] However, little evidence exists about whether prenatal exposure to PPIs increases the risk of asthma development. Concurrently with asthma, the prevalence and incidence of the autoimmune diseases, inflammatory bowel disease (IBD) and celiac disease, have also been increasing.[10-12] Furthermore, individuals with IBD and celiac disease are more likely to have asthma than individuals without these diseases.[13-20] This evidence suggests that asthma may share genetic and environmental risk factors with IBD and/or celiac disease. In individuals with IBD and celiac disease, the risk of asthma may be passed on to the next generation via a pathway of shared risk factors, but studies examining this potential association are practically non-existent.

This thesis describes three epidemiological studies. In study I, we examined whether prenatal exposure to PPIs is associated with asthma risk. In study II, we examined if parental IBD is associated with a risk of asthma in offspring. In study III, we examined if parental celiac disease is associated with a risk of asthma in offspring. Such knowledge will increase our understanding of asthma risk factors, which is important for developing primary (significant reduction or avoidance of risk factors) and secondary prophylactic strategies (identifying individuals at risk, facilitating treatment, and limiting disease progression[21-23]) to reduce the burden of asthma.

Before providing a more detailed description of the rationale behind the three studies, a short presentation of relevant clinical and epidemiological aspects of asthma will be introduced.

1.1 Clinical and epidemiological aspects of asthma

1.1.1 Asthma: The historical perspective

Asthma is currently recognized as a common chronic disease worldwide, and in the Western world asthma is *the* most common chronic disease in children.[2] The term "asthma" originates from the Greek verb *aazein*, meaning "panting" or "breathing hard", and the *Corpus Hippocraticum* by Hippocrates (460-360 BC) is the earliest text in which the word asthma is found as a medical term.[24] At the beginning of the 20th century, asthma was considered to be a psychosomatic disease and treated primarily through psychoanalysis. Asthma was not truly recognized as an inflammatory disease until the 1960s, when anti-inflammatory medications started being used.[25]

1.1.2 Asthma: Definition

Asthma is a complex and heterogeneous chronic inflammatory disease characterized by local inflammation, reversible obstruction of the bronchial airways, and bronchial hyper-reactivity.[26] Figure 1 illustrates how the airways are affected during an asthma attack. Asthma exists as two types: allergic (the most common type in children) and non-allergic.[26,27] Cytokines, which play an important role in asthma, are primarily derived from T helper type 2 (Th2) cells.[28-30] However, Th17 cell responses were also recently associated with asthma.[31] Asthma symptoms include coughing, particularly at night, wheezing, shortness of breath, and chest tightness.[26,32] The severity of the disease varies based on contributing risk factors and the individual's general health condition, and can range from mild to life-threatening.[26]

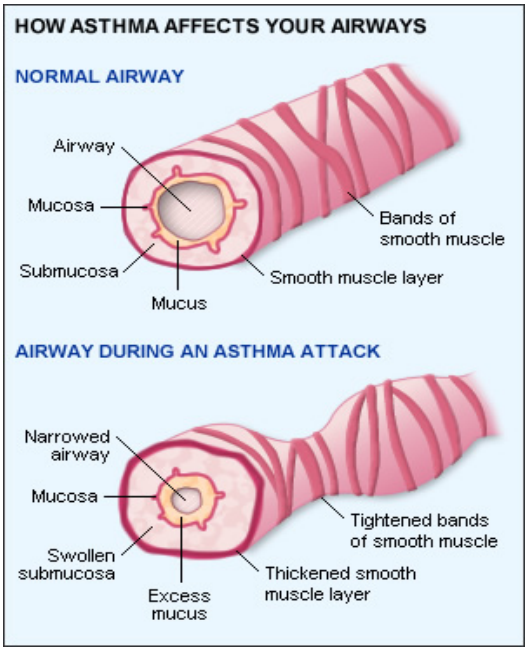

Figure 1: The effect of asthma on the airways

1.1.3 Asthma: The epidemic in numbers

During the second half of the 20th century, the prevalence and incidence of asthma increased worldwide, particularly in children, and asthma has now reached epidemic proportions in industrialized countries.[21,33-35] Notably, the prevalence of asthma is increasing in developing countries as they become more Westernized.[36] The World Health Organization (WHO) estimates that approximately 235 million people worldwide currently suffer from asthma and approximately 250,000 people die annually from the disease.[2,37] An estimated additional 100 million people will suffer from asthma by 2025.[1] High asthma prevalence has been reported in the United Kingdom,

New Zealand, Australia, the Republic of Ireland, Canada, and the United States, reaching up to 15%.[38] In some specific areas the prevalence is even higher in children.[1] The current mean prevalence of asthma among European children is nearly 10%,[39] but the reported prevalence varies according to the definition of asthma.

1.1.4 Asthma: Diagnosis and treatment

Asthma is diagnosed based on family history, clinical symptoms, lung function tests, and the response to medical treatment.[32,40] The lung function test includes peak expiratory flow (PEF) and forced expiratory flow in the first second (FEV1).[40] The lung function test is not reliable before 5-6 years of age.[5] Therefore, in small children the diagnosis of asthma is usually based on the child's history, symptoms, and response to anti-asthmatic therapy.[32] The goal of asthma treatment is to keep the symptoms under control. Symptoms can be alleviated using medications, including inhaled corticosteroids (long-term control) and inhaled beta2-agonists (symptom relief).[32,40] Avoidance of triggers is also a key component of improving control and preventing attacks.

1.1.5 Asthma: Risk factors

Risk refers to the probability that an individual will develop asthma, and exposures associated with an increased risk of asthma are referred to as risk factors.[41] Despite years of research, little is understood about the risk factors for asthma, but the key time period for asthma development is thought to occur between conception and early childhood.[42] Asthma has both genetic and environmental components, a so-called gene-by-environment interaction.[2,43,44] The increasing prevalence and incidence of asthma suggest that the impact of environmental factors is of major importance.[45] Some known risk factors involved in asthma development include low birth weight, being born preterm or by Caesarean section, allergies, childhood respiratory infections, high body mass index (BMI), and exposure to tobacco smoke.[24,42] In recent years, increasing attention has been paid to the impact of prenatal factors on asthma development, particularly prenatal exposure to certain drugs and toxicants, such as endocrine disrupting compounds, because these exposures may cause disturbances in fetal immune and/or lung development.[6,7,46-48] Moreover, vitamin D deficiency may explain some of the asthma epidemic because vitamin D is an important immune system regulator.[49,50] Furthermore, maternal vitamin D deficiency during pregnancy may lead to an imbalance in the developing fetal immune system and cause structural effects on fetal lung development, ultimately leading to asthma.[50-52]

The three studies in this thesis were designed as observational etiological studies, not to be confused with prediction studies. Etiological studies are characterized by a defined hypothesis about a potential causal association between exposure and outcome.[53] Association refers to a

statistical dependence between the relevant exposure and outcome and may be positive (exposure associated with higher risk), negative (exposure associated with lower risk), or no association at all.[41] Each study included in this thesis examines a specific exposure to evaluate if it is associated with an increased asthma risk in offspring. In contrast to an etiological study, a prediction study does not involve the cause of the outcome. Briefly, the purpose of a prediction study is to predict the outcome for future patients based on a number of clinical and/or non-clinical characteristics that do not necessarily affect the outcome.[53]

The three exposures included in this thesis are prenatal exposure to PPIs, parental IBD, and parental celiac disease. These exposures will be presented in the following section, as well as the rationale leading to our hypothesis. The existing literature relating to each study as identified through a structured literature search will also be presented.

2 Background

2.1 Prenatal exposure to proton pump inhibitors and asthma in offspring (study I)

2.1.1 Proton pump inhibitors

PPIs were introduced on the Danish market during the 1980s. All PPIs except omeprazole and lanzoprazole, which became over-the-counter drugs in December 2006 and May 2007, respectively,[54] are dispensed only by prescription. PPIs are used in the treatment of a number of gastrointestinal disorders, including gastroesophageal reflux disease (GERD) and ulcers of the stomach and duodenum.[55] These drugs are named “proton pump inhibitors” because they work by inhibiting the hydrogen-potassium adenosine triphosphatase enzyme system (K+/H+ - ATPase, also called the proton pump) of the gastric parietal cells (stomach acid producers). The proton pump is directly responsible for secreting H+ ions into the stomach cavity, and PPIs are the most potent inhibitors available.[55,56] A drug type alternative to PPIs in the treatment of GERD and ulcers is histamine 2 receptor antagonists (H2RAs), which decrease acid secretion by interfering with the h2-receptor.[57]

2.1.2 Hypothesis

During pregnancy, the intra-abdominal pressure increases, which may lead to the appearance or exacerbation of symptoms of GERD; GERD symptoms occur in up to 80% of pregnancies.[58] These symptoms are often treated with lifestyle changes and antacids,[59] but sometimes treatment with PPIs may be necessary. PPIs are generally considered safe to use during pregnancy because they are not associated with an increased risk of major congenital birth defects, perinatal mortality, morbidity, or spontaneous abortions.[60,61] However, some PPIs are now sold as over-the-counter drugs, which may cause the initiation of self-treatment, stressing the need for further investigation of their safety during pregnancy.

Although PPIs may alleviate asthma in patients with GERD,[62,63] acid-suppressive drugs, including PPIs, are also associated with allergic sensitization,[9,64] but the underlying mechanisms are speculative. Gastric acid suppression may interfere with the normal digestion of peptides/antigens in the adult stomach, inducing a Th2 response and immune (Ig)-E sensitization of the immune system.[9,64] Potentially, these antigens could be transferred to the fetus through the placenta and cause a Th2 bias in the fetus. A study in pregnant mice showed that exposure to acid-suppressive drugs induced a Th2 dominant immune response in their offspring,[65] a condition that may predispose the fetus to allergies and asthma.

We hypothesized that prenatal exposure to PPIs may be associated with an increased risk of asthma.

2.1.3 Existing literature

To conduct a review of the existing literature, we searched the MEDLINE database for studies examining the association between prenatal exposure to PPIs and asthma. The following search was limited to studies in humans that were written in English and last conducted in January 2012, before the publication of our study I. We used the following query:

"Proton Pump Inhibitors"[MeSH] AND ("Prenatal Exposure Delayed Effects"[MeSH] OR "Pregnancy"[MeSH]) AND "Asthma"[MeSH]

This search resulted in only one hit, but it was a relevant study.[66]

Next, we expanded our literature search by broadening the exposure and outcome definitions using the following query:

("Gastroesophageal Reflux/drug therapy"[MeSH] OR "Antacids"[MeSH] OR "Anti-Ulcer Agents"[MeSH]) AND ("Pregnancy"[MeSH] OR "Prenatal Exposure Delayed Effects"[MeSH]) AND "Immune System Diseases"[MeSH]

This resulted in three additional hits, none of which were relevant.

Given the few hits produced by the previous searches, we used a free-text search as follows:

"acid-suppressive" AND ("pregnancy" OR "prenatal exposure") AND "asthma".

This search resulted in only one study, which had already been found in the initial search.[66]

Finally, we reviewed the reference list of the included article and articles that cite it, but no additional studies were identified.

In summary, in a Swedish population-based cohort study, the authors examined the association between prenatal exposure to acid-suppressive drugs and childhood allergies, including asthma[66] (Table 1). The authors found that prenatal exposure increases the risk of allergies (odds ratio [OR]=1.43, 95% confidence interval [CI]: 1.29-1.59). However, when examining asthma and other allergies separately, the risk was only present for asthma (OR=1.51, 95% CI: 1.35-1.69). Estimates were adjusted for year of birth, parity, maternal age, maternal smoking during pregnancy, and maternal BMI. Although stratified ORs were not included for asthma, the authors reported that the association was unaffected by the type of acid-suppressive drug (PPIs, H2RAs, and others) or the timing of exposure during gestation (1st trimester versus later in pregnancy).

2.1.4 Limitations of the existing literature

We were only able to identify one study examining the association between prenatal exposure to PPIs and risk of asthma in offspring.[66] The relevant study by Dehlink et al., though large, was limited by potential measurement error associated with the use of the mothers' self-reported use of acid-suppressive drugs. The study did not completely ascertain asthma diagnosis due to a lack of information on outpatient diagnoses, and asthma medication was used to define asthma only for the last two years of the study period.[66] Therefore, the study primarily represents severe asthma leading to hospitalization. Based on the sparse evidence and relevant limitations, conclusions about the potential association cannot be drawn.

2.1.5 Studies published recently

In February/March 2014, when initiating the writing of this thesis, we updated our literature search to look for studies published after January 2012. We used the same search strategy in MEDLINE as described in section 2.1.3. This search revealed four new relevant studies examining the association between prenatal exposure to acid-suppressive drugs and asthma risk, all of which suggest an increased risk of asthma[6,67-69] (Table 1). One study examined several prenatal drug exposures and did not specify the type of acid-suppressive drugs included. The authors reported an aOR of 1.32 (95% CI: 1.12-1.55) when excluding women using asthma drugs during pregnancy.[6] The three studies that explicitly included PPIs[67-69] reported an association within the range of aHR=1.35 (95% CI: 0.94-1.94)[68] to aOR=2.76 (95% CI: 0.93-8.17).[69] Estimates for prenatal exposure to other acid-suppressive drugs were largely similar to those of PPIs. The studies will be discussed in section 6.3.1.

Table 1. Studies of the association between prenatal exposure to acid-suppressive drugs and asthma in offspring

Author/year of publication	Design/data source[a]/ country	Study population/ study-period	Type of acid-suppressive drug exposure	Asthma definition (outcome)	Main results[b]
Published *before* our study I					
Dehlink et al., 2009.[66]	Nationwide population-based cohort study/the Swedish Birth Registry/Sweden.	585,716 children born in 1995-2004. Follow up from birth through 2006.	H2RAs, PPIs, and other (other includes: prostaglandins, combinations for eradication of *Helicobacter pylori*, and/or other drugs for GERD).	A hospital diagnosis of asthma or at least two prescriptions for an asthma drug (ATC-code R03) (asthma drugs included from 2005-2006). Note: Other allergies were also examined.	Adjusted OR=1.51 (95% CI: 1.35-1.69).
Published *after* our study I					
Källén et al., 2013.[6]	Nationwide population-based cohort study/the Swedish Birth Registry/Sweden.	685,015 children born in 1999-2007. Follow-up from 2005 (or later) through 2009. Note: Study population partly overlaps the study by Dehlink et al.	Drugs used for GERD. Note: Drugs for GERD not specified. Multiple prenatal drug exposures were examined.	Asthma defined as at least five prescriptions for an asthma drug. Note: Asthma drugs not specified.	Adjusted OR=1.60 (95% CI: 1.40-1.76) and 1.32 (95% CI: 1.12-1.55) when excluding women using asthma drugs during pregnancy.
Hak et al., 2013[69]	Bi-directional cross-over study/ General Practitioners Research Database/UK	1,874 children with asthma and 1,874 siblings without asthma born during 1996-2010.	PPIs, H2RAs, and/or other antacids used for GERD.	A diagnosis of asthma and at least three prescriptions for an asthma drug within 12 months after first asthma diagnosis.	Any acid-suppressive drug: adjusted OR=1.23 (95% CI: 1.01-1.51). PPIs and/or H2RAs: adjusted OR=1.72 (95% CI: 1.00-2.98). PPIs: adjusted OR=2.76 (95% CI: 0.93-8.17). H2RAs: adjusted OR=1.56 (95% CI: 0.85–2.90). Other: adjusted OR=1.16 (95% CI: 0.95-1.42).

Mulder et al., 2013[67]	Bi-directional cross-over study and case control study/pregnancy database from the University of Groningen/ the Netherlands.	Cross-over design: 1,253 children with asthma and 1,253 siblings without asthma. Case control design: 1,235 children and 8771 controls. All born during 1995-2006.	Drugs used to treat acid-related disorders (ATC-code A02B).	Asthma defined as at least two prescriptions for an asthma drug (ATC-code R03) within a 6 month period.	Crossover study: OR=1.85 (95% CI: 1.07-3.13). Case-control study: adjusted OR=1.52 (95% CI: 1.11-2.10).
Mulder et al., 2014[68]	Population-based cohort study/ pregnancy database from the University of Groningen/the Netherlands.	33,536 children born from 1995-2011. Followed from birth and until their 8th birthday or end of study period. Note: Study population overlaps the study by Mulder et al., 2013.	PPIs and/or H2RAs.	Asthma defined as at least two prescriptions for an inhaled corticosteroid within a 12 month period. Note: Other allergies were also examined.	PPIs and/or H2RAs: aHR=1.57 (95% CI: 1.20-2.05). PPIs: aHR=1.35 (95% CI: 0.94-1.94). H2RAs: aHR=1.93 (95% CI=1.25-3.00).

Abbreviations: see list of abbreviations

[a] Data source for identification of study participants.

[b] Only results for asthma outcomes are included in the table.

2.2 Parental inflammatory bowel disease and asthma in offspring (study II)

2.2.1 Inflammatory bowel disease

IBD is an autoimmune disease and a collective term for Crohn's disease (CD) and ulcerative colitis (UC).[10,70] IBD involves chronic inflammation with tissue damage in the gastrointestinal tract. UC only affects the large bowel, whereas CD may affect any part of the gastrointestinal tract.[70,71] A differential diagnosis of CD and UC is achieved through pathognomonic clinical manifestations and endoscopic appearance.[70]

IBD is caused by a complex interaction between genetic and environmental factors, but the exact causes are largely unknown.[70-72] Some known risk factors for IBD are family history, smoking (increases the risk of CD while conferring protection against UC[73]), living in northern areas, use of antibiotics, and specific infections.[70,73,74] Also, vitamin D deficiency and endocrine-disrupting compounds may be implicated in IBD development because they may cause a disruption in the immune system.[75,76] However, whether endocrine-disrupting compounds are involved in IBD development is speculative. Apart from being a proposed risk factor for IBD, vitamin D deficiency may also be a consequence of IBD, particularly in patients with CD.[77,78]

Both CD and UC are more common in the Western world than Africa, Asia, or South America.[70] However, over the past few decades the incidence and prevalence of IBD have been increasing worldwide, and today CD affects up to 0.3% and UC up to 0.5% of the European population.[79] Symptoms of IBD can come and go over long periods of time and vary from mild to severe. The symptoms may be similar for CD and UC and include diarrhea, nutritional deficiency, abdominal pain, weight loss, and fatigue.[70] CD has been thought to be a Th1-mediated disease, whereas UC does not fit as nicely into the Th1/Th2 paradigm.[80,81] More recently, Th17 have been reported to be involved in the pathogenesis of both CD and UC.[72,81]

2.2.2 Hypothesis

Asthma and IBD tend to coexist in individuals, which has been reported in the literature as far back as the 1970s.[82] During the 2000s, evidence from studies of IBD and asthma also suggested that the two diseases may be associated in adult individuals,[13-16] with estimates of association ranging from OR=1.5 (95% CI: 1.4.1.6)[14] to OR=3.0 (95% CI: 0.31-28.84).[13] The coexistence in individuals suggests that the two conditions share genetic risk factors, and some shared genetic loci have been identified.[83] Furthermore, the concurrent increasing incidence of asthma and IBD suggests shared environmental risk factors; although little is known about such potential shared risk factors, they may include endocrine-disrupting compounds and vitamin D deficiency.[47,49,52,76]

It is plausible that the risk of asthma in individuals with IBD may be passed on to their children via a pathway of shared risk factors and/or maternal vitamin D deficiency during pregnancy. Therefore, we hypothesized that parental IBD increases the risk of asthma in offspring.

2.2.3 Existing literature

We searched MEDLINE for studies examining the association between parental IBD and asthma in offspring. We limited the search to studies in humans that were written in English. The following search was last conducted in May 2013 before the publication of study II.

We used the following query:

"Inflammatory Bowel Diseases"[MeSH] AND "Family"[Mesh] AND "Asthma"[MeSH]

This resulted in seven hits, one of which was of some relevance.[84]

Next, we broadened the outcome definition:

"Inflammatory Bowel Diseases"[MeSH] AND "Family"[MeSH] AND ("Respiratory Tract Diseases"[MeSH] OR "Immune System Diseases"[MeSH])

This search resulted in 27 hits. After reviewing all titles, six abstracts, and two full articles, one was found to be relevant.[85]

Based on the first query, we broadened the exposure definition:

("Digestive System Diseases"[MeSH]) OR "Autoimmune Diseases"[MeSH]) AND "Family"[MeSH] AND "Asthma"[MeSH]

This search resulted in 73 hits, and after reviewing all titles and three abstracts, we found no additional relevant studies.

Finally, we searched for more studies using the following free text terms in different combinations: "*Crohn's disease", "ulcerative colitis", "Inflammatory bowel disease", "pregnancy", "parental", "mothers", "fathers", "maternal", "paternal", "asthma", "offspring", and "children".*

The majority of the studies identified in this search focused on short-term birth outcomes in women with IBD. However, one additional study examining health outcomes, including asthma, in children born to mothers with IBD was identified.[86] We decided to not include the study because of a lack of quality; for example, percentage calculations in the paper did not match the actual numbers for asthma, among other outcomes, questioning the underlying basis.

