AARHUS
UNIVERSITY

Asthma and farm exposure in early life and across generations

PhD dissertation

Signe Timm

Health
Aarhus University
2020

Asthma and farm exposure in early life and across generations

PhD dissertation

Signe Timm

Health
Aarhus University
Department of Public Health

PhD student

Signe Timm, MSc in Public Health

Department of Public Health

Aarhus University, Aarhus, Denmark

Supervisors

Main supervisor:

Vivi Schlünssen, MD, PhD (Main supervisor)

Department of Public Health

Aarhus University, Aarhus, Denmark

Co-supervisors:

Morten Frydenberg, MSc, PhD

Department of Public Health

Aarhus University, Aarhus, Denmark

Cecilie Svanes, MD, PhD

Department of Global Public Health and Primary Care

University of Bergen, Bergen, Norway

Assessment committee

Dorte Rytter, MSc, PhD (Chairman and moderator)
Department of Public Health
Aarhus University, Aarhus, Denmark

Jeroen Douwes, MSc, PhD
Centre for Public Health Research
Massey University, Wellington, New Zealand

Tina Kold Jensen, MD, PhD
Institute of Public Health
University of Southern Denmark, Odense, Denmark

Financial support
The Graduate School of Health, Aarhus University, Denmark
Department of Public Health, Aarhus University, Denmark
The Danish Ramazzini Centre
The Oticon Foundation

Papers

This thesis is based on the following papers:

Paper 1: The Gradient Study

Timm S, Frydenberg M, Janson C, Campbell B, Forsberg B, Gislason T, Holm M, Jõgi R, Omenaas E, Sigsgaard T, Svanes C, Schlünssen V: **The urban-rural gradient in asthma: A population-based study in Northern Europe.** *International Journal of Environmental Research in Public Health.* 2016, 13, 93; doi:10.3390/ijerph13010093

Awarded as "*BEST PAPER 2019*" by The Danish Society of Respiratory Medicine

Paper 2: The Generation Study

Timm S, Svanes C, Frydenberg M, Sigsgaard T, Holm M, Janson C, Bråbäck L, Campbell B, Madsen MK, Jõgi NO, Jõgi R, Schiöler L, Bertelsen JR, Johannessen A, Sanchez-Ramos JL, Martinez-Moretalla J, Dratva J, Dharmage S & Schlünssen V (shared last authorship): **Does parental farm upbringing influence the risk of asthma in offspring: A three-generation study.** *International Journal of Epidemiology*. 2020 Aug 3, p1-9. doi: 10.1093/ije/dyaa091

Paper 3: The Selection Study

Timm S, Frydenberg M, Abramson MJ, Bertelsen RJ, Bråbäck L, Benediktsdóttir B, Gislason T, Holm M, Janson C, Jõgi R, Johannessen A, Jeong-Lim K, Malinovschi A, Mishra G, Moratalla J, Sigsgaard T, Svanes C, Schlünssen V: **Asthma and selective migration from farming environments in a three-generation cohort study.** *European Journal of Epidemiology*. 2019 Jun;34(6):601-609. doi: 10.1007/s10654-019-00491-9.

Paper 4: The Agreement Study

Timm S, Schlünssen V, Benediktsdóttir B, Bertelsen RJ, Bråbäck L, Holm M, Jogi R, Malinovschi A, Svanes C, Frydenberg M: **Offspring reports on parental place of upbringing: is it valid?** *Epidemiology*. 2019 May;30(3):e16-e18. doi: 10.1097/EDE.0000000000000988

Additional work during the PhD employment period (not for assessment)

Additional paper 1

Christensen SH, Timm S, Janson C, Benediktsdóttir B, Forsberg B, Holm M, Jogi R, Johannessen A, Omenaas E, Sigsgaard T, Svanes C, Schlünssen V: **A clear urban-rural gradient of allergic rhinitis in a population-based study in Northern Europe.** *European Clinical Respiratory Journal*. 2016 Nov 25;3:33463. doi:10.3402/ecrj.v3.33463.

Additional paper 2

Timm S, Schlünssen V, Olsen J, Ramlau-Hansen CH: **Prenatal antibiotics and atopic dermatitis among 18-month-old children in the Danish National Birth Cohort.** *Clinical & Experimental Allergy.* 2017 Jul;47(7):929-936. doi: 10.1111/cea.12916.

Additional paper 3

Kuiper IN, Svanes C, Benediktsdóttir B, Bertelsen RJ, Bråbäck L, Dharmage SC, Holm M, Janson C, Jõgi R, Malinovschi A, Matheson M, Moratalla JM, Real FG, Sánchez-Ramos JL, Schlünssen V, Timm S, Johannessen A: **Agreement in reporting of asthma by parents or offspring - The RHINESSA Generation Study.** *BMC Pulmonary Medicine*. 2018 27;18(1):122. doi:10.1186/s12890-018-0687-4.

Additional paper 4

Andersen KS, Timm S … Schlünssen V: **Livestock farming exposure and the incidence of asthma in adults. A 15-year follow-up study in Denmark**.
Manuscript in preparation for submission to European Respiratory Journal

Additional paper 5

Madsen MK, Schlünssen V …. Timm S: **The effect of farming environment on asthma; time dependent or universal?**
Manuscript in preparation for submission to International Journal of Epidemiology

Acknowledgements

The research presented in this thesis has been conducted at the Department of Public Health, Aarhus University and Melbourne School of Population and Global Health, University of Melbourne, Australia. This work was made possible with funding from Department of Public Health, and I am especially thankful to the former head of the department, Søren Kjærgaard, for giving me this opportunity. I will also like to thank the assessment committee for their efforts in reviewing this thesis.

I will like to express my gratefulness to my academic supervisors Vivi, Morten and Cecilie. Vivi, thank you for believing in me from the very early stage of my research career in 2013 and throughout the whole journey. Thank you for your endless support, your untiring efforts and enthusiasm, and your recognition of small and big achievements along the way. It has been such a privilege to share both private and professional ups and downs with you! Morten, you have spoiled me with statistical expertise, since I met you for the first time 10 years ago. Thank you for making my skin thicker, and for constantly reminding me to question and refine my own work. Cecilie, thank you for making RHINESSA happen and for letting me into the unique and warm research community you have built up around the project. Thank you for your infectious passion and optimism.

I will like to thank all the members of the ECRHS/RHINE and the RHINESSA network for valuable comments and discussions regarding the four papers included in this thesis. Thank you for fun and friendship, and for countless academic discussions at our Solstrand meetings. I am especially grateful to Shyamali and Brittany for warmly welcoming me during my time in Melbourne, and letting me into the research group at the Allergy and Lung Health Unit. Shyamali, thank you for your thoroughness and dedication in this project.

I will also like to thank all my colleagues at The Department of Public Health throughout the years. Thank you for your help, support and scientific discussions. A special thanks to you Mette Lise, for being my faithful travel partner throughout the whole journey and for your constant support when the road was bumpy. You are an invaluable source of inspiration to me. Thank you, Torben, for your

endless ideas, and thank you Grethe, Gitte, Anneli, Karin and Charlotte for all our coffee breaks with beautiful latte art, and discussions about academia and the life beyond.

Last, but not least, I am grateful to all my family and friends. Thank you for being my source of genuine love and joy. Jens-Peter, thank you for your patience, appreciation and for always keeping me grounded – you are the anchor of my life. Maybe it was not a coincidence that Vivi and I casually swapped our Northside festival tickets, which lead to our very first meeting the summer when I started my PhD enrolment. Thank you for being the best support I could ever imagine. I could not have accomplished this without you. And finally, to the smallest people with the biggest impact: Hans & Luna, thank you for being exactly as you are - you make my life complete.

"As a researcher, you should always ask yourself:
How can we be as severe critical about our own work as possible?"

– Jan Vandenbroucke (epidemiologist)
Personal meeting in 2014

Table of contents

Chapter 1:

Introduction

This chapter provides a short overview of the research field and serves as an appetiser for the continued reading of this thesis.

Asthma prevalence has been rising worldwide during the latest century (1,2). Although genetic risk factors have been identified, they are not able to account for this rapid increase (2–6). A range of potential environmental risk factors has therefore been investigated; however, the aetiology of asthma is still poorly understood (2,7).

One of the leading theories behind the rise in asthma started out as "The Hygiene Hypothesis" suggesting impaired immunological competences as a result of low microbial stimulation in early life (8). Asthma has increased in parallel with the urbanisation that has occurred during the second half of the 20th century, which has changed e.g. the living conditions, hygiene standards and nutrition and thereby the microbial exposure of everyday life. Numerous studies have shown a lower prevalence of asthma among people born and raised on a farm compared to their counterparts in the cities (9–15). This has been ascribed to the greater microbial level and diversity on farms, especially on livestock farms (16). However, not all farming environments seems to be protective against asthma, and the literature shows inconsistent results with regard to timing of exposure, farming activity, geography and asthma phenotypes (17–22).

In recent years, research on gene-environment interactions and epigenetics has added to the field of early life programming (4,23–26). A pilot study on DNA-methylation concluded that farm exposure in early life seems to influence the expression on genes related to asthma (25). This indicates, that farm exposure in early life may have the potential to transfer asthma risk across generations; however, this has never been investigated in epidemiological studies.

One important concern about the potential "farm effect" has been the possibility of selective migration across generation, as studies have shown a "healthy worker effect" in the farming population (27,28). This would mean that the farm effect is merely as a result of asthmatic parents preferentially raising their children in the cities rather than a biological effect of the farming environment.

To develop effective preventive strategies in the future, it is imperative to have more detailed evidence on when and how farm exposure may be protective against development of asthma. This thesis investigates the occurrence of asthma in relation to farm exposure in early life and across generations, with the overall aim to contribute knowledge to the field of asthma aetiology.

Chapter 2:

Background

This chapter comprise a state-of-the-art overview on the knowledge regarding asthma, early life programming and farm exposure. This includes a section on when, what and how the farming environment may influence the development of asthma. Furthermore, the literature on agreement in reporting between family members is summarised. Beside the current knowledge, this chapter also outline the gaps in knowledge related to the aims under study in this thesis.

Asthma

Definition, diagnosis and phenotypes

The Global Initiative for Asthma (GINA) has defined asthma as an airway inflammatory disease. The recent report states: "*Asthma is a heterogenous disease, usually characterised by chronic airway inflammation. It is defined by the history of respiratory symptoms such as wheeze, shortness of breath, chest tightness and cough that vary over time and in intensity, together with variable expiratory airflow limitation."* (29). The chronic airway inflammation leads to airflow obstruction and airway hyperresponsiveness that is often reversible either spontaneously or after medical treatment. The aim of treatment is to control symptoms of respiratory distress, limit exacerbations and reduce accelerated loss of lung function (29,30). Symptoms and severity vary greatly both within and between individuals, and although asthma is defined as a chronic disease, many asthmatics experience remission after appropriate treatment (31).

Despite this general definition, the diagnosis "asthma" covers several phenotypes (31). The major types include allergic and non-allergic asthma, which are characterised by the presence or absence of IgE-mediated airway inflammation (31–33). Furthermore, asthma can be defined according to the time of onset and persistence as early-onset transient, early-onset persistent and late-onset asthma; however, there is no clear consensus on when to divide between early and late onset (34). Most early onset asthma manifests as the allergic phenotype, whereas late onset asthma is primarily the non-allergic phenotype (32,35). Other asthma phenotypes include "irritant-induced asthma" induced by, for instance, some occupational exposures, air pollution, cigarette smoking or exercise (31,36). The diagnosis of asthma is primarily given after clinical examination, and when possible, confirmed by variability in lung function measured by spirometry or peak flow. However, due to the broad definition of asthma and the many facets of the disease, the diagnosing can be subject to misclassification (29,37). Asthma may sometimes be confused with other airway diseases such as chronic obstructive pulmonary disease (COPD), bronchitis or emphysema – especially when testing of lung function is neglected (37–39).

Prevalence

Numerous studies have reported a rising prevalence of asthma across the world (1,40,41). The term "epidemic" has been proposed by several experts to describe the trends in asthma during the recent half-century (2,42,43). Approximately 339 million people have asthma around the world, and children constitute the highest proportion of the asthmatic population (7,29). However, large variations in prevalence exist both globally and regionally (2,44). In general, asthma prevalence seems to rise when countries become more affluent (5,45). Furthermore, it seems that the prevalence has reached a peak and even started to decline slightly in more westernised countries (41,45,46).

When measuring and comparing estimates of prevalence from different epidemiologic studies with different methodologies, one major concern arises: no single instrument can be used to identify asthma with certainty (2). A comparison of three methods to measure asthma among Danish children showed large differences in prevalence when using a prescription registry (32%), a self-reported questionnaire (12%) and a hospitalisation registry (7%) (47). International guidelines for diagnosing asthma endorses a patient's medical history, clinical examination and variable airway obstruction as a response to medical treatment, and despite the challenge in diagnosing asthma, agreements between clinicians seems to be fairly good (29,48). However, most epidemiologic studies use symptom questionnaires to identify asthma cases, and validation of these survey instruments can be difficult as the field is still lacks a "gold standard" for defining asthma (1,49). A recent review of the literature found as many as 60 different definitions of "childhood asthma" in 122 epidemiological papers, suggesting that we are far from reaching a consensus (50).

Ideally, to overcome the challenge of different definitions, diagnostic criteria, study designs etc., the best data on asthma prevalence will come from large multicentre studies applying standardised methods to a large study population across diverse countries with follow-up at appropriate time intervals. These data will provide a framework for comparing global trends in asthma prevalence over time and across countries.

In the early nineties, two large multicentre studies were initiated to study asthma among children and adults: the International Study of Asthma and Allergies in Childhood (ISAAC) and the European Community Respiratory Health Survey (ECRHS).

The ISSAC included more than 700,000 children from 56 countries worldwide in two age-groups (6- to 7-year-olds and 13- to 14-year-olds) based on questionnaires (40,51,52). The overall prevalence of asthma symptoms was 7.3% in the younger group and 7.7% in the older group (53). The results revealed a huge difference in prevalence of asthma symptoms between the study centres, ranging from 2 to 32% (52). The prevalence was highest in English-speaking countries (United States, Australia, New Zealand, United Kingdom and Ireland) and Latin America (Chile, Panama and Costa Rica). Europe showed high prevalence in Western Europe and Scandinavia, and lower prevalence in Eastern and Southern parts of Europe. The lowest prevalence was found among children in Asia and Africa (52). Seven years later, when performing phase three of the study, the overall prevalence was 7.2% for the younger group and 7.9% for the older group, suggesting that the overall prevalence has stabilised (40,53). However, many less affluent countries still experience a rising prevalence of asthma symptoms, and the symptoms also appear to be more severe in these countries (40,54).

In line with the results in children, ECRHS reported a large geographical variation in the prevalence of asthma among 140,000 20- to 44-year-old adults from 22 countries worldwide (1,44). Likewise, asthma was more prevalent in English-speaking countries (United States, Australia, New Zealand, United Kingdom and Ireland) and less prevalent in Iceland, parts of Spain, Germany and Italy (44). The median prevalence was 4.5%, ranging from 2 to 12% (1,44). A comparison of the two studies suggested a strong correlation between the prevalence data among children and adults for the 17 countries represented in both the ISSAC and ECRHS (55).

At follow-up (ECRHS II) 5-11 years later, the prevalence of asthma symptoms was unchanged, but interestingly, the prevalence of diagnosed asthma had increased (46,56). At ECRHS III 20 years later, the prevalence of asthma symptoms had even started to decline slightly; however, largely explained by smoking cessation (57). This may suggest that asthma prevalence has stabilised, whereas diagnostic labelling may have changed over time.

Incidence

Not many studies have been performed on the incidence of asthma across the lifespan. In a Finnish population, the average incidence rate of asthma was 2.2/1,000 person-years among both the childhood-diagnosed (<18 years) and the adult-diagnosed (≥18 years) (58). The incidence rates reach a peak for boys at age 0-9 years, and for women at age 40-49 years (58).

The incidence rates in European adults have shown estimates ranging from 1.0-6.9/1,000 person-years (59–63). The different methods used to measure incidence may partly explain the diverging incidence rates of asthma because prospective study designs seems to show lower incidence rates than annual reports (64,65). This suggest that the incidence may be underestimated if the follow-up time between surveys is too long, as mild cases fail to correctly report their asthma status (64). However, the studies with a prospective design agree fairly well on an overall incidence rate around 2/1,000 person-years in the age-group 20-50 years, with a higher incidence among women than men (58,63,66). The incidence of allergic asthma seems to be highest in early childhood, whereas the incidence of non-allergic asthma increases steadily with age until it peaks in middle adulthood (67). After the age of 40, the majority of incident cases are non-allergic (67).
A recent study comparing incidence rates in Northern Europe over time concluded that the incidence of asthma in adults has stabilised during the past 10-20 years (68).

Comorbidity and gender-differences

"The atopic march" proposes a sequential set of allergic and respiratory diseases starting with the onset of atopic dermatitis in early childhood and continuing with asthma in middle childhood and rhinitis in adolescence (69,70). The term "march" has caused some confusion, as this may be more a phenomenon of allergic comorbidities than one disease leading to another (70,71). Cohort studies have shown that approximately one-third of the infants with allergic dermatitis subsequently develop asthma, rhinitis or both (29,71–73).

Gender differences in asthma prevalence are well-established and change throughout the lifespan. In childhood, boys are more prone to develop asthma than girls. However, during puberty a turnover takes place and in adulthood women have a higher prevalence of asthma than men (74,75). The prevalence reaches a peak in women around the age of 50 – the mean age of menopause onset – indicating that sex hormones play an important role in the gender differences in asthma (76,77). Lung size may also serve as an explanation because boys have smaller lungs than girls but develop larger lungs and stronger respiratory musculature in adulthood (78).

Burden for individuals and society

Living with asthma has a huge impact on both work and private life. Measured as loss of disability, asthma is ranked 23rd among 315 diseases in the Global Burden of Disease Study and accounts for 26.2 million disability-adjusted life years (DALYs) worldwide (79). The burden of DALYs is unequally distributed across the life course, being highest among children and the elderly, and lowest in people in their 30s (7). The social and psychological impact may include limited physical activity, discomfort, depression and anxiety (80). Asthma mortality is uncommon, but of serious concern, as many of these deaths are preventable (7). However, asthma is a strong risk factor for Chronic Obstructive Pulmonary Disease (COPD), which causes 3.2 million deaths annually worldwide (79,81,82).

The economic burden of asthma can be divided into direct costs and indirect costs. Direct costs cover health care services and medicines, while indirect costs cover cost for society e.g. from loss of work productivity for asthmatics and their caregivers (7,80). Annually, the total cost of asthma in Europe is estimated to be €17.7 billion. Developed countries are spending 1-2% of their healthcare budget on asthma, the annual cost per asthma patient being estimated to be €300-1200 (83). When properly treated, asthma should rarely result in hospitalisation; however, asthma still remains one of the most common reasons for hospitalisation in, for example, the United States (2,31). Poor asthma control is also responsible for significantly work impairment, accounting for a further €9.8 billion annually (83). Work disability is common among adults with asthma, and severe asthma is associated with unemployment and premature retirement (84,85). Among children, absenteeism from school is estimated to be on average a loss of 3-5 days due to exacerbation of asthma, while his/her caregiver loses the same amount of working time (80,83).

Risk factors for asthma

Risk factors are usually defined as variables considered to increase the risk of a given disease. However, when considering asthma, it is important to distinguish between aetiological factors causing the onset of asthma and triggers of asthma attacks, with the awareness that some risk factors can do both (7,35). Childhood asthma and adult onset asthma are also known to share many of the same causes and triggers (35).

Twenty-five years ago, we knew exactly what caused asthma. Asthma was an atopic disease caused by allergens, so the preventive strategy was self-evident: no allergens - no asthma (5). Today, we know that the aetiology of asthma is not that simple. From investigating clinical studies on allergens and case reports of exacerbations of asthma patients, recent research has now shifted to epidemiological studies focusing on initial "programming" of asthma susceptibility. This shift has also led to a greater interest in not only risk factors but also protective factors.

In general, the aetiology of asthma must be considered as a complex interplay between genetic factors and environmental exposures (5,35). Furthermore, the timing of exposure seems to be crucial (2).

Genetic factors

Genetic predisposition is considered one of the strongest risk factors for developing asthma (4,35,86,87). Studies on twin pairs show that up to 73% of the variation in asthma susceptibility can be ascribed to genetic factors (6). In line with these findings, a meta-analysis concluded that parental asthma increases the risk of asthma in offspring; however, maternal asthma seems to be of greater importance than paternal asthma (88). Although multiple asthma-related genes and loci have been identified, they are only able to explain a limited proportion of asthma heritability (4). An increased genetic susceptibility due to changes in the genetic pool is unlikely to explain the rapid increase in asthma prevalence over a short period, whereas a change in susceptibility due to host responses to altered environmental exposures may serve as a better explanation (6).

Environmental exposures

In general, there is stronger evidence supporting environmental triggers than causes; however, some of the factors can act both as a trigger of asthma attacks and an underlying cause of the disease (7,35). Air pollution, pollen, animal fur and dander, mould, house dust mites, smoking and physical activity are well-known triggers of asthma symptoms (7,89). Although there is substantial evidence to suggest that allergen exposure is associated with asthma, the literature is not able to explain the rapid rise in asthma prevalence as a result of allergen exposure (45).

The most well-established cause of asthma is tobacco smoke (2,7). Active smoking in adulthood increases the risk of asthma, and as passive smoking exposure during the prenatal period and early life also increases the risk of asthma in the offspring (86,89,90). Outdoor air pollution such as traffic-

related air pollution together with low indoor air quality (mould, dampness etc.) also pose a risk factor for asthma in both children and adults (91–94). Furthermore, occupational exposures in workplaces like bakeries, laboratory animal facilities, detergent enzyme factories and seafood factories are dominated by airborne allergens inducing or worsening existing asthma among the workers (7,35,36,95,96). Also, other prenatal and early life factors are suggested as risk factors for asthma. The leading risk factor is childhood infections, especially respiratory syncytial virus (RSV), which is assumed to impair the balance in regulatory T-cells and increase the risk of asthma (97,98). Further risk factors include maternal antibiotic use and nutrition during pregnancy and birth by caesarean section, low birth weight and antibiotic use in early life (2,7,93,98–102). However, the literature is not consistent, and a common concern, e.g. regarding antibiotic use, is that these results reflects reverse causation, as early manifestations of asthma are mistaken as respiratory tract infections and treated with antibiotics (7). Apart from farm exposure in early life, protective factors include breastfeeding, pet exposure in early life and increasing number of siblings; however, the causal effect of these factors is also debated (22,35,70,86,93,103–105).

Gene-environment interactions

An increasing amount of literature also suggests interactions between environmental and genetic factors. In critical time windows of development, environmental factors have the potential to influence genetic responses and thereby asthma susceptibility (4,23). This epigenetic inheritance of environmental effects across generations, particularly through DNA methylation, has been confirmed in several animal models (23,106,107). However, the literature on humans is scarce and conflicting (4,23). Pre- and postnatal exposure to smoking, pets and respiratory viral infections seems to interact with asthma-related genes, whereas results on, for example, outdoor air pollution is inconclusive (3,4,108–110). The majority of the studies have investigated one or more candidate genes, and only a very few studies have linked these epigenetic effects to the manifestation of asthma in humans (4,23,107).

Early life programming

Hypotheses

"The first 1,000 days" has gained massive attention in modern research aiming to understand the early programming of disease in utero and the first years of life (111–113). This perception of disease development has expanded the understanding of disease origin for a range of non-communicable diseases, including asthma (112). Thus, the pre- and postnatal period has become a core target for health care and prevention strategies in many affluent countries bacause this crucial period may have long-lasting effects on subsequent health (114).

In 1986, Barker et al. proposed the Fetal Origins Hypothesis (initially called the Barker Hypothesis) which suggests that poor prenatal nutrition programmes increase the susceptibility to subsequent coronary heart disease (115–117). Three years later, in 1989, Strachan et al. proposed the Hygiene Hypothesis, which suggests that reduced exposure to infections in early life is associated with subsequent allergic disease (8). These novel hypotheses stimulated a substantial worldwide interest in early life programming, and the Developmental Origin of Health and Disease (DoHAD) approach evolved (117).

In the following years, a substantial amount of research was conducted to investigate the validity of the Hygiene Hypothesis. The Hygiene Hypothesis suggests that as a result of microorganisms binding their antigens to innate immune receptors, a non-allergic Th1-mediated immune system response is elicited (118,119). Although, the hypothesis gained considerable support from researchers worldwide, there was still substantial scepticism about the Hygiene Hypothesis as the primary explanation for the global asthma epidemic (119). First, due to the assumed mechanism, the Hygiene Hypothesis could only be valid for allergic asthma. However, only half of the cases of asthma seems to be attributable to atopy (45,120,121). Thus, the Hygiene Hypothesis may only be able to explain part of the global trends in asthma, or the mechanistic theory behind the hypothesis may not exclusively involve atopic immune responses (121). Second, hygiene in itself, measured as cleanliness and handwash, does not seems to influence asthma risk (122). Finally, the Hygiene Hypothesis may be unable to explain why asthma prevalence has levelled off or even started to decline in countries where the hygienic standards are unchanged (40,46,121).

In the beginning of the millennium, researchers put forward that the Hygiene Hypothesis was "an oversimplification" (123,124). There must be more to it than just infections, and thus, a broader

understanding started to arise: immunological stimulation (125). The protective effect of microbial exposure may not be limited to infections, but may also include the non-pathogenic microbes which inhabit indoor and outdoor environments in addition to the skin, gut and respiratory tract of humans (126,127). These microorganisms have been part of the human surroundings throughout evolution; however, they are now less frequent or even absent in the modern human environment of westernised societies (126,128).

In order to incorporate the importance of exposure to commensal species and symbiotic microorganisms, Rook et al. renamed the hypothesis "The Old Friend's Hypothesis" in 2003 (114,129). From assuming that a Th1/Th2 imbalance acts behind the original Hygiene Hypothesis, the microbial exposure was now assumed to interact with regulatory T-cells to prime immunoregulation and not induce aggressive responses (114,127,128). Furthermore, the Old Friend's Hypothesis was extended to include a number of other inflammatory diseases such as inflammatory bowel diseases, diabetes, affective disorders, some types of cancer, atherosclerosis, rheumatoid arthritis, multiple sclerosis and Alzheimer (111,112,129–132). During the following years, extensive research was conducted and the hypothesis has been given many names: the Microflora Hypothesis, the Gut Commensal Hypothesis, the Biodiversity Hypothesis and the Microbial Diversity hypothesis (35,133–135). All of the hypotheses shared the same perception, that diversity of the microbial exposure is key in establishing a balanced human microbiome during early life.

The microbiome

Once, researched believed the human airways was sterile (71). Today we know that the human body houses trillions of microbes, living in the intestines, on the skin, in the mouth, the respiratory tract and other mucosal surfaces. This microbial community is called *the microbiome* (136,137). In the human intestines alone, the microbiome is estimated to comprise over 1,000 different bacterial species and, in total, outnumber the human host cells by a factor 100 or more (138). The human intestines are the ecological site containing the highest density of bacteria (111,136). The microbiome is able to change more rapidly than the host organism itself, thus making it capable of influencing the evolution of humans. Our modern lifestyle is reduced in exposure to the microbes that humans have co-evolved with, making the microbiome of the modern human race significantly different from that of our ancestors (111,112,125). Studies on germ-free mice, born and raised under sterile conditions, show

extensive defects and systemic immunological imbalance. However, colonisation with murine microbiota reversed the immunological abnormalities in neonates, but failed to achieve homeostasis in mice at more advanced age (111).

The gut microbiome is the main driver of microbial stimulation and may lead to the maturation of immune responses (139,140). During birth, the newborn are exposed to a wide range of microbes, which colonise the gut and become the very beginning of the infant gut microbiome (141). The diversity of the gut microbiome increases with age, especially with the introduction of solid food, and an adult-like gut microbiome is established when the child is approximately 3 years of age (136,137). Recently, immature microbial composition at 1 year of age has been shown to increase the risk of asthma at age 5 years. However, this was only observed among children born to asthmatic mothers, suggesting impaired microbial stimulation in early life can trigger inherent asthma risk (142).

The airway microbiome may also be involved in the maturation and maintenance of homeostasis in the lungs (71,143). The microbiome of the lower respiratory tract seems to be established within the first 2 months of life in a highly dynamic process influenced by environmental exposures (143,144). This process may consequently influence the risk of developing asthma later in life, as asthmatic lungs tend to have a disturbed microbiome compared to healthy lungs (71,145).