In summary, in a Canadian single center matched prevalence study the authors examined the prevalence of 10 immune-related disorders, including asthma, in families of children with IBD compared to those without IBD.[84] Children were matched for age and sex (Table 2). Although examining a different association than what we were searching for, we included the study because of its relevance to shared risk factors. The authors observed no higher prevalence of asthma in relatives of children with UC (OR=1.08, 95% CI: 0.87-3.89) compared to relatives of children without UC. For relatives of children with CD the OR was 1.84 (95% CI: 0.87-3.89). A nationwide Swedish cohort study examined associations between IBD and 32 immune-mediated diseases, including asthma, in family members[85] (Table 3). The authors reported a standardized incidence ratio for asthma in offspring with parental CD of 1.1 (95% CI: 1.0-1.2), and 1.2 (95% CI: 1.1-1.3) in offspring with parental UC. Estimates were adjusted for age, sex, period, region, and socioeconomic status. Maternal and paternal IBD were not examined separately.

When updating the literature search in February/March 2014, we found no additional studies.

2.2.4 Limitations of the existing literature

When reviewing the two studies revealed in the structured literature search, we observed some limitations. The study by Sibtain et al. had a small study size, causing imprecise estimates, and used a comparison cohort that was not identified from the general population.[84] Also, unmeasured confounding, such as smoking status, may be present in both studies.[85,84] In the study by Hemminki et al., outpatient diagnoses were not included in the definition of asthma and IBD.[85] In addition, the study did not use asthma drugs as a proxy for milder asthma not requiring a hospital contact.[85] Based on the sparse evidence and related limitations, no conclusions can be drawn about whether parental IBD is associated with asthma in offspring.

Table 2. Studies examining the association between IBD and asthma among family members

Author/year of publication	Design/data source[a]/country	Study population/period	Asthma definition (outcome)	Main results[b]
Sibtain et al., 2011.[84]	Single center matched prevalence study/IBD clinic and emergency rooms/Canada	108 children (≤18 years of age) with IBD: 59 had CD and 49 had UC, and 108 children free of IBD. The children were identified during 2007-2009. Note: Controls were identified from a general gastrointestinal clinic.	Self-reported asthma in relatives confirmed in patient records. Note: a total of 10 immune-mediated diseases were examined.	ORs for asthma in family members of children with IBD : IBD: 1.32 (95% CI: 0.75-2.30) CD: 1.84 (95% CI: 0.87-3.89) UC: 1.08 (95% CI: 0.34-3.37)
Hemminki et al., 2010.[85]	Population-based cohort study/ multigeneration registry/ Sweden.	Individuals from a multigeneration registry identified and followed during 1964-2004. 25,846 with UC and 18,885 with CD.	Asthma was defined as a first-time in-patient hospital diagnosis of asthma. Note: 32 immune-related diseases, including asthma, were examined.	Standardized incidence ratios for asthma according to CD in: A parent: 1.1 (95% CI: 1.0-1.2) Sibling: 1.1 (95% CI: 0.7-1.7) Both parent and sibling: 1.6 (95% CI: 1.1-2.3) Twins: 1.0 (95% CI: 0.2-3.5) Spouses: 1.0 (95% CI: 0.8-1.2) Standardized incidence ratios for asthma according to UC in: A parent: 1.2 (95% CI: 1.1-1.3). Sibling: 1.1 (95% CI: 0.7-1.8) Both parent and sibling: 1.2 (95% CI: 0.8-1.7) Twins: 0.9 (95% CI: 0.2-2.8) Spouses: 1.1 (95% CI: 0.9-1.2)

Abbreviations: See list of abbreviations

[a] Data source for identification of study participants

[b] Only results for asthma outcomes are included in the table

2.3 Parental celiac disease and asthma in offspring (study III)

2.3.1 Celiac disease

Celiac disease is an autoimmune disease of the small intestine that involves an abnormal immune reaction to the protein gluten and affects genetically susceptible individuals.[87] The disease causes damage to the small intestine by inducing villous atrophy and may prevent the absorption of some nutrients.[87] Celiac disease can be treated by a lifelong gluten-free diet. Patients may present with mild to severe symptoms or no symptoms at all ("a silent course"). Frequent gastrointestinal symptoms are diarrhea, steatorrhea, chronic fatigue, and sometimes weight loss.[88] Because of the location of the disease, patients with celiac disease may suffer from vitamin D deficiency.[89]

The prevalence and incidence of celiac disease are increasing, particularly in Europe and North America.[11,90] Recent screening studies suggest that the prevalence in several European countries ranges from about 0.5 – 1.5%,[90] whereas the prevalence of diagnosed celiac disease is about 0.1% in Denmark.[12,91] The incidence among Danish children and adolescents has increased from 2.8 per 100,000 in 1996 to 10.0 per 100,000 in 2009,[12] which may be due to improvements in detection, as well as changes in causal environmental exposures.[12,92] Celiac disease is primarily regarded as being derived from a Th1 response, but Th17 cells are also now recognized as being involved.[93,94] The etiology of celiac disease is poorly understood, but it involves an interaction between genes and environmental factors.[87,95] Not many risk factors are known besides a family history.[96] Yet, autoimmune diseases (e.g., type 1 diabetes mellitus and thyroid disease), rotavirus infection, and no or early termination of breastfeeding are recognized risk factors.[87,96] Among more speculative risk factors is vitamin D deficiency.[97]

2.3.2 Hypothesis

Although asthma is primarily regarded as a disease with a Th2 response and celiac disease a Th1 response, recent studies have provided convincing evidence that the two diseases are associated in individuals,[17-20] with estimates of relative risk for asthma in individuals with celiac disease ranging from 1.61 (95% CI: 1.50-1.72)[19] to 9.26 (95% CI: 6.02-14.29).[17] Although the reason for this association is not entirely clear, it may be explained by genetics, and the HLA gene family has been found to be involved in the development of both diseases.[98,99] Also, a concurrent increase in incidence has been observed for asthma and celiac disease, suggesting that the two diseases may share environmental risk factors, but little is known about such potential shared risk factors.

Based on the plausibility of a pathway with shared risk factors and/or potential vitamin D deficiency in mothers with celiac disease, we hypothesized that parental celiac disease increases the risk of asthma in offspring.

2.3.3 Existing literature

We searched MEDLINE for studies examining the association between parental celiac disease and asthma in offspring. We used the same limitations and MeSH term algorithms as in study II, replacing the MeSH term *"Inflammatory Bowel Diseases"* with the MeSH term *"Celiac Disease".* The search was last updated in March 2014.

"Celiac Disease"[MeSH] AND "Family"[Mesh] AND "Asthma"[MeSH]

This searched resulted in 0 hits. We repeated the search by 1) searching without the MeSH term "Family" and 2) using the same three terms as free text terms instead of MESH terms. These two searches resulted in 32 and 8 hits, respectively. All titles and a total of three abstracts were reviewed, but none were relevant.

Next, based on the initial search we added to the outcome definition:

Celiac disease AND "Family"[MeSH] AND ("Respiratory Tract Diseases"[MeSH] OR "Immune System Diseases"[MeSH])

This search resulted in 36 hits. We reviewed all titles and eight abstracts, one of which was of some relevance.[100]

Based on the first query, we added to the exposure definition:

("Digestive System Diseases"[MeSH] OR "Autoimmune Diseases"[MeSH]) AND "Family"[MeSH] AND "Asthma"[MeSH]

This search resulted in 75 hits. We reviewed all titles and 11 abstracts, none of which were relevant.

To search for more studies, the following free-text terms were used in different combinations: *"celiac disease", "pregnancy", "parental", "mothers", "fathers", "maternal", "paternal", "asthma", "offspring", and "children".* One additional study with some relevance was found.[101]

When reviewing the reference list of the two relevant articles and of articles that cite them, no further studies were identified.

Although neither of the two identified studies examined parental celiac disease and asthma risk in offspring, we included them because they involved a family comparison[100,101] (Table 3). In an Italian study the authors examined allergy and asthma prevalence in adults with untreated celiac disease compared to the allergy and asthma prevalence in their celiac disease-free first-degree relatives (including children) and spouses.[100] Among those with celiac disease, 38 (3.6%) had asthma, and the corresponding numbers were 102 (3.7%) and 13 (4.1%) among first-degree relatives and spouses, respectively. The authors reported that no statistically significant difference in asthma prevalence was observed. Another, but older, Italian case control study was also identified. The study included 82 children with celiac disease, sex- and age-matched with 180 controls.[101] The prevalence of asthma was compared between first-degree family members of cases and controls. One mother (1.2%) and seven siblings (5.8%) had asthma among the cases, compared to two fathers (1.1%) and nine siblings (5.1%) among controls. The authors reported that no statistically significant difference of asthma prevalence in family members was observed.

2.3.4 Limitations of the existing literature

We were unable to identify any study examining the association between parental celiac disease and asthma in offspring. The two studies used a within family comparison, but interpretation of their results is problematic considering the limitations. Among the limitations of the two studies are small sample size[101] and a lack of relative estimates.[100,101] Decisions regarding differences in asthma prevalence were based solely on significance (p-values).[100,101] Finally, in one study it was not clear how cases and controls were selected. Cases were described as children with confirmed celiac disease, but information on where the children were identified was not provided.[101] Controls were described as being drawn from well-baby clinics and out-patient clinics in which they may be treated for various medical conditions, excluding celiac disease.[101]

Table 3. Studies examining the association between celiac disease and asthma among family members

Author/year of publication	Design/data source[a]/country	Study population/period	Asthma definition (outcome)	Main results[b]
Ciacci et al., 2004[100]	Single center prevalence study/ gastrointestinal unit/Italy.	1044 adults with celiac disease diagnosed during 1992-2002. 2752 relatives and 318 spouses free of celiac disease from the same household as the diseased.	Self-reported asthma confirmed by relevant clinical testing. Note: A total of seven allergies were examined.	Prevalence of asthma: In patients with celiac disease: 38 (3.6%) In first-degree relatives: 102 (3.7%) In spouses: 13 (4.1%) P-value=non-significant when comparing patients with celiac disease to relatives and spouses.
Greco et al., 1990[101]	Case control study/ Italy. Note: Data source described as "clinics" but not specified in the text.	82 cases (2-11 years of age) with celiac disease, 180 controls. Study period is not defined.	Data on asthma were obtained from medical records confirmed by relevant clinical testing. Note: A total of eight allergies were examined.	Prevalence of asthma in relatives of cases: Mothers: 1 (1.2%) Fathers: - Siblings: 7 (5.8%) Prevalence of asthma in relatives of controls: Mothers: - Fathers: 2 (1.1%) Sibling: 9 (5.1%) No overall difference in asthma prevalence was detected between relatives of the case and the control group. P-value=non-significant.

Abbreviations: See list of abbreviations

[a] Data source for identification of study participants

[b] Only results for asthma outcomes are included in the table

3 Aims of the thesis

After conducting a structured literature search of the existing literature, we concluded that evidence concerning the three relevant exposures and risk of asthma in offspring was either very sparse with limitations to account for or virtually non-existent. Consequently, there were gaps to be filled in the literature which warranted further investigation. To address these gaps in the existing literature, we conducted three studies with the following aims based on our hypotheses:

Study I: To examine if prenatal exposure to PPIs is associated with asthma by conducting a population-based cohort study in Northern Denmark comparing children prenatally exposed to PPIs to children not exposed during gestation. Furthermore, a comparison cohort was used to examine if prenatal exposure to H2RAs is associated with asthma.

Study II: To examine if parental IBD is associated with asthma in offspring by comparing offspring with parental IBD to offspring without parental IBD in a nationwide population-based setting.

Study III: To examine if parental celiac disease is associated with asthma in offspring by comparing offspring with parental celiac disease to offspring, matched by birth-year, without parental celiac disease in a population-based nationwide setting.

4 Methods

4.1 Setting

All three studies were conducted as population-based cohort studies. Study I was conducted within the population of Northern Denmark (Central Denmark Region and North Denmark Region), which is a mixed rural/urban area with approximately 1.8 million people and a setting that has been used to conduct a number of epidemiological studies. Studies II and III were conducted within the entire Danish population of approximately 5.5 million people. In Denmark, a tax-financed care system is provided equally to all citizens, including free access to hospital care. All three studies were based on data from Danish administrative and health registries. Each registry is introduced below.

4.2 Data sources: The Danish registries

Because of a long and well established tradition of collecting data, Denmark has a variety of both national and regional medical registries covering various characteristics related to births, deaths, diseases, and drug use, among others. Because of this tradition Denmark has high-quality data covering the whole population during long periods of time.[102] The registries are an essential data source in Danish epidemiology, and thus in this thesis. The registries used in the thesis are listed in Table 4.

4.2.1 The Danish Civil Registration System

The Danish Civil Registration System (CRS) was established in 1968 and is the backbone of all Danish registry-based research.[103-105] All registries are linkable via the 10-digit personal identifier, the CPR number, assigned at birth or emigration by the CRS. The CPR number includes date of birth and a gender-specific code. The CRS holds computerized records regarding all Danish citizens who have been born in or immigrated to Denmark and is updated daily. The CRS includes information about emigration, immigration, and vital status.[104,105]

4.2.2 The Danish Medical Birth Registry

The Danish Medical Birth Registry (DMRB) holds computerized records of all live births and still births from 1973 onwards among women with a permanent residence in Denmark.[105] By law, the attending midwife must send a notification of the birth to the CRS and a medical notification to the DMBR.[106,107] The data include the CPR numbers of the mother and the newborn. Some data about the newborn that are included are sex, date of birth, multiplicity of gestation, birth weight, body length, fetal presentation, gestational age, 1- and 5-minute Apgar scores, and mode of delivery. Maternal data include number of previous stillbirths, live births, age at delivery, marital status, smoking during pregnancy (from 1991 onwards), pre-pregnancy body mass index (from

2004 onwards), and citizenship, among others. Furthermore, fathers' identities (CPR numbers) have been recorded in the DMBR since 1991.[105]

4.2.3 The Danish National Patient Registry

The Danish National Patient Registry (DNPR) was established in 1977 and holds records of all inpatient contacts since that time.[108,109] Contacts to emergency rooms, outpatient clinics, and psychiatric hospitals have been registered since 1995. Data include CPR number, date of admission and discharge, diagnosis code (one primary and up to several secondary diagnoses), major treatments and procedures, and surgical procedures. Diagnoses are assigned by discharging physicians according to the International Classification of Diseases (ICD). The 8th revision (ICD-8) was used until 1994 when the 10th revision (ICD-10) was introduced.[105,108,109]

4.2.4 The Danish Pathology Registry

The National Pathology Registry (DPR) has recorded all pathology diagnoses in Denmark according to Systematized Nomenclature of Medicine (SNOMED)[110] codes since 1997. In addition to the CPR number and diagnosis, the DPR also includes the date of the test, requisition number, and the requesting hospital department or general practitioner. The DPR is based on data from the Danish Pathology Databank, which is updated daily and used in clinical practice by Danish pathologists.[111,112]

4.2.5 Aarhus University Prescription Database

Aarhus University Prescription Database (AUPD) records prescription medications that receive general or conditional reimbursement and are dispensed at the community pharmacies of the Central Denmark Region and North Denmark Region. The main variables in addition to CPR numbers are Anatomical Therapeutic Chemical (ATC) code, date of sale, and package identifier (enabling identification of brand, pack size, and dose units). Information on indications and the prescribed daily dose is not available. The database covers the population of the Central Denmark Region and North Denmark Region. These are two of the five Danish regions and have a combined population of 1.8 million inhabitants, corresponding to one-third of the Danish population. Following the municipal reform that went into effect on 1 January 2007, regions replaced counties as administrative units in Denmark. In the database, data have been available from the former North Jutland County since 1992, from the former Aarhus County since 1996, and from the former Viborg and Ringkjøbing counties since 1998. Since the municipal reform, the Aarhus University Prescription Database has been merging prescription data from the community pharmacies of the Central Denmark Region and North Denmark Region.[105,113]

Table 4. Danish health registries included in this thesis

Registry	Start (year)	Unit of observation	Utilized content
The Civil Registration System (CRS)[103-105]	1968	Person	CPR numbers, follow-up, death and emigration, maternal county of residence at time of giving birth.
The Danish Medical Birth Registry (DMBR)[105,107]	1973	Birth/person	CPR numbers, parental age at child birth, child's sex, mode of delivery, birth weight, mode of delivery, birth order, gestational age, multiple births, birth order, the first day of mother's last menstrual period, maternal smoking during pregnancy, and maternal pre-pregnancy BMI.
The Danish National Patient Registry (DNPR) [108,109]	1977	Hospital contacts	CPR numbers, asthma diagnoses, IBD diagnoses, celiac disease diagnoses, date of diagnoses, operation codes for Caesarean section.
The Danish Pathology Registry (DPR)[111,112]	1997	Histopathologi-cal specimens	CPR numbers, SNOMED codes for celiac disease, and date of diagnosis.
The Register of Medicinal Product Statistics (RMPS)[114]	1994	Prescriptions	CPR numbers, asthma medication prescriptions, antibiotic prescriptions, ATC codes, date of dispensation.
Aarhus University Prescription Database (AUPD) [105,113]	1989-1998 depending on the area	Prescriptions	CPR numbers, PPI prescriptions, H2RA prescriptions, asthma medication prescriptions, antibiotic prescriptions, ATC codes, date of dispensation, and pack size.

Abbreviations: see list of abbreviations

4.2.6 The Register of Medicinal Product Statistics

The Register of Medicinal Product Statistics (RMPS) is a nationwide registry initiated in 1994 that is complete from 1995 onwards.[114] The statistics in the registry are based on data submitted from the pharmacies, hospital pharmacies, and Danish Serum Institute. However, only data from the pharmacies are individual-level information. In addition to the CPR number, the register stores detailed information on the prescribed drugs, such as the drug name and dosage, ATC code, defined daily dose, and number of pills per package. However, information on indications and the prescribed daily dose is not available.

We linked data on children and their parents using the CPR number in all three studies.

4.3 Study design

The three studies were designed as population-based cohort studies using data obtained from the registries mentioned above.

4.4 Study population

In all three studies, we identified children and their parents from the DMBR. In relevant cases where the father's CPR number was missing in the DMBR we used the CRS for identification (e.g. before 1991). In study I we included all singletons born alive in Northern Denmark (the Central and North Regions of Denmark) from 1996 to 2008, but children whose mothers did not have a full medical history during the relevant pregnancy were not included. In study II we included children born alive in Denmark between 1979 and 2009. Finally, in study III we included children born alive in Denmark from 1979-2009 whose parents had a medical history of celiac disease. For each child with a parental history of celiac disease we randomly matched 100 children without such a history that were born in the same calendar year as the children with parental celiac disease. In studies II and III all parents had to have at least a 2-year medical history in Denmark before the relevant child birth for the children to be included in the studies.

4.5 Exposure definitions

4.5.1 Study I: Prenatal exposure to PPIs

In study I, the exposure of interest was prenatal exposure to PPIs. Data on maternal prescriptions dispensed during the relevant pregnancy were extracted from the AUPD using ATC codes. We defined prenatal exposure to PPIs as at least one maternal PPI prescription from 30 days preceding the first day of the last menstrual period until birth. We also defined trimester-specific exposure: the first trimester was defined as the first 12 weeks of pregnancy counted from the first day of the last menstrual period; the second and third trimesters (examined together) were defined as the remainder of the pregnancy.[115] According to our exposure classification, children exposed during the first trimester could also be exposed during the second and third trimester, but not vice versa. We also included H2RAs in the study to determine if the putative association between prenatal exposure to PPIs and asthma is related to acid-suppressing drugs as a class or their indication. Data on maternal use of H2RAs were also obtained from the AUPD using the same criteria as for PPIs.

4.5.2 Study II: Parental IBD

In study II, the exposure of interest was parental IBD. We collected information (ICD codes) from the DNRP on paternal IBD before the relevant pregnancy (the first day of the last menstrual

period +14 days) and on maternal IBD before giving birth. IBD was defined as an inpatient, outpatient, or emergency room diagnosis of CD or UC. If both CD and UC diagnoses were present for the same parent, the most recently recorded diagnosis was used to classify parental disease. Parental IBD was categorized as follows: parental IBD, maternal IBD only, paternal IBD only, parental CD, maternal CD only, paternal CD only, parental UC, maternal UC only, and paternal UC only. To determine if the supposed association may be mediated by pregnancy complications in mothers with IBD, we measured maternal disease activity during pregnancy by counting the number of IBD-related admissions during pregnancy and until the day before delivery. To avoid misclassifying planned follow-up visits as disease flares, we used only IBD-related hospital stays lasting 2 days or longer.