The farming environment – when, what and how

When: the time windows of exposure

Numerous studies have reported a lower prevalence of asthma among people born and raised on a farm. This protective "farm effect" has been shown in a range of affluent countries including Germany (9,10), Austria (11), Poland (146), Sweden (147,148), Finland (149), Britain (19), New Zealand (150), Canada (151) and the USA (152). In contrast, studies in Switzerland (153), New Zealand (154), Australia (13), USA (155) and a combined analysis in 13 ECRHS centres located in Belgium, Sweden, France, the Netherlands and New Zealand (156) did not show any association between early life farm exposure and asthma. These diverging results have raised uncertainty about a true protective effect; however, the heterogeneity of farming practices and asthma phenotypes may also contribute to the inconsistency (17).

Prenatal farm exposure is rarely investigated, apparently due to the difficulties in separating the pre- and postnatal environments in epidemiological studies. A New Zealand study found that prenatal farm

exposure may contribute to the lower prevalence of asthma in farmer's children, but continued exposure may be required to maintain optimal protection (150). In contrast, an American study did not find any associations between asthma and maternal farm living, maternal farm work and maternal animal contact while pregnant (155). Beside these two epidemiological studies, the literature is dominated by mechanistic studies, indicating that prenatal farm exposure stimulates the innate immune responses and shapes the child's immune system at a very early stage (24–26,157).

Some studies indicate that early farm exposure may induce long-lasting effects that persist throughout adulthood. A Finnish study found that contact with farm animals in early life reduced the risk of asthma at age 31, whereas a large study within the ECRHS found no association between early life farm exposure and adult asthma (156,158). A Danish study found lower prevalence of asthma among farmers with a farm childhood compared to farmers without a farm childhood, indicating that an early presence of farm exposure is essential (159). On the other hand, farm exposure has a dual effect across the life course. As opposed to the apparently protective effect from early life exposure, adult farm exposure seems to be a risk factor for asthma – especially the non-allergic phenotype (160–162). However, the dual farm effect has been constantly debated (161). Persistent long-term farm exposure seems to be important to maintain the optimal effect into adulthood, as the accumulated number of years of farm exposure from childhood to adulthood shows an inverse association with asthma symptoms (163). Yet, this may reflect a healthy worker bias, as those who experience asthma symptoms leave the farming environment (22,164). Despite this general concern, evidence on a potential healthy selection into and out of farming is rarely investigated. A Swedish and a Dutch study both found indication of selection because asthmatics were less likely to become farmers (27,28). In contrast, a Norwegian study found a similar asthma prevalence among farming students and their siblings, and a Swedish study found a similar prevalence among farmers' and non-farmers' children (148,165). This inconsistency raises the question whether the apparently protective effect from farm upbringing is the product of selective migration rather than a biological effect of the farming environment.

<u>What: the contributors to the farm effect</u>

Urbanisation has accelerated the loss of microbial exposure from the natural environment that humans co-evolved with (114,166,167). In order to function properly, the immune system learns from

environmental stimuli, and the surroundings in early life are a key player in this "natural" immunotherapy (125,142,166). Numerous studies have sought to identify the potential source of asthma protection in the farming environment. Livestock has been pointed out as the most important contributor because the farm animals comprise a rich source of diverse environmental microorganisms (9,11,17,22,168). These microorganisms have been mainly measured as endotoxin, a major part of the cell-wall of gram-negative bacteria, and found in high concentrations in airborne dust, mattress dust, settled dust, doormats etc. (16,166,169,170). The most prominent effect was observed in association with contact to cattle, pigs and poultry, but contact with animal sheds and fodder also seems to be associated with less asthma (17,168). Interestingly, regularly contact with farm animals also seems to be protective among children not living on a farm (11).

Unpasteurised cow's milk has also been identified as an important contributor to the farm effect (19,171,172). Milk used for commercial purposes has been pasteurised, to minimised the natural level of microorganisms in the milk, whereas raw milk is a rich source of bacteria with potential immune modulating properties (171,172). As with livestock exposure, the effect does not seem to be restricted to farm children, but was also observed among non-farm children consuming unpasteurised milk (19,22). Other differences of lifestyle between the urban and farm population such as duration of breastfeeding, day care, dietary habits, parental education and family history of asthma do not seem to account for the protective farm effect, indicating that the microbial exposure and not the lifestyle in general is the underlying contributor (168). However, it is unclear whether diversity, dose or specific microorganisms are accountable for the protective effect (168). Low diversity of the gut microbiome in early life has been associated with asthma in school-age children in a Swedish birth cohort, whereas a Danish birth cohort did not find any association (173,174). Researchers have speculated that higher exposure of microbial diversity in early life will lead to higher diversity of the infant microbiome and thereby lower the risk of subsequent asthma (140,175). However, the literature on the bacterial influence on asthma development is still at a very early stage, and the question has not yet been investigated in-depth (140).

<u>How: exposure routes and molecular cross-talk</u>

Since livestock and unpasteurised milk appear to be the major contributors of the protective farm effect, inhalation and ingestion are probably the two main routes of exposure (168). Researchers have

assumed an “evolved dependence”, where the induction of appropriate immunostimulation from non-pathogenic microorganisms has become a necessity for the immune system to develop properly in order to reduce inflammatory diseases, including asthma (112). Thus, it is speculated whether some genes involved in appropriate immunoregulation may be located in microbial genomes rather than mammalian genomes (112).

In mechanistic studies, researchers often seek cellular and molecular signals of a given exposure in vivo, but in the case of farming, researchers were already influenced by the assumption that the innate immune system senses the signals derived from the microbial exposure in the farming environment and transmit these to the adaptive immune system (168). Prenatal farm exposure is still rarely investigated, but one study found that the CD14-promoter region was differently methylated in placentae among pregnant women living on a farm compared to non-farm women (157). In addition, maternal farm exposure seems to modulate the neonatal immune system by inducing regulatory T-cells (26). Furthermore, early farm exposure has been found to influence methylation in asthma-related genes detectable at age 4 (25). Thus, farm exposure in prior generations may have the potential to confer asthma risk across generations.

Analyses undertaken in school-aged children showed that farm children had significantly higher levels of CD14 and Toll-like receptor 2 (TLR2) mRNA than non-farm children (176). Maternal exposure to farm animals in pregnancy also showed increased regulatory T-cell activity and increased levels of TLR2, TLR4 and CD14 mRNA in the child’s peripheral blood cells at school-age (24,177). Mouse models investigating the farm effect found that nasal exposure of stable dust extracts provided significant protection from allergen-induced Th2-responses, especially those occurring locally in the lungs (178). The molecular and cellular cross-talk underlying these results still remains unclear. Another study in mice indicated that chronic exposure to low-dose endotoxin from farm dust prevented the mice from developing allergic asthma because endotoxin reduced epithelial cell cytokines that activate dendritic cells, and thus supress Th2-activity through A20 induction (179).

Thus, the rich and diverse microbial exposure in the farming environment is assumed to influence the innate immune system and regulatory T-cells, which in turn will balance the adaptive immune system and dampen the Th2-associated cytokine production and Th2-dependent IgE production (168).

Agreement in reporting between family members

Due to the inherent challenges in studying several generations, information is often reported by family members on behalf of each other. Multi-generation studies have become increasingly popular; however, very little is known about the validity of the second-hand information that these studies often rely on. Two studies investigating reports on pre- and postnatal smoking concluded that offspring reports of parental smoking prenatally and in childhood are in good agreement with parents' own reports (180,181). In addition, two studies investigating second-hand reports on asthma concluded that parental reports on offspring asthma show a good agreement with offspring's own reports; however, for late-onset asthma (>10 years), the agreement was moderate (182,183). Offspring reports on both maternal and paternal asthma also showed good agreement (182). Furthermore, the change in methodology from indirect to direct reports did not seem to affect the results markedly (183). Despite the extensive interest in the early environment in asthma research, the agreement and use of second-hand information on place of upbringing have never been investigated.

Chapter 3:

Thesis objectives

This chapter summarises the overall aim of this thesis and the four hypotheses under study in the original papers.

Despite extensive research in the field of farm exposure and asthma, multiple questions still remain unanswered. Key questions may concern whether the farm effect is restricted to the people living on the farms, whether the next generation may benefit from the farm exposure in their parents' early life and whether the farm effect is just the result of selective migration and not a biological effect of the farming environment. To develop successful preventive strategies in the future, it is crucial to gain more evidence on when and how farm exposure may be protective against the development of asthma.

This thesis aims to investigate the occurrence of asthma in relation to farm exposure in early life and across generations in a large multi-generation cohort study. The work conducted in this thesis will contribute to a better understanding of the early origin of asthma.

Study aims and hypotheses

Paper 1 (The Gradient Study) aimed to investigate the gradual association between place of upbringing and subsequent development of asthma. We hypothesised that the occurrence of asthma was gradually increasing across six levels of urbanisation from farm to city corresponding to the decreasing microbial exposure.

Paper 2 (The Generation Study) aimed to study generational effects from parental and grandparental place of upbringing on offspring asthma. We hypothesised that the occurrence of asthma was lower in offspring with parents or grandparents from a farm compared to offspring with parents or grandparents from city.
Furthermore, based on the results in paper 4, the paper employed a quantitative bias analysis to investigate potential bias from using second-hand information on parental place of upbringing from the offspring instead of direct information from the parent him-/herself.

Paper 3 (The Selection Study) aimed to explore the patterns of selective migration from the farming environment by investigating whether parents with asthma are less likely to raise their children on a farm. We hypothesised that selective migration persists across generations.

Paper 4 (The Agreement Study) aimed to evaluate the use of second-hand information by investigating the agreement between parental and offspring reports of parental place of upbringing. We hypothesised a good agreement between offspring and parental reports.

Chapter 4:

Material and methods

This chapter describes the material and methods used. The chapter comprises a description of the study population, data collection and statistical methods. A more detailed description of material and methods can be found in the original papers.

The study population: ECRHS/RHINE/RHINESSA

This thesis is nested within the three cohort studies ECRHS (European Community Respiratory Health Survey), RHINE (Respiratory Health In Northern Europe) and RHINESSA (Respiratory Health In Northern Europe Spain and Australia).

The ECRHS study was planned to investigate the distribution of asthma and potential risk factors for asthma in the European Community (184). The original ECRHS study population included >150,000 randomly selected participants born 1945-1973 who participated in the ECRHS stage 1 questionnaire during 1989-1992. Each of the 48 study centres across 22 countries recruited at least 1,500 men and 1,500 women (1,184). The ECRHS was followed up in 2002 and 2012.

The RHINE study was initiated in 1999-2001 as a sub-study following up on the seven study centres located in Northern Europe: Aarhus (Denmark), Bergen (Norway), Reykjavik (Iceland), Tartu (Estonia) and Umeå, Uppsala and Gothenburg (Sweden). All RHINE subjects received a postal questionnaire (RHINE II), and 16,106 subjects responded (74%). At follow-up in 2010-2012, 13,499 subjects (62%) responded to the RHINE III questionnaire (185).

The RHINESSA cohort was established in 2013 in order to investigate respiratory health across generations (186). The study was designed to identify susceptible time windows of exposure before conception, with the ultimate aim to facilitate efficient prevention strategies (186). The RHINESSA cohort comprises adult offspring of the RHINE/ECRHS participants from ten study centres: Aarhus (Denmark), Bergen (Norway), Reykjavik (Iceland), Tartu (Estonia), Umeå, Uppsala and Gothenburg (Sweden), Melbourne (Australia) and Huelva and Albacete (Spain). At baseline, 8,814 subjects aged 18-50 years were enrolled based on postal questionnaires (186).

The present thesis involves three generations: participants in the ERCHS/RHINE (denoted Generation 1: G1), their parents participating via second-hand information (Generation 0: G0) and their adult offspring participating in RHINESSA (Generation 2: G2), Figure 4.1.

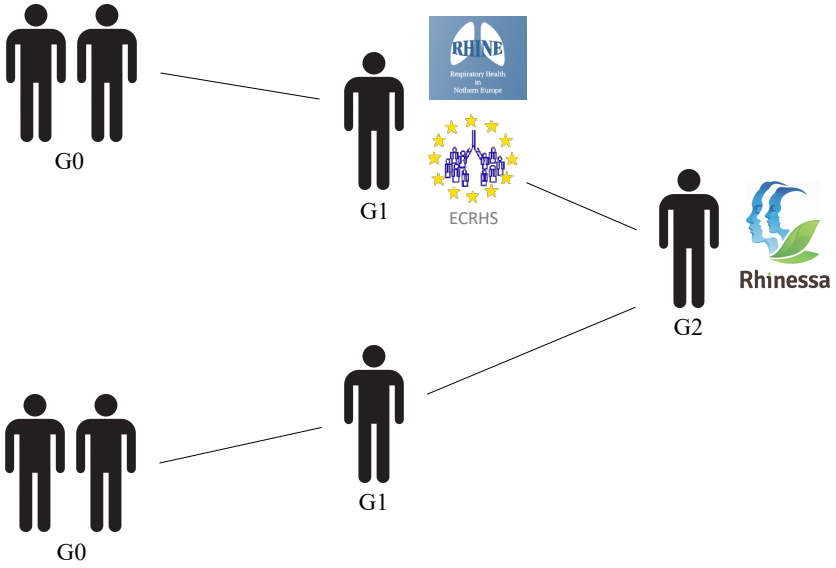


Figure 4.1: Illustration of the three generations under study. From left: G0 (included in the studies based on information given by G1 and G2), G1 (either father or mother participated in the ECRHS/RHINE study) and G2 (RHINESSA participants). The G1 parent who was not part of the ECRHS/RHINE study was included based on information given by G2.

Data collection

G1 participants provided information via the ECRHS/RHINE III questionnaire in 2010-2012, and G2 participants provided information via the RHINESSA questionnaire in 2013-2016. The G1 participants, not included in the ECRHS/RHINE study, participated via information provided by G2. G0 participated based on information provided by either G1 or G2.

All questionnaires had undergone a formal forward-backward translation to ensure homogeneity between study centres.

Farm exposure measurement

Information on place of upbringing was obtained from the ECRHS/RHINE III and the RHINESSA questionnaire by the question *"What term best describes the place you (your (grand)father, your (grand)mother) lived most of the time before the age of five years?",* with response categories: (a) farm with livestock, (b) farm without livestock, (c) village in a rural area, (d) small town, (e) suburb of city,

and (f) inner city. The same phrasing was used in both questionnaires. RHINESSA responders reported about themselves (G2), their parents (G1) and their grandparents (G0), whereas the ECRHS/RHINE responders only reported about themselves (G1). The groups were merged and analysed according to Table 4.2.

Paper no	Exposed generation	Levels analysed	Farm with livestock	Farm without livestock	Village in a rural area	Small town	Suburb of city	Inner City
Paper 1	G1	6 levels	-	-	-	-	-	-
Paper 2	G0+G1	3 levels	Farm		Village		City	
Paper 3	G2	2 levels	Farm		City			
Paper 4	G1	2 levels	Farm		Not farm			

Table 4.2: Overview of farm exposure measurement and merging in papers 1-4.

Asthma measurement

Asthma status was self-reported according to Table 4.3, where ECRHS/RHINE and RHINESSA subjects reported on behalf of themselves and their family members. Their own asthma status was accompanied with a retrospectively reported age of onset.

Responder	Data source	Variable	Measurement	Reporting about	Analysed in
G1	RHINE III	G1 asthma	*Do you have or have you ever had asthma?*	G1	Papers 1, 2, 3
G1	RHINE III	G1 doctor diagnosed asthma	*Have you ever had asthma diagnosed by a doctor?*	G1	Paper 1
G1	RHINE III	G1 allergy	*Do you have any nasal allergies including hay fever?*	G1	Paper 1
G1	RHINE III	G1 wheeze	*Have you ever had wheezing or whistling in you chest?*	G1	Paper 1
G1	RHINE III	G0 asthma	*Do your mother/father have or have they ever had asthma?*	G0	Papers 2, 3
G2	RHINESSA	G2 asthma	*Do you have or have you ever had asthma?*	G2	Papers 2, 4
G2	RHINESSA	G2 allergy	*Do you have any nasal allergies including hay fever?*	G2	Papers 1, 2, 4
G2	RHINESSA	G1 asthma	*Do your mother/father have or have they ever had asthma?*	G1 (not part of ECRHS/RHINE)	Paper 3

Table 4.3: Measurement of asthma, wheeze and hay fever in paper 1-4.

Co-variate determination and measurement

In papers 1 and 4 covariates were selected on the basis of a literature review. In papers 2 and 3 covariates were selected on the basis of Directed Acyclic Graphs (DAGs) (187). DAGs are a graphical tool which can be used to visually assess the hypothesised causal network of interest between exposure, outcome and covariates. The DAGs were based on subject matter knowledge and assumptions to form the basis for the analytical decisions. All DAGs were made in the software DAGitty 2.3, and presented in the appendix of the thesis.

Due to the varying prevalence of farm upbringing in the different study centres, it was a priori determined to adjust for centre in papers 1-3.

The selected covariates for paper 1 (The Gradient Study) were centre, age, sex, G1 smoking, body silhouette at 8 years, G0 smoking in G1 childhood and G0 asthma. These were measured according to Table 4.4. G1 smoking was categorised into current, ex- and never-smokers. Body silhouette was used as a marker of anthropometric characteristics and was classified as lean, normal and obese.

Responder	Data source	Variable	Measurement	Reporting about
G1	RHINE III	G1 age	*What is your date of birth?*	G1
G1	RHINE III	G1 sex	*Are you male or female?*	G1
G1	RHINE III	G1 smoking	*Are you a smoker? Are you an ex-smoker?*	G1
G1	RHINE III	G1 body silhouette at 8y	*Which picture best describes your body silhouette at the age of eight years?*	G1
G1	RHINE III	G0 smoking in G1 childhood	*Did your mother/father ever smoke regularly during your childhood?*	G0
G1	RHINE III	G0 asthma	*Do your mother/father have or have they ever had asthma?*	G0

Table 4.4: Covariates included in paper 1.

In paper 2 (The Generation Study), the minimum set of confounders was identified by the DAG. Adjustment for this set of confounders blocks any known backdoor paths between the exposure and the

outcome. Factors such as smoking, asthma status, socioeconomic status, gene expression (as an epigenetic marker), microbial exposure etc. were included in the DAG. The minimal adjustment sets for the association between parental (G1) place of upbringing and offspring (G2) asthma were identified: G0 asthma, G0 place of upbringing and G0 smoking. In addition, the model was adjusted for study centre.

Covariates for paper 2 were measured according to Table 4.5. Please note, that information on place of upbringing was available for all four G0 participants, whereas information on smoking and asthma was only available for half of the G0 participants.

Responder	Data source	Variable	Measurement	Reporting about
G1	ECRHS/ RHINE III	G0 asthma	*Do your mother/father have or have they ever had asthma?*	G0 (only parents for the ECRHS/RHINE participant)
G2	RHINESSA	G0 place of upbringing	*Which term best describes the place your grandmother/grandfather lived most of the time before the age of five years?*	G0
G1	ECRHS/ RHINE III	G0 smoking in G1 childhood	*Did your mother/father ever smoke regularly during your childhood?*	G0 (only parents for the ECRHS/RHINE participant)

Table 4.5: Covariates included in paper 2.

In paper 3 (The Selection Study), the DAG included parental smoking, parental and grandparental socioeconomic status and parental place of upbringing. The minimal adjustment set for the association between parental asthma and offspring place of upbringing was maternal place of upbringing and paternal place of upbringing. These were measured according to Table 4.6 with the following six response categories: (a) farm with livestock, (b) farm without livestock, (c) village in a rural area, (d) small town, (e) suburb of city, and (f) inner city. When possible, the information was obtained from the G1 participant him-/herself via the ECRHS/RHINE III questionnaire, and otherwise the information was obtained from G2 via the RHINESSA questionnaire. In addition, the model was adjusted for study centre.

Responder	Data source	Variable	Measurement	Reporting about
G1/G2	RHINE III and RHINESSA	G1 maternal place of upbringing	*Which term best describes the place you/your mother lived most of the time before the age of five years?*	G1: G1 G2: the G1 parent not part of ECRHS/RHINE
G1/G2	RHINE III and RHINESSA	G1 paternal place of upbringing	*Which term best describes the place you/your father lived most of the time before the age of five years?*	G1: G1 G2: the G1 parent not part of ECRHS/RHINE

Table 4.6: Covariates included in paper 3.

In paper 4 (The Agreement Study), the following covariates were selected: offspring upbringing, sex, asthma and hay fever. These were measured according to Table 4.7.

Responder	Data source	Variable	Measurement	Reporting about
G2	RHINESSA	G2 place of upbringing	*Which term best describes the place you lived most of the time before the age of five years?*	G2
G2	RHINESSA	G2 sex	*Are you male or female?*	G2
G2	RHINESSA	G2 asthma	*Do you have or have you ever had asthma?*	G2
G2	RHINESSA	G2 hayfever	*Do you have any nasal allergies including hay fever?*	G2

Table 4.7: Covariates included in paper 4.

Statistical analyses

In paper 1 (The Gradient Study), statistical analyses were performed on G1 subjects with complete information on all included variables. Data were analysed in Cox regression models with age as time scale and presented as hazard ratios (HRs) with corresponding 95 % confidence intervals (95% CIs). Subjects were assumed to be at risk from birth and were censored at the time of asthma/wheeze onset or at the end of follow-up, whichever occurred first. The Cox proportional hazard assumption was tested in log-log plots and found acceptable. HRs were presented for the six levels of urbanisation including p-values for urban-rural trend. In addition, HRs for comparing two adjacent levels of urbanisation were presented. Data were analysed in three statistical models: *crude*, *adjusted 1* (adjusted

for non-time dependent covariates: age, sex, parental asthma and study centre) and *adjusted 2* (adjusted for both non-time dependent and time dependent covariates: age, sex, parental asthma and study centre + smoking, body silhouette at 8 years and parental smoking in childhood). Analyses were performed for the two outcomes asthma and wheeze.
Additional analyses included estimation of prevalence and incidence and Cox regression on asthma stratified by centre, sex, smoking status, allergy status and time of onset (early/late onset). The cut-off point between early-onset and late-onset asthma was set at 10 years of age to ensure that asthma in puberty was not defined as childhood (early-onset) asthma.

In paper 2 (The Generation Study), statistical analyses were performed on the two parent/grandparent-offspring sets G1-G2 and G0-G2. Data were analysed in Cox regression models with G2 age as time scale and presented as HRs with corresponding 95% CIs. Clusters within families were taking into account by robust standard errors. Subjects were assumed to be at risk from birth and were censored at the time of asthma onset or at the end of follow-up, whichever appeared first. Thus, the Cox model accounts for the fact that the follow-up times of the study participants (G2) were different because of their different ages. The Cox proportional hazard assumption was tested in log-log plots and found acceptable. Analyses on parental (G1) place of upbringing and offspring (G2) asthma were presented in three statistical models: *crude*, *adjusted 1* (adjusted for centre and G0 place of upbringing) and *adjusted 2* (adjusted for centre, G0 place of upbringing, G0 asthma and G0 smoking). Analyses on grandparental (G2) place of upbringing and offspring (G0) asthma were only presented crude, due to lack of data on the great-grandparents.
Additional analyses included stratification by G2 place of upbringing, to isolate the presence of farm upbringing to the prior generation, and analyses on subjects with hay fever, to investigate the allergic asthma phenotype. Lastly, a quantitative bias analysis was performed to explore the potential bias from using second-hand information on parental place of upbringing from the offspring (G2) instead of direct information from the parent him-/herself (G1).

In paper 3 (The Selection Study), statistical analyses were performed as two identical analyses investigating the association between parental asthma and offspring place of upbringing in the two parent-offspring sets G0-G1 and G1-G2, as illustrated by the circles in Figure 4.8. Data were analysed

in binary regression models with log-link and presented as relative risks (RR) with corresponding 95% CIs taking clusters within families into account. Please note that farm upbringing was considered as outcome.

To investigate the centre-specific effects, analyses were presented for each study centre separately. Moving patterns were investigated by stratifying by the previous generation's place of upbringing. Furthermore, sub-analyses were conducted on parental allergy in a clinical subsample of the ECRHS/RHINE in G0-G1 (with information on both parents' allergies) and in G1-G2 (with information on one parent's allergy). A sensitivity analysis including repetition of G1-G2 analyses using only direct reports from the parent in ECRHS/RHINE instead of indirect reports from the RHINESSA participant was performed to explore the robustness of the results.

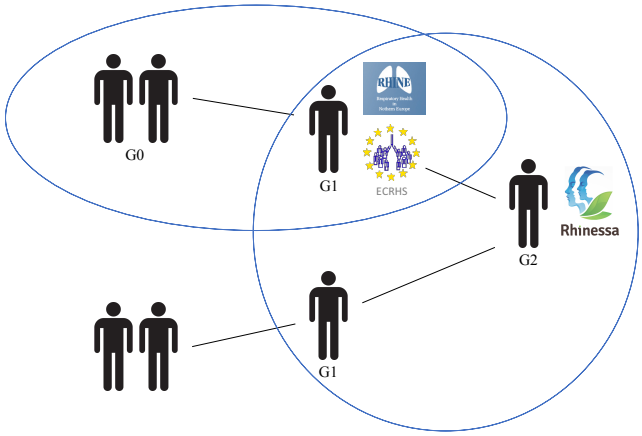


Figure 4.8: The three generations under study. The circles illustrate the two subsets of analyses, denoted G0-G1 and G1-G2, performed in paper 3.

In paper 4 (The Agreement Study), statistical analyses were performed on parent-offspring pairs from RHINE and RHINESSA (G1-G2). Data were presented as percentage of offspring misclassifying their parent's upbringing. A parent was considered as misclassified, if the offspring reported the opposite place of upbringing (farm/not farm) as being the opposite of that reported by the parent him-/herself. Agreement was presented for all and stratified by parental-reported upbringing, and offspring characteristics (place of upbringing, sex, asthma and hay fever).

Ethical approvals

For all studies included in this thesis, principles for good epidemiological practice were fulfilled (188). This means that for Denmark the project was approved by the Danish Data Protection Agency (J.nr. 2013-41-2213) and Danish Ethics Committee (Ref. no.: 1-10-72-301-15)

The local Science Ethics Committees approved the study for each study centre in both ECRHS/RHINE and RHINESSA, and informed consent was obtained from all study participants.

Chapter 5:

Overview of results

This chapter comprises a short overview of the main results from each of the four papers included in this thesis. A more detailed description of key- and sub-analyses can be found in the original papers.

Paper 1: The Gradient Study

The G1 study population (N = 11,123) comprised 1,181 cases with asthma (11%) and 2,133 cases with wheeze (19%). This corresponds to an incidence of 2.14 per 1,000 person years for asthma (95% CI 2.02-2.27) and 3.94 per 1,000 person years for wheeze (95% CI 3.78-4.11).

In Cox regression analyses, subjects who were born and raised on a livestock farm had significantly less asthma compared to their counterparts growing up in a city (HR 0.72, 95% CI 0.57-0.91). Furthermore, an urban-rural gradient was observed across the six levels of urbanisation (p for urban-rural trend 0.02). In sub-analyses, the urban-rural gradient in asthma was most clear among women, smokers and for late-onset asthma. The occurrence of allergic and non-allergic asthma did not follow an urban-rural gradient. The main results are shown in Figure 5.1.

Analyses on wheeze and place of upbringing showed similar results.

Based on these results, the hypothesis of a gradually increasing asthma occurrence across six levels of urbanisation from farm to city was confirmed, however, not consistently for all subgroups.

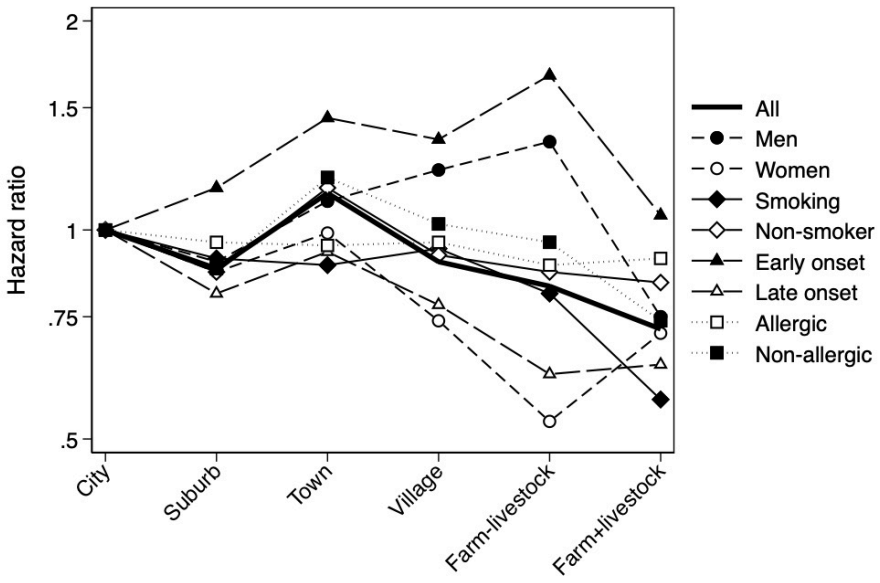


Figure 5.1: Cox regression analyses on asthma presented as HRs adjusted for age, sex, centre, parental asthma, smoking, body silhouette at 8 years of age and parental smoking in childhood (adjusted 2 model).

Paper 2: The Generation Study

The G2 study population comprised a total of 8,260 RHINESSA offspring including 1,490 cases with asthma. This corresponds to a prevalence of 18%.

In Cox regression models, parental (G1) farm upbringing was not associated with offspring (G2) asthma either among all offspring (HR 1.12, 95% CI 0.74-1.69) or when stratified by offspring's own place of upbringing. The main findings are shown in Figure 5.2. Similar results were found for the allergic asthma phenotype seperately (HR 0.96, 95% CI 0.54–1.70).

Quantitative bias analyses revealed the same picture regardless of whether the information on G1 place of upbringing was provided by G1 themselves or their offspring (G2). Similarly, grandparental (G0) farm upbringing was not associated with offspring (G2) asthma either in the maternal (HR 1.05, 95% CI 0.67-1.65) or paternal line (HR 1.02, 95% CI 0.62-1.68).

Thus, the hypothesis that the occurrence of asthma was lower in offspring with parents or grandparents from a farm compared to offspring with parents or grandparents from a city was not confirmed.

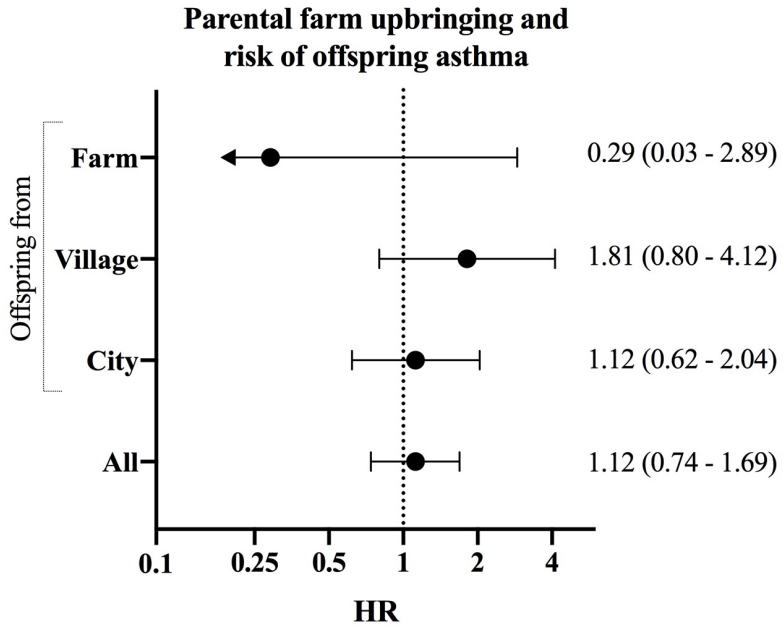


Figure 5.2: HRs with 95% CI for offspring asthma according to parental farm upbringing (both parents from farm vs. both parents from city) among offspring in RHINESSA adjusted for centre, grandparental asthma, grandparental upbringing and grandparental smoking (adjusted 2 model), for all (N = 4,279) and stratified by offspring upbringing.

Paper 3: The Selection Study

The study population included 6,045 G1 subjects participating in ECRHS/RHINE and 8,260 G2 subjects participating in RHINESSA.

In binary regression, parental asthma was not associated with offspring farm upbringing in either of the two parent-offspring sets G1-G2 (RR 1.11, 95% CI 0.81-1.52) and G0-G1 (RR 0.99, 95% CI 0.85-1.15). The main findings are presented in Figure 5.3.

With regard to moving patterns, asthmatic G1 parents born in the city tended to move and raise their G2 offspring on a farm (RR 2.00, 95% CI 1.12-3.55), whereas asthmatic G1 parents born on a farm tended to move and raise their G2 offspring in the city (RR 0.34, 95% CI 0.11-1.06). This pattern was not found among asthmatic G0 parents, who did not seem to change residential area.

Based on these results, the hypothesis of selective migration across generations was not confirmed.

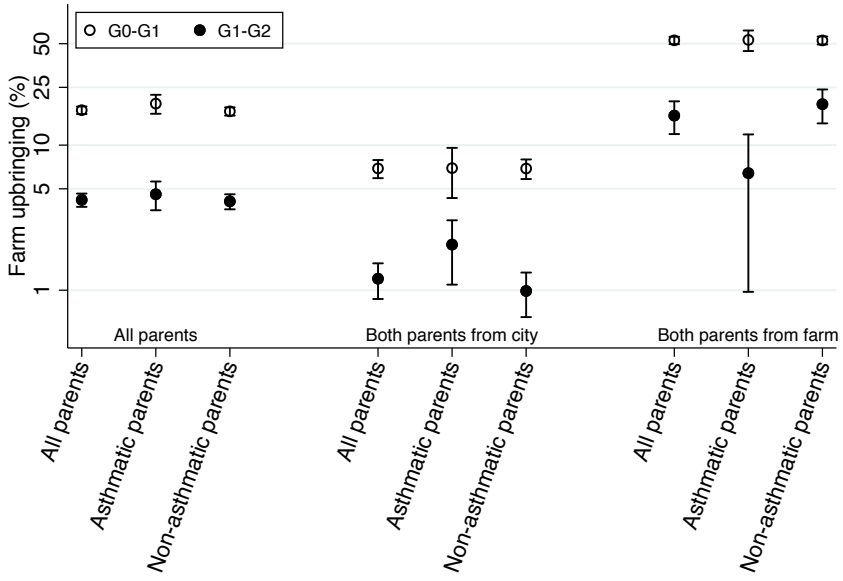


Figure 5.3: Percentage of offspring farm upbringing (outcome) according to parental asthma status (exposure) and parental place of upbringing.

Paper 4: The Agreement Study

The study population included 4,215 parent-offspring pairs.

In general, 10% of the offspring misclassified their parent's place of upbringing. The overall misclassification was higher among offspring reporting about parents from a farm than parents not from a farm (30% vs. 5%). If parental farm upbringing was considered as the target, this corresponds to a specificity of 95% and a sensitivity of 70%.

When stratifying on offspring characteristics, misclassification was highly related to the combination of parent and offspring upbringing. Nonfarm-raised offspring misclassified 33% of their farm-raised parents whereas farm-raised offspring misclassified only 8% of their farm-raised parents. Similarly, farm-raised offspring misclassified 14% of their nonfarm-raised parents whereas nonfarm-raised offspring only misclassified 4% of their nonfarm-raised parents. Misclassification was not related to offspring asthma or hay fever status. The main findings are shown in Figure 5.4.

Thus, the hypothesis of a good agreement between offspring and parental reports was partly confirmed.

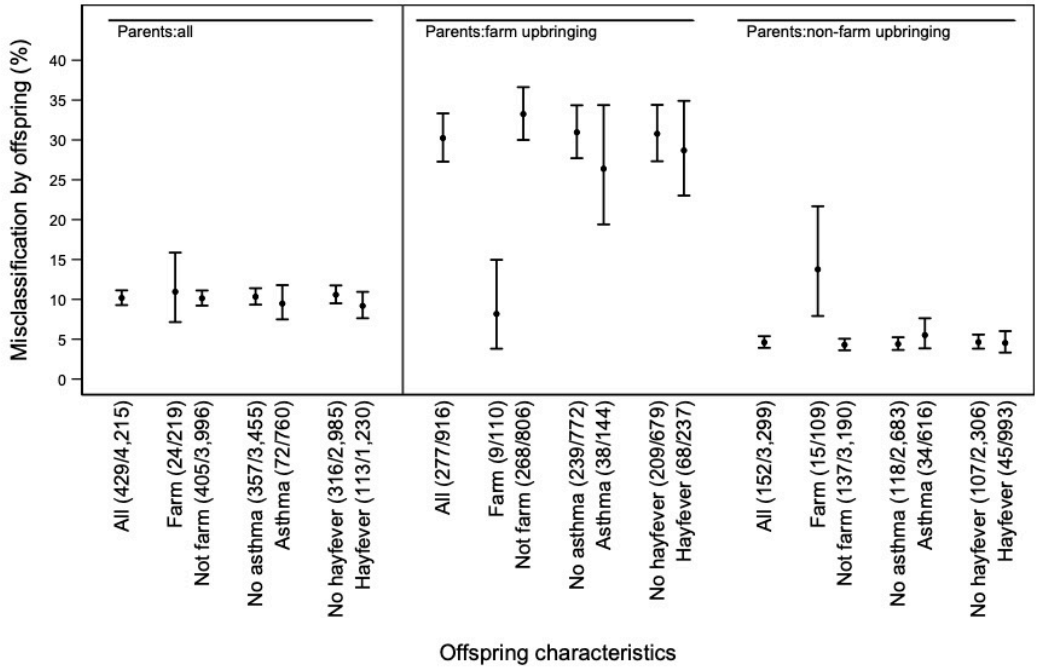


Figure 5.4: Percentage of offspring misclassifying their parent's upbringing (farm or not) sub-divided according to the parent's own report and offspring characteristics. Numbers in each group are in brackets (misclassified/total) and the vertical bars shows 95% CI.

Chapter 6:

Discussion

This chapter gives a reflective summary of the results and their relation to the international state-of-the-art research. This is followed by a discussion on methodological strengths and limitations that must be kept in mind when interpreting the results. Further details and methodological reflections can be found in each of the four original papers.

Interpretation of main findings in light of current evidence

Paper 1: The Gradient Study

This study found that subjects growing up on a livestock farm had significantly less asthma compared to subjects growing up in a city, and an urban-rural gradient was observed across six levels of urbanisation. The urban-rural gradient was most clear among smokers and women and for the late-onset phenotype.

The asthma prevalence found in the present study (11%) was in line with asthma prevalence estimates for other Northern European countries (57,59,96) as was the prevalence for wheeze (57).

Similar to the present study, Lawson et al. found an urban-rural gradient for asthma across three levels of urbanisation in a cross-sectional study among adolescents in Canada (189). Similarly, two studies within the RHINE cohort showed an urban-rural gradient for allergic rhinitis and the two inflammatory bowel diseases ulcerative colitis and Crohn's disease (132,190). In contrast with the present study, Elholm et al. found an urban-rural gradient for only allergic asthma among adult Danish men (191).

Urban upbringing has been shown to increase the risk of subsequent asthma, which has been ascribed to, apart from urban air pollution, the decreased level of microbial exposure in modern urban homes compared to the farming environment (16,169). In a systematic review and meta-analysis, childhood exposure to traffic-related air pollution (measured as black carbon, NO_2, $PM_{2.5}$ and PM_{10}) highly present in urban settings, showed increased risk of asthma across various disease definitions (192). In another review, farm upbringing was associated with less asthma and asthma-like symptoms in several studies performed in the three European paediatric cohorts ALEX, PARSIFAL and GABRIELA (193). In contrast, farm upbringing was not associated with asthma or wheeze in a study within the large ECRHS cohort (156). Heterogeneity with regard to farming locations, farming practices, farm sizes etc. within Europe may contribute to partly explain the diversity of the results. As an example, studies in the Alpine area and Poland has suggested that Polish farm children conferred less protection from asthma than German, Swiss and Austrian farm children (17,146,193).

The findings on a more pronounced effect from farm upbringing among smokers were unexpected. Smoking is less prevalent in farming areas (189), which is confirmed in this study, but smoking as an effect modifier for the association between farm upbringing and asthma may just be a chance finding. A Danish study found farm upbringing to be protective and smoking to be a risk factor for the development of late-onset asthma, but this study did not investigate smoking as a potential effect

modifier (162). A study on early-onset asthma and wheeze found farm upbringing to be protective even if the child was co-exposed to maternal smoking (194).

The sex-specific effects from farm upbringing are in line with the findings from the GABRIELA study in Germany showing a slightly more prominent effect of farm upbringing on girls (195). However, the GABRIELA study did not take livestock into account, and thus, their "farm" group may be analogous to "farm with livestock", "farm without livestock" and "village in a rural area" in the present study. In contrast, a study in Danish farming students did not find gender to be an effect modifier for the association between farm upbringing and asthma (162). The results regarding a more pronounced effect for late-onset asthma, may reflect the same underlying patterns because late-onset asthma more often occurs among women (75). In line with the present study, Omland et al. found a lower prevalence of late-onset asthma among 16-26 year old Danes born and raised on a farm; however, Ege et al. also found farm upbringing to be protective against early-onset asthma in a cross-sectional study in 5- to 13-year-old European children (17,162). Exposures in adulthood, including occupational exposures and air pollution, may also contribute to a higher risk of late-onset asthma among city dwellers, as place of residence in childhood and adulthood may be highly correlated.

Paper 2: The Generation Study

These multi-generation analyses suggest no evidence of an association between parental or grandparental farm upbringing and offspring asthma.

No other study has investigated farm exposure before conception and asthma in offspring. Studies on prenatal farm exposure are inconsistent, but a cross-sectional survey on New Zealand found that prenatal farm exposure may be associated with lower asthma risk in offspring (150). However, this was not replicated in an American nested case-control study investigating maternal farm living in pregnancy (155). A murine study showed that exposure to the farm-derived gram-negative bacterium *A. lwoffii F78* caused alternation in histone acetylation in specific genomic loci among pregnant mice, and thus decreased the risk of an asthmatic phenotype in offspring (196).

Studies on prenatal air pollution and offspring asthma indicate that the urban environment may induce transgenerational transmission of asthma susceptibility (197–199). In a recent multi-generational study within the RHINESSA cohort, Kuiper et al. found that maternal air pollution exposure (NO_2, $PM_{2.5}$, PM_{10}) from birth and up to 18 years of age was associated with increased risk of asthma in her

offspring (199). No association was found for paternal air pollution exposure in the same period (199). Baiz et al. investigated maternal air pollution exposure before and during pregnancy, and found that it may alter the immune competences in offspring and thus increase the risk of asthma (197). The relative distribution of NK cells and T-lymphocytes including CD4, CD25 and regulatory T-cells in cord blood was significantly changed after exposure to ambient air pollutants. A murine study by Gregory et al. showed that maternal exposure to diesel exhaust particles (DEP) and concentrated urban air particles (CAP) during pregnancy led to maternal transmission of increased asthma risk in offspring (198). As with air pollution, smoking is a significant risk factor for asthma and more pronounced in urban environments compared to farm environments. Accordini et al. investigated three generations within the RHINESSA cohort, and found that maternal smoking during pregnancy was significantly associated with a higher risk of asthma in offspring (109). However, compared to farm upbringing, smoking is a more potent exposure in terms of duration, magnitude and exposure route directly into the lungs. This may lead to a stronger impact on generational transmission of asthma risk than place of upbringing.

Paper 3: The Selection Study

This three-generation study suggest that selective migration from farming environments is not an important explanatory factor for the lower risk of asthma in people growing up on a farm. The results showed that parental asthma was not associated with offspring farm upbringing either in younger or older generations.

The current evidence on selective migration and asthma is inconsistent both with regard to selection into farming and selection away from farming. Bråbäck et al. investigated 43,234 Swedish men with farm parents, and found that at the age of 35-39 years, the asthmatic men were significantly less likely to live on a farm (27). Thus, they concluded that selective migration into farming could possibly explain the lower prevalence of asthma among farmers' children. Comparably, Vogelzang et al. investigated a population of Dutch 18- to 65-year-old men, and found that asthmatics were less likely to become a pig farmer compared to non-asthmatics, and thus also suggesting selection into farming (28). In contrast, Eduard et al. found no evidence of selective migration into farming, finding instead a similar prevalence of asthma among Danish farming students and their non-farming siblings (165). However, in a sub-analyses Eduard et al. found indications of selection out of farming among the

Norwegian farmers, as the asthma prevalence among early retired farmers was twice as high compared to active farmers. Klintberg et al. found no difference in family history of asthma among farmers' and non-farmers' children on the Swedish Island of Gotland (148). Interestingly, the studies by Bråbäck et al. and Vogelzang et al. both suggest patterns of selection into farming in populations comparable in age to G1 in the present study, whereas the studies of Eduard et al. suggest selection out of farming for that age-group. Klintberg et al. suggest no patterns of selection among populations comparable to G2 in the present study. The farming industry has undergone huge structural changes during the latest century, which may have influenced the likelihood of settling down on a farm. In addition with lower ability to move, G0 may not have been enlightened about the potential health consequences of being a farmer, whereas G1 may have been more aware and suspected that work-related respiratory exposures could cause or worsen potential asthma. Despite the literature showing different patterns of selective migrations into and away from farming in different generations, the present study showed the same patterns for both the younger and the older generations.

Paper 4: The Agreement Study

This study on parent-offspring pairs revealed that systematic misclassification within subgroups may be masked behind an overall low rate of misclassification because what the offspring reported about their parents tended to be the same as what they reported about themselves.

No other study has investigated the agreement between parental and offspring reports of parental upbringing. Overall, the literature shows good agreement regarding what offspring report about their parents and what they report about themselves. Two studies investigating parental smoking showed that offspring reports on maternal/paternal smoking in childhood and maternal smoking in pregnancy showed good agreement with parental reports (180,181). In addition, two studies investigated agreement between parental and offspring reports on offspring asthma and found good agreement (182,183). Kuiper et al. also investigated parental and offspring reports on parental asthma within the RHINESSA cohort, and found that parents seem to have more knowledge about the asthma status of their offspring than the offspring had about their parents' asthma (182). In line with the present study, Kuiper et al. also found patterns of misclassification within subgroups because mothers and never-smokers were more likely to report offspring asthma correctly (182).

Despite the systematic patterns of misclassification, the regression analyses showed similar results in both the quantitative bias analyses in paper 2 and the sensitivity analyses in paper 3, both of which compared estimates using direct or indirect reports of parental place of upbringing. Thus, in the absence of direct reports, second-hand information may be useful in epidemiological studies.

Methodological considerations

The four papers included in this thesis share several strength and limitations, and the present results must be interpreted with the following methodological considerations in mind. Thus, the three main components of the internal validity – information bias, selection bias and confounding –will be discussed in the following along with statistical considerations and external validity.

Internal validity

Misclassification and information bias

Misclassification of information is a general concern in epidemiology, and it may arise from systematic or random measurement errors. In descriptive statistics, misclassification may lead to an under- or overestimation of, for example, disease prevalence, and in comparative statistics, misclassification may induce bias depending on whether the misclassification is differential or non-differential.

The most important strength of the ECRHS/RHINE/RHINESSA studies is their population-based multi-generation study design. The study design is prospective because exposure (place of upbringing) takes place before the occurrence of disease. However, all the data are self-reported and collected retrospectively, participants having to recall their place of upbringing, asthma status and other lifestyle factors. The potential impact of recall bias and misclassification from this measurement of exposure and outcome is discussed in the following.

Information on place of upbringing

Studies have shown, that the microbial level and diversity is higher on farms compared to urban homes (16,166,169). In the present studies, place of upbringing is measured as a self-reported proxy of the microbial exposure, and is therefore less specific than objective measures such as direct measure of endotoxin exposure. Ambient air concentrations of endotoxin appear with variance both within and between farms, which is not captured by the present proxy of microbial exposure (200). This variation

may also be substantial across the long time period under study (for G1 and G2 equalling 1945-1973 and 1963-1998, respectively). Beside this time-dependent variation in microbial exposure, a large geographical variation is also likely both within and between study centres. This variation may be determined by e.g. the development from traditional small farms to industrialised bigger farms, but also the national regulations of the farming industry in each country. Studies on Amish and Hutterite children, who are comparable in lifestyle, but differ in farming tradition, showed that the traditional Amish farmers had 6.7 times higher endotoxin levels in their homes compared to the industrialised Hutterite families (201). The composition of bacteria also differed between the two communities, and interestingly the two types of farming practice showed various impact on asthma risk as the Amish children had substantially lower prevalence (5%) compared to the Hutterite children (21%) (201).
The type and number of livestock on the farms may also have an impact on the microbial exposure on the specific farms, as farms with poultry and swine contain higher exposures of dust and bacteria compared to i.e. farms with cattle (170,202). The type of stables may also vary between cattle farmers (both open and closed stables) and swine farmers (mainly closed stables) influencing the emission of dust from the stables.
The categories of place of upbringing was not accompanied with any objective characteristics, and the definition of e.g. “farm”, “village”, “small town” and “city” was left open to the responder. This may have caused some random error in the measurement of exposure. In addition, responders may think of the place as it is today and not as it was when they grew up. As a result of the increasing urbanisation, this may have resulted in an overreporting of urban or sub-urban upbringing in the groups from “village in a rural area” and “small town”. However, the data on place of upbringing was not validated with, for instance, geocoded residence or register data on rural and urban zones, so this is purely speculative.
The characteristics of inner city (e.g. presence of traffic related air pollution and green space) may also differ according to the country and population size, in example, between Denmark and Australia. However, this potential misclassification is not believed to be related to asthma, and hence the bias will be non-differential.
The measured place of upbringing is only valid for the first 5 years of life with an underlying assumption that the early exposure will have life-long effects. Studies suggest, that early life exposures may induce long lasting effects on immunological properties, however, this assumption may still be questioned (167). In addition, a study within a Danish farmer cohort shows that 59% of the farmers

were raised on a farm compared to 17% of the non-farmer controls, indicating a high correlation between childhood and adulthood farm exposure. Assuming an additive protective effect from lifelong farm exposure, this high correlation will potentially lead to an overestimation of the effect of early life farm exposure, if the subjects were considered to only spend their first 5 years on a farm but have actually stayed there for many more years. However, the dual effect of farm exposure across the life course are still widely debated (161).

To summarize, the measure of microbial exposure is crude and does not include information on e.g. farming practice or type of livestock on the farms, neither the size or the biodiversity of the city, which may also have an impact on the actual microbial exposure with a potential association to asthma development. This crude measurement is cost-effective, but does not provide any explanation of what it is in the farming environment that lowers the asthma risk. The issue of confounding from this exposure assessment is discussed further below.

Information on asthma

Asthma is a complex disease to measure due to the many facets of asthma phenotypes in addition to a large variation in severity and prognosis. A Danish study from the Danish National Birth Cohort comparing three different measurements of asthma found highly different prevalence when using different sources of information (47). The prevalence among children aged 7 years was highest when using the prescription registry (32%), followed by self-report (12%) and the hospitalization registry (7%) (47). Some definitions of asthma are more sensitive (efficient for defining true asthma cases) and some are more specific (efficient for defining true healthy controls), however, both scenarios causing misclassification of asthma status. There is no consensus for defining asthma diagnosis in epidemiological studies, but, self-reported asthma seem to have a high specificity and moderate sensitivity (49). Studies also indicate that too long time between follow-ups may cause some of the mild cases to report their asthma status incorrectly (64). The present definition of asthma cases may therefore include false negatives and, in addition, the use of a dichotomous variable to categorise asthma will not capture neither the severity nor the duration and current state of the disease. Another approach is to define asthma by a symptom-score, e.g. as the sum of positive answers to symptom questions as measured in the ECRHS study (203). In paper 2, a post-hoc comparison of ever-asthma and asthma score (defined by a positive answer to three symptom questions) showed the same

distribution of cases across the different categories of upbringing, suggesting that the bias from using ever-asthma as outcome definition is non-differential.

Defining asthma by wheezing, as done in paper 1, is a more sensitive but less specific measure of asthma, and consequently, this definition may also include people with bronchial hyperresponsiveness and other respiratory disorders in the group of cases. Thus, the analyses on wheeze in paper 1 showed higher prevalence compared to asthma, but similar tendencies on the urban-rural gradient.

One of the most specific measures of asthma may be doctor-diagnosed asthma, however, this definition rely on the individuals to seek health advice with their asthma symptoms (204). The medical and personal awareness of asthma and thereby the likelihood of receiving an asthma diagnosis may have increased over time, and may thus be lowest in G0 and highest in G2. Although asthma is measure with the same question in both ECRHS/RHINE and RHINESSA, this may have contributed to the increasing prevalence observed across generations. The diagnostic procedure may also have varied markedly between study centres over time. In addition, studies show, that among past doctor-diagnosed asthmatics, up to 33% did not qualify for a current diagnosis of asthma (205).

The reported age of asthma onset may also be subject to misclassification and recall bias. However, a validation study within the RHINE cohort showed that approximately 90% reported the correct year of disease onset (± 1 year) compared to their clinical diagnosis (66).

Second-hand reports of parental asthma may also be subject to both recall bias and misclassification. Offspring may not be aware if their parents have or have had mild asthma or if their parents only had asthma in childhood long before the offspring was born. This is in line with a previous study within the RHINESSA cohort showing that offspring were less likely to report if their parent had asthma after they had grown up (182).

To summarize, this thesis is based on information on asthma that is more specific than sensitive resulting in some false negatives while leaving the mildest and earliest cases out. However, this misclassification is unlikely to be associated with place of upbringing, and thus the bias from this will most likely be non-differential. This may have blurred the contrasts between exposure groups and induced bias towards the null.

Information on covariates

Information on all covariates included was self-reported and obtained from questionnaires. Taken together, this information comprises centre, sex, smoking, body silhouette and hay fever. No misclassification was expected in the information on centre and sex. However, smoking – both own smoking and parental smoking in childhood – may be subject to misclassification. In a systematic review, self-reported smoking information showed a trend towards underestimation and low sensitivity when compared to objective markers of nicotine in saliva, urine or blood (206). In addition, smokers with asthma-like symptoms are less likely to be diagnosed with asthma than symptomatic non-smokers, which will lead to an underestimation of asthma among smokers (207). Speculations on a "healthy smoker effect", implying that those who take up smoking may have more robust lungs, will also contribute to an underestimation of smoking on asthma (208). Likewise, offspring reports on parental smoking in childhood seem to have a low sensitivity and thus, misclassification was most pronounced among parents with low tobacco consumption (181). However, such misclassification may most likely not be associated with place of upbringing and is therefore non-differential.

Body silhouettes have shown a substantial overlap with anthropometric measurements and thus, a high prediction ability for identifying obesity (209). In addition, a study within the ECRHS/RHINE cohort comparing previously measured BMI and retrospective reports on body silhouettes at 30 and 45 years of age found that body silhouettes comprised a good epidemiological tool for reporting body composition in the past (210).

The use of self-reported hay fever as a marker of sensitisation may lead to misclassification in phenotyping cases into allergic and non-allergic asthmatics. An Australian study comparing self-reported hay fever with skin prick test as the "gold standard" to define allergic asthma showed an acceptable sensitivity and a moderate specificity (211). Likewise, an American study using specific IgE in blood as the "gold standard" to measure atopy, found that self-reported hay fever was only a modest predictor of allergic asthma (212). Consequently, the use of self-reported hay fever may have caused an overestimation of the allergic phenotype as only a part of the "allergic" were sensitised to allergens.

To summarize, some misclassification is expected on the covariates, which will most likely leave some residual confounding in the presented results.

Selection bias

Recruitment and loss to follow-up

The recruitment procedure of ECRHS/RHINE may have varied substantially since each study centre could tailor the recruitment strategy most likely to maximize the response rate (184). The default was to make a random sample of 1,500 men and women in each study centre, but how this random sample was selected may have varied markedly depending on the health registers available for randomization. Despite the inconsistent recruitment procedure, the ECRHS reached a median response rate of 78% (44).

The ECRHS questionnaire was conducted as an interview at the ECRHS clinical examinations, while the RHINE questionnaire was postal. For the RHINESSA questionnaire, all centers used a web-based questionnaire portal except for the three centers in Sweden, who used postal questionnaires. The RHINESSA offspring was either recruited from parents participating in ECRHS 1 (Bergen, Aarhus, Reykjavik, Tartu) or from parents participating in ECRHS 3 (Albacete, Huelva, Melbourne, Gothenburg, Umeå, Uppsala). The drop-out in the ECRHS study has been substantial (approx. 50%) (185), so the study centers concerning the ECRHS III participants as the parents with the offspring source population, may theoretically be missing half of the offspring. This inconsistency in the definition of the RHINESSA offspring across study centers, may therefore have left out some of the eligible offspring, who have not been invited to participate in RHINESSA. This may also apply to offspring from the 22%, who never entered the study due to non-response at the ECRHS/RHINE I questionnaire. In addition, only one third of the invited RHINESSA offspring returned the questionnaire meaning that the actual RHINESSA population is only a fraction of the eligible offspring. A non-response study within the RHINE cohort suggest a similar asthma prevalence among baseline participants and long-term responders (185). In addition, sub-analyses in paper 1 restricted to incident cases occurring between baseline and RHINE III showed similar results as the main analyses. These data are not available for the RHINESSA study yet, but similar patterns are plausible. Non-response analyses related to residence in Denmark and Belgium shows a higher likelihood of non-participation among urban dwellers compared to rural (213,214). To summarize, the non-response from self-selection may be related to place of upbringing, but probably not to asthma, and is therefore non-differential.