4.5.3 Study III: Parental celiac disease

In study III, the exposure of interest was parental celiac disease. We used the DNRP to collect information (ICD codes) on paternal celiac disease before the relevant pregnancy and on maternal celiac disease before giving birth. We defined celiac disease as an inpatient, outpatient, or emergency room diagnosis of celiac disease. We divided parents with celiac disease into the following categories: parental celiac disease, maternal celiac disease only, and paternal celiac disease only. To refine the definition of celiac disease, we linked biopsy data recorded in the DPR and, from 1997 onwards, defined celiac disease in parents using both hospital diagnoses and biopsy results.

4.6 Outcome/asthma definition

In study I, we defined asthma based on an algorithm combining a hospital diagnosis of asthma and data on asthma medication. The inclusion of asthma medication to define asthma enabled us to include children with less severe asthma treated in general practice.[116] For the medication-based criterion, we required a minimum of two dispensations of an inhaled β-agonist *and* a minimum of two dispensations of an inhaled corticosteroid (i.e., minimum four dispensations in total) to ensure ongoing use as a proxy for chronic disease. A child was considered to have asthma if the child had an inpatient, outpatient, or emergency room diagnosis of asthma recorded in the DNRP *or* if the child fulfilled the dispensation algorithm in the AUPD. The date of asthma onset was the date of the first hospital diagnosis or fulfilled medication algorithm, whichever was earliest.

In studies II and III, asthma was defined as an inpatient, outpatient, or emergency room asthma diagnosis recorded in the DNRP and the date of asthma was the date of the first hospital diagnosis of asthma. However, in a restricted population of children born from 1996 onwards, we used the same definition of asthma based on a hospital diagnosis of asthma and data on asthma

medication as in study I. In this restricted population the date of asthma onset was the same as defined in study I.

4.7 Covariates

To describe the children's characteristics and perform adjusted analyses relevant to each study we included *a priori* selected risk factors for asthma identified from relevant registries (Table 4). In study I, we used the DMBR to define the covariate *mode of delivery* (vaginal birth vs. Caesarean section). In studies II and III this covariate was created by combining data from both the DMBR and the DNPR to allow for the inclusion of surgical procedure codes.

All codes used to define the study variables are listed in Table 5.

Table 5. Utilized codes by classification system

Disease, drugs, and procedures	**Codes according to applied classifications**	
Diagnosis	**ICD-8**	**ICD-10**
Crohn's disease	563.01	K50
Ulcerative colitis	563.19, 569.04	K51
Celiac disease	269.00	K90
Asthma	493	J45, J46
Drug use	**ATC**	
PPIs	A02BC	
H2RAs	A02BA	
Inhaled β-agonists	R03AC	
Inhaled corticosteroids	R03BA	
Systemic antibiotics	J01	
Biopsy	**SNOMED**	
Celiac disease (small intestine)	T64020+M58018 T65110+M58018 T64310+M58018 S62180	
Operation		
Caesarean section	636.20, 651.90, 660.20, 660.40	KMCA (except KMCA20)

Abbreviations: See list of abbreviations

4.8 Permissions

All three studies were approved by the Danish Data Protection Agency and conducted in accordance with the guidelines for Good Epidemiological Pratice.[117] No further permissions are needed to conduct studies without intervention or participant contact in Denmark.

4.9 Statistical analyses

In study I, statistical analyses were performed using SAS software version 9.2 (SAS Institute INC., Cary, North Carolina). In studies II and III, analyses were performed using Stata software version 12.0 (StataCorp LP, College Station, TX, USA).

4.9.1 Descriptive statistics

In all three studies we examined the distribution of children's characteristics at birth as relevant to each study according to exposure status. We also estimated the median follow-up time and age at asthma onset.

4.9.2 Absolute risk

In study I, we used the Kaplan-Meier method (1-KM) to estimate the 2- and 10-year (time from birth) absolute risk of asthma according to prenatal PPI and H2RA exposure. In studies II and III, we estimated the 2- and 10-year cumulative incidence of asthma as an estimate of the absolute risk according to parental IBD and celiac disease status, respectively, treating death as a competing risk.[118]

4.9.3 Cox proportional hazards regression analyses

In all three studies, follow-up started at the time of birth and ended on the date of asthma onset, emigration, death, or the end of the study period, whichever came first. In study I, follow-up was continued throughout 2009; in studies II and III, follow-up continued throughout 2010. Therefore, in study I we had a maximum follow-up of 14 years (1996-2009), and in studies II and III the maximum follow-up was 32 years (1979-2010).

In studies I and II, we calculated incidence rates (IRs) and used Cox proportional hazards regression to compute crude and adjusted hazard ratios with 95% CIs as estimates of incidence rate ratios (IRRs/aIRRs). In study III, we calculated IRs and used stratified Cox proportional hazards regression (children were matched by year of birth) to compute IRRs and aIRRs with 95% CIs. The Cox proportional hazards model does not make any assumption about the shape of hazards over time, whether it is decreasing, increasing, or constant, but it assumes that the general shape is the same for everyone.[119] In each study, the assumption of proportional hazards was checked graphically by log (-log) plots and found to be fulfilled.

In study I, we examined the risk of asthma in children with prenatal exposure to PPIs anytime during gestation by comparing exposed children to the reference cohort of children not exposed to PPIs at any time during gestation. Analyses were adjusted for year of birth, mother's county of residence when giving birth, sex of child, gestational age, birth order, mode of delivery, maternal smoking and use of antibiotics during pregnancy, mother's age at delivery, and maternal asthma

(using the same asthma definition as for the children). We also examined asthma risk according to trimester-specific exposure and stratified based on number of pills dispensed (≤ 28 pills vs. > 28 pills). Using the same methodology and analyses, we also examined asthma risk in children with prenatal exposure to H2RAs any time during gestation. However, when stratifying based on number of pills dispensed, we used the categories ≤20 vs. >20. Finally, we examined if maternal PPI and H2RA use in the year after birth, but not during pregnancy, was associated with an increased risk of asthma in the children. Children whose mothers did not use PPIs or H2RAs during either of those periods were the reference groups.

In study II, we examined the risk of asthma by comparing children with a parental history of IBD to children without such a parental history. We adjusted for year of birth, sex of child, mode of delivery, mother's age at delivery, birth order, multiple births, and maternal and paternal asthma (defined by hospital asthma diagnoses). Asthma risk was also examined according to maternal disease activity during pregnancy based on the categories of the mother's CD- and UC-related hospital admissions during the relevant pregnancy (number of admissions: 0, 1, or ≥2). This analysis was performed in the entire cohort and separately among children born at term (week 37 or later).

In study III, we examined asthma risk by comparing children with a parental history of celiac disease to children without this parental history with adjustment for child's sex, mode of delivery, mother's age at delivery, birth order, multiple births, and paternal and maternal asthma (defined by hospital asthma diagnoses).

4.9.4 Sensitivity analyses

We conducted a number of sensitivity analyses to determine the robustness of our findings by examining how "sensitive" the observed results are to changes in the definitions of methods, values of variables, or assumptions.[41]

In study I, we conducted the following sensitivity analyses among children exposed to PPIs: 1) we defined asthma as a hospital diagnosis of asthma (i.e., excluding asthma prescriptions), 2) we changed the earliest PPI exposure period from 30 days to 0 days and 60 days before pregnancy, and 3) as a diagnosis of asthma cannot be made with certainty before 5 years of age, we repeated all PPI and H2RA analyses for starting follow-up at 5 years.

In studies II and III, we conducted the following sensitivity analyses: 1) we stratified the analyses according to children born before and after 1995 using a period of inclusion of outpatient diagnoses (study II), 2) because celiac disease may have a silent course for years, we changed the window for parental medical history from 2 years to 10 years before the relevant child birth

(study III), and 3) we repeated the analyses with a cohort restricted to children born in 1996 or later to allow for inclusion of asthma medication in the asthma definition. In these analyses we also adjusted for maternal smoking and antibiotic use during pregnancy (studies II and III). In addition, 4) we repeated the analyses starting follow-up at the age of 5 years. These later analyses were performed for both the entire cohort and for children born in 1996 or later (studies II and III).

Finally, in studies I and II we also adjusted for maternal pre-pregnancy BMI in a subset of children born in 2004 or later. Data on maternal BMI were not available from the DMBR before 2004.

5 Results

5.1 Study I: Prenatal exposure to PPIs

5.1.1 Descriptive data

We identified 197,060 singletons (51.3% boys) born in 1996 to 2008. A total of 2,238 (1.1%) children had been exposed prenatally to PPIs, including 1,238 (55.3%) exposed during the first trimester. High maternal age at delivery, maternal asthma, maternal use of antibiotics during pregnancy, and high pre-pregnancy BMI were more prevalent in children prenatally exposed to PPIs. The distribution of characteristics is shown in Table 6. The median follow-up time was 6.8 years, and by the end of follow-up 24,506 (12.4%) children had asthma. The median age at asthma diagnosis was similar among the unexposed and exposed children: 1.5 years and 1.6 years, respectively.

5.1.2 Absolute risk estimates

Among children without prenatal exposure to PPIs, the 2-year risk of asthma was 7.5% and the 10-year risk was 14.4%. The corresponding estimates in prenatally exposed children were 12.2% and 21.1%, respectively. Estimates were similar among those prenatally exposed to H2RAs (results not shown).

5.1.3 Cox proportional hazards regression analyses

The aIRR for asthma when comparing children exposed prenatally to PPIs to those who were not exposed was 1.41 (95% CI: 1.27-1.56). The risk was elevated among children exposed to more than 28 pills compared to those exposed to 28 or fewer PPI pills (Table 7). When examining the risk of asthma according to trimester of exposure, no notable differences were observed: the aIRR was 1.46 (95% CI: 1.27-1.67) when exposed in the first trimester and 1.34 (95% CI: 1.15-1.56) when exposed in the second and/or third trimester.

Among the 197,060 children, 1,605 (0.8%) had been exposed prenatally to H2RAs. Among these children we observed estimates similar to those for PPIs. However, we noted that the association did not vary according to the cumulative dose as measured by the number of pills (Table 7).

Table 6. Characteristics of 197,060 children born in Northern Denmark in 1996-2008 according to prenatal exposure to proton pump inhibitors (PPIs).

	Exposed to PPIs during gestation (N=2,238)		Not exposed to PPIs during gestation (N=194,822)	
Characteristics	**N**	**%**	**N**	**%**
Maternal use of PPI pills				
≤ 28 pills	837	37.4	-	-
> 28 pills	1,401	62.6	-	-
Sex of child				
Girl	1,127	50.4	94,864	48.7
Boy	1,111	49.6	99,958	51.3
Gestational age (weeks)				
<37	144	6.4	9,638	5.0
37-41	1,962	87.7	170,707	87.6
≥42	132	5.9	14,477	7.4
Birth order				
1	861	38.5	80,109	41.1
2	771	34.5	73,699	37.8
≥3	606	27.1	41,014	21.1
Mode of delivery				
Missing	14	0.6	3,821	2.0
Caesarean	535	23.9	31,673	16.3
Vaginal	1,689	75.5	159,328	81.8
Maternal age at delivery				
<25	267	11.9	24,281	12.5
25-29	684	30.6	71,193	36.5
30-34	779	34.8	69,144	35.5
≥35	508	22.7	30,204	15.5
Maternal smoking during pregnancy				
Missing	53	2.4	4,055	2.1
No	1,647	73.6	153,634	78.9
≤10 cigarettes/day	378	16.9	28,317	14.5
>10 cigarettes/day	160	7.2	8,816	4.5
Maternal use of antibiotics during pregnancy				
Yes	1,147	51.3	62,060	31.9
Maternal asthma				
Yes	205	9.2	8,309	4.3
Maternal BMI[1]	(N=1,431)		(N=77,240)	
15-19.9	161	11.3	10,364	13.4
20-24.9	610	42.6	39,831	51.6
25-29.9	378	26.4	17,238	22.3
≥30	282	19.7	9,807	12.7

Abbreviations: see list of abbreviations
[1] Children born in 2004 or later, N=78,671

Maternal use of PPIs in the year after birth, but not during pregnancy, was associated with an increased risk of asthma among offspring, with estimates similar to those in the main analysis. However, the association was weak for maternal use of H2RAs (Table 8).

Table 7. Incidence rates and incidence rate ratios for asthma in children born in 1996-2008 in Northern Denmark according to prenatal exposure to PPIs and H2RAs any time during gestation (N=197,060)

	N=197,060 n	Children with asthma N=24,506 n (%)	IR (per 1000 PY)	IRR (95% CI)	aIRR* (95% CI)
Exposure					
No PPI exposure	194,822	24,125 (12.4)	19.4	1.00	1.00
PPI exposure	2,238	381 (17.0)	39.8	1.63 (1.48-1.81)	1.41 (1.27-1.56)
≤28 pills	837	132 (15.8)	30.0	1.37 (1.16-1.63)	1.20 (1.01-1.43)
>28 pills	1,401	249 (17.8)	48.1	1.82 (1.60-2.06)	1.54 (1.36-1.75)
No H2RA exposure	195,455	24,191 (12.4)	19.5	1.00	1.00
H2RA exposure	1,605	315 (19.6)	31.4	1.61 (1.44-1.80)	1.47 (1.32-1.65)
≤20 pills	223	43 (19.3)	28.5	1.54 (1.14-2.08)	1.44 (1.06-1.95)
>20 pills	1,382	272 (19.7)	31.9	1.62 (1.44-1.82)	1.48 (1.31-1.67)

Abbreviations: See list of abbreviations

*Adjusted for year of birth, sex of child, gestational age, mode of delivery, birth order, mother's age, county, maternal smoking during pregnancy, maternal asthma, and maternal use of antibiotics during pregnancy.

Table 8. Incidence rate ratios for asthma in children born in Northern Denmark according to maternal use of proton pump inhibitors (PPIs) and H2-receptor antagonists (H2RAs) in the year after birth but not during pregnancy

	n	Children with asthma n (%)	IRR (95% CI)	aIRR* (95% CI)
Exposure				
No PPI use during pregnancy or the year after birth	192,307	23,698 (12.3)	1.00	1.00
Maternal PPI use in the year after birth but not during pregnancy	2,515	427 (17.0)	1.55 (1.41-1.71)	1.32 (1.20-1.46)
No H2RA use during pregnancy or the year after birth	194,760	24,077 (12.4)	1.00	1.00
Maternal H2RA use in the year after birth but not during pregnancy	695	114 (16.4)	1.24 (1.03-1.49)	1.13 (0.93-1.36)

Abbreviations: See list of abbreviations

*Adjusted for year of birth, sex of child, gestational age, mode of delivery, birth order, mother's age, county, maternal smoking during pregnancy, maternal asthma, and maternal use of antibiotics during pregnancy.

5.1.4 Sensitivity analyses

The analyses defining asthma by only a hospital diagnosis of asthma (10,632 children with asthma) yielded fairly similar results for PPI exposure as the full cohort analyses (aIRR = 1.19, 95% CI: 1.01-1.40; other results not shown). The estimates were not affected much after changing the earliest PPI exposure to 0 and 60 days before pregnancy (results not shown). Finally, when starting follow-up at 5 years of age (N=129,888, children with asthma=4966), we observed results similar to those of the full cohort analyses (overall aIRR=1.38, 95% CI: 1.00-1.89; other results not shown). When starting follow up at 5 years for children prenatally exposed to H2RAs, the results were similar to those for PPIs (overall aIRR=1.58, 95% CI: 1.24-2.00). Additional adjustment for maternal pre-pregnancy BMI did not change the estimates (results not shown).

5.2 Study II: Parental IBD

5.2.1 Descriptive data

We identified 1,845,281 children (51.3% boys), 14,952 (0.8%) of whom had a parent with a history of IBD. Maternal IBD comprised 57% of all parental IBD. Advanced maternal age at birth, being born by Caesarean section, and parental asthma were more prevalent among children with parental IBD compared to children without parental IBD. Maternal smoking, use of antibiotics during pregnancy, and low maternal pre-pregnancy BMI were more prevalent in children with parental CD than among children with parental UC and no parental IBD. The distribution of characteristics is shown in Table 9. A total of 106,939 (5.8%) children were diagnosed with asthma during follow-up, and the median follow-up time was 14.9 years. Median age at asthma onset was 1.6 years for children with parental CD, 1.9 years for children with parental UC, and 3.2 years for children without parental IBD.

5.2.2 Absolute risk estimates

Among children with parental CD, the 2-year risk of asthma was 3.7% and the 10-year risk was 6.9%. Corresponding results among children with parental UC were 2.8% and 5.6%, respectively, and among children without parental IBD 2.3% and 5.0%, respectively.

Table 9. Characteristics of children born in Denmark during 1979-2009 according to the presence of parental IBD type (N=1,845,281)

	Parental Crohn's disease (n=5,106)	Parental ulcerative colitis (n=9,846)	No parental IBD (n=1,830,329)
Characteristics	**n (%)**	**n (%)**	**n (%)**
Parental IBD			
Maternal	3,102 (60.8)	5,473 (55.6)	-
Paternal	2,004 (39.2)	4,373 (44.4)	-
Sex of child			
Female	2,478 (48.5)	4,816 (48.9)	890,959 (48.7)
Male	2,628 (51.5)	5,030 (51.1)	939,370 (51.3)
Gestational age (weeks)			
<37	416 (8.3)	754 (7.7)	103,264 (5.6)
37-41	4,387 (85.9)	8,334 (84.6)	1,538,939 (84.1)
≥ 42	279 (5.5)	674 (6.5)	145,334 (8.0)
Missing	24 (0.5)	84 (0.9)	42,792 (2.3)
Mode of delivery			
Vaginal	3,776 (74.0)	7,618 (77.4)	1,548,727 (84.6)
Caesarean	1,330 (26.1)	2,228 (22.6)	281,602 (15.4)
Birth order[1]			
1	2,374 (46.5)	4,115 (41.8)	827,582 (45.2)
≥2	2,732 (53.5)	5,731 (58.2)	1,002,747 (54.8)
Multiple birth			
Yes	183 (3.6)	359 (3.7)	59,838 (3.3)
Mother's age at delivery (years)			
<25	626 (12.3)	973 (9.9)	371,149 (20.3)
25-34	3,885 (76.1)	7,585 (77.0)	1,296,539 (70.8)
≥35	595 (11.7)	1,288 (13.1)	162,641 (8.9)
Maternal asthma			
Yes	143 (2.8)	195 (2.0)	23,424 (1.3)
Paternal asthma			
Yes	107 (2.0)	179 (1.8)	17,830 (1.0)
	(n=4,001)	(n=7,018)	(n=860,655)
Prenatal exposure to antibiotics[2]			
Yes	1,474 (36.8)	2,326 (33.1)	273,913 (31.8)
Maternal smoking during pregnancy[2]			
No	2,974 (74.3)	5,860 (83.5)	665,964 (77.4)
≤10 cigarettes/day	640 (16.0)	703 (10.0)	122,659 (14.3)
>10 cigarettes/day	235 (5.9)	210 (3.0)	40,790 (4.7)
Missing	152 (3.8)	245 (3.5)	31,242 (3.6)
	(N=2185)	(N=3586)	(N=363,381)
Maternal pre-gravid body mass index[3]			
<18.5	127 (5.8)	184 (5.1)	16,577 (4.6)
18.5-24	1,269 (58.1)	2,116 (59.0)	209,956 (57.8)
25-29	424 (19.4)	656 (18.3)	71,261 (19.6)
≥30	220 (10.1)	388 (10.8)	39,881 (11.0)
Missing	145 (6.6)	242 (6.8)	25,706 (7.1)

Abbreviations: see list of abbreviations

[1]Children of multiple births are coded in same birth order

[2]Children born from 1996 and after, N=871,674

[3]Children born from 2004 and after, N=369,152

5.2.3 Cox proportional hazards regression analyses

The aIRR was 0.98 (95% CI: 0.91-1.04) for asthma associated with parental IBD, with similar results for parental/maternal/paternal CD and UC (Table 10).

We observed an increased risk of asthma among children born to mothers with two or more CD admissions during pregnancy (aIRR=1.74, 95% CI: 1.03-2.94; Table 11). However, when restricting this analysis to children born at 37 weeks or later, the aIRR decreased to 1.42 (95% CI: 0.76-2.64; other results not shown).

Table 10. Incidence rates and crude and adjusted incidence rate ratios for asthma in Danish children born during 1979-2009 according to parental IBD status (N=1,845,281)

	N	Children with asthma n (%)	IR (per 1,000 PY)	Crude IRR (95% CI)	aIRR* (95% CI)
Parental IBD					
No parental IBD	1,830,329	106,083 (5.8)	3.8	1.00	1.00
Parental IBD	14,952	856 (5.7)	5.6	1.20 (1.12-1.28)	0.98 (0.91-1.04)
Maternal IBD	8,575	492 (5.7)	5.4	1.19 (1.09-1.30)	0.97 (0.89-1.06)
Paternal IBD	6,377	364 (5.7)	5.8	1.22 (1.10-1.35)	0.99 (0.89-1.09)
Parental CD	5,106	328 (6.4)	6.9	1.41 (1.27-1.57)	1.09 (0.98-1.22)
Maternal CD	3,102	203 (6.5)	6.9	1.43 (1.25-1.64)	1.10 (0.95-1.26)
Paternal CD	2,004	125 (6.3)	6.8	1.38 (1.16-1.65)	1.09 (0.91-1.29)
Parental UC	9,846	528 (5.4)	5.0	1.10 (1.01-1.20)	0.92 (0.84-1.00)
Maternal UC	5,473	289 (5.2)	4.7	1.06 (0.94-1.19)	0.89 (0.80-1.00)
Paternal UC	4,373	239 (5.5)	5.4	1.15 (1.01-1.30)	0.94 (0.83-1.07)

Abbreviations: See list of abbreviations

*Adjusted for sex of child, year of birth, mode of delivery, multiple birth, birth order, mother's age at delivery, asthma in mother, and asthma in father.