Sampling area

The original ECRHS population was sampled in and around bigger cities in different countries, mainly in Europe. This may have resulted in an overrepresentation of urban-dwellers, for instance, in paper 3 (Figure 2). However, paper 3 also shows a tendency towards high mobility in G1 when raising their children, which may have balanced the geographical skewness towards more diverse geographical areas in G2.

Missing data

Subjects with missing data were excluded from the performed statistical analyses. Drop-out caused by missing data may induce bias if the subjects with missing data differ on the association between exposure and outcome compared to the analysed study population. In general, the issue of missing data was more pronounced among G1 than G2 (for instance, 16% vs. 2% missing on own upbringing in paper 3). As an example, in paper 1 was 1,318 subjects (G1) excluded due to missing data on smoking. However, the prevalence of missing data was equally distributed across the six exposure categories, and thus the drop-out is non-differential.

Potential sources of confounding – a farm effect or a farmer effect?

There has been a continued debate on the dual effect of farming, that both seem to cause and prevent allergic and non-allergic asthma and asthma-like symptoms such as such as wheeze or bronchial hyper-responsiveness primarily among farmers and residents on farms, but interestingly also among the neighbouring residents (21,161,193,215–217). One may therefore question whether this is a "farm effect" or a "farmer effect" – or rather: if the protective effect is a matter of the exposures on the farm or the underlying health and lifestyle among the rural population.

Place of upbringing is considered as a marker of the microbial exposure in early life. However, the farming environment comprise a complex mixture of exposures such as airway irritants (dust, endotoxin, ammonia, mould and fungal spores) but also air pollution, gasses, allergens and various chemicals including pesticides (202). In addition, cities also harbour airborne pollutants due to traffic-related air pollution including $PM_{2.5}$, gaseous pollutants (ozone, nitrogen dioxide, sulphur dioxide) etc., which may also have an impact on asthma development in both children and adults (91). Comparing the farm and city environment may therefor include several underlying exposures beside the microbial

exposure, as the two environments may also differ with regard to, for instance, dietary habits, smoking, antibiotic use and lifestyle in general. The farm dwellers may more often have pets (e.g. cats, dogs), however, the urban dwellers may have their pets more indoor than outdoor in contrast with the farm dwellers. This mixture of exposures may have confounded the results on place of upbringing and asthma.

Statistical considerations

Statistical methods

The data used in papers 1 and 2 are cross-sectional as information on exposure and outcome are measured at the same time. However, the information given includes the time of exposure (first 5 years of life) and age of asthma onset, which warrants the use of survival analyses on retrospectively reported data as time of risk starting at birth. In addition, the Cox regression model assumes subjects to be at risk for their whole lives, despite their exposure being valid only for the first 5 years of life. Furthermore, data on smoking and asthma are collected at the same time, and the adjustment may therefore be problematic as we cannot rule out if they started smoking before or after they got asthma. However, this issue has been accounted for when analysing smoking as a time-dependent covariate in the Cox regression models in paper 1.

In addition, the participants are included at various ages and thus with various follow-up times. G1 mean age at follow-up in 2011 was 53 years and G2 mean age at recruitment in 2013 was 31 years. This will lead to an underestimation of the effects from farm upbringing if the effect is most pronounced for late-onset asthma because especially the G2-population may still be at risk of developing asthma in the years that follow. In analyses on G1 (Paper 1), this was actually the case because the sub-analyses showed farm upbringing to be significantly related to late-onset asthma (HR 0.64, 95% CI 0.49-0.83) and not related to early-onset asthma (HR 1.05, 95% CI 0.65-1.68). It is reasonable to assume that the same is true among G2. However, the Cox proportional hazard models take the various follow-up times into account by only comparing cases and controls with the same person-years at risk, and thus this statistical model is a more robust method to investigate participants with use of various follow-up times.

Statistical power and chance findings

The prevalence of farm upbringing is very low, especially among G2 (4%), resulting in reduced statistical power when stratifying by, in example, study centre or asthma phenotypes. This limitation also applies when investigating parental upbringing, as only 5% of the offspring had both of their parents raised on a farm. Thus, the results must be interpreted with these power limitations in mind. The hypotheses were a priory defined as well as a detailed analytical plan. Type 1 errors (false positive results) may occur with multiple testing, however, a large number of tests were not performed in any of the papers, and the likelihood of type 1 errors may therefore be small. However, the results with a more pronounced effect of farming among smokers in paper 1, may have been a chance finding.

Competing risks

Theoretically, competing risks, e.g. from death, may inhibit the possibility of observing the event of interest. Thus, this possibility is depending on time at risk and would affect the detection of especially the late-onset asthma phenotype. If one imagines, that subjects might have died from severe asthma attacks before entering the study, this will lead to an underestimation of both the prevalence and incidence. However, the asthma mortality is low, and the Cox regression model handled censoring due to death or emigration. Consequently, the estimates are apparently not affected by competing risks.

External validity

The homogeneity of the 7 to 11 ECRHS/RHINE/RHINESSA study centres in this thesis may influence the external validity and generalisability. The three Scandinavian countries (Denmark, Sweden and Norway) may be more similar to each other than they are to Iceland, Estonia, Spain and Australia with regard to farming practice, health systems and health-seeking behaviour. Furthermore, the three study centres in Sweden represented 43% of the RHINESSA population, whereas the two study centres in Spain represented 2%. This may have contributed to an overrepresentation of the Swedish population, or the Scandinavian population in general, and may have skewed the results with regard to descriptive estimates and associations. In addition, the distribution of participants living on a farm and those living in a city differed markedly between the study centres. In G1, farm upbringing ranged from 6% in Gothenburg (SE) to 29% in Umeå (SE), and likewise in G2, it ranged from 0% in Albacete (ES), Huelva (ES) and Melbourne (AU) to 10% in Umeå (SE). To overcome this potential skewness,

analyses in papers 1-3 were stratified by study centre, however, with very low power especially for the small study centres.

If the skewed proportions of study participants are taken into account, the results presented in this thesis may reasonably be generalised to other modern westernised countries.

Chapter 7:

Main conclusions and future research

This chapter briefly summarises the main conclusions of this thesis, and reflects on future opportunities and challenges for further research.

Overall, this thesis confirms and extend the current evidence on asthma and place of upbringing. Paper 1 (The Generation Study) confirmed the existing literature on the lower asthma risk among people born and raised on a farm, and extend the understanding of the urban-rural gradient with more detailed exposure groupings. Paper 2 (The Generation Study) explored an unexplored time window of farm exposure, and suggest that farm exposure in prior generations may not have a substantial influence on the asthma risk in offspring. Paper 3 (The Selection Study) confirmed existing literature suggesting no selective migration from farming environment, indicating that healthy selection is not an important explanatory factor behind the protective effect from farm upbringing on asthma. Paper 4 (The Agreement Study) investigated the agreement in second-hand information, and concluded that offspring misclassification was highly dependent on offspring own upbringing, as offspring tended to report the same for their parents as for themselves.
To summarize, this thesis has contributed unique knowledge on the association between asthma and place of upbringing by investigating more detailed exposure groupings in addition with exposure windows and selection patterns across generations. In addition, this thesis also adds valuable knowledge on the patterns and implications of misclassification from using second-hand information.

Further research in this field should preferably include several focus areas and different research disciplines. First, the potential farm effect must be confirmed in nationwide studies using, for instance, geocoded residence to better quantify the characteristics of place of upbringing in combination with registry information on asthma. Next, the underlying exposures in the farming environment should be investigated more, to specify the key sources of asthma protection. This may be done in large birth cohorts with a broad range of exposure data from conception and into late childhood. Third, the relationship between environmental microbiome and microbial composition e.g. in the gut and lungs should be elucidated in close collaboration with environmental scientists and microbiologists. This research may also include a better understanding of the crosstalk between the gut microbiome and development of the immune system during the early childhood period. Fourth, exposure to either farm-derived bacteria or probiotics should be evaluated in randomised controlled trials or intervention studies, to explore the impact of the microbial exposure in different doses, compositions and time windows. These studies may also reveal if the immune maturation is driven by certain microbial species to be present or the diversity of the microbial community. Fifth, the genetic component across

generations and gene-environment interactions have to be explored further in well-designed exposure studies with multiple generations and genome wide data available. Lastly, future studies may also address the current inconsistent evidence by attempting to replicate findings using repeated measurements. Given the time-dependent variability of both environmental exposures, the human microbiome and the epigenetic marks, this may not be an easy task.

Translating and combining epidemiological research and mechanistic studies regarding immune maturation seems key in the primary prevention of asthma. Future health strategies may therefore include cross-disciplinary work with involvement of both microbiologists, urban architects and almost everything in between.

Chapter 8:

English summary

This chapter summarises the research field on farm exposure and asthma, and outlines the contributions of the four original papers included in this thesis.

Asthma prevalence has been rising worldwide during the latest century, and the underlying causes are still largely unknown. Numerous studies have shown lower prevalence of asthma among people born and raised on a farm than their counterparts in the cities. Despite extensive research, it still remains unanswered whether the farm effect is restricted to the people living on the farms, whether the next generation may benefit from the farm exposure in their parents' early life and whether the farm effect is just the result of selective migration and not a biological effect of the farming environment. The aim of the work conducted in this thesis aims was to contribute to a better understanding of the early origin of asthma.

In *The Gradient Study* (Paper 1) we investigated the gradual association between place of upbringing and subsequent development of asthma. Overall, we found that the occurrence of asthma was gradually increasing across six levels of urbanisation from farm to city corresponding to the decreasing microbial exposure. However, this was not consistent within sub-groups.

In *The Generation Study* (Paper 2) we investigated the generational effects of parental and grandparental farm upbringing on offspring asthma. Overall, we observed no evidence for an association between farm upbringing in previous generations and offspring asthma, either for parental or grandparental farm upbringing. Findings remained similar when stratified by offspring's own upbringing and asthma phenotypes.

In *The Selection Study* (Paper 3) we investigated selective migration from farming environments, and thus, if parents with asthma were less likely to raise their children on a farm. Overall, we found that selective migration is not an important explanatory factor for the protective effect of farm upbringing on asthma. Results show that parental asthma was not associated with offspring farm upbringing, either in analyses of the younger generations or analyses of the older generations.

In *The Agreement Study* (Paper 4) we investigated the agreement between offspring's and parent's reporting of parental place of upbringing. Overall, the misclassification rate was acceptable; however, the misclassification rate was highly influenced by the offspring's own place of upbringing.

Chapter 9:

Dansk resumé

Dette kapitel opsummerer den nuværende viden om landbrugseksponering og astma, og sammenfatter det videnskabelige bidrag fra de fire originalartikler i denne PhD-afhandling.

Forekomsten af astma er steget markant over hele verden, og de bagvedliggende årsager for denne stigning er stadig ukendte. Flere studier har vist en lavere forekomst af astma blandt personer, der er født og opvokset på landet sammenlignet med byen, og denne effekt tilskrives den øgede mikrobielle stimulering af immunsystemet ved et liv på landet. Til trods for meget forskning på feltet, står det stadig ubesvaret hvorvidt den beskyttende effekt af en opvækst på landet kun er gældende for personer på gårdene, hvorvidt den beskyttende effekt kan videreføres til næste generation via epi-genetiske mekanismer, samt hvorvidt den beskyttende effekt i højere grad er et udtryk for selektive flyttemønstre og ikke en biologisk effekt af landbrugseksponering. Formålet med dette PhD-projekt er således at bidrage til en bedre forståelse af landbrugseksponering og den tidlige manifestation af astma.

I Gradient Studiet (artikel 1) undersøgte vi den gradvise sammenhæng mellem hvor man er født og opvokset og udvikling af astma. Studiet viste at forekomsten af astma var gradvist stigende på tværs af seks urbaniseringsgrupper fra gård til by svarende til en faldende mikrobiel eksponering. Dette fund var dog ikke konsistent i alle sub-analyser.

I Generations Studiet (artikel 2) undersøgte vi generationseffekter af forældres og bedsteforældres opvækst på landet for astma blandt børnene. Vi fandt ingen sammenhæng mellem landbrugseksponering i tidligere generationer og børnenes astma, hverken for forældres eller bedsteforældres landbrugseksponering. Dette fund var uændret for sub-grupper af børnenes egen opvækst og forskellige fænotyper af astma.

I Selektions Studiet (artikel 3) undersøgt vi selektive flyttemønstre for personer med astma og dermed om forældre med astma er mindre tilbøjelige til at opfostre deres børn på en gård. Studiet viste, at selektive flyttemønstre ikke er en vigtig forklarende faktor for sammenhængen mellem landbrugs-eksponering og astma. Resultaterne var sammenlignelige for de yngre og de ældre generationer.

I Overensstemmelses Studiet (artikel 4) undersøgte vi overensstemmelsen mellem børns og forældres afrapportering af hvor forældrene er født og opvokset. Generelt var der god overensstemmelse, men sub-analyser viste, at misklassifikation var associeret med hvor barnet selv er født og opvokset.

Chapter 10:

Thesis Q&A

In this chapter, research questions and corresponding answers are presented in their simplest form, while being aware that the truth is much more complicated than this.

Research questions and answers at a glance:

Paper 1: The Gradient Study

Is the occurrence of asthma gradually increasing across six levels of urbanisation from farm to city corresponding to the decreasing microbial exposure?

YES

Paper 2: The Generation Study

Is the farm-effect detectable across generations?

NO

Can offspring report of parental place of upbringing be used as a valid measure in the absence of direct measures?

YES

Paper 3: The Selection Study

Can the protective effect of farm upbringing be explained by selective migration away from farming environments?

NO

Paper 4: The Agreement Study

Is the agreement between offspring and parental reports of parental place of upbringing influenced by the offspring's own upbringing?

YES

References

1. Burney P, Chinn S, Jarvis D, Luczynska C, Lai E. Variations in the prevalence of respiratory symptoms, self-reported asthma attacks, and use of asthma medication in the European Community Respiratory Health Survey (ECRHS). Eur Respir J. 1996;9(4):687–95.

2. Eder W, Ege MJ, von Mutius E. The Asthma Epidemic. N Engl J Med. 2006;355:2226–35.

3. Ege MJ, Strachan DP, Cookson WOCM, Moffatt MF, Gut I, Lathrop M, et al. Gene-environment interaction for childhood asthma and exposure to farming in Central Europe. J Allergy Clin Immunol. 2011;127(1).

4. Morales E, Duffy D. Genetics and Gene-Environment Interactions in Childhood and Adult Onset Asthma. Front Pediatr. 2019;7(December):1–14.

5. Douwes J, Pearce N. Asthma and the westernization 'package.' Int J Epidemiol. 2002;31:1098–102.

6. Skadhauge LR, Christensen K, Kyvik KO, Sigsgaard T. Genetic and environmental influence on asthma: a population-based study of 11,688 Danish twin pairs. Eur Respir J. 1999;13:8–14.

7. World Health Organisation. The Global Asthma Report 2018. 2018.

8. Strachan DP. Hay fever, hygiene and household size. BMJ. 1989;299(November):1259–60.

9. Riedler J, Braun-Fahrländer C, Eder W, Schreuer M, Waser M, Maisch S, et al. Exposure to farming in early life and development of asthma and allergy: a cross-sectional survey. Lancet. 2001 Oct 6;358(9288):1129–33.

10. Von Ehrenstein OS, Von Mutius E, Illi S, Baumann L, Böhm O, Von Kries R. Reduced risk of hay fever and asthma among children of farmers. Clin Exp Allergy. 2000;30(2):187–93.

11. Riedler J, Eder W, Oberfeld G, Schreuer M. Austrian children living on a farm have less hay fever, asthma and allergic sensitization. Clin Exp Allergy. 2000;30(2):194–200.

12. Merchant J a., Naleway AL, Svendsen ER, Kelly KM, Burmeister LF, Stromquist AM, et al. Asthma and farm exposures in a cohort of rural Iowa children. Environ Health Perspect. 2005;113(3):350–6.

13. Downs SH, Marks GB, Mitakakis TZ, Lëuppi JD, Car NG, Peat JK. Having lived on a farm and protection against allergic diseases in Australia. Clin Exp Allergy. 2001;31(4):570–5.

14. Campbell B, Lodge C, Lowe A, Burgess J, Matheson M, Dharmage S. Exposure to 'farming' and objective markers of atopy: a systematic review and meta-analysis. Clin Exp Allergy. 2015;45(4):744–57.

15. Sozańska B, Pearce N, Błaszczyk M, Boznański a., Cullinan P. Changes in atopy prevalence and sibship effect in rural population at all ages. Allergy. 2015;(14)

16. Ege M, Mayer M. Exposure to environmental microorganisms and childhood asthma. N Engl J Med. 2011;364(8):701–9.

17. Ege MJ, Frei R, Bieli C, Schram-Bijkerk D, Waser M, Benz MR, et al. Not all farming environments protect against the development of asthma and wheeze in children. J Allergy Clin Immunol. 2007;119(5):1140–7.

18. Wlasiuk G, Vercelli D. The farm effect, or: when, what and how a farming environment protects from asthma and allergic disease. Curr Opin Allergy Clin Immunol. 2012;12(5):461–6.

19. Perkin MR, Strachan DP. Which aspects of the farming lifestyle explain the inverse association with childhood allergy? J Allergy Clin Immunol. 2006;117(6):1374–81.

20. Illi S, Depner M, Genuneit J, Horak E, Loss G, Strunz-Lehner C, et al. Protection from childhood asthma and allergy in Alpine farm environments—the GABRIEL Advanced Studies. J Allergy Clin Immunol. 2012;129(6):1470-1477.e6.

21. Douwes J, Travier N, Huang K, Cheng S, McKenzie J, Le Gros G, et al. Lifelong farm exposure may strongly reduce the risk of asthma in adults. Allergy. 2007;62(10):1158–65.

22. von Mutius E, Radon K. Living on a Farm: Impact on Asthma Induction and Clinical Course. Immunol Allergy Clin North Am. 2008;28(3):631–47.

23. Karmaus W, Ziyab A, Everson T, Holloway JW. Epigenetic mechanisms and models in the origin of asthma. Curr Opin Allergy Clin Immunol. 2013;13:63–9.

24. Ege MJ, Bieli C, Frei R, van Strien RT, Riedler J, Ublagger E, et al. Prenatal farm exposure is related to the expression of receptors of the innate immunity and to atopic sensitization in school-age children. J Allergy Clin Immunol. 2006 Apr;117(4):817–23.

25. Michel S, Busato F, Genuneit J, Pekkanen J, Dalphin J-C, Riedler J, et al. Farm exposure and time trends in early childhood may influence DNA methylation in genes related to asthma and allergy. Allergy. 2013;68(3):355–64.

26. Schaub B, Liu J, Höppler S, Schleich I, Huehn J, Olek S, et al. Maternal farm exposure modulates neonatal immune mechanisms through regulatory T cells. J Allergy Clin Immunol. 2009;123(4):774-82.e5.

27. Bråbäck L, Hjern A, Rasmussen F, Van Schayck CP, Knottnerus JA. Selective migration contributes to a healthy worker effect in the farming population. J Clin Epidemiol. 2006;59(1):102–3.

28. Vogelzang PFJ, van der Gulden JWJ, Tielen MJ., Folgering H, van Schayck CP. Health based selection for asthma, but not for chronic bronchitis, in pig farmers: an evidence based hypothesis. Eur Respir J. 1999;13(1):187.

29. Boulet L, Reddel HK, Bateman E, Pedersen S, Fitzgerald JM, Byrne PMO. The Global Initiative for Asthma (GINA): 25 years later. Eur Respir J. 2019;54:1–9.

30. Kwah JH, Peters AT. Asthma in adults: Principles of treatment. Allergy Asthma Proc. 2019;40(6):396–402.

31. Lockey RF. Asthma Phenotypes : An Approach to the Diagnosis and Treatment of Asthma. J Allergy Clin Immunol Pract. 2014;2(6):682–5.

32. Schatz M, Rosenwasser L. The Allergic Asthma Phenotype. J Allergy Clin Immunol Pract. 2014;2(6):645–8.

33. Peters SP, Winston-salem F. Asthma Phenotypes : Nonallergic (Intrinsic) Asthma. J Allergy Clin Immunol Pract. 2014;2(6):650–2.

34. London SJ, Gauderman WJ, Avol E, Rappaport EB, Peters JM. Family History and the Risk of Early-Onset Persistent, Early-Onset Transient, and Late-Onset Asthma. Epidemiology. 2001;12:577–83.

35. Dharmage SC, Perret JL, Custovic A. Epidemiology of Asthma in Children and Adults. Front Pediatr. 2019;7(June):1–15.

36. Vandenplas O, Wiszniewska M, Raulf M, Blay F De, Wijk RG Van, Moscato G. EAACI position paper: irritant-induced asthma. Allergy. 2014;69:1141–53.

37. Hargreave FE, Parameswaran K. Asthma, COPD and bronchitis are just components of airway disease. Eur Respir J. 2006;28(2):264–7.

38. Tinkelman DG, Price DB, Nordyke RJ, Halbert RJ, Tinkelman DG, Price DB, et al. Misdiagnosis of COPD and Asthma in Primary Care Patients 40 Years of Age and Over. J Asthma. 2006;43(1):75–80.

39. Wells CD, Joo MJ. COPD and asthma: Diagnostic accuracy requires spirometry. J Fam Pract. 2019;68(2):76–81.

40. Asher MI, Montefort S, Björkstén B, Lai CKW, Strachan DP, Weiland SK, et al. Worldwide time trends in the prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and eczema in childhood: ISAAC Phases One and Three repeat multicountry cross-sectional surveys. Lancet. 2006 Aug 26;368(9537):733–43.

41. Pearce N, Douwes J. Commentary : Asthma time trends - mission accomplished? Int J Epidemiol. 2005;34:1018–9.

42. Kauczor H, Heussel CP, Herth FJF. Asthma in the global NCD agenda: a neglected epidemic. Lancet. 2013;1(April 2013):96–8.

43. Sony A El. Asthma: the increasing, unchecked epidemic. Int J Tuberc Lung Dis. 2017;21(10):5588.

44. Janson C, Anto J, Burney P, Chinn S, de Marco R, Heinrich J, et al. The European Community Respiratory Health Survey: what are the main results so far? Eur Respir J. 2001 Sep 1;18(3):598–611.

45. Douwes J, Brooks C, Dalen C Van. Importance of Allergy in Asthma : An Epidemiologic Perspective. Curr Allergy Asthma Rep. 2011;11:434–44.

46. Chinn S, Jarvis D, Burney P, Cerveri I, Marco R De, Gislason T, et al. Increase in diagnosed asthma but not in symptoms in the European Community Respiratory Health Survey. Thorax. 2004;59:646–51.

47. Hansen S, Strøm M, Maslova E, Mortensen EL, Granström C, Olsen SF. A comparison of three methods to measure asthma in epidemiologic studies: Results from the Danish National Birth Cohort. PLoS One. 2012;7(5):3–7.

48. Marco R De, Cerveri I, Bugiani M, Ferrari M, Verlato G. An undetected burden of asthma in Italy: the relationship between clinical and epidemiological diagnosis of asthma. Eur Respir J. 1998;11:599–605.

49. Pekkanen J, Pearce N. Defining asthma in epidemiological studies. Eur Respir J. 1999;14(4):951–7.

50. Wonderen KE Van, Mark LB Van Der, Mohrs J, Riet G. Different definitions in childhood asthma: how dependable is the dependent variable? Eur Respir J. 2010;36(1):48–56.

51. Asher MI, Keil U, Anderson HR, Beasley R, Crane J, Martinez F, et al. International study of asthma and allergies in childhood (ISAAC): rationale and methods. Eur Respir J. 1995;8:483–91.

52. ISSAC Steering Committee. Worldwide variations in the prevalence of asthma symptoms : the International Study of Asthma and Allergies in Childhood (ISAAC). Eur Respir J. 1998;12:315–35.

53. Asher MI, Stewart AW, Wong G, Strachan DP, García-marcos L, Anderson HR, et al. Changes over time in the relationship between symptoms of asthma, rhinoconjunctivitis and eczema: A global perspective from the International Study of Asthma and Allergies in Childhood (ISAAC). Allergol Immunopathol. 2012;40(5).

54. Lai CKW, Beasley R, Crane J, Foliaki S, Shah J, Weiland S, et al. Global variation in the prevalence and severity of asthma symptoms: Phase Three of the International Study of Asthma and Allergies in Childhood (ISAAC). Tho. 2009;64(1):476–83.

55. Pearce N, Sunyer J, Cheng S, Chinn S, Bjo B. Comparison of asthma prevalence in the ISAAC and the ECRHS. Eur Respir J. 2000;16:420–6.

56. Weiland SK, Pearce N. Asthma prevalence in adults: good news? Thorax. 2004;59:637–8.

57. Jarvis D, Newson R, Janson C, Corsico A, Heinrich J, Anto JM, et al. Prevalence of asthma-like symptoms with ageing. Thorax. 2017;0:1–12.

58. Honkamäki J, Hisinger-mölkänen H, Ilmarinen P, Piirilä P, Tuomisto LE, Andersén H, et al. Age- and gender-specific incidence of new asthma diagnosis from childhood to late adulthood. Respir Med. 2019;154(June):56–62.

59. Torén K, Hermansson B a. Incidence rate of adult-onset asthma in relation to age, sex, atopy and smoking: A Swedish population-based study of 15 813 adults. Int J Tuberc Lung Dis. 1999;3(3):192–7.

60. Thomsen SF, Ulrik CS, Kyvik KO, Larsen K, Skadhauge LR, Steffensen I, et al. The Incidence of Asthma in Young Adults. Chest. 2005;127(6):1928–34.

61. Torén K, Ekerljung L, Kim J, Hillström J, Rönmark E, Lötvall J, et al. Adult-onset asthma in west Sweden - Incidence, sex differences and impact of occupational exposures. Respir Med. 2011;105:1622–8.

62. Basagaña X, Sunyer J, Zock J, Kogevinas M, Urrutia I. Incidence of Asthma and Its Determinants among Adults in Spain. Am J Respir Crit Care Med. 2001;164:1133–7.

63. Marco R De, Locatelli F, Cazzoletti L, Bugianio M, Carosso A, Marinoni A. Incidence of asthma and mortality in a cohort of young adults: a 7-year prospective study. Respir Res. 2005;10:1–10.

64. Hedman L, Bjerg A, Lundbäck B, Rönmark E. Conventional epidemiology underestimates the incidence of asthma and wheeze-a longitudinal population-based study among teenagers. Clin Transl Allergy. 2012;2(1):1.

65. Lundbäck B, Rönmark E, Jönsson E, Larsson K, Sandström T. Incidence of physician-diagnosed asthma in adults - a real incidence or a result of increased awareness? Report from the Obstructive Lung Disease in Northern Sweden Studies. Respir Med. 2001;95:685–92.

66. Torén K, Gislason T, Omenaas E, Jögi R, Forsberg B, Nyström L, et al. A prospective study of asthma incidence and its predictors: the RHINE study. Eur Respir J. 2004;24(6):942–6.

67. Pakkasela J, Ilmarinen P, Honkamäki J, Tuomisto LE, Andersén H, Piirilä P, et al. Age-specific incidence of allergic and non- allergic asthma. BMC Pulm Med. 2020;2:1–9.

68. Larsson K, Sundblad B, Ekerljung L, Ro E, Lundba B. No further increase of incidence of asthma : Incidence , remission and relapse of adult asthma in Sweden. Respir Med. 2008;102:1730–6.

69. Davidson WF, Leung DYM, Beck LA, Cecilia M, Boguniewicz M, Busse WW, et al. Report from the National Institute of Allergy and Infectious Diseases workshop on “Atopic dermatitis and the atopic march: Mechanisms and interventions.” J Allergy Clin Immunol. 2019;143(3):894–913.

70. Khan SJ, Dharmage SC, Matheson MC, Gurrin LC. Is the atopic march related to confounding by genetics and early-life environment? A systematic review of sibship and twin data. Allergy. 2017;73(June):17–28.

71. Nibbering B, Ubags NDJ. Microbial interactions in the atopic march. Clin Exp Immunol. 2020;199:12–23.

72. Kapoor R, Menon C, Hoffstad O, Bilker W, Leclerc P, Margolis DJ, et al. The prevalence of atopic triad in children with physician-confirmed atopic dermatitis. J Am Acad Dermatol. 2008;58(1):68–73.

73. Hulst AE Van Der, Klip H, Brand PLP. Risk of developing asthma in young children with atopic eczema : A systematic review. J Allergy Clin Immunol. 2007;120:565–9.