Table 11. Risk of hospital (inpatient or outpatient) diagnosed asthma in Danish children born during 1979-2009 according to the number of maternal IBD-related admissions during pregnancy

Maternal IBD admissions during pregnancy	N	Children with asthma n (%)	Crude IRR (95% CI)	aIRR* (95% CI)
All children (N=1,845,281)				
No maternal IBD	1,836,706	106,447 (5.8)	1.00	1.00
Maternal CD				
No admissions	1,729	107 (6.2)	1.30 (1.08-1.57)	1.05 (0.87-1.27)
1 admission	1,235	82 (6.6)	1.54 (1.24-1.91)	1.08 (0.87-1.35)
≥ 2 admissions	138	14 (10.1)	2.15 (1.27-3.63)	1.74 (1.03-2.94)
Maternal UC				
No admissions	3,229	178 (5.5)	1.05 (0.91-1.22)	0.94 (0.81-1.09)
1 admission	2,015	97 (4.8)	1.06 (0.87-1.24)	0.80 (0.66-0.98)
≥ 2 admissions	229	14 (6.1)	1.18 (0.70-2.00)	1.04 (0.61-1.75)

Abbreviations: See list of abbreviations

*Adjusted for sex of child, year of birth, mode of delivery, multiple birth, birth order, mother's age at delivery, asthma in mother, and asthma in father.

5.2.4 Sensitivity analyses

Stratifying the results by calendar period (births before/after 1995) did not substantially change the estimates (results not shown). When restricting to children born in 1996 and later, adding asthma medication to the definition of asthma (N=871,674, children with asthma=106,732), and with additional adjustments for maternal smoking and use of antibiotics during pregnancy, we observed aIRRs similar to those of the full cohort analyses. The aIRR was 1.05 (95 % CI: 0.97-1.14) for parental IBD, 1.11 (95% CI: 0.98–1.25) for parental CD, and 1.01 (95% CI: 0.92–1.12) for parental UC. Similar results were observed for maternal and paternal CD/UC.

When starting follow-up at 5 years of age in the full cohort (N=1,581,040, children with asthma=50,978), the estimates remained unaffected. However, the association between two or more maternal CD-related admissions during pregnancy and asthma was no longer present (aIRR=1.08, 95% CI: 0.35-3.34; other results not shown). Restricting the analyses with follow-up starting at 5 years to children born in 1996 or later, no association was observed in the parental IBD groups (results not shown). Finally, additional adjustments for maternal pre-pregnancy BMI did not affect the results (results not shown).

5.3 Study III: Parental celiac disease

5.3.1 Descriptive data

We identified 1,107 children with a parental history of celiac disease and 110,700 children without such a history and matched by year of birth. Maternal celiac disease comprised 69% of all parental celiac disease. We observed only minor differences in the distribution of covariates (Table 12).

During up to 32 years of follow-up, 68 (6.1%) children with parental celiac disease and 6,057 (5.5%) children without parental celiac disease had an asthma diagnosis. The median follow-up was 7.4 years and the median age at asthma onset was 1.5 years for both children with and children without parental celiac disease.

5.3.2 Absolute risk estimates

The 2-year risk of asthma was 4.3% among children with parental celiac disease and 3.6% among children without parental celiac disease. After 10 years, the risks were 7.4% and 6.4%, respectively.

5.3.3 Cox proportional hazards regression analyses

We observed an overall aIRR of 1.10 (95% CI: 0.86-1.39) for asthma in offspring associated with parental celiac disease. Adjustments had practically no effect on the results. Corresponding estimates were observed for children with a mother or father with celiac disease (Table 13).

Table 12. Characteristics of children born in Denmark in 1979-2009 according to the presence of parental celiac disease (N=111,807)

	Parental celiac disease (n=1,107)		No parental celiac disease (n=110,700)	
Characteristics	n	%	n	%
Parental celiac disease				
Maternal	759	68.6	-	-
Paternal	348	31.4	-	-
Sex of child				
Male	568	51.3	56,742	51.3
Female	539	48.7	53,958	48.7
Birth order[1]				
1	536	48.4	48,149	43.5
≥2	571	51.6	62,551	56.5
Birth weight (kilograms)				
<2500	58	5.2	5,751	5.2
2500-3499	501	45.3	46,558	42.1
≥3500	543	49.1	57,914	52.3
Missing	5	0.5	477	0.4
Gestational age (weeks)				
<3700	68	6.1	7,133	6.4
37-41	967	87.4	95,432	86.2
≥42	66	6.0	7,543	6.8
Missing	6	0.5	592	0.5
Mode of delivery				
Vaginal	864	78.1	89,067	80.5
Caesarean section	243	21.9	21,633	19.5
Multiple gestation				
Yes	33	3.0	4,565	4.1
Mother's age at birth (years)				
<25	156	14.1	14,518	13.1
25-34	852	76.0	82,332	74.4
≥35	99	8.9	13,850	12.5
Maternal asthma				
Yes	39	3.5	2,648	2.4
Paternal asthma				
Yes	43	3.9	2,235	2.0
	(n=982)		(n=98,200)	
Maternal use of antibiotics during pregnancy[2]				
Yes	355	36.1	31,857	32.4
Maternal smoking during pregnancy[2]				
No	815	83.0	78,525	80.0
≤ 10 cigarettes/day	107	10.9	12,451	12.7
>10 cigarettes/day	40	4.1	4,289	4.4
Missing	20	2.0	2,935	3.0

Abbreviations: See list of abbreviations

[1]Children of multiple births are coded as having the same birth order

[2]Children born in 1996 and later

Table 13. Incidence rates and crude and adjusted incidence rate ratios for asthma in Danish children born during 1979-2009 according to parental celiac disease status (N=111,807)

	N	Children with asthma	IR (per 1,000 PY)	Crude IRR (95% CI)	Adjusted IRR* (95% CI)
Parental celiac disease					
No parental celiac disease	110,700	6,057	7.6	1.00	1.00
Parental celiac disease	1,107	68	8.5	1.12 (0.88-1.48)	1.10 (0.86-1.39)
Maternal	759	47	8.5	1.11 (0.83-1.48)	1.09 (0.82-1.45)
Paternal	348	21	8.5	1.13 (0.83-1.48)	1.10 (0.82-1.45)

Abbreviations: See list of abbreviations.
*Adjusted for sex of child, mode of delivery, multiple gestation, birth order, mother's age at delivery, asthma in mother, and asthma in father.

5.3.4 Sensitivity analyses

When changing the criteria for a parental medical history from a minimum of 2 years to a minimum of 10 years before the relevant child birth, we also observed similar results (results not shown). When repeating the analyses for children born in 1996-2009 and including asthma medication in the asthma definition (N=99,182, children with asthma=11,249), we found an aIRR of 1.14 (95% CI: 0.95-1.35) for parental celiac disease, with similar estimates for maternal and paternal celiac disease. Finally, when starting follow-up at 5 years of age in the full cohort (N=63,732, children with asthma=1,239), we observed an overall aIRR of 0.80 (95% CI: 0.43-1.48) for parental celiac disease and 1.21 (0.82-1.80) in children born in 1996 or later.

6 Discussion

This chapter covers the conclusions followed by a thorough discussion of the methodology used, and a discussion of the results in light of the existing literature.

6.1 Conclusions

6.1.1 Study I: Prenatal exposure to PPIs

In this population-based cohort study, we found that prenatal exposure to PPIs is associated with an increased risk of asthma. We also found a similar association for H2RAs. Because the observed association is not drug specific and also observed for maternal use in the year after birth, we could not rule out a manifestation of the 'class effect' of acid-suppressive medications or confounding by underlying maternal disease.

6.1.2 Study II: Parental IBD

In this population-based nationwide cohort study, our results reassuringly suggest that, even though IBD may be associated with asthma in individuals, parental IBD does not increase the risk of asthma in offspring. Our results were robust, even with several changes in the definitions of study variables.

6.1.3 Study III: Parental celiac disease

In this population-based nationwide cohort study, we observed no convincing evidence that parental celiac disease is associated with asthma in offspring. Our results did not change with changes in the definitions of study variables. Therefore, although asthma and celiac disease tend to be associated in individuals, our results encouragingly suggest that parental celiac disease does not increase the risk of asthma in offspring.

6.2 Methodological considerations

The three studies in this thesis aimed to examine whether prenatal exposure to PPIs, parental IBD, and parental celiac disease is associated with asthma in offspring. The estimates obtained in each study were the result of the methodology used, namely the study design, study conduct, and data analysis.[120] Our overall methodological goal in each study was to obtain precise and valid estimates of the association between the relevant exposure and the outcome of asthma, and estimates that are generalizable to relevant populations.[120] Valid estimates have little systematic error (commonly referred to as bias), whereas precise estimates have few random errors (or play of chance). Generalizability refers to the degree to which results hold true in other settings.[121] Given the population-based design in the setting of a health care system that guarantees free access to uniform health care, our results are likely to be highly generalizable.

Because several factors could potentially influence the validity of our results they must be carefully considered before confirming or rejecting a causal relationship.[122,123] The following encompasses an evaluation of how problems in the selection of and information on study participants, confounders, and chance may have influenced our results (Figure 2).

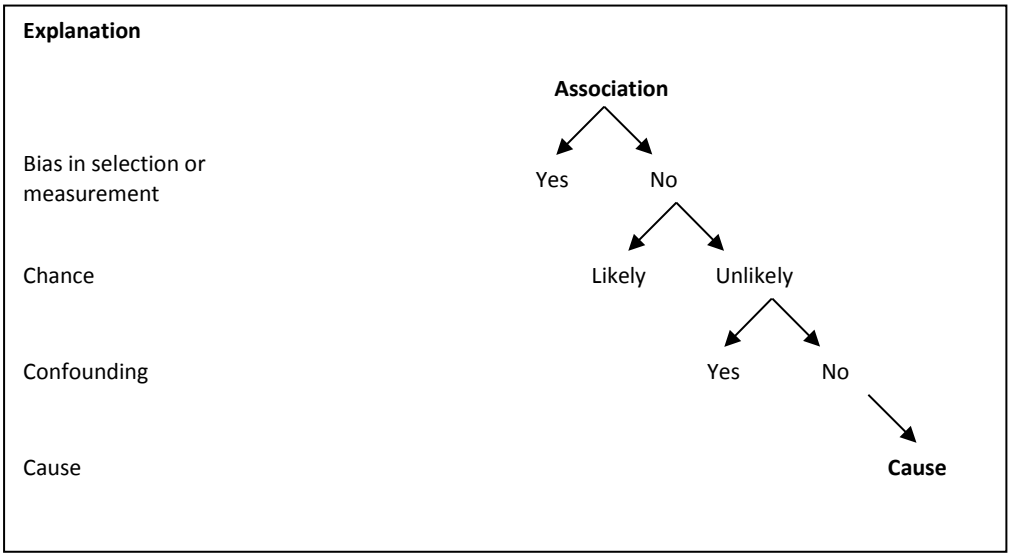

Figure 2. Association and cause (adapted from Fletcher and Fletcher[123])

6.2.1 Selection bias

Selection bias is usually defined as systematic error stemming from the procedures used to select subjects and from factors that influence study participation. This bias arises when the association between exposure and outcome is different for study participants and non-participants. As the association among non-participants is rarely known, selection bias cannot be observed, but only inferred.[124] A general strength when using population-based registries for research is the possibility of reducing selection bias when including study participants.

The three studies in this thesis were conducted in well-defined and fully enumerated study populations identified from the DMBR with universal access to health care and by using high-quality administrative and medical databases. Our studies involved no contact with the study participants and were conducted without their knowledge or acceptance, but with permission from relevant Danish authorities. Furthermore, complete follow-up of all children was ensured by the CRS. Taken together, these features virtually eliminate selection bias.

6.2.2 Information bias

Data quality is the Achilles heel of using registry data (or any other data) for research purposes. Erroneous information about the exposure and/or outcome can rarely be ruled out and may introduce bias in a study. Information about the relevant exposures and asthma in our studies was considered in categories and any information error would cause children and/or parents to be misclassified into incorrect categories. If, for example, the rate of misclassification of asthma depends on the status of the relevant exposure, such misclassification will be differential; if not, the misclassification is non-differential.[124] Differential misclassification may bias the estimate in an unpredictable manner. If there is an association, non-differential misclassification generally biases the relative estimate towards a null effect. However, when the exposure is measured in more than two categories, an association may be exaggerated.[124,125]

Proton pump inhibitors

In study I, the primary exposure was prenatal PPIs as identified from prescription data in the AUPD containing high-quality prescription data.[113] Because maternal drug use was measured by reimbursement records, we can rule out any misclassification caused by maternal recall bias. However, the use of prescriptions as a proxy for actual drug use is an imperfect measure of actual drug intake and its timing, potentially leading to misclassification of prenatal exposure to the drugs. As we do not expect such misclassification to be related to asthma, this could have underestimated the association. However, we expect this to only have had a minor impact on estimates because changing the earliest PPI exposure period from 30 days to 0 days and 60 days before pregnancy did not change the results. The same considerations apply to H2RAs.

Parental IBD

In study II, the exposure was parental IBD as defined by a hospital diagnosis of either CD or UC in the DNRP. A previous IBD validation study was carried out in the former county of North Jutland in Denmark based on hospital diagnoses of CD and UC from 1988-1992. The completeness of the regional hospital system when using the pathology data as a reference standard was 94% for both CD and UC. The positive predictive value (PPV) of DNRP-recorded diagnoses compared to a reference standard of either pathology data or fulfilled clinical criteria in medical records was 97% for CD and 90% for UC.[126] Thus, both CD and UC diagnosis have been proven to have high validity. Therefore, in our study the misclassification of CD and UC is limited, and non-differential misclassification of parental IBD is not expected to explain the observed null result.

Parental celiac disease

In study III, the exposure was parental celiac disease as defined by a hospital diagnosis of celiac disease in the DNRP and from 1997 onwards also by biopsy data from the DPR. To the best of our knowledge there are no substantial validation studies of celiac disease diagnoses.[12] Some degree of misclassification of parental celiac disease status is unavoidable, as is the case in any epidemiologic study; however, our results did not materially change after extending the minimum medical history requirement from 2 years to 10 years to define parental celiac disease. Although misclassification is likely to be non-differential and is therefore expected produce bias towards the null, only very severe misclassification would be expected to produce the null result in the present study.[125]

Asthma

In study I, asthma was defined as a hospital diagnosis of asthma and/or fulfillment of the asthma medication algorithm. In studies II and III, asthma was defined as a hospital diagnosis of asthma in the full cohort analyses, but it also included the asthma medication algorithm in analyses restricted to children born in 1996 or later.

To the best of our knowledge, no validation study of asthma diagnosis in the DNPR has been carried out in children under the age of 6 years. However, in children aged 6 to 14 years, the PPV of ICD-10 asthma diagnoses in the DNPR is 85% and the sensitivity is 90% when using hospital records as the reference standard (Table 14). This finding demonstrates high-quality recording of asthma diagnoses in the DNRP.[127] Still, when defining asthma based solely on a hospital diagnosis of asthma in the full cohort analyses in studies II and III, the misclassification of children with less severe asthma symptoms (e.g., those children treated in general practice) as non-asthmatic children is unavoidable. Such a misclassification is likely to be non-differential.

We sought to reduce this potential problem by using an asthma drug algorithm from 1996 onwards. Our medication algorithm originated from a study in the U.S. that included patients aged 5-45 years.[128] The U.S. study included individuals from a Health Maintenance Organization and found that at least one dispensation of an inhaled β-agonist and one inhaled corticosteroid have a PPV of 100% for identifying "any asthma" (definitive asthma, wheezing, chronic obstructive pulmonary disease, or allergy) and a PPV of 80% for "definitive asthma" when using medical records as the reference standard (Table 14). Though the U.S. is not directly comparable to a Danish setting, inhaled β-agonists and inhaled corticosteroids are the first choice treatment for asthma in children worldwide.[2] Furthermore, prescriptions for asthma drugs, including inhaled β-agonists and inhaled corticosteroids, were validated in a Danish setting among children aged 6-14 years and found useful in identifying asthmatic children[129] (Table 14).

To reduce the potential misclassification of children under 5 years of age with wheezing symptoms but not actual asthma, and to ensure the ongoing use of asthma drugs as a proxy for chronic disease, we required that the medication algorithm of at least one inhaled β-agonist and one inhaled corticosteroid be dispensed twice in order for a child to be counted as having asthma. Nonetheless, this algorithm is still likely to have captured some children who did not have asthma which could have caused bias towards the null. Still, as we observed similar results when starting follow-up at 5 years of age, such misclassification is not very likely to have had a major impact on the results in the three studies.

Table 14. Relevant asthma validation studies including children

Author, year of publication	Diagnosis/drug models validated	Reference standard	Validity estimates
Moth et al., 2007[127]	Discharge diagnoses of asthma in the DNPR in children aged 6-14 in 2002.	Medical records from 10 Danish hospitals.	Sensitivity: 0.90 Specificity: 0.99 PPV: 0.85 NPV: 0.99
Osborne et al., 1995[128]	Prescription data on asthma drugs in 1987-1990 among residents of the Kaiser Permanente Northwest Region aged 5-45 in 1987. Three models were validated: Model 1: At least two dispensations for inhaled β-agonists. Model 2: At least one dispensation for an inhaled β-agonists *and* one dispensation for an inhaled corticosteroid. Model 3: At least five dispensations for any asthma drug.	Medical records identified through a Health Maintenance Organization[a.]	Definitive asthma[b] Model 1: PPV: 0.42 Model 2: PPV: 0.80 Model 3: PPV: 0.78 For any asthma[c] Model 1, 2, and 3: PPV: 1.00
Moth et al., 2007[129]	Prescription data during 2002 on asthma drugs from two regional Danish prescription registries (Aarhus and Odense) for children aged 6-14. Three models were validated: Model 1: At least one dispensation for any asthma drug excluding β-agonists as liquid. Model 2: At least one dispensation for any asthma drug except for β-agonists as liquid and inhaled β-agonists only once. Model 3: A dispensation for any asthma drug except for β-agonists as liquid and inhaled β-agonists only once or inhaled corticosteroids only once.	Asthma discharge diagnoses from the DNRP or asthma diagnoses confirmed by the general practitioner.	Model 1: Sensitivity: 0.96 Specificity:0.43 Model 2: Sensitivity: 0.83 Specificity: 0.73 Model 3: Sensitivity: 0.63 Specificity: 0.86

Abbreviations: See list of abbreviations

[a] An organization that provides health coverage with providers under contract

[b] A clinical diagnosis of asthma

[c] Any clinical picture consistent with asthma including definitive asthma, chronic obstructive pulmonary disease with reactive airway disease and a history of allergy and infection with wheezing[128]

6.2.3 Confounding

Confounding is an essential issue in etiological studies. Confounding can be thought of as a confusion of effect, implying that the effect of an exposure is mixed with the effect of another

exposure that is extraneous to the research question under study. A confounder is usually defined as a variable that is: 1) associated with the exposure, 2) an extraneous risk factor for the outcome, and 3) not an intermediate step on the causal path between the exposure and outcome.[121] In observational studies, confounding can be handled in the study design through restriction or matching and/or in analyses through adjustment, standardization, and stratification.[120] In all three studies we sought to eliminate confounding by adjusting for various factors chosen *a priori,* and we also used matching in study III. A drawback of using registry data is the limited availability of information on potential confounders, as a registry may not collect all data relevant to a given hypothesis.

In general, the misclassification of confounders will result in residual confounding, meaning that confounding is still present after adjustments.[130] We cannot completely rule out residual confounding in our studies; for example, information on maternal smoking during pregnancy was based on self-reported data and may not be accurate. Also, for obvious reasons we were not able to rule out unknown confounding in our studies.

In study I, the main issue was the major challenge that any pharmacoepidemiological study meets, namely the phenomenon referred to as *confounding by indication.* Confounding by indication can rarely, if ever, be ruled out.[131] In study I, confounding by indication arose from the fact that the pregnant women who were using PPIs differed from those who were not using them according to the medical indication for which the drug was prescribed.[124] Because we also observed increased asthma risk in offspring prenatally exposed to H2RAs, we could not disentangle whether the observed effect was caused by a class effect of the drugs or by confounding by indication. GERD is associated with asthma in adults,[62,63,132] and even though we adjusted the analysis for maternal asthma, maternal GERD may also be associated with asthma in offspring. However, to the best of our knowledge this has not yet been examined. Furthermore, we observed an increased risk of asthma in the postnatal analyses of maternal use in the year after giving birth but not during pregnancy, arguing that our main results may be affected by confounding by indication. On the other hand, the year after birth, or part of it, is the breastfeeding period and we cannot rule out that the observed effect during this period stems from exposure through breastfeeding.

In study II, we also handled the issue of confounding by adjusting for potential confounders. However, we were not able to adjust for maternal smoking during pregnancy in the full cohort analyses and, as previously mentioned, residual confounding of maternal smoking could potentially be present in analyses from 1996 onwards. Still, when considering the overall null result, a confounder would have to be protective of asthma to explain this result. We cannot rule

out that the mothers with IBD may have taken vitamin supplements during pregnancy and, thus, an effect of maternal vitamin D deficiency during pregnancy would be difficult to detect.

In study III, we matched the children based on birth year and adjusted for potential confounders, but this adjustment had nearly no effect on estimates. Again, if confounding explains our results, we should look for a confounder with a protective effect on asthma. Breastfeeding may protect against the development of both asthma and celiac disease,[133] and women with celiac disease may be more aware of breastfeeding. However, such data are not available from the registries. In addition, we have no information on maternal use of vitamin supplements, as these are mainly bought over the counter.

6.2.4 Chance

Chance refers to random error that cannot be predicted, but it can be quantified by a measure of the statistical precision of the point estimate.[134] A small study has less statistical precision than a large study and would be subject to more random error.[135] In general, the statistical precision was high in our studies as reflected by the 95% CIs. In study III, however, we noted that our results were more prone to imprecision and, thus, more sensitive to chance.

Given the overall null findings, the association observed for two or more maternal CD admissions during pregnancy is likely non-causal and could stem from a chance finding. The risk disappeared when starting follow-up at 5-years of age, but these estimates had low statistical precision. Other explanations may be upward detection bias of asthma in small children with asthma-like symptoms. Furthermore, the association was reduced when restricting the analyses to children born at term suggesting that the association could be due to maternal pregnancy complications causing respiratory symptoms in children.

6.2.5 Summary: methodological considerations

The main strengths of the studies in this thesis are their population-based designs, large sizes, prospective data collection from medical registries, and virtually complete follow-up. Confounding cannot be completely ruled out in any of the three studies, but it is not likely to explain the null results observed in studies II and III. In study I, we were unable to rule out confounding by maternal indication. Non-differential exposure and outcome misclassification is to some extent present in our studies. Still, based on our discussion, we do not expect that such potential methodological explanations account for the observed null results in studies II and III. Finally, in studies I and II, chance, or random error, is an unlikely explanation for the results; however, our results in study III were more sensitive to chance.

6.3 Main findings in light of the existing literature

In the following three subsections, the findings of our three studies will be discussed in relation to the existing literature.

6.3.1 Study I: Prenatal exposure to PPIs

One previous study by Dehlink et al. examined the association between prenatal exposure to PPIs and asthma risk.[66] We aimed to examine the same association while addressing some of the limitations present in the Swedish study by Dehlink et al. by: 1) including outpatient asthma diagnoses; 2) including asthma prescriptions for the entire study period; 3) including data on PPIs and H2RAs based on reimbursement records; and 4) adjusting for some additional potential confounders. Our results clearly corroborate the results of the previous study. However, neither of the studies could rule out confounding by indication.

Since the publication of our study, four studies examining the association between prenatal exposure to acid-suppressive drugs and asthma have been conducted[6,67-69] (Table 1). All four studies reported an increased risk of asthma. In two studies, a sibling design was used as an attempt to address unmeasured confounding. Mulder et al. conducted a study using *both* a case-control design and a bidirectional case-crossover design, comparing the results from each design.[67] The latter design compared siblings (i.e., they had the same mother), and this study design is expected to address the issue of unmeasured confounding, such as maternal medical conditions. Interestingly, in this study both designs yielded similar results, increased asthma risk in exposed children. Hak et al. also designed a study using the sibling design/bidirectional case-crossover design.[69] The authors also reported increased asthma risk among children prenatally exposed to PPIs. In both studies, the authors concluded that the results are unlikely to be explained by unmeasured confounding. However, the sibling design may address confounding by indication to some extent, but only as long as discordant PPI status does not closely correlate with GERD symptoms.

Taken together, irrespective of design and setting, all relevant studies have reported results similar to ours, supporting that prenatal exposure to both PPIs and H2RAs is associated with an increased risk of asthma. However, no study has effectively been able to rule out confounding by indication, raising the question of whether a true causal relationship exists.

6.3.2 Study II: Parental IBD

As described earlier, previous studies have found that IBD and asthma are associated in individuals.[13-16] However, very few studies have examined whether this association is present within family members, and to the best of our knowledge we are the first to provide an exhaustive examination of the association between parental IBD and asthma in offspring.

In a small study, Sibtain et al. studied the prevalence of asthma in relatives of children with IBD[84] (Table 3), but a comparison to our study is difficult because of different exposure and outcome. However, the study by Sibtain et al. addressed an association between IBD and asthma by examining generations of the same family. The study found no increased risk of asthma in relatives when the exposure was UC in children. However, the study suggested an association between CD in children and asthma in their relatives. Notably, their results suffered from low statistical precision. Also, confounding could potentially explain the association with CD. The Swedish cohort study by Hemminki et al. suggested that parental UC may slightly increase the risk of asthma in offspring[85] (Table 3), which was not replicated in our study. However, our results virtually corroborate their results for CD. The slight differences in study results may be explained by differences in the factors adjusted for in the two studies. For example, the Hemminki study did not adjust for parental asthma or smoking. Also, Hemminki et al. examined the risks of 31 immune-related diseases in addition to asthma, increasing the risk of a chance finding. Thus, only minor differences were observed between previous study results and our study results, and these differences are likely explained by confounding factors. Though IBD and asthma are associated in individuals, parental IBD does not seem to increase the risk of asthma in offspring.

6.3.3 Study III: Parental celiac disease

Although earlier studies reported an association between celiac disease and asthma in individuals,[17-20] to the best of our knowledge, our study was the first to examine the association between parental celiac disease and asthma in offspring. Therefore, there is no study with which we can directly compare our results. However, we identified two studies that examined the occurrence of celiac disease and asthma among relatives[100,101] (Table 3). In a case-control study, the authors reported that the prevalence of asthma in relatives of children with celiac disease was not different from the prevalence of asthma in relatives of children without celiac disease.[101] However, some of the controls were from outpatient clinics, which could potentially bias the results toward no difference in asthma prevalence among relatives if asthma is more common in relatives of children with relevant disorders than among relatives of children from the general population. In another study, the authors reported that the prevalence of asthma in adult

individuals with celiac disease was similar to the prevalence among their first-degree relatives and spouses free of celiac disease but living in the same household.[100] Because individuals with celiac disease were not compared with the general population, the study did not provide any insight into shared environmental risk factors. However, the study may suggest that celiac disease and asthma do not share genetic risk factors. Although these studies, including ours, argue against the existence of shared risk factors for celiac disease and asthma, the results should be interpreted cautiously given the limitations. Based on our results, though celiac disease and asthma are associated in individuals, parental celiac disease does not seem to increase the risk of asthma in offspring.

7 Perspectives

Asthma is a common disease, inflicting a substantial burden on patients, families, and health care systems. To decrease this burden, evidence-based prevention strategies are essential and the identification of asthma risk factors warranted. This thesis has explored the intergenerational origins of asthma by examining prenatal exposure to PPIs and gastrointestinal morbidity in parents, addressing issues that were poorly documented in the literature.

Our work suggests that prenatal exposure to both PPIs and H2RAs is associated with an increased risk of asthma, which is corroborated by other studies. This finding highlights that the benefit-risk balance of the drugs should always be considered in pregnant women. However, because no study could effectively rule out confounding by indication, it may be premature for our results to have clinical implications. Also, because the potential biological mechanisms underlying the observed association are still speculative and unclear, more studies examining mechanisms are warranted before final conclusions can be drawn. We were not able to demonstrate an association between parental IBD or celiac disease and asthma in offspring. Therefore, future studies could examine other potential intergenerational risk factors.

Future research may address the following questions:

- If a true association exists, what is the biological mechanism underlying the association between prenatal exposure to PPIs and H2RAs and asthma development?
- Is maternal GERD associated with an increased risk of asthma in offspring?
- Can we identify other prenatal drug exposures associated with increased asthma risk in offspring?
- Could other parental immune-mediated disorders with increasing incidence and prevalence (e.g., diabetes type 1[136]) be associated with an increased risk of asthma in offspring?
- What can explain the coexistence of IBD and celiac disease and asthma in individuals?

The Danish health care system provides a unique and important source for large population-based studies on asthma by linking medical and administrative registries with the possibility to adjust for various potential confounders. However, the indication for prescribed drugs is currently not available in prescription registries; therefore, a desirable step forward in future pharmacoepidemiologic research would be reliable data on the indication for prescriptions in the registries. Also, because children with less severe asthma and pregnant women with GERD are often treated in general practice, future asthma research would be strengthened if diagnoses

made by general practitioners were linked with registry data. In Denmark in 1998, the International Classification of Primary Care was implemented in the electronic health record systems in general practice[137] and linking to these data would be highly relevant. Adding this information may also allow for even larger study sizes, increasing the precision of results. Multinational studies linking data from several registries, e.g. from the Nordic countries,[138] will also strengthen future research by producing larger study sizes.

8 Summary

For decades the prevalence and incidence of asthma has been increasing worldwide, and asthma has now reached epidemic proportions in industrialized countries. Increasing attention has been paid to prenatal drug exposures and their role in asthma development, but evidence regarding PPIs is sparse. Asthma is known to coexist with IBD and celiac disease in individuals and the prevalence and incidence of both IBD and celiac disease have been increasing concurrently with asthma, suggesting shared risk factors. However, studies examining the association between parental IBD and celiac disease and asthma risk in offspring are virtually non-existent.

We conducted three studies to examine if: I) prenatal exposure to PPIs is associated with asthma; II) parental IBD is associated with asthma in offspring; and III) parental celiac disease is associated with asthma in offspring.

Study I was conducted in Northern Denmark and included singletons born alive between 1996 and 2008 and followed from birth throughout 2009. Study II and III were conducted as nationwide studies in Denmark and included children born alive between 1979 and 2009 and followed from birth throughout 2010. We used the unique CPR number to link relevant data from Danish population-based administrative and medical registries.

In study I we identified 197,060 singletons. A total of 2,238 (1.1%) children had been exposed prenatally to PPIs, and during follow-up 24,505 (12.4%) children had asthma (defined by a hospital diagnosis and/or asthma drugs). Among children without prenatal exposure to PPIs the 10-year risk was 14.4%, and 21.1% among children with prenatal PPI exposure. We found an aIRR of 1.41 (95% CI: 1.27-1.56) when comparing children who were prenatally exposed to children who were not exposed. In a comparison cohort of children with prenatal exposure to H2RAs we found a similar increase in risk compared to children without prenatal exposure to H2RAs. The aIRRs for maternal PPI and H2RA use in the year after, but not during, pregnancy was 1.32 (95% CI: 1.20-1.46) and 1.13 (0.93-1.36. Starting follow-up at age 5 years did change the estimates.

In study II we identified 1,845,281 children. A total of 14,952 (0.8%) children had a parent with a history of IBD, and by the end of follow-up in 2010 a total of 106,939 (5.8%) children had a hospital diagnosis of asthma. The 10-year risk of asthma was 6.9% among offspring of parents with CD and 5.6% among offspring of parents with UC, and 5.0% among offspring of parents without IBD. The aIRR for asthma associated with parental IBD was 0.98 (95% CI: 0.91–1.04).

The aIRR was 1.09 (95% CI: 0.98–1.22) for parental CD and 0.92 (95% CI: 0.84–1.00) for parental UC. The results were similar regardless of parent of origin, when including asthma medication to define asthma, and when starting follow-up at 5 years of age.

In study III we identified 1,107 children with a parental history of celiac disease and 110,700 children without such parental history, matched by year of birth. By the end of follow-up in 2010, 68 (6.1%) children with parental celiac disease had a diagnosis of asthma. The corresponding number was 6,057 (5.5%) among those without parental celiac disease. The 10-year risk of asthma after birth was 7.4% among children with parental celiac disease and 6.5% among children without parental celiac disease. We found an aIRR of 1.10 (95% CI: 0.86-1.39) when comparing children with parental celiac disease to children without. Results were similar for maternal and paternal celiac disease, when including asthma medication in the asthma definition, and when starting follow-up at 5 years of age.

The results in study I suggested that prenatal exposure to PPIs may increase the risk of asthma, but we could not rule out a “class effect” of acid-suppressive drugs or underlying maternal condition. The results in study II reassuringly suggest that parental IBD is not associated with an increased risk of asthma in offspring. Finally, in study III we observed no convincing evidence that parental IBD is associated with an increased risk of asthma in offspring.

9 Dansk resume

I løbet af de seneste årtier har incidensen og prævalensen af astma været stigende verden over og sygdommen har nu nået epidemiske proportioner. Der er en øget opmærksomhed mod prænatal medicin eksponering og udviklingen af astma, men evidensen for PPI er sparsom. Samtidigt med astma har også incidensen og prævalensen af IBD og cøliaki være stigende. Astma forekommer ofte blandt personer med IBD og cøliaki og deler således muligvis genetiske og miljømæssige risikofaktorer med disse to sygdomme. På trods af dette er studier vedrørende associationen mellem IBD og cøliaki blandt forældre og risikoen for astma blandt deres børn storset ikke at finde i litteraturen.

På baggrund af ovenstående gennemførte vi 3 studier med følgende formål: I) at undersøge om prænatal eksponering for PPI er associeret med astma, II) at undersøge om IBD hos forældre er associeret med astma blandt børn, og III) at undersøge om cøliaki hos forældre er associeret med astma blandt børn.

Studie I blev udført i det tidligere Aarhus og Nordjyllands Amt og inkluderede børn født i live fra 1996 til og med 2008. Børnene blev fulgt fra fødslen til udgangen af 2009. Studie II og III inkluderede børn født i live i Danmark fra 1979 til og med 2009. Disse børn blev fulgt fra fødslen til udgangen af 2010. Data fra eksisterende danske administrative og medicinske registre blev koblet ved hjælp af CPR numre.

I studie 1 identificerede vi 197.060 børn. I alt havde 2.238 (1.1%) børn været prænatalt eksponeret for PPI. Ved slutningen af studieperioden i 2009 havde 24.505 (12.4%) børn udviklet astma (defineret ved en hospitalsdiagnose og/eller astma medicin). 10 år efter fødslen var den absolutte risiko for astma 14.4% blandt børn uden prænatal eksponering og den tilsvarende risiko var 21.1% blandt eksponerede børn. Vi fandt en justeret IRR på 1.41 (95% CI: 1.27-1.56) for astma når vi sammenlignede eksponerede børn med ikke-eksponerede børn. Vi anvendte en sammenlignings-kohorte af børn prænatalt eksponeret for H2RA og fandt lignende resultater. Justerede IRR'er for mødres brug af PPI og H2RA i året efter graviditet men ikke under graviditeten var 1.32 (95% CI: 1.20-1.46) og 1.13 (0.93-1.36), henholdsvis. Vi fandt lignende resultater, når vi startede follow-up ved 5 års alderen.

I studie 2 identificerede vi 1.845.281 børn. I alt 14.952 (0.8%) børn havde en forælder med IBD, og ved slutningen af studieperioden havde i alt 106.952 (5.8%) børn fået en hospitalsdiagnose for astma. Den absolutte risiko for astma 10 år efter fødslen var 6.9% blandt børn af forældre med Crohn's sygdom og 5.6% blandt børn af forældre med colitis ulcerosa og 5.0% blandt børn

af forældre uden IBD. Den justerede IRR var 0.98 (95% CI: 0.91–1.04) når vi sammenlignede børn af forældre med IBD med børn af forældre uden IBD. Vi fandt ens resultater uanset om det var mor eller far der havde IBD, når vi inkluderede astmamedicin i astma definitionen og når vi startede follow-up ved 5-års alderen.

I studie 3 identificerede vi 1.107 børn som havde en forælder med cøliaki og 110.700 børn med forældre uden cøliaki. Børnene var matchet på fødselsår. Ved slutningen af studieperioden havde 68 (6.1%) børn af forældre med cøliaki fået en hospitalsdiagnose for astma mod 6.057 (5.5%) børn af forældre uden cøliaki. Den absolutte risiko for astma 10 år efter fødslen var 7.4% blandt børn af forældre med cøliaki of 6.5% blandt børn af forældre uden. Vi fandt en justeret IRR for astma på 1.10 (95% CI: 0.86-1.39) og resultaterne var tilsvarende uanset om det var mor eller far som havde cøliaki. Resultaterne var også tilsvarende når vi inkluderede astmamedicin i astma definitionen og startede follow-up ved 5-års alderen.

I studie 1 fandt vi, at børn prænatalt eksponeret for PPI havde en øget risiko for at udvikle astma. Dog kunne vi ikke udelukke, at dette skyldes en effekt af syrehæmmende medicin som gruppe eller den underliggende sygdom hos moderen. Vores studier kunne ikke påvise en sammenhæng mellem IBD og cøliaki blandt forældre og astma blandt børn.

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Study I

AP&T Alimentary Pharmacology and Therapeutics

Prenatal exposure to acid-suppressive drugs and the risk of childhood asthma: a population-based Danish cohort study

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SUMMARY

Background
Proton pump inhibitors (PPIs) may activate the immune system and cause asthma.

Aim
To investigate the association of prenatal exposure to PPIs and histamine 2-receptor antagonists (H2RAs) with risk of asthma.

Methods
In this cohort study, 197 060 singletons born between 1996 and 2008 in northern Denmark were followed until the end of 2009. Data were obtained through Danish medical registries. Asthma in offspring was defined as at least two prescriptions of both a β-agonist and an inhaled glucocorticoid and/or a hospital diagnosis of asthma during the follow-up. Cox proportional-hazard regression was used to compute incidence rate ratios, adjusting for covariates.

Results
A total of 2238 (1.1%) children were prenatally exposed to PPIs and 24 506 (12.4%) children developed asthma during follow-up (median follow-up = 6.8 years). The adjusted IRR (aIRR) of asthma associated with prenatal exposure to PPIs was 1.41 (95% confidence interval (CI): 1.27–1.56), compared with those unexposed. The association did not vary by trimester of exposure, and prenatal exposure to H2RAs was associated with similar increase in risk. The aIRR for maternal PPI and H2RA use in the year after, but not during pregnancy was 1.32 (95% CI: 1.20–1.46) and 1.13 (0.93–1.36), respectively, compared with non-use during and in the year after pregnancy.

Conclusions
Prenatal exposure to both PPIs and H2RAs was associated with an increased risk of asthma in our study. Because the observed association is not drug specific and also observed for maternal postnatal use it may be explained by a 'class effect' or maternal underlying condition.

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INTRODUCTION

The prevalence of asthma is increasing both in industrialised and in developing countries,[1–5] although the aetiology of asthma is not well understood. Prenatal exposures, such as maternal smoking and use of antibiotics, are some of the speculated contributing causes.[2, 6–8]

Proton pump inhibitors (PPIs) are used to treat gastro-oesophageal reflux disease (GERD) and peptic ulcers.[9, 10] The PPIs are generally considered safe to use in pregnancy [11, 12] and are sometimes used by pregnant women to treat symptoms of GERD, which are reported in up to 80% of pregnancies.[10, 13, 14]

In adults, use of acid-suppressive drugs may alleviate asthma in patients with GERD,[15, 16] but the drugs are also associated with allergic sensitisation.[17, 18] Although little is known about PPI exposure and risk of asthma, acid-suppressing medications may interfere with denaturation of food antigens in the stomach, making food proteins act like allergens causing allergic sensitisation and ultimately possibly asthma.[19–21] Furthermore, a study in pregnant mice showed that gastric acid suppression induced in their offspring a type 2 T-helper (Th2) bias, a condition that might predispose to allergies and asthma.[17, 19] This suggests that prenatal exposure to PPIs may play a role in aetiology of asthma.

Recently, a Swedish registry-based cohort study reported a 51% increase in risk of childhood asthma after *in utero* exposure to acid-suppressive drugs, including PPIs.[19] Maternal medication intake was ascertained at prenatal visits, and a majority of asthma outcomes were represented by severe cases leading to hospitalisation. In the current study, we examined whether or not prenatal exposure to PPIs, based on routinely registered maternal prescription dispensations, was associated with risk of asthma of any severity.

METHODS

Study population

The study cohort and data collection have been previously described.[22] This population-based cohort study included all singletons born alive from 1 January 1996 to 31 December 2008 in northern Denmark, a region representing approximately 33% of the Danish population. The study population was identified through the Danish Medical Birth Registry, which has recorded all births in Denmark since 1973.[6, 23] The Danish National Health Service provides tax-supported health care to all residents of the country and refunds a portion of patient expenditures for a wide range of prescribed drugs.