74. Shah R, Newcomb DC. Sex Bias in Asthma Prevalence and Pathogenesis. Front Immunol. 2018;9(December):1–11.

75. Naeem A, Silveyra P. Sex Differences in Paediatric and Adult Asthma. Eur Med J. 2019;4(2):27–35.

76. Balzano G, Fuschillo S, Melillo G, Bonini S. Asthma and sex hormones. Allergy. 2001;56:13–20.

77. Real F, Svanes C, Macsali F, Omenaas E. Hormonal factors and respiratory health in women – a review. Clin Respir J. 2008;(2):111–9.

78. Ekström M, Schiöler L, Grønseth R, Johannessen A. Absolute values of lung function explain the sex difference in breathlessness in the general population. Eur Respir J. 2017;49:1–9.

79. Global Burden of Disease 2015 Chronic Respiratory Disease Collaborators. Global, regional, and national deaths, prevalence, disability-adjusted life years, and years lived with disability for chronic obstructive pulmonary disease and asthma, 1990 – 2015: a systematic analysis for the Global Burden of Disease Study 2015. Lancet Respir Med. 2017;5:691–706.

80. Ferrante G, LaGrutta S. The Burden of Pediatric Asthma. Front Pediatr. 2018;6:186(JUNE).

81. Aanerud M, Carsin A, Sunyer J, Dratva J, Gislason T, Jarvis D, et al. Interaction between asthma and smoking increases the risk of adult airway obstruction. Eur Respir J. 2015;45:635–43.

82. Bui DS, Walters HE, Burgess JA, Perret JL, Bui MQ, Bowatte G, et al. Childhood Respiratory Risk Factor Profiles and Middle-Age Lung Function: A Prospective Cohort Study from the First to Sixth Decade. Ann Am Thorac Soc. 2018;15(9):1057–66.

83. Braman SS. The Global Burden of Asthma. Chest. 2006;130(1):4S-12S.

84. Taponen S, Lehtimäki L, Karvala K, Luukkonen R, Uitti J. Correlates of employment status in individuals with asthma: a cross-sectional survey. J Occup Med Toxicol. 2017;12(19):1–7.

85. Blanc ED, Besson C, Katz E, Ph D, Yelin E, Ph D. Work Disability Among Adults With Asthma. Chest. 1993;104(5):1371–7.

86. Grabenhenrich LB, Gough H, Reich A, Eckers N, Zepp F, Nitsche O, et al. Early-life determinants of asthma from birth to age 20 years : A German birth cohort study. J Allergy Clin Immunol. 2014;133(4):979-988.e3.

87. Lockett GA, Patil VK, Soto-Ramírez N, Ziyab A, Holloway JW, Karmaus W. Epigenomics and allergic disease. Epigenomics. 2013;5(6):685–99.

88. Lim RH, Kobzik L, Dahl M. Risk for Asthma in Offspring of Asthmatic Mothers versus Fathers : A Meta-Analysis. PLoS One. 2010;5(4).

89. Burke AH, Leonardi-bee J. Prenatal and Passive Smoke Exposure and Incidence of Asthma and Wheeze: Systematic Review and Metaanalyses. Pediatrics. 2012;129(4).

90. Mcleish AC, Zvolensky MJ, Mcleish AC, Ph D, Zvolensky MJ, Ph D. Asthma and Cigarette Smoking: A Review of the Empirical Literature. J Asthma. 2010;47(4):345–61.

91. Guarnieri M, Balmes JR. Outdoor air pollution and asthma. Lancet. 2015;383(9928):1581–92.

92. Bentayeb M, Norback D, Bednarek M, Bernard A, Cai G, Cerrai S, et al. Indoor air quality , ventilation and respiratory health in elderly residents living in nursing homes in Europe. Eur Respir J. 2015;45(2006343):1228–38.

93. Beasley R, Semprini A, Mitchell EA. Risk factors for asthma: is prevention possible? Lancet. 2015;386(9998):1075–85.

94. Holst GJ, Pørneki A, Lindgreen J, Thuesen B, Bønløkke J, Hyvärinen A, et al. Household dampness and microbial exposure related to allergy and respiratory health in Danish adults. Eur Clin Respir J. 2020;7(1).

95. Raulf M, Buters J, Chapman M, Cecchi L, De Blay F, Doekes G, et al. Monitoring of occupational and environmental aeroallergens - EAACI Position Paper: Concerted action of the EAACI IG occupational allergy and aerobiology & air pollution. Allergy Eur J Allergy Clin Immunol. 2014;69(10):1280–99.

96. Eagan TML, Gulsvik A, Eide GE, Bakke PS. Occupational airborne exposure and the incidence of respiratory symptoms and asthma. Am J Respir Crit Care Med. 2002;166(7):933–8.

97. Krishnamoorthy N, Khare A, Oriss TB, Raundhal M, Morse C, Yarlagadda M, et al. Early infection with respiratory syncytial virus impairs regulatory T cell function and increases susceptibility to allergic asthma. Nat Med. 2012;18(10).

98. McKeever TM, Lewis S a., Smith C, Collins J, Heatlie H, Frischer M, et al. Early exposure to infections and antibiotics and the incidence of allergic disease: A birth cohort study with the West Midlands General Practice Research Database. J Allergy Clin Immunol. 2002;109(1):43–50.

99. Bobolea I, Arismendi E, Valero A, Agustí A. Early Life Origins of Asthma: A Review of Potential Effectors. J Investig Allergol Clin Immunol. 2019;29(3):168–79.

100. Sevelsted A, Stokholm J, Bonnelykke K, Bisgaard H. Cesarean Section and Chronic Immune Disorders. Pediatrics. 2014;135(1):e92–8.

101. Stensballe LG, Simonsen J, Jensen SM, Bønnelykke K, Bisgaard H. Use of antibiotics during pregnancy increases the risk of asthma in early childhood. J Pediatr. 2013;162(4):832-838.e3.

102. Bisgaard H, Northman Hermansen M, Buchvald F, Loland L, Brydensholt Halkjaer L, Bønnelykke K, et al. Childhood Asthma after Bacterial Colonization of the Airway in Neonates. N Engl J Med. 2007;357:1487–95.

103. Lodge C, Tan D, Lau M, Dai X, Tham R, Bowatte G. Breastfeeding and asthma and allergies: a systematic review and. Acta Paediatr. 2015;104:38–53.

104. Svanes C, Heinrich J, Jarvis D, Chinn S, Omenaas E, Gulsvik A, et al. Pet-keeping in childhood and adult asthma and hay fever: European community respiratory health survey. J Allergy Clin Immunol. 2003;112(2):289–300.

105. Hesselmar B, Åberg N, Åberg B, Eriksson B, Björkstén B. Does early exposure to cat or dog protect against later allergy development? Clin Exp Allergy. 1999;29(5):611–7.

106. Prescott S, Saffery R. The role of epigenetic dysregulation in the epidemic of allergic disease. Clin Epigenetics. 2011;2(2):223–32.

107. Krauss-Etschmann S, Meyer KF, Dehmel S, Hylkema MN. Inter-and transgenerational epigenetic inheritance: Evidence in asthma and COPD? Clin Epigenetics. 2015;7(1):1–13.

108. Lau MYZ, Dharmage SC, Burgess J a, Lowe a J, Lodge CJ, Campbell B, et al. CD14 polymorphisms, microbial exposure and allergic diseases: a systematic review of gene-environment interactions. Allergy. 2014;69(11):1440–53.

109. Accordini S, Calciano L, Johannessen A, Portas L, Benediktsdóttir B, Bertelsen RJ, et al. A three-generation study on the association of tobacco smoking with asthma. Int J Epidemiol. 2018;(March):1106–17.

110. Stokholm J, Chawes BL, Vissing N, Bønnelykke K, Bisgaard H. Cat exposure in early life decreases asthma risk from the 17q21 high-risk variant. J Allergy Clin Immunol. 2017;1–9.

111. Wahn U, Sampson HA. Allergy, immunity and tolerance in early childhood - the first steps of the atopic march. Elsevier Inc.; 2016.

112. Rook G a W. The hygiene hypothesis and Darwanian medicine. Birkhäuser - Progress in inflammation research; 2009.

113. Gluckman PD, Hanson MA, Cooper C, Thornburg KL. Effect of in utero and early-life conditions and adult health and disease. N Engl J Med. 2008;359:1523–4;

114. Bloomfield F S, Rook AW G, Scoot A E, Shanahan F, Stanwell-smith R, Turner P. Time to abandon the hygiene hypothesis: new perspectives on allergic disease , the human microbiome , infectious disease prevention and the role of targeted hygiene. Perspect Public Health. 2016;136(4):213–24.

115. Barker D, Osmond C. Infant Mortality, Childhood Nutrition, and Ischaemic Heart Disease in England and Wales. Lancet. 1986;327(8489):1077–81.

116. Barker DJ. Fetal origins of coronary heart disease. BMJ. 1995;311(6998):171–4.

117. Wadhwa P, Buss C, Entringer S, Swanson J. Developmental Origins of Health and Disease: Brief History of the Approach and Current Focus on Epigenetic Mechanisms. Semin Reprod Med. 2009;27(5):358–68.

118. Linneberg A. Hygiene hypothesis : wanted — dead or alive Author ' s Response Hygiene hypothesis : wanted — dead or alive. Int J Epidemiol. 2010;39(1):313–4.

119. Douwes J, Pearce N. Commentary: The end of the hygiene hypothesis? Int J Epidemiol. 2008;37(May):570–2.

120. Pearce N, Pekkanen J, Beasley R. How much asthma is really attributable to atopy? Thorax. 1999;54:268–72.

121. Brooks C, Pearce N, Douwes J. The hygiene hypothesis in allergy and asthma: an update. Curr Opin Allergy Clin Immunol. 2013;13(1).

122. Weber J, Illi S, Nowak D, Schierl R, Holst O, Mutius E Von, et al. Asthma and the Hygiene Hypothesis - Does Cleanliness Matter? Am J Respir Crit Care Med. 2015;191(5):522–9.

123. Sheikh A. There is no evidence of an inverse relationship between T H 2-mediated atopy and T H 1-mediated autoimmune disorders : Lack of support for the hygiene hypothesis. 2003;(1):131–5.

124. Sheikh A, Strachan DP. The hygiene theory: fact or fiction? Curr Opin Otolaryngol Head Neck Surg. 2004 Jun;12(3):232–6.

125. Rook G a, Stanford JL. Give us this day our daily germs. Immunol Today. 1998;19(3):113–6.

126. Rook G a W, Adams V, Hunt J, Palmer R, Martinelli R, Brunet LR. Mycobacteria and other environmental organisms as immunomodulators for immunoregulatory disorders. Springer Semin Immunopathol. 2004;25(3–4):237–55.

127. Rook G a W, Brunet LR. Old friends for breakfast. Clin Exp Allergy. 2005;35(7):841–2.

128. Guarner F, Bourdet-Sicard R, Brandtzaeg P, Gill HS, McGuirk P, van Eden W, et al. Mechanisms of disease: the hygiene hypothesis revisited. Nat Clin Pract Gastroenterol Hepatol. 2006;3(5):275–84.

129. Rook G a W. 99th Dahlem conference on infection, inflammation and chronic inflammatory disorders: darwinian medicine and the “hygiene” or “old friends” hypothesis. Clin Exp Immunol. 2010;160(1):70–9.

130. Rook G a W. Hygiene hypothesis and autoimmune diseases. Clin Rev Allergy Immunol. 2012;42(1):5–15.

131. Rook G a W. The hygiene hypothesis and the increasing prevalence of chronic inflammatory disorders. Trans R Soc Trop Med Hyg. 2007;101(11):1072–4.

132. Timm S, Svanes C, Janson C, Sigsgaard T, Johannessen A, Gislason T, et al. Place of upbringing in early childhood as related to inflammatory bowel diseases in adulthood: A population-based cohort study in Northern Europe. Eur J Epidemiol. 2014;29:429–37.

133. Hanski I, von Hertzen L, Fyhrquist N, Koskinen K, Torppa K, Laatikainen T, et al. Environmental biodiversity, human microbiota, and allergy are interrelated. Proc Natl Acad Sci U S A. 2012 May 22;109(21):8334–9.

134. Hertzen L Von, Hanski I, Haahtela T. Natural immunity. EMBO Rep. 2011;12(11):1089–93.

135. Matricardi PM. 99 th Dahlem Conference on Infection , Inflammation and Chronic Inflammatory Disorders: Controversial aspects of the ‘ hygiene hypothesis .’ Clin Exp Immunol. 2010;160:98–105.

136. Munyaka PM, Khafipour E, Ghia J-E. External influence of early childhood establishment of gut microbiota and subsequent health implications. Front Pediatr. 2014;2(October):109.

137. Arrieta M-C, Stiemsma LT, Amenyogbe N, Brown EM, Finlay B. The Intestinal Microbiome in Early Life: Health and Disease. Front Immunol. 2014 Sep;5(September):1–18.

138. Gilbert J, Blaser MJ, Caporaso JG, Jansson J, Susan V. Current understanding of the human microbiome. Nat Med. 2018;24(4):392–400.

139. Noverr MC, Huffnagle GB. Does the microbiota regulate immune responses outside the gut? Trends Microbiol. 2004;12(12):562–8.

140. Jatzlauk G, Bartel S, Heine H, Schloter M, Krauss-Etschmann S. Influences of environmental bacteria and their metabolites on allergies, asthma, and host microbiota. Allergy. 2017;3(June):1859–67.

141. Sbihi H, CT Boutin R, Chelsea C, Suen M, Brett Finlay B, Turvey SE. Thinking bigger : How early-life environmental exposures shape the gut microbiome and influence the development of asthma and allergic disease. Allergy. 2019;(November 2018):2103–15.

142. Stokholm J, Blaser MJ, Thorsen J, Rasmussen MA, Waage J, Vinding RK, et al. Maturation of the gut microbiome and risk of asthma in childhood. Nat Commun. 2018;(JAN):1–10.

143. Pattaroni C, Watzenboeck ML, Schneidegger S, Marsland CP, Roth-kleiner M, Marsland BJ. Early-Life Formation of the Microbial and Immunological Environment of the Human Airways. Cell Host Microbe. 2018;24:857–65.

144. Man WH, Piters WAADS, Bogaert D. The microbiota of the respiratory tract: gatekeeper to respiratory health. Nat Rev Microbiol. 2017;15(May):259–70.

145. Hilty M, Burke C, Pedro H, Cardenas P, Bush A, Bossley C, et al. Disordered Microbial Communities in Asthmatic Airways. PLoS One. 2010;5(1).

146. MacNeill SJ, Sozanska B, Danielewicz H, Debinska A, Kosmeda A, Boznanski A et al. Asthma and allergies: Is the farming environment (still) protective in Poland? the GABRIEL Advanced Studies. Allergy Eur J Allergy Clin Immunol. 2013;68(6):771–9.

147. Bråbäck L, Hjern A, Rasmussen F. Trends in asthma, allergic rhinitis and eczema among Swedish conscripts from farming and non-farming environments. A nationwide study over three decades. Clin Exp Allergy. 2004 Jan;34(1):38–43.

148. Klintberg B, Berglund N, Lilja G, Wickman M, van Hage-Hamsten M. Fewer allergic respiratory disorders among farmers' children in a closed birth cohort from Sweden. Eur Respir J. 2001;17(6):1151–7.

149. Kilpeläinen M, Terho EO, Helenius H, Koskenvuo M. Farm environment in childhood prevents the development of allergies. Clin Exp Allergy. 2000;30(2):201–8.

150. Douwes J, Cheng S, Travier N, Cohet C, Niesink A, McKenzie J, et al. Farm exposure in utero may protect against asthma, hay fever and eczema. Eur Respir J. 2008;32(3):603–11.

151. Ernst P, Cormier Y. Relative scarcity of asthma and atopy among rural adolescents raised on a farm. Am J Respir Crit Care Med. 2000;161(5):1563–6.

152. Adler A, Tager I, Quintero DR. Decreased prevalence of asthma among farm-reared children compared with those who are rural but not farm-reared. J Allergy Clin Immunol. 2005;115(1):67–73.

153. Braun-Fahrländer C, Gassner M, Grize L, Neu U, Sennhauser FH, Varonier HS, et al. Prevalence of hay fever and allergic sensitization in farmer's children and their peers living in the same rural community. Clin Exp Allergy. 1999;29(1):28–34.

154. Wickens K, Lane JM, Fitzharris P, Siebers R, Riley G, Douwes J, et al. Farm residence and exposures and the risk of allergic diseases in New Zealand children. Allergy. 2002;57(12):1171–9.

155. House JS, Wyss AB, Hoppin JA, Richards M, Long S, Umbach DM, et al. Early-life farm exposures and adult asthma and atopy in the Agricultural Lung Health Study. J Allergy Clin Immunol. 2017;140(1):249-256.e14.

156. Leynaert B, Neukirch C, Jarvis D, Chinn S, Burney P, Neukirch F. Does living on a farm during childhood protect against asthma, allergic rhinitis, and atopy in adulthood? Am J Respir Crit Care Med. 2001;164(10 I):1829–34.

157. Slaats GGG, Reinius LE, Alm J, Kere J, Scheynius A, Joerink M. DNA methylation levels within the CD14 promoter region are lower in placentas of mothers living on a farm. Allergy. 2012;67(7):895–903.

158. Lampi J, Canoy D, Jarvis D, Hartikainen A. Farming environment and prevalence of atopy at age 31: prospective birth cohort study in Finland. Clin Exp Allergy. 2011;41:987–93.

159. Portengen L, Sigsgaard T, Omland Ø, Hjort C, Heederik D, Doekes G. Low prevalence of atopy in young Danish farmers and farming students born and raised on a farm. Clin Exp allergy. 2002;32(2):247–53.

160. Schenker MB. Farming and asthma. Occup Environ Med. 2005;62:211–2.

161. Eduard W, Douwes J, Omenaas E, Heederik D. Do farming exposures cause or prevent asthma? Results from a study of adult Norwegian farmers. Thorax. 2004 May;59(5):381–6.

162. Omland Ø, Hjort C, Pedersen OF, Miller MR, Sigsgaard T. New-onset asthma and the effect of environment and occupation among farming and nonfarming rural subjects. J Allergy Clin Immunol. 2011;128(4):761–5.

163. Douwes J, Travier N, Huang K, Cheng S, Mckenzie J, Gros G Le, et al. Lifelong farm exposure may strongly reduce the risk of asthma in adults. Allergy. 2007;62(17):1158–65.

164. Le Moual N, Kauffmann F, Eisen E, Kennedy S. The healthy worker effect in asthma: work may cause asthma, but asthma may also influence work. Am J Respir Crit Care Med. 2008;177(Jan (1)):4–10.

165. Eduard W, Schlünssen V, Sigsgaard T, Omland Ø, Pearce N, Douwes J. Can selection explain the protective effects of farming on asthma? Ann Agric Environ Med. 2015;22(3):467–9.

166. Parajuli A, Grönroos M, Siter N, Puhakka R, Vari HK. Urbanization Reduces Transfer of Diverse Environmental Microbiota Indoors. Front Microbiol. 2018;9(February):1–13.

167. Rook G a W. Hygiene and other early childhood influences on the subsequent function of the immune system. Dig Dis. 2011;29(2):144–53.

168. Mutius E Von, Vercelli D. Farm living: effects on childhood asthma and allergy. Nat Publ Gr. 2010;10(12):861–8.

169. Vestergaard D V, Holst GJ, Basinas I, Elholm G, Sigsgaard T, Marshall IPG, et al. Pig Farmers' Homes Harbor More Diverse Airborne Bacterial Communities Than Pig Stables or Suburban Homes. Front Microbiol. 2018;9(May):1–14.

170. Basinas I, Sigsgaard T, Kromhout H, Heederik D, Wouters IM, Schlünssen V. A comprehensive review of levels and determinants of personal exposure to dust and endotoxin in livestock farming. J Expo Sci Environ Epidemiol. 2015;25(2):123–37.

171. Sozanska B. Raw Cow's Milk and Its Protective Effect on Allergies and Asthma. Nutrients. 2019;11:469–80.

172. Perkin MR. Unpasteurized milk: health or hazard? Clin Exp Allergy. 2007;37:627–30.

173. Abrahamsson TR, Jakobsson HE, Andersson AF, Bj B. Low gut microbiota diversity in early infancy precedes asthma at school age. Clin Exp Allergy. 2013;44:842–50.

174. Bisgaard H, Li N, Bonnelykke K, Chawes BLK, Skov T, Paludan-Müller G, et al. Reduced diversity of the intestinal microbiota during infancy is associated with increased risk of allergic disease at school age. J Allergy Clin Immunol. 2011;128(3).

175. Heederik D, Von Mutius E. Does diversity of environmental microbial exposure matter for the occurrence of allergy and asthma? J Allergy Clin Immunol. 2012;130(1):44–50.

176. Lauener RP, Birchler T, Adamski J. Expression of CD14 and Toll-like receptor 2 in farmers' and non- farmers' children. Lancet. 2002;360:465–6.

177. Schaub B, Liu J, Höppler S, Schleich I, Huehn J, Olek S, et al. Maternal farm exposure modulates neonatal immune mechanisms through regulatory T cells. J Allergy Clin Immunol. 2009;123(4):774–82.

178. Peters M, Kauth M, Swarze J, Körner-Rettberg C, Riedler J, Nowak D. Inhalation of stable dust extract prevents allergen induced airway inflammation and hyperresponsiveness. Thorax. 2006;61:134–9.

179. Schuijs MJ, Willart MA, Vergote K, Gras D, Deswarte K, Ege MJ, et al. Farm dust and endotoxin protect against allergy through A20 induction in lung epithelial cells. Science. 2015;349(6252):1907–11.

180. Simard JF, Rosner BA, Michels KB. Exposure to cigarette smoke in utero - comparison of reports from mother and daughter. Epidemiology. 2008;19(4):628–33.

181. Pape K, Svanes C, Malinovschi A, Benediktsdottir B, Lodge C. Agreement of offspring-reported parental smoking status: the RHINESSA generation study. BMC Public Health. 2019;19(94):1–9.

182. Kuiper IN, Svanes C, Benediktsdottir B, Bertelsen RJ, Bråbäck L, Dharmage SC, et al. Agreement in reporting of asthma by parents or offspring - the RHINESSA generation study. BMC Pulm Med. 2018;18(122).

183. Hedman L, Lindgren B, Perzanowski M. Agreement between parental and self-completed questionnaires about asthma in teenagers. Pediatr Allergy Immunol. 2005;16:176–81.

184. Burney PGJ, Luczynska C, Chinn S, Jarvis D. The European Community Respiratory Health Survey. Eur Respir J. 1994;7(5):954–60.

185. Johannessen A, Verlato G, Benediktsdottir B, Forsberg B, Franklin K, Gislason T, et al. Longterm follow-up in European respiratory health studies - patterns and implications. BMC Pulm Med. 2014 Jan;14(1):63.

186. Svanes C, Johannessen A, Bertelsen R, Dharmage S, Malinovschi A, Bråbäck L, et al. Manuscript in preperation "Cohort profile: The multi-generation Respiratory Health in Northern Europe, Spain and Australia (RHINESSA) cohort."

187. Glymour MM, Greenland S. Causal Diagrams. In: Modern Epidemiology. Lippincott Williams & Wilkins; 2012. p. 183–212.

188. IEA European Federation. Good epidemiological practice (GEP). 2004;1–10.

189. Lawson J a., Janssen I, Bruner MW, Madani K, Pickett W. Urban-rural differences in asthma prevalence among young people in Canada: The roles of health behaviors and obesity. Ann Allergy, Asthma Immunol. 2011;107(3):220–8.

190. Christensen S, Timm S, Janson C, Benediktsdottir B, Forsberg B, Holm M, et al. A clear urban-rural gradient of allergic rhinitis in a population-based study in Northern Europe. Eur Clin Respir J. 2016;3(33463).

191. Elholm G, Linneberg A, Husemoen LLN, Omland Ø, Grønager PM, Sigsgaard T, et al. The Danish urban-rural gradient of allergic sensitization and disease in adults. Clin Exp Allergy. 2015;12(June).

192. Khreis H, Kelly C, Tate J, Parslow R, Lucas K, Nieuwenhuijsen M. Exposure to traffic-related air pollution and risk of development of childhood asthma: A systematic review and meta-analysis. Environ Int. 2017;100:1–31.

193. Wells AD, Poole J a., Romberger DJ. Influence of farming exposure on the development of asthma and asthma-like symptoms. Int Immunopharmacol. 2014;23(1):356–63.

194. Horak E, Morass B, Ulmer H, Genuneit J, Braun-Fahrländer C, von Mutius E. Prevalence of wheezing and atopic diseases in Austrian schoolchildren in conjunction with urban, rural or farm residence. Wien Klin Wochenschr. 2014;1–5.

195. Genuneit J. Sex-specific development of asthma differs between farm and nonfarm children: A cohort study. Am J Respir Crit Care Med. 2014;190(5):588–90.

196. Brand S, Teich R, Dicke T, Harb H, Yildirim AÖ, Tost J, et al. Epigenetic regulation in murine offspring as a novel mechanism for transmaternal asthma protection induced by microbes. J Allergy Clin Immunol. 2011;128(3).

197. Baiz N, Slama R, Béné M, Charles M, Kolopp-Sarda M, Magnan A, et al. Maternal exposure to air pollution before and during pregnancy can induce changes in newborn's cord blood lymphocytes involved in asthma and allergies. Allergy. 2011;66:707.

198. Gregory DJ, Kobzik L, Yang Z, McGuire CC, Fedulov A V. Transgenerational transmission of asthma risk after exposure to environmental particles during pregnancy. Am J Physiol - Lung Cell Mol Physiol. 2017;313;395-413.

199. Kuiper IN, Markevych I, Accordini S, Bertelsen RJ. Associations of Preconception Exposure to Air Pollution and Greenness with Offspring Asthma and Hay Fever. Int J Environ Res Public Health. 2020;17(2).

200. Rooij MMT De, Heederik DJJ, Borlée F, Hoek G, Wouters IM. Spatial and temporal variation in endotoxin and PM10 concentrations in ambient air in a livestock dense area. Environ Res. 2017;153(September 2016):161–70.

201. Ober C, Sperling A l, von Mutius E, Vercelli D. Immune Development and Environment: Lessons from Amish and Hutterite Children. Curr Opin Immunol. 2017;October(48):51–60.

202. Omland Ø. Exposure and respiratory health in farming in temperate zones - a review of the litterature. Ann Agric Env Med. 2002;9(2):119–36.

203. Pekkanen J, Sunyer J, Anto JM, Burney P. Operational definitions of asthma in studies on its aetiology. Eur Respir J. 2005;26(1):28–35.

204. Jenkins MA, Clarke JR, Carun JB, Robertson CF, Hopper JL, Dalton MF, et al. Validation of Questionnaire and Bronchial Hyperresponsiveness against Respiratory Physician Assessment in the Diagnosis of Asthma. Int J Epidemiol. 1996;25(3).

205. Aaron SD, Vandemheen KL, Fitzgerald JM, Ainslie M, Gupta S, Lemière C, et al. Reevaluation of Diagnosis in Adults With Physician-Diagnosed Asthma. JAMA. 2017;317(3):269–79.

206. Gorber SC, Schofi S, Hardt J, Levasseur G, Tremblay M. The accuracy of self-reported smoking: A systematic review of the relationship between self-reported and cotinine- assessed smoking status. Nicotine Tob Res. 2009;11(1):12–24.

207. Vesterinen E, Kaprio J, Koskenvuo M. Prospective study of asthma in relation to smoking habits among 14,729 adults. Thorax. 1988;43(7):534–9.

208. Becklake MR, Lalloo U. The 'Healthy Smoker': A Phenomenon of Health Selection? Respiration. 1990;57:137–44.

209. Dratva J, Bertelsen R, Janson C, Johannessen A, Bråbäck L, Dharmage SC, et al. Validation of self-reported figural drawing scales against anthropometric measurements in adults. Public Health Nutr. 2016;19(11):1944–51.

210. Marianne L, Svanes C, Igland J, Franklin KA, Bentouhami H, Blanco AG, et al. Body silhouettes as a tool to reflect obesity in the past. PLoS One. 2018;13(4):1–13.

211. Pape K, Schlünssen V, Lodge C, Perret J, Walters HE, Bui DS, et al. Is self-reported history of eczema and hay fever a valid measure of atopy in those who report current asthma? Allergy. 2020;(February):1–5.

212. Abraham CM, Ownby DR, Peterson EL, Wegienka G, Zoratti EM, Williams LK, et al. The relationship between seroatopy and symptoms of either allergic rhinitis or asthma. J Allergy Clin Immunol. 2007;May:1099–104.

213. Kjøller M, Thoning H. Characteristics of non-response in the Danish Health Interview Surveys, 1987-1994. Eur J Public Health. 2005;15(5):528–35.

214. Demarest S, Van Der Heyden J, Charafeddine R, Tafforeau J, Van Oyen H, Van Hal G. Socio-economic differences in participation of households in a Belgian national health survey. Eur J Public Health. 2013;23(6):981–5.

215. Smit LAM, Heederik D, Doekes G, Lammers JWJ, Wouters IM. Occupational endotoxin exposure reduces the risk of atopic sensitization but increases the risk of bronchial hyperresponsiveness. Int Arch Allergy Immunol. 2010;152(2):151–8.

216. Smit LAM, Hooiveld M, van der Sman-de Beer F, Opstal-van Winden AWJ, Beekhuizen J, Wouters IM, et al. Air pollution from livestock farms, and asthma, allergic rhinitis and COPD among neighbouring residents. Occup Environ Med. 2014;71(2):134–40.

217. Timm S, Frydenberg M, Janson C, Campbell B, Forsberg B, Gislason T, et al. The urban-rural gradient in asthma: A population-based study in Northern Europe. Int J Environ Res Public Health. 2015;13(1):1–14.

Appendices: Paper 1-4

List of appendices:

Paper 1: The Gradient Study

Timm S, Frydenberg M, Janson C, Campbell B, Forsberg B, Gislason T, Holm M, Jõgi R, Omenaas E, Sigsgaard T, Svanes C, Schlünssen V: **The urban-rural gradient in asthma: A population-based study in Northern Europe.** *International Journal of Environmental Research in Public Health.* 2016, 13, 93; doi:10.3390/ijerph13010093

Paper 2: The Generation Study

Timm S, Svanes C, Frydenberg M, Sigsgaard T, Holm M, Janson C, Bråbäck L, Campbell B, Madsen MK, Jõgi NO, Jõgi R, Schiöler L, Bertelsen JR, Johannessen A, Sanchez-Ramos JL, Martinez-Moretalla J, Dratva J, Dharmage S & Schlünssen V (shared last authorship): **Does parental farm upbringing influence the risk of asthma in offspring: A three-generation study.** *International Journal of Epidemiology*. 2020 Aug 3, p1-9. doi: 10.1093/ije/dyaa091

Paper 3: The Selection Study

Timm S, Frydenberg M, Abramson MJ, Bertelsen RJ, Bråbäck L, Benediktsdóttir B, Gislason T, Holm M, Janson C, Jõgi R, Johannessen A, Jeong-Lim K, Malinovschi A, Mishra G, Moratalla J, Sigsgaard T, Svanes C, Schlünssen V: **Asthma and selective migration from farming environments in a three-generation cohort study.** *European Journal of Epidemiology*. 2019 Jun;34(6):601-609. doi: 10.1007/s10654-019-00491-9.

Paper 4: The Agreement Study

Timm S, Schlünssen V, Benediktsdóttir B, Bertelsen RJ, Bråbäck L, Holm M, Jogi R, Malinovschi A, Svanes C, Frydenberg M: **Offspring reports on parental place of upbringing: is it valid?** *Epidemiology*. 2019 May;30(3):e16-e18. doi: 10.1097/EDE.0000000000000988

Paper 1: The Gradient Study

Timm S, Frydenberg M, Janson C, Campbell B, Forsberg B, Gislason T, Holm M, Jõgi R, Omenaas E, Sigsgaard T, Svanes C, Schlünssen V: **The urban-rural gradient in asthma: A population-based study in Northern Europe.** *International Journal of Environmental Research in Public Health.* 2016, 13, 93; doi:10.3390/ijerph13010093

Paper 1

International Journal of
Environmental Research and Public Health

MDPI

Article

The Urban-Rural Gradient In Asthma: A Population-Based Study in Northern Europe

Signe Timm [1,*], Morten Frydenberg [1], Christer Janson [2], Brittany Campbell [3], Bertil Forsberg [4], Thorarinn Gislason [5,6], Mathias Holm [7], Rain Jogi [8], Ernst Omenaas [9], Torben Sigsgaard [1], Cecilie Svanes [9] and Vivi Schlünssen [1,10]

Received: 5 October 2015; Accepted: 15 December 2015; Published: 30 December 2015
Academic Editors: Helena Solo-Gabriele and Alesia Ferguson

1 Department of Public Health, Danish Ramazzini Centre, Aarhus University, DK-8000 Aarhus C, Denmark; morten@ph.au.dk (M.F.); ts@ph.au.dk (T.S.); vs@ph.au.dk (V.S.)
2 Department of Medical Sciences: Respiratory, Allergy and Sleep Research, Uppsala University, 751 05 Uppsala, Sweden; christer.janson@medsci.uu.se
3 Allergy and Lung Health Unit, Centre for Epidemiology and Biostatistics, Melbourne School of Population and Global Health, The University of Melbourne, Victoria 3010, Australia; brittany.campbell@unimelb.edu.au
4 Division of Occupational and Environmental Medicine, Department of Public Health and Clinical Medicine, Umeå University, 901 87 Umeå, Sweden; bertil.forsberg@envmed.umu.se
5 Department of Respiratory Medicine and Sleep, Landspitali University Hospital, Reykjavik IS-108, Iceland; thorarig@landspitali.is
6 Faculty of Medicine, University of Iceland, Reykjavik IS-101, Iceland
7 Department of Occupational and Environmental Medicine, Sahlgrenska University Hospital, Gothenburg 405 30, Sweden; mathias.holm@amm.gu.se
8 Lung Clinic, Tartu University Hospital, Tartu 50406, Estonia; Rain.Jogi@kliinikum.ee
9 Institute of Clinical Science, University of Bergen, 5020 Bergen, Norway; ernst.omenaas@helse-bergen.no (E.O.); cecilie.svanes@helse-bergen.no (C.S.)
10 National Research Center for Working Environment, Copenhagen DK-2100, Denmark
* Correspondence: signe.timm@ph.au.dk

Abstract: The early life environment appears to have a persistent impact on asthma risk. We hypothesize that environmental factors related to rural life mediate lower asthma prevalence in rural populations, and aimed to investigate an urban-rural gradient, assessed by place of upbringing, for asthma. The population-based Respiratory Health In Northern Europe (RHINE) study includes subjects from Denmark, Norway, Sweden, Iceland and Estonia born 1945–1973. The present analysis encompasses questionnaire data on 11,123 RHINE subjects. Six categories of place of upbringing were defined: farm with livestock, farm without livestock, village in rural area, small town, city suburb and inner city. The association of place of upbringing with asthma onset was analysed with Cox regression adjusted for relevant confounders. Subjects growing up on livestock farms had less asthma (8%) than subjects growing up in inner cities (11%) (hazard ratio 0.72 95% CI 0.57–0.91), and a significant urban-rural gradient was observed across six urbanisation levels ($p = 0.02$). An urban-rural gradient was only evident among women, smokers and for late-onset asthma. Analyses on wheeze and place of upbringing revealed similar results. In conclusion, this study suggests a protective effect of livestock farm upbringing on asthma development and an urban-rural gradient in a Northern European population.

Keywords: asthma; early life environment; farming; microbial exposure; urban-rural gradient; hygiene hypothesis; RHINE

1. Introduction

Knowledge of the aetiology of asthma remains poor, although asthma prevalence has risen steeply in recent years. This rise has been ascribed to the 20th century environmental changes, including extensive urbanisation. Growing up in cities is reported to be associated with higher asthma risk than growing up on farms; this observation has contributed to the hypothesis that limited exposure to microbial diversity plays a key role in asthma aetiology [1,2]. Supporting this hypothesis, Ege *et al.* found a wider range of microbial species in children growing up on a farm, and several studies suggests that endotoxin levels in the indoor environment varies according to the level of urbanisation [3–5]. Moreover, living in rural areas in close distance to neighbouring farms seems to lower the asthma risk too [6]. Protective effects on asthma seems to be limited to early life exposure, since occupational farm exposure later in life may cause increased risk of respiratory symptoms and also asthma [7]. However, recent evidence of the effect of farm upbringing on asthma is inconsistent [8–13]. Growing up on a farm may reflect factors in a farm environment, however, it may also reflect not growing up in an urban environment with presence of several risk factors for asthma such as smoking and air pollution [14]. Current evidence tends to focus on the effect of farm upbringing compared with city or non-farm upbringing, and it remains unclear whether an urban-rural gradient exists for asthma as suggested for IgE mediated sensitisation and inflammatory bowel diseases [15,16]. Investigating the urban-rural gradient in asthma will push our knowledge a step further by revealing if the relative size of the urban-rural environments also is a decisive factor. If observed, an urban-rural gradient may indicate a causal relationship and raise evidence for a dose-response like relationship between microbial diversity and development of asthma. This will provide a more detailed understanding of the early life origin of asthma. The aim of this study was to investigate the urban-rural gradient in place of upbringing for asthma in a population-based multi-centre study in Northern Europe.

2. Material and Methods

2.1. Study Population

This study is based on data from a subpopulation of The European Community Respiratory Health Survey (ECRHS). The original study population included >150,000 randomly selected men and women born between 1945 and 1973, who participated in ECRHS stage 1 during 1989–1992. Each of the 48 participating centres recruited at least 1500 men and 1500 women aged 20–44 years [17]. The Respiratory Health in Northern Europe (RHINE) study followed up on 21,659 subjects from the seven study centres located in Northern Europe—Reykjavik in Iceland; Bergen in Norway; Umeå, Uppsala and Gothenburg in Sweden; Tartu in Estonia; and Aarhus in Denmark. In 1999–2001, all RHINE subjects were sent a postal questionnaire (RHINE II), which was answered by 16,106 subjects (74%). At follow-up in 2010–2012, altogether 13,499 subjects (62%) responded to the RHINE III questionnaire [18]. The present analysis is based on this follow-up. The local Science Ethics Committees approved the study for each study centre, and informed consent was obtained from all study participants.

2.2. Questionnaire Information

All information was obtained from the RHINE III questionnaire in 2010–2012. Information was obtained from a standardised postal questionnaire. A formal forward/backward translation of the questionnaires was performed to ensure validity and homogeneity between study centres.

Asthma was obtained in RHINE III and defined as an affirmative answer to either "Do you have or have you ever had asthma?" or "Have you ever had asthma diagnosed by a doctor?" and a retrospectively reported age of onset. Allergic asthma was defined as an affirmative answer to both hay fever and asthma. *Wheeze* was defined as an affirmative answer to "Have you ever had wheezing or whistling in your chest?" and a retrospectively reported age of onset.

Place of upbringing was obtained in RHINE III and defined as the place the subject lived most of the time under the age of five years with response categories (1) farm with livestock; (2) farm without livestock; (3) village in rural area; (4) small town; (5) suburb of city; and (6) inner city.

Confounding variables were selected a priori on the basis of a literature search. These were age, sex, centre, smoking, body silhouette at 8 years of age, parental smoking in childhood and parental asthma. Smoking exposures were categorised as current, ex- and never-smokers. Anthropometric characteristics were measured by recalled body silhouette at age 8 years classified as lean, normal and obese. Parental smoking was defined by regular smoking by either parent during childhood, and parental asthma was defined by either biological parent ever suffering from asthma.

2.3. Statistical Analysis

Eligible subjects had information on the basic variables place of upbringing, asthma/wheeze and time of onset. However, the final analyses were conducted on subjects with complete information on all variables included in the models (hereafter called the study population) to ensure a constant number throughout all analyses. Data were analysed by Cox regression models with age as time scale and presented by hazard ratios (HR) with corresponding 95% confidence intervals (95% CI). The proportional hazard assumption was tested by log-log plots and found acceptable. Although the data collection in this study is cross sectional, the information given by the subjects specifies the exact time of exposure and outcome, which warranting the data to be analysed as longitudinal data. Subjects were assumed to be at risk from birth and censored at the time of asthma/wheeze onset, respectively, or at the end of follow up, whichever appeared first. HRs were presented for each urbanisation level respectively, and furthermore HRs comparing two adjacent urbanisation levels were presented including p values for an urban-rural trend. Data were analysed as crude, adjusted for non-time dependent covariates (age, sex, centre and parental asthma) and further adjusted for time-dependent covariates (smoking, body silhouette at 8 years of age and parental smoking in childhood). Smoking was analysed as a time-dependent covariate. Adjustment for centre was done by stratifying. It takes large strength to get significant interactions, and as there are differences between centres with regard to farming environment, language, translations *etc.* it was therefore, a priori, determined to stratify by centre, which is usually required for all the analyses of this multi-centre study, whether interaction by centre for the particular associations investigated in this paper would be significant or not.

Analyses were conducted separately for the two outcomes, asthma and wheeze. Additional analyses included estimation of prevalence and incidence (with time at risk starting from birth), and Cox regression stratified by sex, time of onset, smoking status, centre and allergy status, respectively. As there is no strong consensus in the literature about the cut-off point for early-onset *versus* late-onset asthma, the cut-off was set at 10 years of age in line with previous studies in the ECRHS/RHINE cohort, to ensure that asthma in puberty was not defined as early-onset (childhood) asthma. Interaction was tested by adding the interaction to the model and testing it by Wald test. A sensitivity analysis on incident cases in the follow-up period 1989–2010 was performed to explore the impact of selection bias. Statistics were calculated using STATA 12.1 (STATA Corp., College Station, TX, USA) and all *p*-values were two-sided with significance level 5%.

3. Results

Of the 13,499 RHINE III responders, data on exposure and outcomes were available for 12,441 eligible subjects. Basic characteristics of the eligible subjects and the study population (N = 11,123) are shown in Table 1 and characteristics according to centre are given as online supplementary (Table S1, Supplementary Material). The study population included 1181 cases with asthma (10.6%) and 2133 cases with wheeze (19.1%). This corresponds to an incidence of 2.14 (95% CI 2.02–2.27) per 1000 person-years for asthma and 3.94 (95% CI 3.78–4.11) per 1000 person-years for wheeze. Subjects in the six exposure groups were comparable regarding age, sex, parental asthma and body silhouettes at 8 years.

Table 1. Characteristics of the study population and the eligible subjects.

	Inner City	Suburb of City	Small Town	Village in Rural Area	Farm without Livestock	Farm with Livestock	Study Population	Eligible Subjects *
Subjects, N	1725	3337	2720	1599	250	1492	11,123	12,441
Age in 2011, mean ± SD	53.5 ± 7.1	52.0 ± 7.1	52.2 ± 7.0	54.14 ± 7.1	52.5 ± 6.7	55.6 ± 6.6	53.1 ± 7.1	53.0 ± 7.1
Sex, N (%F)	872 (51%)	1765 (53%)	1441 (53%)	915 (57%)	126 (50%)	850 (57%)	5969 (54%)	6612 (53%)
Smoking status								
Current smoker, N (%)	494 (29%)	824 (24%)	581 (21%)	311 (20%)	64 (26%)	328 (22%)	2602 (23%)	2757 (22%)
Ex-smoker, N (%)	409 (34%)	822 (25%)	647 (24%)	428 (26%)	60 (24%)	348 (23%)	2714 (24%)	2842 (23%)
Never smokers, N (%)	670 (39%)	1378 (41%)	1281 (47%)	744 (47%)	108 (43%)	702 (47%)	4883 (44%)	5061 (41%)
Age at smoke start, mean ± SD	16.9 ± 4.4	17.2 ± 4.3	17.4 ± 4.3	17.0 ± 4.1	17.3 ± 4.3	17.9 ± 4.9	17.3 ± 4.4	17.3 ± 4.4
Parental smoking:								
No parents smoke, N (%)	430 (25%)	976 (29%)	875 (32%)	538 (34%)	74 (30%)	641 (43%)	3534 (32%)	3976 (32%)
One parent smoke, N (%)	630 (37%)	1284 (39%)	942 (35%)	623 (39%)	95 (38%)	597 (40%)	4171 (38%)	4637 (37%)
Both parents smoke, N (%)	593 (34%)	972 (29%)	815 (30%)	373 (23%)	73 (29%)	193 (13%)	3019 (27%)	3343 (27%)
Don't know, N (%)	72 (4%)	105 (3%)	88 (3%)	65 (4%)	8 (3%)	61 (4%)	399 (4%)	462 (4%)
Body silhouette at 8y								
1–3 (lean), N (%)	1422 (82%)	2742 (82%)	2203 (81%)	1312 (82%)	211 (84%)	1194 (80%)	9084 (82%)	9743 (78%)
4–6 (normal), N (%)	281 (16%)	552 (17%)	479 (18%)	269 (17%)	37 (15%)	269 (18%)	1887 (17%)	1996 (16%)
7–9 (obese), N (%)	22 (1%)	43 (1%)	38 (1%)	18 (1%)	2 (1%)	29 (2%)	152 (1%)	159 (1%)

Table 1. *Cont.*

	Inner City	Suburb of City	Small Town	Village in Rural Area	Farm without Livestock	Farm with Livestock	Study Population	Eligible Subjects *
Centre								
Aarhus (DK), N (%)	351 (20%)	600 (18%)	475 (17%)	271 (17%)	26 (11%)	229 (15%)	1952 (18%)	2182 (18%)
Reykjavik (IS), N (%)	297 (17%)	664 (20%)	454 (17%)	68 (4%)	20 (8%)	131 (9%)	1634 (15%)	1862 (15%)
Bergen (NO), N (%)	343 (20%)	580 (17%)	488 (18%)	79 (5%)	131 (52%)	231 (15%)	1852 (17%)	2050 (16%)
Gothenburg (SE), N (%)	256 (15%)	660 (20%)	235 (9%)	185 (12%)	15 (6%)	95 (6%)	1446 (13%)	1631 (13%)
Umeaa (SE), N (%)	94 (5%)	137 (4%)	464 (17%)	499 (31%)	28 (11%)	432 (29%)	1654 (15%)	1840 (15%)
Uppsala (SE), N (%)	256 (15%)	380 (11%)	462 (17%)	375 (23%)	24 (10%)	192 (13%)	1689 (15%)	1859 (15%)
Tartu (EE), N (%)	128 (7%)	316 (9%)	142 (5%)	122 (8%)	6 (2%)	182 (12%)	896 (8%)	1017 (8%)
Parental asthma								
Mother, N (%)	144 (8%)	280 (8%)	208 (8%)	120 (8%)	23 (9%)	123 (8%)	898 (8%)	1021 (8%)
Father, N (%)	80 (5%)	162 (5%)	125 (5%)	87 (5%)	17 (7%)	78 (5%)	549 (5%)	615 (5%)
No parental asthma, N (%)	1492 (86%)	2873 (86%)	2379 (87%)	1378 (86)	208 (83%)	1282 (86%)	9612 (86%)	9292 (75%)
Both parents asthma, N (%)	9 (1%)	22 (1%)	8 (1%)	14 (1%)	2 (1%)	9 (1%)	64 (1%)	70 (1%)
Hay fever								
Yes, N (%)	466 (27%)	805 (24%)	706 (26%)	364 (23%)	55 (22%)	301 (20%)	2697 (24%)	2998 (24%)

* = Numbers may vary due to missing data.

Subjects who grew up in a city were more likely to be current smokers and exposed to parental smoking in childhood. Growing up on a farm without livestock was more common among Norwegians than other participating centres. In Cox regression analyses, subjects who grew up on a livestock farm were significantly less likely to suffer from asthma than subjects who grew up in a city, and a significant urban-rural gradient was observed across the six urbanisation levels (Table 2). The incidence rates according to place of upbringing varied from 1.59 (95% CI 1.34–1.90) for living on farms with livestock to 2.55 (95% CI 2.29–2.84) for living in small towns (Table 2). Sub-analyses revealed that farm upbringing was protective only among smokers, and an urban-rural gradient was also only present among smokers (Table 3). The sex-specific analyses showed that the effect of livestock farming was similar among men and women, however, upbringing in rural areas and on farm without livestock was only protective in women. An urban-rural gradient was only detected among women (Table 3). Furthermore, livestock farming was protective only against late-onset asthma, and an urban-rural gradient was only present for this phenotype (Table 4). Adjusted urban-rural gradients for the certain subgroups and phenotypes are shown in Figures 1 and 2.

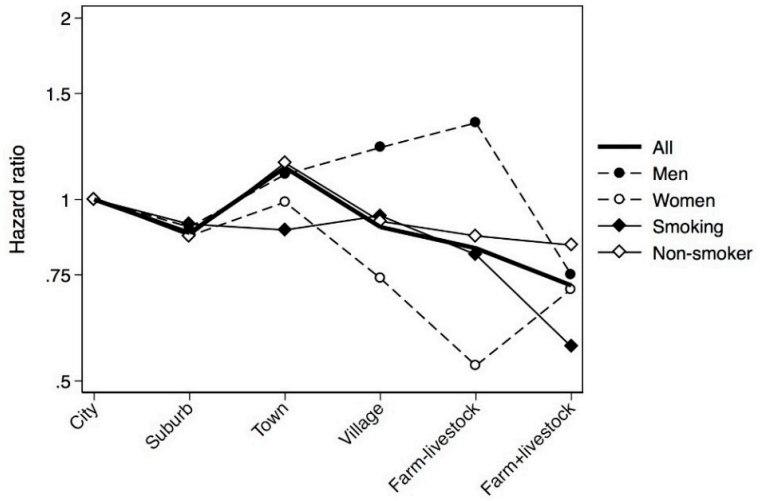


Figure 1. Cox regression subgroup analyses on asthma presented as HR adjusted for age, sex, centre, parental asthma, smoking, body silhouette at 8 years of age and parental smoking in childhood.

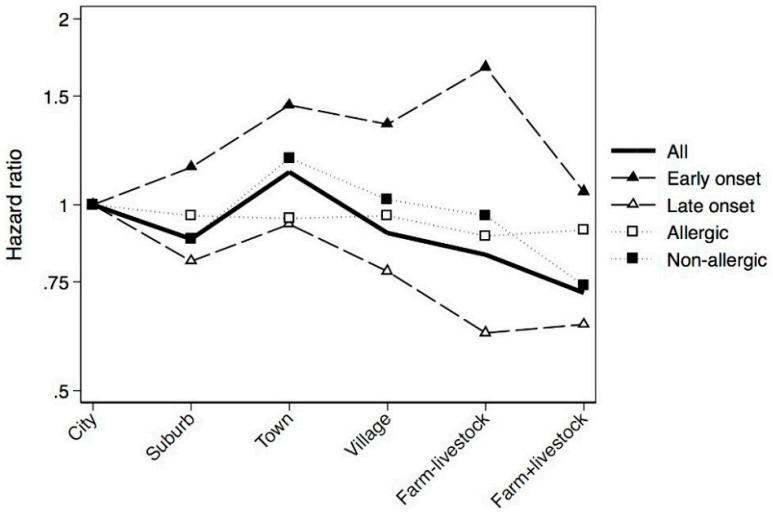


Figure 2. Cox regression analyses on asthma phenotypes presented as HR adjusted for age, sex, centre, parental asthma, smoking, body silhouette at 8 years of age and parental smoking in childhood.

Table 2. Descriptive data on asthma and wheeze and Cox regression analyses on asthma for all presented as HR (95% CI).

	Inner City	Suburb of City	Small Town	Village in Rural Area	Farm without Livestock	Farm with Livestock	HR for Urban-Rural Trend "	*p* for Urban-Rural Trend
Cases with asthma N (%)	194 (11%)	334 (10%)	334 (12%)	167 (10%)	27 (11%)	125 (8%)		
Incidence of asthma per 1000 pyr (95% CI)	2.25 (1.95–2.59)	2.06 (1.85–2.29)	2.55 (2.29–2.84)	2.07 (1.78–2.41)	2.22 (1.52–3.24)	1.59 (1.34–1.90)		
Mean age of asthma onset ± SD	26.6 ± 15.8	24.5 ± 15.6	23.6 ± 16.4	24.9 ± 17.6	22.1 ± 17.3	27.3 ± 17.9		
Cases with wheeze N (%)	368 (21%)	628 (19%)	541 (20%)	288 (18%)	47 (19%)	261 (17%)		
All								
Crude	1	0.91 (0.76–1.09)	1.13 (0.95–1.35)	0.92 (0.75–1.13)	0.98 (0.66–1.47)	0.71 (0.57–0.89)	0.95 (0.92–0.99)	0.01
Adjusted 1 °	1	0.87 (0.73–1.04)	1.03 (0.86–1.23)	0.90 (0.72–1.11)	0.83 (0.55–1.24)	0.71 (0.56–0.89)	0.95 (0.91–0.99)	0.01
Adjusted 2 *	1	0.88 (0.73–1.05)	1.04 (0.87–1.24)	0.90 (0.73–1.12)	0.83 (0.56–1.25)	0.72 (0.57–0.91)	0.95 (0.92–0.99)	0.02

° = Adjusted for sex, age, centre and parental asthma; * = Adjusted for sex, age, centre, parental asthma, smoking, bodyshape at 8 years and parental smoking in childhood; " = comparing two adjacent levels of urbanisation.

Table 3. Cox regression analyses on asthma stratified by sex and smoking status presented as HR (95% CI).

	Inner City	Suburb of City	Small Town	Village in Rural Area	Farm without Livestock	Farm with Livestock	HR for Urban-Rural Trend "	*p* for Urban-Rural Trend
Men								
Crude	1	0.90 (0.68–1.20)	1.20 (0.91–1.59)	1.17 (0.85–1.61)	1.60 (0.95–2.70)	0.70 (0.48–1.02)	0.98 (0.93–1.04)	0.62
Adjusted 1 °	1	0.90 (0.67–1.19)	1.11 (0.83–1.47)	1.22 (0.88–1.71)	1.34 (0.79–2.28)	0.75 (0.51–1.10)	0.99 (0.93–1.06)	0.77
Adjusted 2 *	1	0.90 (0.67–1.19)	1.10 (0.83–1.46)	1.22 (0.87–1.71)	1.34 (0.79–2.28)	0.75 (0.51–1.11)	0.99 (0.93–1.06)	0.81
Women								
Crude	1	0.90 (0.72–1.13)	1.07 (0.85–1.34)	0.76 (0.58–0.99)	0.59 (0.31–1.12)	0.69 (0.52–0.91)	0.93 (0.88–0.97)	<0.01
Adjusted 1 °	1	0.86 (0.69–1.08)	0.97 (0.77–1.22)	0.73 (0.55–0.97)	0.52 (0.27–0.99)	0.68 (0.51–0.91)	0.93 (0.88–0.97)	<0.01
Adjusted 2 *	1	0.87 (0.70–1.10)	0.99 (0.79–1.25)	0.74 (0.56–0.98)	0.53 (0.27–1.00)	0.71 (0.53–0.94)	0.93 (0.88–0.98)	<0.01
Smoking								
Crude	1	0.96 (0.72–1.26)	0.93 (0.70–1.25)	0.90 (0.65–1.26)	0.96 (0.50–1.86)	0.59 (0.40–0.87)	0.92 (0.86–0.98)	0.01
Adjusted 1 °	1	0.90 (0.69–1.19)	0.88 (0.66–1.19)	0.94 (0.66–1.33)	0.80 (0.41–1.56)	0.58 (0.39–0.86)	0.92 (0.86–0.98)	0.02
Adjusted 2 *	1	0.91 (0.69–1.19)	0.89 (0.66–1.19)	0.94 (0.66–1.33)	0.81 (0.42–1.58)	0.57 (0.38–0.85)	0.92 (0.86–0.98)	0.01
Not smoking								
Crude	1	0.89 (0.70–1.12)	1.26 (1.01–1.57)	0.95 (0.73–1.23)	1.01 (0.61–1.68)	0.80 (0.60–1.06)	0.98 (0.93–1.02)	0.28
Adjusted 1 °	1	0.87 (0.69–1.09)	1.14 (0.91–1.43)	0.91 (0.69–1.20)	0.85 (0.51–1.43)	0.82 (0.61–1.10)	0.97 (0.93–1.02)	0.29
Adjusted 2 *	1	0.87 (0.69–1.10)	1.15 (0.92–1.45)	0.92 (0.70–1.21)	0.87 (0.51–1.45)	0.84 (0.63–1.12)	0.98 (0.93–1.03)	0.39

° = Adjusted for sex, age, centre and parental asthma; * = Adjusted for sex, age, centre, parental asthma, smoking, bodyshape at 8 years and parental smoking in childhood; " = comparing two adjacent levels of urbanisation.

Table 4. Cox regression analyses on asthma phenotypes presented as HR (95% CI). Allergic asthma was defined as presence of both hay fever and asthma.