Data on PPIs and other drugs

Data on maternal prescriptions dispensed during relevant pregnancy were extracted from the Aarhus University Prescription Database (AUPD), which tracks outpatient dispensations of reimbursed prescribed medicines in the northern Denmark region.[24] Recorded data include personal identifiers, type of medication, coded according to the Anatomical Therapeutic Chemical classification, and the date of dispensation. From 1996 on, children's prescriptions have been recorded under their own, rather than their parents', identifiers. For this reason we restricted the study to births beginning in 1996. In Denmark, all PPIs, except omeprazole and lanzoprazole, which became over-the-counter drugs in December 2006 and May 2007, respectively,[25] are dispensed only by prescription. Their cost is partially reimbursed by the National Health Service.

We searched AUPD for maternal prescriptions for PPIs and histamine 2-receptor antagonists (H2RAs). H2RAs were used to examine whether or not the putative association between prenatal exposure to PPIs and asthma is related to acid-suppressing drugs as a class (or their indication).

Data on asthma

Presence of asthma among the children in the study population was defined based on an algorithm combining data on anti-asthma medication dispensations [26] and a hospital diagnosis of asthma. A child was considered to have asthma if the child had a record of a hospitalisation, outpatient visit, or an emergency-room visit with a diagnosis of asthma or if the child had a dispensation record for an anti-asthma medication. For the medication-base criterion, we required prescriptions of both a β-agonist and an inhaled glucocorticoid.[26] In patients aged 5–45 years, this algorithm has a positive predictive value (PPV) of 100% for 'any asthma' (including definitive asthma, wheezing, chronic obstructive pulmonary disease or allergy), and a PPV of 80% for a definitive asthma diagnosis.[6, 26] To ensure ongoing use of anti-asthma medication and to avoid possible misclassification of small children with wheezing, we additionally required that a given medication regimen be dispensed twice for a child to be counted as having asthma.[22]

Asthma diagnoses were ascertained from records of inpatient, outpatient, and emergency-room visits, as recorded in the Danish National Registry of Patients (DNRP). The diagnoses were coded using the Eighth Revision of the International Classification of Diseases (ICD-10) before 1994 and ICD-10 thereafter. The DNRP

has tracked all discharges from nonpsychiatric acute care hospitals since 1977; reporting of emergency-room and outpatient clinic contacts started in 1995. Prescription dispensations were ascertained from the AUPD.

Data on covariates

We included the following covariates, selected *a priori,* as risk factors for asthma identified in previous studies and measureable using, as appropriate, DNRP, Birth Registry or AUPD: year of birth, county of residence, gender of child, gestational age, birth order, mode of delivery, mother's age at delivery, maternal smoking in pregnancy, maternal use of systemic antibiotics during pregnancy, maternal history of asthma recorded at any time prior to delivery, and maternal pregravid body mass index (BMI).[6, 8, 19, 27–32] BMI has been recorded in the Birth Registry from 2004 onwards. We identified maternal history of asthma using the same hospitalisation- and prescription-based algorithm as the one used for children but also included ICD-8 codes. Data from all sources were linked on an individual level using the Civil Registration number, a 10-digit unique number assigned to all Danish residents at birth or immigration and used in all Danish healthcare registries.[33] All relevant diagnostic and medication codes are listed in the Appendix S1.

Statistical analysis

We defined prenatal exposure to PPIs as at least one maternal PPI prescription from 30 days preceding the giving first day of the last menstrual period (LMP) and until giving birth. We subsequently examined trimester-specific exposure: the first trimester was defined as the first 12 weeks of pregnancy counted from the first day of LMP; the second and third trimesters (examined together) were defined as the remainder of the pregnancy.[22, 34] According to this exposure classification, children exposed during the first trimester could also be exposed during second and/or third trimester, but not *vice versa*. To examine whether the cumulative dose of PPIs had any impact on subsequent asthma risk, we stratified PPI exposure according to the number of pills dispensed: $\leq$28 pills vs. >28 pills. We defined the reference group as children unexposed to PPIs at any time during gestation, as evidenced by absence of maternal PPI prescriptions.

The children were followed up from their date of birth until the date of asthma diagnosis, death, emigration, or the end of follow-up on 31 December 2009, whichever came first. We calculated incidence rates of asthma as the number of children with asthma divided by total follow-up time. We used the Kaplan–Meier method to estimate 2-year and 10-year risk of asthma according to PPI exposure. We used Cox proportional-hazards regression to compute crude and adjusted incidence rate ratios (IRRs and aIRRs) with 95% confidence intervals (95% CI). The assumption of proportional-hazards was assessed graphically and found appropriate. GERD in children may be related to asthma development.[35] Additional adjustment for the children's postnatal use of PPIs and/or H2RAs as a proxy for GERD did not change the estimates; therefore reported estimates do not include this adjustment but are available from the authors on request.

In addition to the primary analyses described above, we performed a number of secondary analyses. Since a definitive diagnosis of asthma cannot be made before the age of 5–6 years,[2, 36] we performed an analysis restricted to children diagnosed with asthma from age 5 years or older. To examine whether or not the putative association varied by severity of asthma, we also performed an analysis restricted to children with a hospital diagnosis of asthma (excluding the definition of asthma requirement of outpatient prescriptions). We also conducted the following separate sub-analyses: (i) adjusting for maternal BMI for children born in 2004 or later; and (ii) changing the earliest PPI exposure period from 30 days to 0 days and 60 days before the first day of the last menstrual period, to examine sensitivity of results to modifications of definition of the exposure. Furthermore, we examined the association between prenatal exposure to maternal use of H2RAs and asthma (with the number of pills dispensed categorised as $\leq$20 vs. >20); and association with offspring's asthma of maternal PPI use in the year after giving birth, but not during pregnancy; children whose mothers did not use PPI in either of those periods were the reference group for this latter analysis. The latter analysis was also performed with H2RAs.

All analyses were carried out using SAS version 9.2 (SAS Institute Inc., Cary, NC, USA). The study was approved by the Danish Data Protection Agency.

RESULTS

Descriptive data

There were 197 060 eligible children, of whom 101 069 (51.3%) were boys. A total of 2238 (1.1%) children had been exposed prenatally to PPIs, including 1238 (55.3%) exposed during the first trimester. Advanced maternal age at delivery, maternal asthma and high pregravid BMI

Table 1 | Characteristics of 197 060 children born in northern Denmark in 1996–2008, according to prenatal exposure to proton pump inhibitors (PPIs)

	Exposed to PPIs during gestation (N = 2238)		Non-exposed to PPIs (during gestation) N = (194 822)	
Characteristics	N	%	N	%
Maternal use of PPIs				
+PPIs ≤28 pills	837	37.4	–	–
+PPIs >28 pills	1401	62.6	–	–
Included risk factors				
Gender of child				
Girl	1127	50.4	94 864	48.7
Boy	1111	49.6	99 958	51.3
Gestational age (weeks)				
<37	144	6.4	9 638	5.0
37–41	1962	87.7	170 707	87.6
≥42	132	5.9	14 477	7.4
Birth order				
1	861	38.5	80 109	41.1
2	771	34.5	73 699	37.8
≥3	606	27.1	41 014	21.1
Maternal age at delivery				
<25	267	11.9	24 281	12.5
25–29	684	30.6	71 193	36.5
30–34	779	34.8	69 144	35.5
≥35	508	22.7	30 204	15.5
Maternal smoking during pregnancy				
Missing	53	2.4	4055	2.1
No	1647	73.6	153 634	78.9
≤10 cigarettes/day	378	16.9	28 317	14.5
>10 cigarettes/day	160	7.2	8816	4.5
Maternal use of antibiotics during pregnancy				
No	1091	48.8	132 762	68.2
Yes	1147	51.3	62 060	31.9
Maternal asthma				
No	2033	90.8	186 513	95.7
Yes	205	9.2	8309	4.3
Mode of delivery				
Missing	14	0.6	3821	2.0
Caesarean	535	23.9	31 673	16.3
Vaginal	1689	75.5	159 328	81.8
Maternal BMI*	(N = 1431)		(N = 77 240)	
15–19.9	161	11.3	10 364	13.4
20–24.9	610	42.6	39 831	51.6
25–29.9	378	26.4	17 238	22.3
≥30	282	19.7	9807	12.7

BMI, body mass index.

* N = 78 671, data on maternal BMI were only available from 2004 and forth.

were more frequent among PPI-exposed children than among unexposed children. So were maternal use of antibiotics during pregnancy (Table 1).

The maximum follow-up time was 14 years, with a median follow-up of 6.8 years. By the end of follow-up, 24 506 (12.4%) children were identified as having asthma. The median age at asthma diagnosis was similar among unexposed and exposed to PPIs: 1.5 and 1.6 years respectively. The prevalence rate of maternal PPI prescriptions over time increased continuously from 1996 to 2008 and no substantial change was observed in 2006 or 2007 (during which some PPIs became over-the-counter drugs).

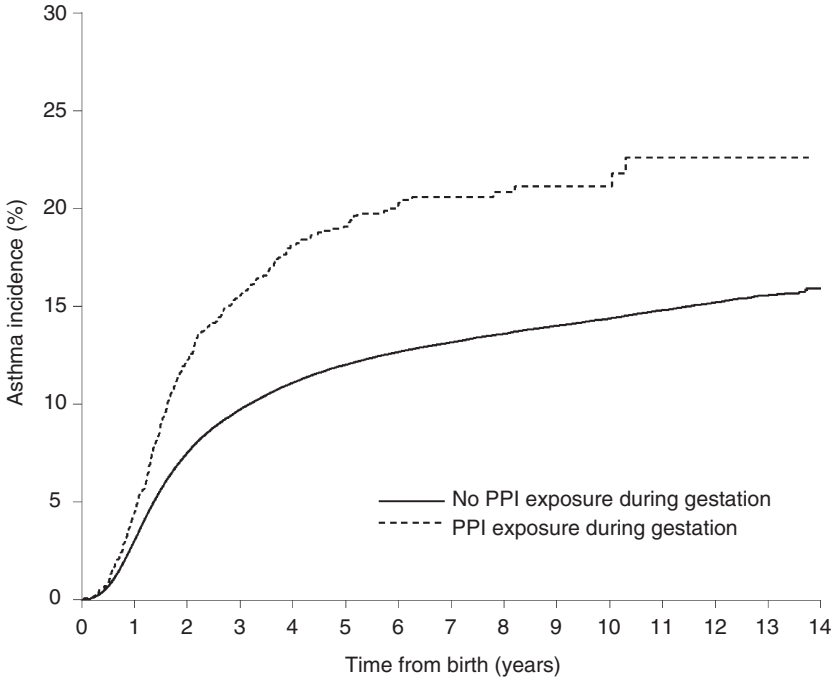

Figure 1 | Cumulative incidence of asthma in children according to prenatal exposure to proton pump inhibitors (PPIs).

Risk estimates

Among the children without prenatal exposure to PPIs, the 2-year risk of asthma was 7.5% and the 10-year risk was 14.4%. The corresponding estimates were 12.2% and 21.1% among the children prenatally exposed to PPIs (Figure 1). We found similar estimates among those exposed prenatally to H2RAs (results not shown).

After adjustment for potential risk factors, risk of asthma among children exposed prenatally to PPIs was 41% higher than among unexposed children (aIRR = 1.41, 95% CI: 1.27–1.56). Risk of asthma was also elevated among children exposed to more than 28 pills compared with 28 or fewer PPI pills (Table 2). When examining the risk of asthma according to trimester of exposure the aIRR was 1.46 (95% CI: 1.27–1.67) when exposed in first trimester and 1.34 (95% CI: 1.15–1.56) when exposed in second and/or third trimester.

In the analysis restricted to children aged 5 years and older (N = 129 888), 4966 children had asthma (3.8%) and the aIRR was 1.38 (95% CI: 1.00–1.89). The analyses restricted to children with a hospitalisation with asthma [10 632 children with asthma (43.4% of all asthma cases)] also yielded similar associations for PPI exposure as the main results (aIRR = 1.19, 95% CI: 1.01–1.40).

After adjusting for maternal BMI in the subset of women with available BMI data (N = 78 671), the crude IRR was 1.41 (95% CI: 1.23–1.62) and the aIRR was 1.25 (95% CI: 1.09–1.44) when exposed during gestation.

The estimates did not change substantially after varying the earliest PPI exposure between 0 and 60 days before pregnancy (results not shown).

Among the 197 060 children, 1605 (0.8%) had been exposed prenatally to H2RAs, as measured by maternal prescription dispensations. We found that 315 (19.6%) of the exposed children and 24 191 (12.4%) of the unexposed children had asthma, a result which was similar to the main results for PPIs (Table 2). The association did not vary according to the cumulative dose as measured by the number of pills.

Maternal use of PPIs in the year after giving birth, but not during pregnancy [2515 (1.3%) women], was associated with increased risk of asthma among offspring, with estimates similar to those in the main analysis. Corresponding results were observed for maternal postnatal use of H2RAs [695 (0.4%) women] Table 3.

DISCUSSION

In this large population-based cohort study, prenatal exposure to PPIs was associated with an increased risk of

Table 2 | Incidence rates and incidence rate ratios for asthma in children born in 1996–2008 in northern Denmark, according to prenatal exposure to proton pump inhibitors (PPIs) and H2-receptor antagonists (H2RAs) any time during gestation. *N* = 197 060

	N = 197 060 *n*	Children with asthma *N* = 24 506 *n* (%)	IR (per 1000 PY)	IRR (95% CI)	aIRR* (95% CI)
No PPI exposure	194 822	24 125 (12.4)	19.4	1.00	1.00
PPI exposure	2238	381 (17.0)	39.8	1.63 (1.48–1.81)	1.41 (1.27–1.56)
≤ 28 pills	837	132 (15.8)	30.0	1.37 (1.16–1.63)	1.20 (1.01–1.43)
>28 pills	1401	249 (17.8)	48.1	1.82 (1.60–2.06)	1.54 (1.36–1.75)
No H2RA exposure	195 455	24 191 (12.4)	19.5	1.00	1.00
H2RA exposure	1605	315 (19.6)	31.4	1.61 (1.44–1.80)	1.47 (1.32–1.65)
≤ 20 pills	223	43 (19.3)	28.5	1.54 (1.14–2.08)	1.44 (1.06–1.95)
>20 pills	1382	272 (19.7)	31.9	1.62 (1.44–1.82)	1.48 (1.31–1.67)

aIRR, adjusted incidence rate ratio; IR, incidence rate; IRR, incidence rate ratio; PY, person-years.

* Adjustment was made for year of birth, county, gender of child, gestational age, birth order, mother's age, maternal smoking during pregnancy, maternal asthma, mode of delivery, and maternal use of antibiotics during pregnancy.

Table 3 | Incidence rate ratios for asthma in children born in northern Denmark, according to maternal use of proton pump inhibitors (PPIs) and H2-receptor antagonists (H2RAs) in the year after giving birth but not during pregnancy

	n	Children with asthma *n* (%)	IRR (95% CI)	aIRR* (95% CI)
No PPI use during pregnancy or the year after birth	192 307	23 698 (12.3)	1.00	1.00
Maternal PPI use in the year after birth but not during pregnancy	2515	427 (17.0)	1.55 (1.41–1.71)	1.32 (1.20–1.46)
No H2RA use during pregnancy or the year after birth	194 760	24 077 (12.4)	1.00	1.00
Maternal H2RA use in the year after birth but not during pregnancy	695	114 (16.4)	1.24 (1.03–1.49)	1.13 (0.93–1.36)

aIRR, adjusted incidence rate ratio; IRR, incidence rate ratio.

* Adjustment was made for year of birth, county, gender of child, gestational age, birth order, mother's age, maternal smoking during pregnancy, maternal asthma, mode of delivery, and maternal use of antibiotics during pregnancy.

childhood asthma. The association was not specific to prenatal exposure and was observed for maternal use of PPI at any time during gestation and outside relevant pregnancy. Prenatal exposure to H2RAs and outside relevant pregnancy was also associated with childhood asthma. However, the association was weaker in the latter analysis. This suggests a class effect of gastric acid-suppressive drugs or confounding by indication, with maternal GERD as a potential risk factor for asthma in offspring.

Only one epidemiological study has previously examined the association between prenatal exposure to acid-suppressive drugs and childhood asthma.[19] Delink *et al.* conducted a population-based registry study in Sweden, which included 585 716 children born between 1995 and 2004, with follow-up through 2006. They used data from three Swedish registries, with self-reported information on both PPIs and H2RAs and registry-recorded hospital diagnoses of asthma. However, registry-recorded prescribed asthma medications were available only for 2005 and 2006. The study reported a 51% increased risk of childhood asthma in children exposed *in utero* to acid-suppressive medications irrespective of the time of exposure during pregnancy.[19] Despite the short follow-up duration for children receiving anti-asthma medications, the study's findings are in accordance with ours. The biological mechanisms behind a causal relation between prenatal exposure to PPIs and childhood asthma are not entirely clear. It has been suggested that acid-suppressive medications interfere with normal digestion of peptides in the adult stomach, resulting in a Th2 dominant

response. This response is thought to be caused by preservation of epitopes that are normally degraded by exposure to the acidic environment in the stomach.[19, 21] If transferred to the foetus, these epitopes could cause allergic sensitisation of the foetus.[19, 37] This hypothesis is supported by a study in pregnant mice showing that gastric acid suppression caused a Th2 dominant immune response in the offspring.[17, 19] Furthermore, in a human study of cord blood, maternal sensitisation to allergens was associated with reduced maternal production of the Th2 antagonist interferon-γ and with elevated production of the specific Th2 cytokine interleukin-13 in the offspring.[38] The development of the foetal immune system begins in early gestation and continues throughout gestation and beyond.[39] That could explain the increased risk observed for prenatal PPI exposure in all trimesters.

Our study has strengths and limitations. We relied on information from medical databases for a population with universal medical coverage, thus minimising some types of biases. For example, all births, including maternal information are recorded in the Medical Birth Registry, allowing one to rule out selection bias at enrolment. Selection bias at follow-up is also unlikely since deaths, and migrations are also subject to routine recording. Recall bias with respect to maternal use of medication is not present since medication was measured by reimbursement records.

At the same time, prescription dispensations are an imperfect measure of the fact and the timing of the actual drug intake, leading to misclassification of children with respect to prenatal drug exposure. We used a validated algorithm for identifying children with asthma,[6, 26] and made it stricter by requiring two prescriptions of anti-asthma medications. Still, since these medications also may be used to treat small children without asthma (e.g. wheezers),[2, 4] we may have included some children without actual asthma among those categorised as having asthma in the main analysis, causing us to overestimate the absolute risks of asthma. However, we do not expect that rates of measurement error of medication use and asthma are related to each other and thus the resulting bias would be expected to dilute associations. An earlier Danish study among children aged 6–8 years found that 4.2% had received prescriptions for both a β-agonist and an inhaled glucocorticoid, which is in agreement with our findings for children aged 5 years or older at the time of asthma diagnosis.[40]

Although we adjusted the analyses for several risk factors for asthma and conducted additional analyses adjusting for maternal BMI we cannot rule out unknown and unmeasured confounding. The results therefore may be confounded by unmeasured factors such as alcohol use or socioeconomic factors. Confounding by indication and class effect are both suggested by the analyses conducted for prenatal exposure to H2RAs. Maternal GERD could explain the association observed for maternal postnatal use of PPIs. GERD is associated with asthma in adults due to inflammatory effects on the upper and lower airways caused by reflux material.[15, 16, 41] Even though we adjusted the analysis for maternal asthma, maternal GERD may be also associated with asthma in offspring, but to our knowledge this has not yet been investigated. The high frequency of maternal use of antibiotics during pregnancy among PPI-exposed children could indicate maternal *Helicobacter pylori* infections. Still, several studies have suggested an inverse relation between asthma and *H. pylori*,[42–44] which in this case would argue against the confounding by indication conjecture.

An important limitation for the postnatal analyses is our inability to remove the potential effect of exposure through breastfeeding, since such data are not recorded in the routine registries in Denmark.

In conclusion, prenatal exposure to PPIs is associated with an increased risk of asthma in offspring, but explanations alternative to causal cannot be ruled out. The observed association could be caused by a manifestation of the 'class effect' of the acid-suppressive medications or by confounding by maternal underlying disease.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1. ATC-codes for medications examined.

Please note: Wiley-Blackwell are not responsible for the content or functionality of any supporting materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.

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SUPPORTING INFORMATION

Appendix S1

ATC-codes for medications examined

Drug	**ATC-code**
Proton pump inhibitors (PPI)	A02BC
Omeprazole	A02BC01
Histamine 2-receptor antagonists (H2RAs)	A02BA
β-agonist	R03AC
Inhaled glucocorticoids	R03BA
Systemic antibiotics	J01

ICD-8 and ICD-10 codes for asthma

Diagnoses	**ICD classification**
Asthma	ICD-8: 493
Asthma	ICD-10: J45, J46

Study II

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Parental Inflammatory Bowel Disease and Risk of Asthma in Offspring: A Nationwide Cohort Study in Denmark

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OBJECTIVES: Common genetic and environmental risk factors may explain the concurrent increase in the incidence of both inflammatory bowel disease (IBD) and asthma. We examined whether IBD in a parent is associated with an increased asthma risk in offspring.

METHODS: This was a registry-based cohort study of all children born alive in Denmark in 1979–2009, followed through 2010. IBD and asthma were identified using hospital diagnoses; antiasthma medication was also used to identify asthma. We computed risk of asthma and estimated adjusted incidence rate ratios (aIRRs) with 95% confidence intervals (CIs) using Cox proportional-hazards regression. We evaluated asthma risk according to maternal and paternal IBD, Crohn's disease (CD), and ulcerative colitis (UC). Children without parental IBD were the comparison cohort for all comparisons.

RESULTS: We identified 1,845,281 children, of whom 14,952 (0.8%) had a parent with IBD. The 10-year risk of asthma was 6.9% among offspring of parents with CD, 5.6% among offspring of parents with UC, and 5.0% among offspring of parents without IBD. The aIRR for asthma associated with parental IBD was 0.98 (95% CI: 0.91–1.04). The aIRR was 1.09 (95% CI: 0.98–1.22) for parental CD and 0.92 (95% CI: 0.84–1.00) for parental UC. Results were similar regardless of parent of origin or inclusion of antiasthma medication to define asthma.

CONCLUSIONS: Our data do not provide evidence for an increased risk of asthma in offspring with a parental history of IBD.

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INTRODUCTION

There have been concurrent increases in the occurrence of both inflammatory bowel disease (IBD) and asthma.[1–4] IBD is a collective term used for two diseases, Crohn's disease (CD) and ulcerative colitis (UC). During the first decade of the 2000s, in Europe, the population prevalence was up to 0.5% for UC and up to 0.3% for CD,[3] whereas asthma has become a leading chronic disease in industrialized countries, with prevalence in children reaching up to 20%.[1,2] The two conditions share genetic susceptibility loci,[5] and their concomitantly increasing incidence suggests common environmental risk factors.

Potential environmental risk factors implicated in the development of IBD and asthma include exposures to antibiotics[6,7] and endocrine-disrupting chemicals, which have immunomodulatory properties.[8] Active and passive cigarette smoking[9,10] also increase the risk of both CD and asthma.

IBD and asthma are immune-mediated diseases and can co-occur in the same individual.[11–14] A recent study based on inpatient hospital admissions reported a slightly increased risk of asthma among children with parental CD and UC.[15] However, to our knowledge, this is the only study to have addressed this potential parent/offspring association.

We therefore examined the association between IBD in parents and the risk of asthma in offspring in a cohort study in Denmark. We used prospectively registered data on all types of hospital contacts and on medication use to define study variables.

METHODS

For this cohort study, we linked individual-level data of children and their parents from different population health registries in Denmark. The linkage was possible due to the personal registration number (CPR number), which is a 10-digit unique identifier assigned at birth or immigration and used in all public records. The CPR number has been assigned since 1968 by the Civil Registration System, which uses the number to track residence and vital status.[16]

Study population. The study cohort included all children born alive in Denmark from 1 January 1979 to 31 December 2009 as recorded in the Danish Medical Birth Registry (DMBR). The DMBR has recorded all births in Denmark since 1973, including CPR numbers of the newborn, the mother, and, since 1991, the father.[17] Thus, we used the Civil Registration System to identify fathers of children born before 1991 and the DMBR thereafter. To ensure a minimum of 2-year availability of data on parental medical history (recorded since 1977), we started the cohort assembly from 1979 and excluded children whose parents had not been residents of Denmark for at least 2 years before the child's birth or whose parents had no valid CPR number.

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Data on IBD. From the Danish National Registry of Patients (DNRP),[18] we collected information on paternal IBD before the relevant pregnancy and on maternal IBD before or during the relevant pregnancy. IBD was defined as an inpatient, outpatient, or emergency-room diagnosis of CD or UC. If both CD and UC diagnoses were present for the same parent, the most recently recorded diagnosis was used to classify parental disease. To measure maternal disease activity during pregnancy, we counted the number of IBD admissions recorded from the estimated date of conception and until (and excluding) the date of delivery. To avoid misclassifying planned follow-up visits as disease flare-ups, we required that IBD-related hospital stays lasting $\geq$2 days to be considered indicative of the disease activity. The DNRP records all inpatient hospitalizations at nonpsychiatric public hospitals in Denmark since 1977 and emergency and outpatient contacts since 1995. Diagnoses have been coded using the eighth revision of the International Classification of Diseases (ICD-8) until the end of 1993 and the tenth revision (ICD-10) thereafter. Positive predictive values of DNRP-recorded diagnoses are 97% for CD and 90% for UC.[19]

Data on asthma. Asthma in children was defined as an inpatient, outpatient, or emergency-room asthma diagnosis recorded in the DNRP.[18] The positive predictive values of DNRP-recorded asthma diagnoses in children aged 6–14 years are 85%.[20]

Furthermore, in a restricted population of children born from 1996 onwards, we added to the asthma definition an algorithm based on filled prescriptions for both inhaled β-agonists and inhaled glucocorticoids. In the United States, in patients aged 5–45 years, the algorithm based on at least one prescription of an inhaled β-agonist and an inhaled glucocorticoid has a positive predictive value of 100% for identifying ''any asthma'' (definitive asthma, wheezing, chronic obstructive pulmonary disease, or allergy), and a positive predictive value of 80% for ''definitive asthma.''[21] In the Danish setting, antiasthma prescriptions have also been found useful in identifying asthma in children (6–14 years of age).[22]

We used the Danish Registry of Medicinal Product Statistics (RMPS) to identify prescriptions. The RMPS records all prescriptions filled at Danish outpatient pharmacies, including patient CPR numbers, Anatomical Therapeutic Chemical code, amount dispensed, and date of sale. The RMPS is complete from 1995 onward.[23] To reduce potential misclassification of children with wheezing as asthmatics, we required that a given medication regimen be dispensed twice in order for a child to be counted as having asthma. Date of asthma onset in the restricted population was the date of the first hospital inpatient or outpatient diagnosis or fulfilled prescription algorithm, whichever was earlier.[21]

Data on covariates. From the available data sources, we identified information on known risk factors for asthma.[7,24–27] From the DMBR, we obtained data on sex of child, birth order, multiple birth, gestational age, mother's age at delivery, mode of delivery, maternal smoking during pregnancy (recorded from 1991), and maternal pregravid body mass index (recorded from 2004). From the RMPS, we obtained data on maternal use of antibiotics during pregnancy, and from the DNRP, data on parental asthma, defined on the basis of hospital diagnoses. The algorithms used to define study variables are provided in the Appendix.

Statistical analyses. We excluded 66 children with IBD in both parents (including four children with asthma) as this group was too small to allow a meaningful interpretation.

In our study cohort, the follow-up started on the day of birth and ended on the date of asthma onset, emigration, death, or 31 December 2010, whichever came first. First, we examined distributions of perinatal characteristics at birth according to parental IBD status. Second, we estimated 2-year and 10-year risk of asthma according to parental IBD status, with death as a competing risk.[28] Third, using Cox proportional-hazards regression, we computed crude and adjusted hazard ratios as estimates of crude and adjusted incidence rate ratios (IRRs and aIRRs) with 95% confidence intervals (CIs) for asthma. We used the following categories of IBD: parental IBD, maternal IBD only, paternal IBD only, parental CD, maternal CD only, paternal CD only, parental UC, maternal UC only, and paternal UC only. Children without a record of IBD in mother or father served as the comparison cohort for all comparisons. The IRRs were adjusted for year of birth, child's sex, mode of delivery, mother's age at delivery, birth order, multiple birth, and parental asthma. The assumption of proportional hazards was assessed graphically and found valid. To assess whether the risk of asthma varied by maternal disease activity during pregnancy, we estimated IRRs and aIRRs in categories defined by the number of mother's CD- and UC-related hospital admissions during the relevant pregnancy (0, 1, and $\geq$2). This analysis was done in the entire cohort and separately among children born at term (week 37 or later) to remove potential effect of prematurity on early-life respiratory complications.

We conducted analyses to address differences in recording practices over time. We stratified by calendar period of availability of outpatient diagnoses in the DNRP (before 1995/1995 onwards). We also conducted an analysis restricting to children born in 1996 or later, including the antiasthma medication in the definition of asthma and also adjusting for maternal smoking and use of antibiotics during pregnancy. In addition, we restricted the latter analysis to the subset of children born in 2004 onwards to adjust for maternal pregravid body mass index. Finally, as a definitive diagnosis of asthma cannot be made until a child is at least 5 years old,[29] we repeated the analyses whereby follow-up for each child started at age 5 years. This analysis was done both for the entire cohort and for children born in 1996 or late, to allow inclusion of antiasthma medication in the asthma definition. We used Stata software version 12 to analyze the data (StataCorp LP, College Station, TX). The study was approved by the Danish Data Protection Agency (record no. 2013-41-1790).

RESULTS

Descriptive data. We identified 1,845,281 children born between 1979 and 2009 (51.3% boys) of whom 14,952 (0.8%) had a parent with IBD. Children born to parents with CD or UC were more likely than children of parents without

IBD to have had older mothers, to be born preterm or by caesarean delivery, and to have a parent with asthma. Maternal smoking or use of antibiotics during pregnancy and low pregravid body mass index were more prevalent among children with parental CD than among children with parental UC or no parental IBD (Table 1).

During follow-up 106,939 children were diagnosed with asthma of whom 856 had a parent with IBD. The median follow-time was 14.9 years (quartiles: 7.3–22.7). Median age at asthma onset was 1.6 years (quartiles: 0.9–4.4) for children with parental CD, 1.9 years (quartiles: 1.0–5.1) for children with parental UC, and 3.2 years (quartiles: 1.2–8.5) for children without parental IBD. Corresponding observed values when starting follow-up at age 5 years were 8.0 years (quartiles: 5.8–11.2), 8.7 years (quartiles: 6.7–11.6), and 9.5 years (quartiles: 6.7–14.2), respectively.

Risk of asthma. Among children with parental CD, the 2-year risk of asthma was 3.7% and the 10-year risk was 6.9%. Among children with parental UC, the respective risks were 2.8 and 5.6%. Among children without parental IBD, the 2- and 10-year risks were 2.3 and 5.0%.

Incidence rate ratios. Overall, the aIRR for asthma associated with parental IBD was 0.98 (95% CI: 0.91–1.04). For parental CD, the aIRR was 1.09 (95% CI: 0.98–1.22), and for parental UC, the aIRR was 0.92 (95% CI: 0.84–1.00). Results did not change when maternal and paternal CD and UC were examined separately (Table 2) or stratified by calendar period (results not shown). Risk of asthma was elevated among children born to mothers with two or more CD admissions during pregnancy (aIRR 1.74; 95% CI: 1.03–2.94; Table 3). This aIRR decreased to 1.42 (95% CI: 0.76–2.64) after restricting this analysis to children born in gestational week 37 or later (results not shown). There was no association between UC-related admissions during pregnancy and asthma (Table 3). Adding antiasthma medication to the definition of asthma (for children born in 1996–2009, $N = 871{,}674$) obtained aIRR for parental IBD of 1.05 (95% CI: 0.97–1.14), for parental CD of 1.11 (95% CI: 0.98–1.25), and for parental UC of 1.01 (95% CI: 0.92–1.12). The estimates did not vary by parent of CD/UC origin (results not shown).

The estimates for parental CD and parental UC remained unaffected by starting follow-up at age 5 years in the full cohort (estimates not shown); however, the association between two or more maternal CD-related admissions during pregnancy and asthma was no longer present (Table 3). When restricting the analyses with follow-up starting at age 5 years to children born in 1996 or later, no association was observed for either parental UC or CD (results not shown).

Additional adjustment in subcohorts with available data for pregravid body mass index did not affect the estimates (data not shown). All estimates are available from the authors upon request.

DISCUSSION

In this nationwide population-based cohort study of nearly 2 million individuals, we found no evidence for an overall association between parental IBD and asthma in offspring. This finding did not vary by calendar period and was unaffected by choice of asthma-defining algorithms.

The strengths of this study are its large size and setting in a universal healthcare system, allowing long and complete follow-up based on routinely recorded health-related events. The validity of the registries used in this study is high,[23,30] including quality of IBD diagnostic coding in the DNRP.[19] Furthermore, data on IBD and asthma were collected prospectively and independently of each other. Independent and routine data collections reduce the risk of recall, selection, and diagnostic biases. Available data sources allow for adjustment for confounding by parental asthma and maternal smoking during pregnancy. Furthermore, we can 100% identify persons who claim being the father and assume that rate of nonbiological paternity is random across the status of asthma and IBD.[16] If there were an association between parental IBD and asthma in offspring, nondifferential error in classifying IBD or asthma status could dilute the estimates of association to create an apparent null effect. For example, our asthma algorithm and databases could mistakenly capture some small children with wheezing and no asthma. However, this seems unlikely given that similar null results were observed when starting follow-up at age 5 years.

Taken together, the epidemiologic studies of IBD and asthma in the same individual suggested the presence of an association,[11–14] and this evidence provided the rationale for this study. Sibtain *et al.*[31] reported a 53% prevalence of a family history of asthma among children with IBD in a hospital-based cross-sectional study. To the best of our knowledge, the association between parental IBD and asthma in offspring was addressed in one epidemiologic study. Using the cohort design, Hemminki *et al.*[15] examined familial risks of 32 different immune-related diseases among 441,642 individuals with these diseases based on inpatient hospital diagnoses in Sweden. The standardized IRRs were 1.1 (95% CI: 1.0–1.2) for asthma among offspring of a parent with CD and 1.2 (95% CI: 1.1–1.3) for asthma among offspring of a parent with UC. Estimates for maternal and paternal IBD were not reported. Hemminki *et al.*[15] studied multiple outcomes and did not include outpatient asthma diagnoses or medications in asthma algorithms.

Similar to the Swedish study, we observed a weak association of parental CD with asthma in some analyses. Hemminki *et al.*[15] could not adjust for smoking, and as we were only able to adjust for maternal smoking during pregnancy in children born from 1996 onwards, not fully measured confounding by parental smoking could explain some of the weak association observed in our study.

In the light of the overall null findings, the explanation for an observed increased risk of asthma in the subgroup of children with two or more maternal CD-related admissions during pregnancy is probably noncausal. Possible explanations are chance or upward detection bias of asthma among young children with asthma-like symptoms and more frequent contact with health care because of maternal CD. Furthermore, restriction of the analyses to children born at term reduced the association for maternal CD-related admissions, suggesting that the association may be attributable to maternal pregnancy complications, rather than IBD, causing respiratory symptoms in small children.[32]

Table 1 Characteristics of children born in Denmark during 1979–2009 according to parental type of inflammatory bowel disease (IBD), $N = 1{,}845{,}281$

Characteristics	Parental IBD		No parental IBD ($n = 1{,}830{,}329$) *n* (%)
	Parental Crohn's disease ($n = 5{,}106$) *n* (%)	Parental ulcerative colitis ($n = 9{,}846$) *n* (%)	
Parental IBD			
Maternal	3,102 (60.8)	5,473 (55.6)	—
Paternal	2,004 (39.2)	4,373 (44.4)	—
Sex of child			
Female	2,478 (48.5)	4,816 (48.9)	890,959 (48.7)
Male	2,628 (51.5)	5,030 (51.1)	939,370 (51.3)
Year of birth			
1979–1984	170 (3.3)	551 (5.6)	314,398 (17.2)
1985–1989	251 (4.9)	787 (8.0)	272,798 (14.9)
1990–1994	522 (10.2)	1,195 (12.1)	316,007 (17.3)
1995–1999	936 (18.3)	1,752 (17.8)	318,426 (17.4)
2000–2004	1,393 (27.3)	2,537 (25.8)	306,827 (16.7)
2005–2009	1,834 (35.9)	3,024 (30.7)	301,873 (16.5)
Mother's age at delivery (years)			
<25	626 (12.3)	973 (9.9)	371,149 (20.3)
25–34	3,885 (76.1)	7,585 (77.0)	1,296,539 (70.8)
≥ 35	595 (11.7)	1,288 (13.1)	162,641 (8.9)
Gestational age (weeks)			
<37	416 (8.3)	754 (7.7)	103,264 (5.6)
37–41	4,387 (85.9)	8,334 (84.6)	1,538,939 (84.1)
≥ 42	279 (5.5)	674 (6.5)	145,334 (8.0)
Missing	24 (0.5)	84 (0.9)	42,792 (2.3)
Mode of delivery			
Vaginal	3,776 (74.0)	7,618 (77.4)	1,548,727 (84.6)
Cesarean	1,330 (26.1)	2,228 (22.6)	281,602 (15.4)
Birth order[a]			
1	2,374 (46.5)	4,115 (41.8)	827,582 (45.2)
≥ 2	2,732 (53.5)	5,731 (58.2)	1,002,747 (54.8)
Maternal asthma			
No	4,963 (97.2)	9,651 (98.0)	1,806,905 (98.7)
Yes	143 (2.8)	195 (2.0)	23,424 (1.3)
Paternal asthma			
No	4,999 (98.0)	9,667 (98.2)	1,812,499 (99.0)
Yes	107 (2.0)	179 (1.8)	17,830 (1.0)
Multiple birth			
No	4,923 (96.4)	9,487 (96.4)	1,770,491 (96.7)
Yes	183 (3.6)	359 (3.7)	59,838 (3.3)
Prenatal exposure to antibiotics[b]	($n = 4{,}001$)	($n = 7{,}018$)	($n = 860{,}655$)
No	2,527 (63.2)	4,692 (66.9)	586,742 (68.2)
Yes	1,474 (36.8)	2,326 (33.1)	273,913 (31.8)
Maternal smoking during pregnancy[b]			
No	2,974 (74.3)	5,860 (83.5)	665,964 (77.4)
≤ 10 cigarettes/day	640 (16.0)	703 (10.0)	122,659 (14.3)
>10 cigarettes/day	235 (5.9)	210 (3.0)	40,790 (4.7)
Missing	152 (3.8)	245 (3.5)	31,242 (3.6)
Maternal pregravid body mass index[c]	($N = 2{,}185$)	($N = 3{,}586$)	($N = 363{,}381$)
<18.5	127 (5.8)	184 (5.1)	16,577 (4.6)
18.5–24	1,269 (58.1)	2,116 (59.0)	209,956 (57.8)
25–29	424 (19.4)	656 (18.3)	71,261 (19.6)
≥ 30	220 (10.1)	388 (10.8)	39,881 (11.0)
Missing	145 (6.6)	242 (6.8)	25,706 (7.1)

[a]Children of multiple births are coded in same birth order.
[b]Children born from 1996 onwards, $N = 871{,}674$.
[c]Children born from 2004 onwards, $N = 369{,}152$.

Table 2 Incidence rates and crude and adjusted incidence rate ratios for asthma in Danish children born during 1979–2009 according to parental IBD status (*N* = 1,845,281)

	N	Children with asthma, *n* (%)	IR (per 1,000 PY)	Crude IRR (95% CI)	aIRR[a] (95% CI)
Parental IBD					
No parental IBD	1,830,329	106,083 (5.8)	3.8	1.00 (Ref.)	1.00 (Ref.)
Parental IBD	14,952	856 (5.7)	5.6	1.20 (1.12–1.28)	0.98 (0.91–1.04)
Maternal IBD	8,575	492 (5.7)	5.4	1.19 (1.09–1.30)	0.97 (0.89–1.06)
Paternal IBD	6,377	364 (5.7)	5.8	1.22 (1.10–1.35)	0.99 (0.89–1.09)
Parental CD	5,106	328 (6.4)	6.9	1.41 (1.27–1.57)	1.09 (0.98–1.22)
Maternal CD	3,102	203 (6.5)	6.9	1.43 (1.25–1.64)	1.10 (0.95–1.26)
Paternal CD	2,004	125 (6.3)	6.8	1.38 (1.16–1.65)	1.09 (0.91–1.29)
Parental UC	9,846	528 (5.4)	5.0	1.10 (1.01–1.20)	0.92 (0.84–1.00)
Maternal UC	5,473	289 (5.2)	4.7	1.06 (0.94–1.19)	0.89 (0.80–1.00)
Paternal UC	4,373	239 (5.5)	5.4	1.15 (1.01–1.30)	0.94 (0.83–1.07)

aIRR, adjusted incidence rate ratio; CD, Crohn's disease; CI, confidence interval; IBD, inflammatory bowel disease; IR, incidence rate; IRR, incidence rate ratio; PY, person-years; Ref., reference; UC, ulcerative colitis.
[a]Adjustment: sex of child, year of birth, mother's age at delivery, mode of delivery, multiple birth, birth order, asthma in mother, and asthma in father.