	Inner City	Suburb of City	Small Town	Village in Rural Area	Farm without Livestock	Farm with Livestock	HR for Urban-Rural Trend "	*p* for Urban-Rural Trend
Early onset (⩽10 years of age)								
Crude	1	1.14 (0.78–1.65)	1.56 (1.08–2.25)	1.43 (0.95–2.16)	1.91 (0.98–3.73)	0.98 (0.62–1.55)	1.02 (0.95–1.09)	0.58
Adjusted 1 °	1	1.15 (0.79–1.68)	1.44 (1.00–2.09)	1.34 (0.88–2.04)	1.65 (0.84–3.23)	1.03 (0.65–1.64)	1.02 (0.95–1.10)	0.61
Adjusted 2 *	1	1.15 (0.79–1.68)	1.45 (1.00–2.10)	1.35 (0.89–2.06)	1.67 (0.85–3.27)	1.05 (0.65–1.68)	1.02 (0.95–1.11)	0.54
Late onset (>10 years of age)								
Crude	1	0.85 (0.70–1.04)	1.02 (0.83–1.24)	0.79 (0.62–1.00)	0.74 (0.44–1.24)	0.64 (0.50–0.83)	0.93 (0.89–0.97)	<0.01
Adjusted 1 °	1	0.80 (0.66–0.98)	0.92 (0.75–1.13)	0.78 (0.60–1.00)	0.61 (0.36–1.02)	0.63 (0.48–0.82)	0.92 (0.88–0.97)	<0.01
Adjusted 2 *	1	0.81 (0.66–0.99)	0.93 (0.76–1.15)	0.78 (0.61–1.01)	0.62 (0.37–1.04)	0.64 (0.49–0.83)	0.93 (0.88–0.97)	<0.01
Allergic asthma								
Crude	1	0.96 (0.76–1.22)	1.06 (0.83–1.35)	1.10 (0.83–1.45)	1.00 (0.55–1.81)	0.95 (0.70–1.29)	1.00 (0.95–1.06)	0.90
Adjusted 1 °	1	0.96 (0.75–1.22)	0.95 (0.74–1.21)	0.96 (0.72–1.28)	0.89 (0.49–1.63)	0.90 (0.66–1.23)	0.98 (0.93–1.04)	0.52
Adjusted 2 *	1	0.96 (0.75–1.22)	0.95 (0.75–1.22)	0.96 (0.72–1.29)	0.89 (0.49–1.63)	0.91 (0.67–1.25)	0.98 (0.93–1.04)	0.59
Non-allergic asthma								
Crude	1	0.91 (0.70–1.20)	1.25 (0.95–1.63)	0.89 (0.65–1.22)	1.13 (0.64–1.99)	0.66 (0.48–0.94)	0.95 (0.89–1.00)	0.05
Adjusted 1 °	1	0.87 (0.66–1.14)	1.18 (0.90–1.55)	1.01 (0.72–1.40)	0.93 (0.52–1.66)	0.72 (0.51–1.02)	0.96 (0.91–1.02)	0.21
Adjusted 2 *	1	0.88 (0.67–1.15)	1.19 (0.91–1.56)	1.02 (0.73–1.42)	0.96 (0.54–1.70)	0.74 (0.52–1.04)	0.97 (0.91–1.03)	0.28

° = Adjusted for sex, age, centre and parental asthma; * = Adjusted for sex, age, centre, parental asthma, smoking, bodyshape at 8 years and parental smoking in childhood; " = comparing two adjacent levels of urbanisation.

The centre-specific analyses showed geographic variation in the effect of livestock farm upbringing (Figure 3). For Tartu livestock farm upbringing was protective (HR 0.35 95% CI 0.14–0.88), while no association was found for Gothenburg (HR 1.02 95% CI 0.45–2.30). However, there was no significant interaction between place of upbringing and centre (p = 0.92), and no urban-rural gradient for the respective centres.

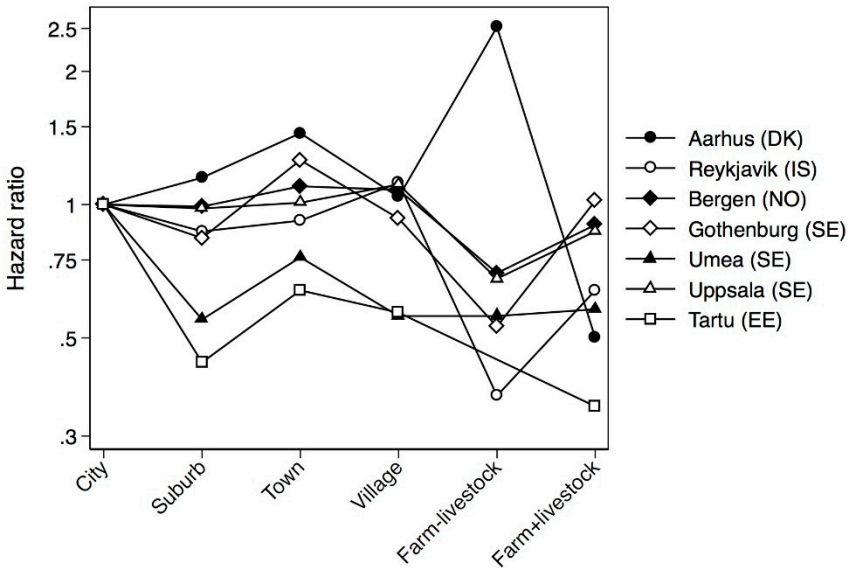


Figure 3. Centre specific Cox regression analyses on asthma presented as HR adjusted for age, sex, centre, parental asthma, smoking, body silhouette at 8 years of age and parental smoking in childhood.

Analyses on wheeze and place of upbringing revealed similar results except for suburb of city, which seems to be protective among non-smokers (HR 0.80 95% CI 0.65–0.98) and allergic subjects (HR 0.78 95% CI 0.64–0.96). Furthermore, the urban-rural gradient for smokers and for late-onset asthma was not confirmed (Table S2 and S3, Supplementary Material).

Both crude and partly adjusted sub-analyses on all eligible subjects (N = 12,441) revealed similar results as the fully adjusted analyses. In addition, the sensitivity analysis performed on incident asthma cases during the follow-up period 1989–2010 (487 cases) showed similar results, however the results were not statistically significant. Furthermore, removal of parental asthma from the adjusted model barely changed the results.

4. Discussion

In this population-based study, subjects growing up on livestock farms had significantly less asthma than subjects growing up in cities, and an urban-rural gradient in asthma development was observed across six levels of urbanisation. This urban-rural gradient was evident only among women, smokers and only for a late-onset asthma phenotype.

To our knowledge, this is the first study to investigate the urban-rural gradient of asthma applying survival analyses in a population-based study. Although the data collection in this present study is cross-sectional, the given information includes the exact time of exposure and occurrence of outcome, which warrant survival analyses of retrospectively reported onset of asthma with time at risk starting from birth.

As a potential limitation of the present study, information on all variables of interest was self-reported and a potential risk of recall bias and misclassification therefore exists. However, an analysis of the ECRHS showed consistent, long-term repeatability in adults reporting of childhood events, and that the misclassification was not associated with asthma [19]. However, this analyses was for instance not on anthropometric characteristics and we believe that silhouette at 8 years of age

might be subject to recall bias. While some misclassification in reporting early life factors is likely, it seems unlikely that such error should be differential with regard to current symptoms although such error cannot be excluded.

Another weakness of the study is the lack of information about where the subjects lived after their first 5 years of life, as this might also have an impact on the risk of developing asthma. Farming exposure can be protective even after 5 years of age as shown by Douwes *et al.* who have found adults to be protected from asthma regardless of the timing of farming exposure. The strongest protection was found in those with current (adult) and childhood exposure, and least in those with only childhood exposure [20]. However, it is likely that most people exposed to farming after the age of 5 years also were exposed before that.

Both self-reported asthma and doctor-diagnosed asthma have a high specificity and a low sensitivity [21]. Asthma prevalence in the present study (10.6%) was comparable to asthma prevalence estimates from other Nordic studies [22,23]. Age of onset may also be subject to misclassification and recall bias. However, a previous analysis of the RHINE population showed approximately 90% reporting the correct year of asthma onset ($\pm$1 year) according to their clinical asthma diagnosis [24].

The 1318 subjects excluded from this study due to missing data were primarily excluded due to missing smoking data (812 subjects, among them 89 cases with asthma and 144 cases with wheeze). The prevalence of missing data was equally distributed across the six exposure categories, and we therefore assess the dropout to be non-differential. Furthermore, an analysis of loss to follow-up in the RHINE population suggested that asthma prevalence was somewhat higher among long-term responders, but risk associations were not affected by the non-response [18]. In addition, the sensitivity analysis performed on incident cases during the follow-up period 1989–2010 showed similar results, which supports a minimal impact of selection bias at baseline. However, this analysis will only make inferences about late-onset asthma and did not take into account that asthma-cases may be more likely to participate in the follow-up as well.

A methodological limitation is the retrospective procedures for estimates of risk and occurrence of disease. These estimates require that subjects were alive and reachable for follow-up in 2010–2012, and that they were able to retrospectively report the time of onset for asthma or wheeze. The Cox regression models assume subjects to be at risk during their whole life, even though the marker of microbial exposure is only valid for the first 5 years of life. Recent studies suggest that early life exposures may induce life-long effects on immunoregulatory properties, but it may still be questioned whether this is a reasonable assumption [25]. Because of these conditions the estimates must be interpreted with this limitation in mind.

The findings from this study are overall comparable to current evidence. In line with our findings, Lawson *et al.* found an urban-rural gradient of asthma but not wheeze in a cross-sectional study among adolescents in Canada with only three categories of residence [26].

In a recent review, farm upbringing was found to be protective against asthma and asthma-like symptoms in several studies performed in the three large European cohorts ALEX, PARSIFAL and GABRIELA [2]. However, no association was found between farm upbringing and the risk of asthma or wheeze in the ECRHS study [13]. Different farm locations and farming practices within Europe may explain the heterogeneity in these results. Furthermore, Bråbäck *et al.* observed a cohort effect when investigating trends of asthma through three decades in Sweden, as the protective effect of farm living on asthma was only observed in cohorts born after 1970 [11]. Similarly, a cohort effect of farming was observed for inflammatory bowel disease in the RHINE population, where only subjects born after 1952 gained protection from livestock farm upbringing [15]. Post hoc analyses comparing subjects born before and after 1959 did not confirm a cohort effect of asthma in this study.

The sex-specific effects among subjects growing up on a farm without livestock or in a village in a rural area are in line with findings from the GABRIELA study in Germany, showing the protective effect of growing up on a farm to be slightly more pronounced among girls [27]. The GABRIELA study did not take livestock into account, and we therefore assess the subjects in their "farm" group to

be comparable with the subjects from farms with livestock, farms without livestock and villages in rural area in our study. The explanation for these sex specific findings is unknown, however, we can imagine girls growing up in rural areas to be more in contact with "farming exposure" from horses, stables ect. and boys being more exposed to motor exhaust from tractors *etc.* both before and after the age of 5 years, but this is purely speculative. A Danish study on farming students did not find any differences in asthma risk according to sex [7].

The protective effect from livestock farming being strongest among smokers was an unexpected finding, although the interaction was not statistically significant ($p > 0.1$). Smokers with asthma-like symptoms are less likely to receive a diagnosis of asthma than their non-smoking peers, and together with the healthy smoker effect, this will induce bias tending to underestimate asthma among smokers [28,29]. However, it is unlikely that such misclassification is differential with regard to place of upbringing, and we therefore expect the misclassification to be non-differential. A recent Danish study suggests smoking to be a risk factor and farm upbringing to be a protective factor for development of late-onset asthma, but this study did not explore smoking as a potential effect modifier [7].

Smoking is less prevalent in farming areas [26], which is confirmed in this study (Table 1), but adjustment for smoking did not change the results markedly, and is probably not an explanation for the association between farm upbringing and asthma. Furthermore, a study on children shows a protective effect from farm exposure despite the children being exposed to maternal smoking as well [30].

Our results on specific phenotypes suggest a tendency towards a stronger protective effect from livestock farming on late-onset asthma and non-allergic asthma. Comparable to our findings, Omland *et al.* found farm upbringing to be protective against development of late onset asthma among 16–26 year old Danes, but in contrast with our findings, Ege *et al.* also found farm living to be protective against early onset asthma among 5–13 year old European children in a cross-sectional study [7,12]. Adult exposures and particularly occupational factors and air pollution may play a role in our findings and lead to higher risk of late-onset asthma among city-dwellers, hence we believe that place of residence during childhood and adulthood are correlated. In contrast with our findings, Ege *et al.* and Omland *et al.* found farm upbringing to be protective against both allergic and non-allergic asthma [7,12], and Elholm *et al.* found an urban-rural gradient only for allergic asthma [16].

The centre-specific variations are in line with findings from the GABRIELA study showing the farm effect not to be universal as Polish farm children were less protected from asthma than German, Swiss and Austrian farm children [31]. In our study, the negative finding with regard to the urban-rural gradient may in part be due to the six categories of upbringing not being proportional to the microbial load and diversity. For instance, recent research suggests the microbial load from neighbouring farms to lower the risk of asthma among residents in rural areas not living on farms [6]. In addition, the categories may overlap and the subjects' own definition of, for instance, a small town may vary between centres. We believe the measurement of upbringing reflects the urban-rural relationship in differing ways according to centre because of different population density, industrialization, lifestyle, distance between farms and city areas *etc.* This may in part explain the variations between centres, however any objective characteristics given for the different exposure categories would have allowed us a better understanding and interpretation of the patterns and the incremental differences when comparing two adjacent levels of urbanisation. In addition, behind the trend analyses lies a strong assumption that the difference in risk between two adjacent levels of urbanization is the same across the whole spectrum, and it may be questioned whether this is a reasonable assumption or not. Differences in consumption of unpasteurized milk may also contribute to centre variation, as it is suggested to be a part of the protective effect in many studies [12]. However, we believe that subjects growing up on livestock farms are more similar across the centres, and the variation in effect may be a result of differences in farming practice and size, as suggested by MacNeill *et al.* [31]. Apart from low power, we do not have any explanation for the outliers at the Danish farms without livestock.

The homogeneity of the five countries within the study population may influence the external validity. The Scandinavian countries (Denmark, Norway and Sweden) may be more similar to one another than to Estonia and Iceland. In addition, three of the seven centres are located in Sweden which means that Sweden accounts for 49% of the participants compared to, for instance, 9% from Estonia. This may contribute to an overrepresentation of Sweden, or the Scandinavian countries in general, and may skew the results. However, this skewing is met in the centre specific analyses.

5. Conclusions

In conclusion, this population-based study suggests an urban-rural gradient of asthma in a Northern European population, so that subjects growing up on a livestock farm had significantly less late-onset asthma than subjects growing up in cities. This finding supports the hypothesis that the microbial environment in early childhood may be of importance for subsequent development of asthma, as has been previously shown for sensitisation and allergy.

Acknowledgments: The study was funded by The Faculty of Health, Aarhus University, Denmark (Project No. 240008), The Wood Dust Foundation (Project No. 444508795), The Danish Lung Association, the Swedish Heart and Lung Foundation, the Swedish Association Against Asthma and Allergy, the Swedish Association against Heart and Lung Disease, the Swedish Council for Working Life and Social Research, The Bror Hjerpstedt Foundation, The Vårdal Foundation for Health Care and Allergic Researce, The Norwegian Research Council project 135773/330, The Norwegian Asthma and Allergy Association, The Icelandic Research Council and the Estonian Science Foundation (Grant No. 4350).Vivi Schlünssen, Thorarinn Gislason and Cecilie Svanes are members of the COST BM1201 network.

Author Contributions: Vivi Schünssen and Signe Timm had the original idea for the study and, with all co-authors carried out the design. Christer Janson (SE), Cecilie Svanes (NO), Mathias Holm (SE), Bertil Forsberg (SE), Thorarinn Gislason (IS), Rain Jogi (EE) and Vivi Schlünssen (DK) were responsible for recruitment and follow-up of study participants. Signe Timm and Morten Frydenberg were responsible for data cleaning and carried out the analyses. Torben Sigsgaard and Brittany Campbell provided feedback during the process due to their expert knowledge in this field. Signe Timm drafted the manuscript, which was revised by all authors. All authors read and approved the final manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Wlasiuk, G.; Vercelli, D. The farm effect, or: When, what and how a farming environment protects from asthma and allergic disease. *Curr. Opin. Allergy Clin. Immunol.* **2012**, *12*, 461–466. [CrossRef] [PubMed]
2. Wells, A.D.; Poole, J.A.; Romberger, D.J. Influence of farming exposure on the development of asthma and asthma-like symptoms. *Int. Immunopharmacol.* **2014**, *23*, 356–363. [CrossRef] [PubMed]
3. Ege, M.; Mayer, M. Exposure to environmental microorganisms and childhood asthma. *N. Engl. J. Med.* **2011**, *364*, 701–709. [CrossRef] [PubMed]
4. Tischer, C.; Casas, L.; Wouters, I.M.; Doekes, G.; Garcia-Esteban, R.; Gehring, U.; Hyvärinen, A.; Oldenwening, M.; Kerkhof, M.; Sunyer, J.; *et al*. Early exposure to bio-contaminants and asthma up to 10 years of age: Results of the HITEA study. *Eur. Respir. J.* **2014**, 328–337. [CrossRef] [PubMed]
5. Karvonen, A.M.; Hyvärinen, A.; Gehring, U.; Korppi, M.; Doekes, G.; Riedler, J.; Braun-Fahrländer, C.; Bitter, S.; Schmid, S.; Keski-Nisula, L.; *et al*. Exposure to microbial agents in house dust and wheezing, atopic dermatitis and atopic sensitization in early childhood: A birth cohort study in rural areas. *Clin. Exp. Allergy* **2012**, *42*, 1246–1256. [CrossRef] [PubMed]
6. Smit, L.A.M.; Hooiveld, M.; van der Sman-de Beer, F.; Opstal-van Winden, A.W.J.; Beekhuizen, J.; Wouters, I.M.; Yzermans, C.J.; Heederik, D. Air pollution from livestock farms, and asthma, allergic rhinitis and COPD among neighbouring residents. *Occup. Environ. Med.* **2014**, *71*, 134–140. [CrossRef] [PubMed]
7. Omland, Ø.; Hjort, C.; Pedersen, O.F.; Miller, M.R.; Sigsgaard, T. New-onset asthma and the effect of environment and occupation among farming and nonfarming rural subjects. *J. Allergy Clin. Immunol.* **2011**, *128*, 761–765. [CrossRef] [PubMed]
8. Downs, S.H.; Marks, G.B.; Mitakakis, T.Z.; Lëuppi, J.D.; Car, N.G.; Peat, J.K. Having lived on a farm and protection against allergic diseases in Australia. *Clin. Exp. Allergy* **2001**, *31*, 570–575. [CrossRef] [PubMed]

9. Riedler, J.; Braun-Fahrländer, C.; Eder, W.; Schreuer, M.; Waser, M.; Maisch, S.; Carr, D.; Schierl, R.; Nowak, D.; von Mutius, E.; *et al*. Exposure to farming in early life and development of asthma and allergy: A cross-sectional survey. *Lancet* **2001**, *358*, 1129–1133. [CrossRef]
10. Adler, A.; Tager, I.; Quintero, D.R. Decreased prevalence of asthma among farm-reared children compared with those who are rural but not farm-reared. *J. Allergy Clin. Immunol.* **2005**, *115*, 67–73. [CrossRef] [PubMed]
11. Bråbäck, L.; Hjern, A.; Rasmussen, F. Trends in asthma, allergic rhinitis and eczema among Swedish conscripts from farming and non-farming environments. A nationwide study over three decades. *Clin. Exp. Allergy* **2004**, *34*, 38–43. [CrossRef] [PubMed]
12. Ege, M.J.; Frei, R.; Bieli, C.; Schram-Bijkerk, D.; Waser, M.; Benz, M.R.; Weiss, G.; Nyberg, F.; van Hage, M.; Pershagen, G.; *et al*. Not all farming environments protect against the development of asthma and wheeze in children. *J. Allergy Clin. Immunol.* **2007**, *119*, 1140–1147. [CrossRef] [PubMed]
13. Leynaert, B.; Neukirch, C.; Jarvis, D.; Chinn, S.; Burney, P.; Neukirch, F. Does living on a farm during childhood protect against asthma, allergic rhinitis, and atopy in adulthood? *Am. J. Respir. Crit. Care Med.* **2001**, *164*, 1829–1834. [CrossRef] [PubMed]
14. Wegienka, G.; Zoratti, E.; Johnson, C.C. The role of the early-life environment in the development of allergic disease. *Immunol. Allergy Clin. North Am.* **2015**, *35*, 1–17. [CrossRef] [PubMed]
15. Timm, S.; Svanes, C.; Janson, C.; Sigsgaard, T.; Johannessen, A.; Gislason, T.; Jogi, R.; Omenaas, E.; Forsberg, B.; Torén, K.; *et al*. Place of upbringing in early childhood as related to inflammatory bowel diseases in adulthood: A population-based cohort study in Northern Europe. *Eur. J. Epidemiol.* **2014**, *29*, 429–437. [CrossRef] [PubMed]
16. Elholm, G.; Linneberg, A.; Husemoen, L.L.N.; Omland, Ø.; Grønager, P.M.; Sigsgaard, T.; Schlünssen, V. The Danish urban-rural gradient of allergic sensitization and disease in adults. *Clin. Exp. Allergy* **2015**, *12*. [CrossRef] [PubMed]
17. Burney, P.; Chinn, S.; Jarvis, D.; Luczynska, C.; Lai, E. Variations in the prevalence of respiratory symptoms, self-reported asthma attacks, and use of asthma medication in the European Community Respiratory Health Survey (ECRHS). *Eur. Respir. J.* **1996**, *9*, 687–695.
18. Johannessen, A.; Verlato, G.; Benediktsdottir, B.; Forsberg, B.; Franklin, K.; Gislason, T.; Holm, M.; Janson, C.; Jögi, R.; Lindberg, E.; *et al*. Longterm follow-up in European respiratory health studies—Patterns and implications. *BMC Pulm. Med.* **2014**, *14*, 63. [CrossRef] [PubMed]
19. Svanes, C.; Dharmage, S.; Sunyer, J.; Zock, J.P.; Norbäck, D.; Wjst, M.; Heinrich, J.; Jarvis, D.; de Marco, R.; Plana, E.; *et al*. Long-term reliability in reporting of childhood pets by adults interviewed twice, 9 years apart. Results from the European Community Respiratory Health Survey i and II. *Indoor Air* **2008**, *18*, 84–92. [CrossRef] [PubMed]
20. Douwes, J.; Travier, N.; Huang, K.; Cheng, S.; McKenzie, J.; le Gros, G.; von Mutius, E.; Pearce, N. Lifelong farm exposure may strongly reduce the risk of asthma in adults. *Allergy Eur. J. Allergy Clin. Immunol.* **2007**, *62*, 1158–1165. [CrossRef] [PubMed]
21. Pekkanen, J.; Pearce, N. Defining asthma in epidemiological studies. *Eur. Respir. J.* **1999**, *14*, 951–957. [CrossRef] [PubMed]
22. Eagan, T.M.L.; Gulsvik, A.; Eide, G.E.; Bakke, P.S. Occupational airborne exposure and the incidence of respiratory symptoms and asthma. *Am. J. Respir. Crit. Care Med.* **2002**, *166*, 933–938. [CrossRef] [PubMed]
23. Torén, K.; Hermansson, B.A. Incidence rate of adult-onset asthma in relation to age, sex, atopy and smoking: A Swedish population-based study of 15813 adults. *Int. J. Tuberc. Lung Dis.* **1999**, *3*, 192–197. [PubMed]
24. Torén, K.; Gislason, T.; Omenaas, E.; Jögi, R.; Forsberg, B.; Nyström, L.; Olin, A.-C.; Svanes, C.; Janson, C. A prospective study of asthma incidence and its predictors: The RHINE study. *Eur. Respir. J.* **2004**, *24*, 942–946. [CrossRef] [PubMed]
25. Rook, G.A.W. Hygiene and other early childhood influences on the subsequent function of the immune system. *Dig. Dis.* **2011**, *29*, 144–153. [CrossRef] [PubMed]
26. Lawson, J.A.; Janssen, I.; Bruner, M.W.; Madani, K.; Pickett, W. Urban-rural differences in asthma prevalence among young people in Canada: The roles of health behaviors and obesity. *Ann. Allergy Asthma Immunol.* **2011**, *107*, 220–228. [CrossRef] [PubMed]
27. Genuneit, J. Sex-specific development of asthma differs between farm and nonfarm children: A cohort study. *Am. J. Respir. Crit. Care Med.* **2014**, *190*, 588–590. [CrossRef] [PubMed]

28. Vesterinen, E.; Kaprio, J.; Koskenvuo, M. Prospective study of asthma in relation to smoking habits among 14,729 adults. *Thorax* **1988**, *43*, 534–539. [CrossRef] [PubMed]
29. Eisner, M.D. Smoking and adult asthma—A healthy smoker effect? *Am. J. Respir. Crit. Care Med.* **2002**, *165*, 1566–1567. [CrossRef] [PubMed]
30. Horak, E.; Morass, B.; Ulmer, H.; Genuneit, J.; Braun-Fahrländer, C.; von Mutius, E. Prevalence of wheezing and atopic diseases in Austrian schoolchildren in conjunction with urban, rural or farm residence. *Wien. Klin. Wochenschr.* **2014**, *126*, 532–536. [CrossRef] [PubMed]
31. MacNeill, S.J.; Sozanska, B.; Danielewicz, H.; Debinska, A.; Kosmeda, A.; Boznanski, A.; Illi, S.; Depner, M.; Strunz-Lehner, C.; Waser, M.; *et al*. Asthma and allergies: Is the farming environment (still) protective in Poland? the GABRIEL Advanced Studies. *Allergy Eur. J. Allergy Clin. Immunol.* **2013**, *68*, 771–779. [CrossRef] [PubMed]

Supplementary material, paper 1

Table S1: Characteristics of the study population according to centre

	Aarhus (DK)	Reykjavik (IS)	Bergen (NO)	Gothenburg (SE)	Umeaa (SE)	Uppsala (SE)	Tartu (EE)	Study population	All eligible *
Subjects, N	1,952	1,634	1,852	1,446	1,654	1,689	894	11,123	12,441
Age in 2011, mean ± SD	52.9 ± 67.0	53.9 ± 6.9	52.5 ± 6.78	53.3 ± 7.2	53.6 ± 7.4	53.5 ± 7.3	51.2 ± 7.0	53.1 ± 7.1	53.0 ± 7.1
Sex, N (%F)	1,030 (53%)	904 (55%)	953 (51%)	782 (54%)	880 (53%)	913 (54%)	507 (57%)	5,969 (54%)	6,612 (53%)
Smoking status									
Current smoker, N (%)	520 (27%)	399 (24%)	564 (30%)	336 (23%)	274 (17%)	258 (15%)	251 (28%)	2,602 (23%)	2,757 (14%)
Ex-smoker, N (%)	444 (23%)	455 (28%)	444 (24%)	372 (26%)	410 (25%)	427 (25%)	162 (18%)	2,714 (24%)	2,842 (23%)
Never smokers, N (%)	793 (41%)	563 (34%)	655 (35%)	627 (43%)	892 (54%)	944 (56%)	409 (46%)	4,883 (44%)	5,061 (41%)
Age at smoke start, mean ± S	16.8 ± 3.9	17.6 ± 4.4	17.5 ± 4.3	16.8 ± 4.3	16.9 ± 4.5	17.0 ± 4.4	19.4 ± 4.8	17.3 ± 4.4	17.3 ± 4.4
Parental smoking:									
No parents smoke, N (%)	345 (18%)	467 (29%)	541 (29%)	475 (33%)	687 (42%)	659 (39%)	360 (40%)	3,534 (32%)	3,976 (32%)
One parent smoke, N (%)	755 (39%)	622 (38%)	724 (39%)	530 (37%)	561 (34%)	569 (34%)	410 (46%)	4,171 (38%)	4,637 (37%)
Both parents smoke, N (%)	803 (41%)	476 (29%)	547 (30%)	385 (27%)	345 (21%)	399 (24%)	64 (7%)	3,019 (27%)	3,343 (27%)
Don't know, N (%)	49 (3%)	69 (4%)	40 (2%)	56 (4%)	61 (4%)	62 (4%)	62 (7%)	399 (4%)	462 (4%)
Body silhouette at 8y									
1-3 (lean), N (%)	1,617 (83%)	1,334 (82%)	1,536 (83%)	1,209 (84%)	1,313 (79%)	1,329 (79%)	746 (82%)	9,084 (82%)	9,743 (78%)
4-6 (normal), N (%)	312 (16%)	273 (17%)	293 (16%)	218 (15%)	319 (19%)	334 (20%)	138 (15%)	1,887 (17%)	1,996 (16%)
7-9 (obese), N (%)	23 (1%)	27 (2%)	23 (1%)	19 (1%)	22 (1%)	26 (2%)	12 (1%)	152 (1%)	159 (1%)
Parental asthma									
Mother, N (%)	159 (8%)	150 (9%)	172 (9%)	94 (6%)	131 (8%)	138 (8%)	54 (6%)	898 (8%)	1,021 (8%)
Father, N (%)	98 (5%)	104 (6%)	90 (5%)	71 (5%)	83 (5%)	74 (4%)	29 (3%)	549 (5%)	615 (5%)
No parental asthma, N (%	1,683 (86%)	1,369 (84%)	1,582 (85%)	1,271 (88%)	1,428 (86%)	1,471 (87%)	808 (90%)	9,612 (86%)	9,292 (75%)
Both parents asthma, N (%	12 (1%)	11 (1%)	8 (1%)	10 (1%)	12 (1%)	6 (1%)	5 (1%)	64 (1%)	70 (1%)
Hay fever									
Yes, N (%)	496 (26%)	388 (24%)	432 (23%)	337 (23%)	391 (24%)	425 (25%)	228 (25%)	2,697 (24%)	2,998 (24%)