Table 3 Risk of asthma in Danish children born during 1979–2009 according to the number of maternal IBD-related admissions during pregnancy

Maternal IBD admissions during pregnancy	*N*	Children with asthma, *n* (%)	Crude IRR (95% CI)	aIRR[a] (95% CI)
*All children (*N = *1,845,281)*				
No maternal IBD	1,836,706	106,447 (5.8)	1.00 (Ref.)	1.00 (Ref.)
Maternal CD				
No admissions	1,729	107 (6.2)	1.30 (1.08–1.57)	1.05 (0.87–1.27)
1 admission	1,235	82 (6.6)	1.54 (1.24–1.91)	1.08 (0.87–1.35)
≥2 admissions	138	14 (10.1)	2.15 (1.27–3.63)	1.74 (1.03–2.94)
Maternal UC				
No admissions	3,229	178 (5.5)	1.05 (0.91–1.22)	0.94 (0.81–1.09)
1 admission	2,015	97 (4.8)	1.06 (0.87–1.24)	0.80 (0.66–0.98)
≥2 admissions	229	14 (6.1)	1.18 (0.70–2.00)	1.04 (0.61–1.75)
*Children at age ≥5 years (*N = *1,581,040)*				
No maternal IBD	1,574,750	50,816 (3.2)	1.00 (Ref.)	1.00 (Ref.)
Maternal CD				
No admissions	1,226	34 (2.8)	1.11 (0.80–1.56)	1.07 (0.76–1.50)
1 admission	834	26 (3.1)	1.57 (1.07–2.30)	1.48 (1.00–2.17)
≥2 admissions	101	3 (3.0)	1.14 (0.37–3.55)	1.08 (0.35–3.34)
Maternal UC				
No admissions	2,514	71 (2.8)	0.99 (0.78–1.25)	0.95 (0.76–1.20)
1 admission	1,444	22 (1.5)	0.72 (0.47–1.09)	0.71 (0.46–1.07)
≥2 admissions	171	6 (3.5)	1.18 (0.53–2.63)	1.16 (0.52–2.59)

aIRR, adjusted incidence rate ratio; CD, Crohn's disease; CI, confidence interval; IBD, inflammatory bowel disease; IRR, incidence rate ratio; Ref., reference; UC, ulcerative colitis.
[a]Adjustment: sex of child, year of birth, mother's age at delivery, mode of delivery multiple birth, birth order, asthma in mother, and asthma in father.

Although IBD and asthma may be associated in individuals, the overall findings of our study reassuringly suggest that IBD in a parent does not increase the risk of asthma in offspring.

CONFLICT OF INTEREST

Guarantor of the article: Ane Birgitte Telén Andersen, MPH.
Specific author contributions: study concept and design, analyses, interpretation of data, manuscript writing, manuscript revision, editing, and decision to publish: Ane Birgitte Telén Andersen; study concept and design, supervising in analyses, interpretation of data, revision of manuscript, editing, and decision to publish: Vera Ehrenstein, Rune Erichsen, and Henrik Toft Sørensen; study concept and design, participated in data analysis, interpretation of data, revision of manuscript, editing, and decision to publish: Trine Frøslev. All authors had full access to all data.
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Potential competing interests: None.

Study Highlights

WHAT IS CURRENT KNOWLEDGE

Inflammatory bowel disease (IBD) and asthma may co-occur in the same individual.

The association between parental IBD and asthma in the offspring is poorly documented.

WHAT IS NEW HERE

Of the 1,845,281 children born between 1979 and 2009 in Denmark, 14,952 (0.8%) had a parent with IBD.

Our study provides no evidence of an association between parental IBD and asthma in the offspring.

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APPENDIX 1

ATC-codes for included medications

Drug	ATC-code
β-agonist	R03AC
Inhaled glucocorticoids	R03BA
Systemic antibiotics	J01

ICD-8 and ICD-10 codes for IBD and asthma

Diagnoses	ICD classification
Crohns Disease	ICD-8: 563.01 ICD-10:K50
Ulcerative Colitis	ICD-8:563.19, 569.04 ICD-10: K51
Asthma	ICD-8: 493 ICD-10: J45, J46

Study III

Risk of asthma in children of parents with celiac disease: A Danish nationwide cohort study

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Keywords: Asthma, celiac disease, children, cohort study, parents.

ABSTRACT

Background: The incidences of celiac disease (CD) and asthma are increasing and the two conditions are associated in individuals. Risk of asthma may be passed on to the next generation through shared risk factors.

Aim: To examine whether parental CD is associated with risk of asthma in offspring.

Methods: We conducted a population-based Danish nationwide cohort study, using medical databases, covering the period 1 January 1979 to 31 December 2009. In a restricted cohort of children (1996-2009), we also defined asthma according to use of asthma-medication. For each child with a parental history of CD, we randomly sampled 100 children without this history from the children born in the same calendar year. We computed the 2- and 10-year risk of asthma from birth and used stratified Cox proportional-hazards regression to estimate adjusted incidence rate ratios (aIRR) for asthma, adjusting for measured covariates.

Results: We identified 1,107 children with a parental history of CD and 110,700 birth-year-matched children without this parental history. During up to 32 years of follow-up 6,125 children developed asthma. The 2-year risk of asthma was 4.3% among children with parental CD and 3.6% among children without parental CD. The corresponding 10-year estimates were 7.4% and 6.5%. The aIRR for asthma associated with a parental history of CD was 1.10 (95% CI: 0.86-1.39) and were similar for maternal and paternal CD. Inclusion of asthma-medication did not substantially change the results.

Conclusion: There was no convincing evidence of an increased risk of asthma among offspring of parents with CD.

INTRODUCTION

Celiac disease (CD) is a chronic immune-mediated disorder associated with villous atrophy and inflammation of the small intestine.[1] It is caused by an immune reaction to gluten in genetically susceptible individuals.[2] The prevalence and incidence of CD are increasing, particularly in Europe and North America,[3,4] with an estimated current prevalence of 0.5% - 1.5%.[4,5] This trend probably results from a combination of better detection and true incidence increase, presumably in response to environmental exposures.[4,6] The prevalence and incidence of asthma have been increasing concurrently with CD. Asthma is the most common childhood disease in Europe, affecting, on average, 10% of children.[7,8]

While asthma is a disease with a T-helper cell type 2 expression and CD has a T-helper cell type 1 expression, studies have shown that the two diseases are associated in individuals,[9-12] suggesting shared genetic risk factors. The concurrent rapid increase in incidence suggests that the two diseases also share environmental risk factors. The shared risk factors remain largely unknown, although vitamin D deficiency may be implicated in the development of both CD and asthma.[13,14] Another consideration is that patients with CD may suffer from vitamin D deficiency as a consequence of the disease.[15] Vitamin D is a critical regulator of the immune system and may also improve fetal lung growth and maturation.[16] Therefore, deficiency during pregnancy may increase risk of asthma in offspring.[16-18] Currently, there is no epidemiologic evidence addressing the association between parental CD and offspring asthma. We hypothesized that offspring of parents with CD have an increased risk of asthma. If an association is confirmed, it will help foster the understanding of shared risk factors. If an association exists only for maternal CD, it may point towards a pathway through maternal vitamin D deficiency. Such knowledge may contribute to early detection of children at risk of developing asthma, thus preventing under-diagnosis and under-treatment.[7] We therefore conducted a population-based nationwide cohort study using data linked from Danish medical registries.

METHODS

Setting and study population

This nationwide registry-based matched cohort study included children born alive from 1 January 1979 to 31 December 2009, as recorded in the Danish Medical Birth Registry (DMBR). Follow-up started on the date of birth and ended on the date of asthma onset, emigration, death, or 31 December 2010, whichever came first.

The DMBR has recorded all births since 1973, including data on child, maternal, and paternal characteristics.[19] We linked individual-level data on children and their parents to other Danish population health registries using the civil personal registration (CPR) number. The CPR number is a 10-digit unique identifier, assigned at birth or immigration since 1969 by the Civil Registration System and used in all public records.[20] For children with missing father's CPR number in the DMBR, we identified fathers in the Civil Registration System. To ensure a minimum of 2 years of parental medical history, we only included children whose parents had lived in Denmark for minimum 2 years before the relevant child birth. For each child with a parental history of CD, we randomly selected 100 children born in the same calendar year without parental history of CD.

Data on parental celiac disease

Data on parental CD came from the Danish National Registry of Patients (DNRP).[21] We searched the DNRP for CD diagnoses before the relevant pregnancy for the fathers, and before the relevant birth for the mothers. The DNRP has tracked all inpatient stays at non-psychiatric hospitals in Denmark since 1977. Reporting of emergency room and outpatient clinic contacts was added in 1995. We used inpatient, outpatient or emergency-room diagnoses of CD among the parents. The diagnoses were coded in the DNRP using the Eighth Revision of the *International Classification of Diseases* (ICD-8) before 1994 and the Tenth Revision (ICD-10) thereafter. To refine the CD definition, we also accessed data on biopsies recorded in the National Pathology Registry (NPR). The NPR has a complete record of results of pathology procedures since 1997, classified according to the Danish version of the Systemized Nomenclature of Medicine (SNOMED).[22] Thus, from 1997 onwards, confirmed CD biopsies were also used to define CD.

Data on asthma

We identified asthma in children based on inpatient, outpatient and emergency-room discharge diagnoses recorded in the DNRP. We defined asthma onset as the date of the first asthma diagnosis. Using medical records as the gold standard, the positive predictive value (PPV) and sensitivity of asthma diagnoses in children aged 6 to 14 years has been shown to be 85% and 90%, respectively, in the DNRP.[23]

For the subgroup of children born in 1996 or later, the availability of prescription data from the Danish Registry of Medicinal Product Statistics (RMPS)[24] allowed us to add an algorithm to the asthma definition based on minimum two dispensations of an inhaled β-agonist and minimum two dispensations of an inhaled corticosteroid, to ensure ongoing use. We have described and used this algorithm in previous studies.[25-27] In this subgroup of children, we thus defined asthma onset as the date of the first inpatient or outpatient asthma diagnosis or fulfilled prescription algorithm, whichever came first. The RMPS has recorded all prescriptions filled at Danish outpatient pharmacies since 1995, including the type of drug prescribed (according to the Anatomical Therapeutic Chemical [ATC] classification system) and the date of dispensation.[24] In a study from the US, a prescription for at least one inhaled β-agonist and one inhaled corticosteroid has a PPV of 80% for identifying definitive asthma in individuals 5-45 years of age.[28]

Data on covariates

We obtained information on measured risk factors for asthma from Danish medical registries.[29-35] From the DMBR, we procured data on sex of child, birth order, birth weight, gestational age, multiple gestation, mode of delivery, mother's age at birth, and maternal smoking during pregnancy (recorded from 1991). From the DNRP, we obtained hospital diagnoses of maternal and paternal asthma, and from the RMPS, data on maternal use of antibiotics during the relevant pregnancy. All algorithms used to define the study variables are provided in the Appendix.

Statistical analyses

Descriptive statistics were used to characterize the study population at birth according to parental CD status. We then estimated the two-year and ten-year risk of asthma, treating death as a competing risk.[36] We used stratified Cox proportional-hazards regression to compute crude and adjusted hazard ratios as estimates of incidence rate ratios (IRR and aIRR) with 95% confidence intervals (CI) for asthma, comparing children with a parental history of CD to children without this parental history. We examined the following categories of CD: parental CD, maternal CD only, and paternal CD only, adjusting for child's sex, mode of delivery, birth order, multiple gestation, mother's age at delivery, and paternal and maternal asthma. The proportional-hazards assumption was assessed graphically and found to apply.

Since some patients may have symptoms for several years before being diagnosed with CD,[37] we repeated the analyses changing the parental medical history time window from a minimum of 2 years to a minimum of 10 years prior to their child's birth.

Because hospital-diagnosed asthma may represent the most severe disease, in another sensitivity analysis, we restricted the sample to children born in 1996 or later, in order to include the anti-

asthma medication algorithm in the definition of asthma. This analysis included additional adjustment for maternal smoking and use of antibiotics during pregnancy. Finally, since asthma can be difficult to diagnose in small children,[38] we conducted the analyses starting follow-up at age five, both for the full cohort and for the restricted cohort of children born in 1996 or later, to allow inclusion of the anti-asthma medication algorithm and additional adjustment.

We used Stata software version 12 to analyze the data (StataCorp LP, College Station, TX). The study was approved by the Danish Data Protection Agency (record no. 2013-41-1790).

RESULTS

We identified 1,107 children with parental history of CD and 110,700 children without such a parental history of CD, matched by year of birth. Maternal CD accounted for 69% of all parental CD. First-born status, parental asthma, and maternal use of antibiotics were slightly more prevalent among children with parental CD than among those without (Table 1). All children contributed 806,332 person-years of follow-up and the median follow-up time was 7.4 years.

During up to 32 years of observation, 68 children with parental CD and 6057 children without this parental history had an asthma diagnosis. The median age at asthma onset was 1.5 years in both cohorts. When follow-up was started at the age of 5 years, the median age of asthma onset was 7.4 years. The two-year risk of asthma in the full cohort was 4.3% among children with a parental history of CD and 3.6% among children without this parental history; the 10-year risks were 7.4% and 6.4%, respectively.

Overall, the aIRR for asthma associated with parental CD was 1.10 (95% CI: 0.86-1.39), with nearly no effect from adjustment for the covariates. Results did not vary according to maternal or paternal CD (Table 2).

In the sub-cohort of children born in 1996-2009, for whom we included anti-asthma medication in the asthma definition, we identified 982 children with a parental history of CD and 98,200 without this history. During up to 15 years of follow-up, 129 children with parental CD developed asthma compared to 11,120 children without parental CD. The aIRR for parental CD was 1.14 (95% CI: 0.95-1.35), with similar estimates for maternal and paternal CD (other results not shown).

Changing the time window for a parental medical history of CD from a minimum of 2 years to 10 years before their child's births did not change the results. Finally, starting follow-up at age 5 in the full cohort and in children born in 1996 or later also did not substantially affect the results (results not shown).

DISCUSSION

In this nationwide population-based cohort study with long-term complete follow-up and prospectively collected data, we found no convincing evidence for an association between parental CD and risk of asthma. The results were robust to different approaches to measuring the study variables and our study thus argues against a pathway of shared risk factors from parent to the next generation.

To the best of our knowledge, our study is the first to examine the association between parental CD and the risk of asthma in offspring. The association between CD and asthma within the same individuals has been examined in previous studies. Kero *et al*., in a cohort study of 59,867 children, including 114 children with CD, reported an adjusted relative risk of asthma in children with CD of 9.26 (95% CI: 6.02-14.29).[12] Hemmenki *et al*., using data from 3006 patients hospitalized with asthma, reported standardized incidence ratio for subsequent hospitalization with CD to be 1.97 (95% CI, 1.64-2.34).[11] In a Swedish matched cohort study, including 28,281 individuals with CD and 140,295 individuals without CD, the hazard ratio for asthma associated with CD was 1.61 (95% CI: 1.50-1.72).[10] Still, although examining a different association, our results are consistent with two previous studies examining family associations of CD and asthma. An Italian study compared the prevalence of allergies, including asthma, in individuals with CD (n=1,044) with that of their CD-free first-degree relatives and spouses.[39] The authors reported that no statistically significant difference in asthma prevalence was observed. Also, a small Italian case control study of 82 children with CD and 180 children without, reported no statistically significant difference in asthma prevalence among relatives of the two groups.[40] Neither of the studies reported relative estimates. The observed association of the two conditions in the same individual[9-12] might be explained by host genetics and their interaction with environmental factors or vitamin D deficiency, with subsequent inability to control regulatory T cell responses.[10] Although our study argues against it, we cannot reject that CD and asthma share risk factors, however, a pathway from parent to child is not very likely.

The strengths of our study are its population-based cohort design with long-term and complete follow-up and use of prospectively collected data from population-based medical databases. The setting of a universal, tax-funded healthcare and routine registration of the events of interest virtually removes selection bias. We were also able to control for several important risk factors of asthma, including parental asthma and maternal smoking during pregnancy.

An important limitation of our study may be potential misclassification of the study variables. CD may have a silent clinical course for years with resolution of symptoms through a gluten-free

diet,[37] however, our results did not substantially change after extending the minimum parental medical history requirement from 2 years to 10 years. Any misclassification is likely to be non-differential with respect to development of asthma in offspring, and will bias the risk estimates towards the null. Misclassification of asthma among small children may also occur, and since we would expect it to be unrelated to parental CD, it may produce bias towards the null. However, as our results were the same when we started follow-up at age 5, bias from misclassification of the asthma diagnosis does not appear to be of major importance.

We cannot completely rule out bias from unmeasured confounding. Breastfeeding may protect against development of both asthma and CD,[41] and women with CD may have an increased awareness of the importance of breastfeeding. However, data on this variable are not recorded in Danish registries. Also, we lacked information on maternal use of vitamin supplements. It would be valuable to examine risk of asthma in children of mothers with treated CD during pregnancy (i.e., CD diagnosed more than one year before pregnancy) vs. mothers who were untreated during pregnancy. Finally, despite large sample size, precision of the estimates was suboptimal owing to small number of events among children with parental CD.

In conclusion, in this nationwide cohort study, we found no evidence of an association between parental CD and asthma in offspring.

AUTHORSHIP

Guarantor of the article: A.B.T. Andersen takes the full responsibility for the integrity of the work as a whole, from inception to published article.

Author contributions: All authors have made substantial contributions to the study design, analyses or interpretation of the data, drafted the manuscript or critically revised it for important intellectual content. All authors approved the final version of the manuscript.

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Table 1. Characteristics of children born in Denmark in 1979-2009 according to parental celiac disease. N=111,807

	Parental celiac disease (n=1,107)		No parental celiac disease (n=110,700)	
Characteristics	n	%	n	%
Parental celiac disease				
Maternal	759	68.6		
Paternal	348	31.4		
Sex of child				
Male	568	51.3	56,742	51.3
Female	539	48.7	53,958	48.7
Birth order[1]				
1	536	48.4	48,149	43.5
≥2	571	51.6	62,551	56.5
Birth weight (kilograms)				
<2500	58	5.2	5,751	5.2
2500-3499	501	45.3	46,558	42.1
≥3500	543	49.1	57,914	52.3
Missing	5	0.5	477	0.4
Gestational age (weeks)				
<37	68	6.1	7,133	6.4
37-41	967	87.4	95,432	86.2
≥42	66	6.0	7,543	6.8
Missing	6	0.5	592	0.5
Mode of delivery				
Vaginal	864	78.1	89,067	80.5
Caesarean section	243	21.9	21,633	19.5
Multiple gestation				
Yes	33	3.0	4,565	4.1
Mothers age at birth (years)				
<25	156	14.1	14,518	13.1
25-34	852	76.0	82,332	74.4
≥35	99	8.9	13,850	12.5
Maternal asthma				
Yes	39	3.5	2,648	2.4
Paternal asthma				
Yes	43	3.9	2,235	2.0
	(n=982)		**(n=98,200**	
Maternal use of antibiotics during pregnancy[2]				
Yes	355	36.1	31,857	32.4
Maternal smoking during pregnancy[2]				
No	815	83.0	78,525	80.0
≤ 10 cigarettes/day	107	10.9	12,451	12.7
>10 cigarettes/day	40	4.1	4,289	4.4
Missing	20	2.0	2,935	3.0

[1]Children of multiple births are coded in the same birth order

[2]Children born from 1996 onwards

Table 2. Crude and adjusted incidence rate ratios for asthma in Danish children born in 1979-2009, according to parental celiac disease status, N=111,807

	N	Children with asthma	IR (per 1,000 PY)	Crude IRR (95% CI)	Adjusted IRR* (95% CI)
Parental celiac disease					
No	110,700	6,057	7.6	1.00 (Ref.)	1.00 (Ref.)
Any parent	1,107	68	8.5	1.12 (0.88-1.48)	1.10 (0.86-1.39)
Maternal	759	47	8.5	1.11(0.83-1.48)	1.09 (0.82-1.45)
Paternal	348	21	8.5	1.13 (0.74-1.75)	1.10 (0.72-1.70)

Abbreviations: CI: confidence interval, IR: incidence rate, IRR: incidence rate ratios, PY: person years.
*Adjustment: sex of child, mode of delivery, multiple gestation, birth order, mother's age at delivery, asthma in mother, and asthma in father.

SUPPORTING INFORMATION

Appendix

ATC-codes for included medications

Medication	ATC code
Inhaled β-agonists	R03AC
Inhaled corticosteriods	R03BA
Systemic antibiotics	J01

ICD-8 and ICD-10 codes for celiac disease and asthma

Diagnoses	ICD codes
Celiac disease	ICD-8: 269.00 ICD-10: K90
Asthma	ICD-8: 493 ICD-10: J45, J46

SNOMED-codes

Diagnosis	SNOMED code
Celiac disease	T64020+M58018 T65110+M58018 T64310+M58018 S62180

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