*** = Numbers may vary due to missing data**

Table S2: Cox regression analyses on wheeze for all and stratified by sex and smoking status presented as HR (95% CI)

	Inner city	Suburb of city	Small town	Village in rural area	Farm without livestock	Farm with livestock	HR for urban-rural trend "	P for urban-rural trend
All								
Crude	1	0.92 (0.81-1.04)	0.98 (0.86-1.12)	0.81 (0.70-0.94)	0.92 (0.68-1.24)	0.74 (0.63-0.87)	0.95 (0.92-0.97)	<0.01
Adjusted 1 °	1	0.85 (0.75-0.97)	0.94 (0.82-1.07)	0.85 (0.73-1.00)	0.85 (0.63-1.15)	0.82 (0.70-0.96)	0.97 (0.94-1.01)	0.06
Adjusted 2 *	1	0.86 (0.76-0.98)	0.96 (0.84-1.10)	0.87 (0.74-1.02)	0.88 (0.65-1.20)	0.87 (0.73-1.02)	0.98 (0.95-1.01)	0.22
Men								
Crude	1	0.90 (0.75-1.08)	1.01 (0.83-1.22)	0.83 (0.66-1.04)	1.16 (0.78-1.71)	0.84 (0.67-1.05)	0.98 (0.93-1.02)	0.23
Adjusted 1 °	1	0.83 (0.69-1.00)	0.98 (0.81-1.19)	0.93 (0.73-1.16)	1.10 (0.74-1.63)	0.97 (0.76-1.22)	1.01 (0.97-1.05)	0.58
Adjusted 2 *	1	0.83 (0.69-1.00)	1.00 (0.82-1.21)	0.92 (0.73-1.17)	1.13 (0.76-1.68)	1.02 (0.80-1.28)	1.02 (0.98-1.06)	0.35
Women								
Crude	1	0.93 (0.78-1.12)	0.95 (0.79-1.14)	0.80 (0.65-0.99)	0.69 (0.42-1.12)	0.67 (0.54-0.84)	0.92 (0.89-0.96)	<0.01
Adjusted 1 °	1	0.86 (0.72-1.03)	0.89 (0.73-1.07)	0.79 (0.63-0.98)	0.65 (0.39-1.05)	0.72 (0.57-0.90)	0.94 (0.90-0.98)	<0.01
Adjusted 2 *	1	0.88 (0.74-1.06)	0.92 (0.76-1.11)	0.82 (0.66-1.02)	0.67 (0.41-1.10)	0.76 (0.61-0.96)	0.95 (0.91-0.99)	0.02
Smoking								
Crude	1	0.99 (0.84-1.17)	0.96 (0.80-1.15)	0.82 (0.66-1.01)	0.91 (0.60-1.39)	0.74 (0.59-0.92)	0.93 (0.90-0.97)	<0.01
Adjusted 1 °	1	0.90 (0.76-1.06)	0.91 (0.76-1.09)	0.85 (0.69-1.06)	0.83 (0.54-1.27)	0.79 (0.63-0.99)	0.96 (0.92-1.00)	0.05
Adjusted 2 *	1	0.90 (0.76-1.06)	0.92 (0.76-1.10)	0.86 (0.70-1.08)	0.83 (0.54-1.27)	0.82 (0.65-1.02)	0.96 (0.93-1.01)	0.09
Not smoking								
Crude	1	0.85 (0.70-1.04)	1.07 (0.88-1.30)	0.87 (0.69-1.10)	0.98 (0.63-1.52)	0.83 (0.65-1.04)	0.98 (0.93-1.02)	0.27
Adjusted 1 °	1	0.80 (0.65-0.97)	1.00 (0.82-1.22)	0.87 (0.68-1.09)	0.90 (0.58-1.41)	0.90 (0.71-1.15)	1.00 (0.96-1.04)	0.91
Adjusted 2 *	1	0.80 (0.65-0.98)	1.01 (0.83-1.23)	0.87 (0.69-1.10)	0.91 (0.58-1.41)	0.92 (0.72-1.17)	1.01 (0.96-1.05)	0.95

° = Adjusted for sex, age, centre and parental asthma

*** = Adjusted for sex, age, centre, parental asthma, smoking, bodyshape at 8y and parental smoking in childhood**

" = comparing two adjacent levels of urbanisation

Table S3: Cox regression analyses on wheeze stratified by time of onset and phenotype of asthma presented as HR (95% CI)
Allergic asthma was defined as presence of both hay fever and asthma.

	Inner city	Suburb of city	Small town	Village in rural area	Farm without livestock	Farm with livestock	HR for urban-rural trend "	P for urban-rural trend
Early onset (≤10y)								
Crude	1	0.93 (0.64-1.36)	1.41 (0.97-2.04)	1.26 (0.83-1.91)	1.86 (0.96-3.63)	0.87 (0.55-1.39)	1.02 (0.95-1.10)	0.64
Adjusted 1 °	1	0.91 (0.62-1.34)	1.31 (0.90-1.90)	1.17 (0.76-1.80)	1.64 (0.84-3.22)	0.90 (0.56-1.45)	1.02 (0.94-1.10)	0.67
Adjusted 2 *	1	0.90 (0.61-1.32)	1.30 (0.90-1.90)	1.15 (0.76-1.78)	1.65 (0.84-3.23)	0.89 (0.55-1.43)	1.02 (0.94-1.10)	0.70
Late onset (>10y)								
Crude	1	0.92 (0.80-1.05)	0.92 (0.80-1.06)	0.76 (0.64-0.90)	0.79 (0.56-1.11)	0.73 (0.61-0.86)	0.94 (0.91-0.97)	<0.01
Adjusted 1 °	1	0.84 (0.73-0.97)	0.89 (0.77-1.03)	0.81 (0.68-0.97)	0.74 (0.52-1.04)	0.81 (0.68-0.96)	0.96 (0.93-1.00)	0.03
Adjusted 2 *	1	0.86 (0.75-0.98)	0.92 (0.80-1.06)	0.83 (0.70-0.99)	0.77 (0.55-1.09)	0.86 (0.72-1.02)	0.98 (0.95-1.01)	0.14
Allergic								
Crude	1	0.82 (0.67-1.00)	0.95 (0.76-1.16)	0.90 (0.71-1.13)	0.85 (0.51-1.41)	0.90 (0.71-1.16)	0.99 (0.95-1.04)	0.81
Adjusted 1 °	1	0.79 (0.64-0.96)	0.89 (0.72-1.09)	0.84 (0.66-1.08)	0.89 (0.53-1.50)	0.94 (0.73-1.22)	1.00 (0.95-1.05)	0.99
Adjusted 2 *	1	0.78 (0.64-0.96)	0.89 (0.73-1.10)	0.84 (0.65-1.07)	0.90 (0.53-1.51)	0.95 (0.73-1.23)	1.00 (0.95-1.05)	0.96
Non-allergic								
Crude	1	1.03 (0.86-1.22)	1.02 (0.85-1.22)	0.79 (0.64-0.97)	1.05 (0.71-1.53)	0.74 (0.60-0.91)	0.93 (0.90-0.97)	<0.01
Adjusted 1 °	1	0.95 (0.80-1.12)	1.00 (0.84-1.20)	0.91 (0.74-1.14)	0.95 (0.65-1.40)	0.86 (0.69-1.07)	0.97 (0.93-1.03)	0.20
Adjusted 2 *	1	0.97 (0.81-1.15)	1.05 (0.87-1.26)	0.95 (0.76-1.17)	1.02 (0.70-1.51)	0.94 (0.76-1.17)	0.99 (0.95-1.03)	0.70

° = Adjusted for sex, age, centre and parental asthma

*** = Adjusted for sex, age, centre, parental asthma, smoking, bodyshape at 8y and parental smoking in childhood**

" = comparing two adjacent levels of urbanisation

Paper 2: The Generation Study

Timm S, Svanes C, Frydenberg M, Sigsgaard T, Holm M, Janson C, Bråbäck L, Campbell B, Madsen MK, Jõgi NO, Jõgi R, Schiöler L, Bertelsen JR, Johannessen A, Sanchez-Ramos JL, Martinez-Moretalla J, Dratva J, Dharmage S & Schlünssen V (shared last authorship): **Does parental farm upbringing influence the risk of asthma in offspring: A three-generation study.** *International Journal of Epidemiology*. 2020 Aug 3, p1-9. doi: 10.1093/ije/dyaa091

Paper 2

International Journal of Epidemiology, 2020, 1–9
doi: 10.1093/ije/dyaa091
Original Article

Original Article

Does parental farm upbringing influence the risk of asthma in offspring? A three-generation study

Signe Timm ,[1] Cecilie Svanes,[2,3]* Morten Frydenberg,[1] Torben Sigsgaard,[1] Mathias Holm,[4] Christer Janson,[5] Lennart Bråbäck,[6] Brittany Campbell,[7] Marie Kjaer Madsen,[1] Nils Oskar Jõgi,[8] Rain Jõgi,[9] Linus Schiöler,[10] Randi Jacobsen Bertelsen,[11] Ane Johannessen,[2,3] Jose Luis Sanchez-Ramos,[12] Jesus Martinez-Moretalla,[13] Julia Dratva,[14] Shyamali Dharmage[7] and Vivi Schlünssen[1,15] (shared last authorship)

[1]Department of Public Health, Danish Ramazzini Centre, Aarhus University, Aarhus, Denmark, [2]Centre for International Health, Department of Global Public Health and Primary Care, University of Bergen, Bergen, Norway, [3]Department of Occupational Medicine, Haukeland University Hospital, Bergen, Norway, [4]Department of Occupational and Environmental Medicine, Sahlgrenska University Hospital, Gothenburg, Sweden, [5]Department of Medical Sciences: Respiratory, Allergy and Sleep Research, Uppsala University, Uppsala, Sweden, [6]Section of Sustainable Health, Department of Public Health and Clinical Medicine, Umeå University, Umeå, Sweden, [7]Allergy and Lung Health Unit, Centre for Epidemiology and Biostatistics, The University of Melbourne, Melbourne, Victoria, Australia, [8]Department of Clinical Science, University of Bergen, Bergen, Norway, [9]Tartu University Hospital, Lung Clinic, Tartu, Estonia, [10]Section of Occupational and Environmental Medicine, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden, [11]Institute of Clinical Science, University of Bergen, Bergen, Norway, [12]Department of Nursing, University of Huelva, Huelva, Spain, [13]Department of Internal Medicine, Albacete University Hospital, Albacete, Spain, [14]ZHAW School of Health Professions, Winterthur, Switzerland and [15]National Research Centre for the Working Environment, Copenhagen, Denmark

*Corresponding author. Overlege Danielsens hus, Årstadveien 21, room 536. Postboks 7804, 5020 Bergen, Norway.
E-mail: Cecilie.Svanes@uib.no

Editorial decision 21 April 2020; Accepted 26 May 2020

Abstract

Background: A farm upbringing has been associated with lower risk of asthma and methylation of asthma-related genes. As such, a farm upbringing has the potential to transfer asthma risk across generations, but this has never been investigated. We aimed to study the generational effects from a parental farm upbringing on offspring asthma.

Methods: Our study involved three generations: 5759 participants from the European Community Respiratory Health Survey (ECRHS) study (born 1945–1971, denoted G1), their 9991 parents (G0) and their 8260 offspring (G2) participating in RHINESSA

(Respiratory Health In Northern Europe, Spain and Australia). Questionnaire data were collected on G0 and G1 from G1 in 2010 and on G2 from themselves in 2013. The parental/grandparental place of upbringing was categorized: (i) both parents from farm; (ii) mother from farm, father from village/city; (iii) father from farm, mother from village/city; (iv) both parents from village or one parent from village and one from city; (v) both parents from city (reference group). Grandparental upbringing was equivalently categorized. Offspring asthma was self-reported and data were analysed using Cox-regression models with G2 age as the time scale.

Results: A parental farm upbringing was not associated with offspring asthma when compared with city upbringing [hazard ratio (HR) 1.12, 95% confidence interval (CI) 0.74–1.69]. Findings remained similar when stratified by offspring upbringing and asthma phenotypes. Quantitative bias analyses showed similar estimates for alternative data sources. A grandparental farm upbringing was not associated with offspring asthma in either the maternal (HR 1.05, 95% CI 0.67–1.65) or paternal line (HR 1.02, 95% CI 0.62–1.68).

Conclusions: This multigenerational analysis suggests no evidence of an association between parental/grandparental farm upbringing and offspring asthma.

Key words: Asthma, ECRHS, RHINESSA, farm upbringing, generation study, generational effects

Key Messages

- A farm upbringing has been suggested to reduce the risk of asthma and potentially induce epigenetic changes related to asthma, suggesting that a farm upbringing has the potential to transfer asthma risk across generations, but this has never been investigated.
- In our three-generation study, we observed no evidence of an association between farm upbringing in previous generations and offspring asthma, either for parental or grandparental upbringing.
- These null findings were consistent when stratified by the offspring's own upbringing or by asthma phenotypes.
- A quantitative bias analysis showed that the results were similar regardless of whether the information on upbringing was provided by the parent themselves or as second-hand information by their offspring.

Background

Asthma prevalence has risen steeply during the last decades and several hypotheses have been proposed for this increase.[1] One of the most promising explanations is the Hygiene Hypothesis, subsequently modified to the Microbial Diversity Hypothesis, which suggests the development of a compromised immune system due to low exposure to microbes in early life.[2–4] Studies suggest that farm exposure in early life reduces the risk of asthma, which has been attributed to greater microbial diversity, i.e. from stable dust and unpasteurized farm milk,[5–9] leading to immunomodulatory changes.

Early exposure to a farm environment has been found to influence methylation in asthma-related genes at age 4.[10] Furthermore, one study indicated that the CD14-promoter region was differently methylated in placentae among mothers living on a farm compared with mothers not living on a farm.[11] This suggests that exposure to a farming environment might cause intergenerational effects through the induction of changes to gene expression. Whereas there is accumulating evidence that adverse exposures, such as smoking, prior to conception might play a role in the aetiology of asthma,[12,13] there is little evidence on the potential effect of microbial exposure as a preconception protective factor for offspring asthma.[14]

Evidence for generational effects from farming on asthma mainly arise from epigenome studies in animals[14] but, as of yet, this has never been investigated in an epidemiological study. Such information may help us to identify critical exposure periods and, in the long run, enable targeted intervention strategies for individuals at high risk of

subsequent asthma development. Therefore, the aim of this study was to investigate the generational effect of early farm exposure on asthma in offspring in an international multicentre and generational study.

Methods

Study population

The present study is nested within two cohort studies: the ECRHS (European Community Respiratory Health Survey) and RHINESSA (Respiratory Health in Northern Europe, Spain and Australia). The ECRHS collected information from the parents (G1) of the offspring (G2) who were investigated within RHINESSA (Figure 1).

In 1988–1992, the ECRHS randomly included a population-based sample of 1500 men and 1500 women born in 1945–1973 from each of the participating study centres across Europe.[15] The RHINESSA study included ECRHS participants (G1), their parents (G0) and their offspring (G2) in the following ECRHS centres: Denmark (Aarhus), Norway (Bergen), Sweden (Gothenburg, Umeå, Uppsala), Iceland (Reykjavik), Estonia (Tartu), Spain (Albacete, Huelva) and Australia (Melbourne).

Data collection and definitions

G1 provided information via the ECRHS questionnaire in 2010 and G2 provided information via the RHINESSA questionnaire in 2013. G1 started as a population-based study and did not include spouses of the participants. Therefore, we collected information on the spouse of G1 participants via G2. G2 also provided information on the place of upbringing of G0 via the RHINESSA questionnaire and G1 provided information on G0 smoking and asthma via the ECRHS questionnaire.

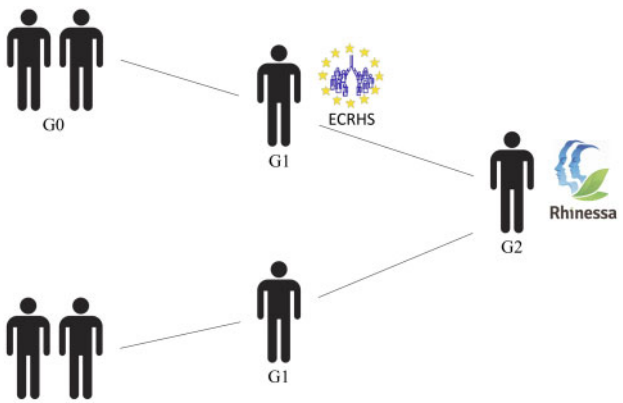


Figure 1. Three generations G0, G1 and G2 derived from the two cohorts ECRHS (European Community Respiratory Health Survey) and RHINESSA (Respiratory Health In Northern Europe, Spain and Australia).

G2 asthma status was defined as an affirmative answer to *'Do you have or have you ever had asthma?'* and a reported age of onset. G1 provided information on their own and their parents' (G0) asthma status via the same question. G2 hay fever was defined as an affirmative answer to *'Do you have any nasal allergies including hay fever?'*. Information on parental place of upbringing was reported by the ECRHS parent themselves (G1) and by their offspring (G2) via the question *'What term best describes the place you (your father, your mother) lived most of the time before the age of five years?'*, with response categories: (i) farm with livestock, (ii) farm without livestock, (iii) village in a rural area, (iv) small town, (v) suburb of city and (vi) inner city. G2 also gave information on grandparental (G0) place of upbringing. The groups were merged as follows: a + b as 'farm', c + d as 'village' and e + f as 'city', assuming the exposure level to be reasonably similar within the merged groups.

As the initial analyses showed similar estimates for maternal and paternal upbringing separately, these two variables were merged into a combined parental exposure variable. Parental (G1) place of upbringing was categorized as five groups after merging father's and mother's upbringing: (i) both parents from farm; (ii) mother from farm, father from village/city; (iii) father from farm, mother from village/city; (iv) both parents from village or one parent from village and one from city; (v) both parents from city (reference group). The grandparental place of upbringing was analysed in the same way.

Statistical analyses

Data were analysed in Cox-regression models with G2 age as the time scale and presented as hazard ratios (HRs) with the corresponding 95% confidence intervals (CIs) adjusted for potential confounders taking clustering within families into account via robust standard errors. Although the data collection is cross-sectional, the information given by the subjects specifies the exact time of exposure and duration and age at onset of the outcome, which provided the opportunity for longitudinal data analysis. Subjects were assumed to be at risk from birth and censored at the time of asthma onset or at the end of follow-up, whichever appeared first. Thereby, the Cox models account for the fact that the study participants (G2) are participating with different follow-up times according to their different ages, which is a more robust method of investigating the relevant associations.

A minimum set of confounders was identified using Directed Acyclic Graphs (DAGs) via the software DAGitty 2.3. Adjustment for this set of confounders blocks any known backdoor paths between the exposure and the

outcome. Factors such as smoking, asthma status, socioeconomic status, gene expression (as an epigenetic marker), microbial exposure, etc. were included in the DAG. From this, the minimal adjustment set for the association between parental (G1) place of upbringing and offspring (G2) asthma were identified to be: G0 asthma, G0 place of upbringing and G0 smoking (Supplementary Figure 1, available as Supplementary data at *IJE* online). As the prevalence of farm upbringing varies markedly between study centres, an a priori decision was made to adjust for study centre. Analyses on parental (G1) place of upbringing and offspring (G2) asthma were presented as crude and adjusted estimates in two models. *Adj1* was adjusted for centre and place of upbringing, available for all four G0 grandparents, and *adj2* was adjusted for centre and all confounders identified in our hypothesized DAG. However, whereas data on the place of upbringing was available for all G0 grandparents, information on smoking and asthma was only available for half of the G0 grandparents.

A separate analysis on grandparental (G0) place of upbringing and offspring (G2) asthma was also performed, but no adjustments were made due to a lack of data on the great-grand generation.

Secondary analyses included stratification by G2 place of upbringing and analyses on subjects with hay fever to specifically investigate the allergic-asthma phenotype. Furthermore, we conducted a quantitative bias analysis to investigate the potential bias from using second-hand information on parental place of upbringing from the offspring (G2) instead of direct information from the parent

Table 1. Characteristics of the study population: participants in the European Community Respiratory Health Survey (ECRHS, G1), their parents (G0) and their offspring participating in Respiratory Health In Northern Europe, Spain and Australia (RHINESSA, G2)

	Parental (G1) place of upbringing					
	Both parents from farm	Mother from farm, father from village/city	Father from farm, mother from village/city	Both parents from village or one from village and one from city	Both parents from city	Missing
Offspring (G2), *N* (%)	405 (5%)	790 (10%)	866 (10%)	3553 (43%)	2246 (27%)	400 (5%)
Offspring (G2) age, mean ± SD	31.95 ± 7.33	31.17 ± 7.63	31.12 ± 7.68	30.21 ± 7.69	30.39 ± 7.57	29.13 ± 7.90
Offspring (G2) sex, *N* (%F)	218 (54%)	474 (60%)	504 (58%)	2069 (58%)	1283 (57%)	233 (58%)
Offspring (G2) smoking status						
Never smoker, *N* (%)	283 (70%)	538 (68%)	590 (68%)	2343 (66%)	1476 (66%)	174 (43%)
Current smoker, *N* (%)	42 (10%)	90 (11%)	99 (11%)	448 (12%)	307 (14%)	59 (15%)
Ex-smoker, *N* (%)	79 (20%)	160 (20%)	172 (20%)	739 (21%)	456 (20%)	58 (15%)
Missing, *N* (%)	1 (0%)	2 (0%)	5 (1%)	23 (1%)	7 (0%)	109 (27%)
Offspring (G2) asthma, *N* (%)	79 (20%)	146 (18%)	171 (20%)	628 (18%)	415 (18%)	51 (13%)
Missing, *N* (%)	2 (0%)	2 (0%)	1 (0%)	15 (0%)	11 (0%)	109 (27%)
Centre						
Aarhus (DK), *N* (%)	35 (9%)	100 (13%)	98 (11%)	349 (10%)	265 (12%)	45 (12%)
Albacete (ES), *N* (%)	3 (1%)	6 (1%)	4 (0%)	53 (1%)	8 (0%)	1 (0%)
Bergen (NO), *N* (%)	69 (17%)	178 (23%)	166 (19%)	698 (19%)	523 (23%)	125 (31%)
Gothenburg (SE), *N* (%)	18 (4%)	49 (6%)	50 (6%)	378 (11%)	423 (19%)	21 (5%)
Huelva (ES), *N* (%)	4 (1%)	0 (0%)	3 (0%)	54 (2%)	7 (0%)	2 (0%)
Melbourne (AU), *N* (%)	6 (1%)	7 (1%)	11 (1%)	54 (2%)	106 (5%)	7 (2%)
Reykjavik (IS), *N* (%)	22 (5%)	93 (12%)	117 (14%)	432 (12%)	477 (21%)	52 (13%)
Tartu (EE), *N* (%)	56 (14%)	73 (9%)	71 (8%)	157 (4%)	124 (6%)	63 (16%)
Umeaa (SE), *N* (%)	144 (36%)	162 (20%)	222 (26%)	676 (19%)	63 (3%)	33 (8%)
Uppsala (SE), *N* (%)	48 (12%)	122 (15%)	124 (14%)	702 (20%)	250 (11%)	51 (13%)
Grandparental (G0) smoking[a]						
No grandparents smoke, *N* (%)	157 (39%)	258 (33%)	272 (31%)	986 (28%)	487 (22%)	90 (23%)
One grandparent smoke, *N* (%)	139 (34%)	266 (34%)	276 (32%)	1099 (31%)	664 (30%)	112 (28%)
Both grandparents smoke, *N* (%)	30 (7%)	139 (18%)	155 (18%)	802 (23%)	520 (23%)	76 (19%)
Don't know, *N* (%)	15 (4%)	16 (2%)	28 (3%)	101 (3%)	70 (3%)	17 (4%)
Missing, *N* (%)	64 (16%)	111 (14%)	135 (16%)	565 (15%)	505 (22%)	105 (26%)

[a]Reported by the ECRHS participant and therefore only available for the grandparents on the ECRHS participants' side.

him/herself (G1). This was in order to establish the likelihood of differential recall of parental place of upbringing according to offspring vs parental reports.[16]

Statistical analyses were performed in Stata 15 (Stata Corp., College Station, TX, USA).

Results

Basic characteristics of the G2 study population in RHINESSA ($N = 8260$) are shown in Table 1.

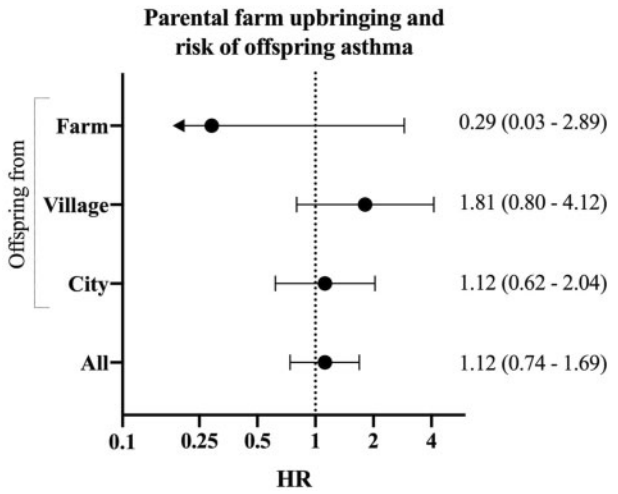


Figure 2 Hazard ratios (HR) with 95% confidence intervals (CI) for offspring asthma according to parental farm upbringing (both parents from farm VS. both parents from city) among offspring in RHINESSA (Respiratory Health In Northern Europe, Spain and Australia) adjusted for centre, grandparental asthma, grandparental upbringing and grandparental smoking (adj2 model), for all ($N = 4279$) and stratified by offspring upbringing (Table 2 and Supplementary Material Table S2).

The number in each exposure group was unevenly distributed, ranging from 405 (5%) offspring with both parents from farms to 2246 (27%) offspring with both parents from cities. This was even more pronounced when dividing by study centres. Offspring (G2) were comparable with regard to age, sex, smoking status and asthma across parental-place-of-upbringing categories (Table 1). Grandparental smoking ranged from 39% G0 non-smokers in the group with both parents from farms to 22% non-smokers in the group with both parents from cities.

In Cox-regression models, parental (G1) farm upbringing was not associated with offspring (G2) asthma when compared with city upbringing, either among all offspring ($_{adj2}$HR 1.12, 95% CI 0.74–1.69) or among the subgroup of offspring born and raised in the city themselves ($_{adj2}$HR 1.12, 95% CI 0.62–2.04) (Table 2A and Figure 2). Similar findings were observed when investigating only allergic asthma ($_{adj2}$HR 0.96, 95% CI 0.54–1.70) (Table 2B). Centre-specific estimates showed some variation in the association, especially for Tartu (EE), although with very wide CIs (Supplementary Figure 2, available as Supplementary data at *IJE* online).

The quantitative bias analyses comparing the association between maternal/paternal (G1) upbringing and offspring (G2) asthma showed similar results when using either G2 or G1 as the source of information (Table 3).

Grandparental (G0) farm upbringing was not associated with offspring (G2) asthma either in the maternal line (HR 0.89, 95% CI 0.73–1.08) or in the paternal line (HR 1.05, 95% CI 0.86–1.29) (Supplementary Table 1, available as Supplementary data at *IJE* online). The results persisted when investigating the subgroup of offspring and parents,

Table 2A Hazard ratios and corresponding 95% confidence intervals for offspring asthma according to parental place of upbringing

	All			Offspring from city		
	$N = 7795$	$N = 5799$	$N = 4279$	$N = 5096$	$N = 3679$	$N = 2522$
Parental upbringing	Crude	Adj1	Adj2	Crude	Adj1	Adj2
Both parents from farm	1.03 (0.81–1.33)	1.06 (0.75–1.49)	1.12 (0.74–1.69)	0.92 (0.64–1.34)	0.99 (0.62–1.59)	1.12 (0.62–2.04)
Mother from farm, father from village/city	0.99 (0.81–1.21)	1.01 (0.77–1.33)	1.09 (0.78–1.51)	0.90 (0.70–1.16)	0.89 (0.62–1.26)	0.96 (0.62–1.49)
Father from farm, mother from village/city	1.03 (0.85–1.25)	1.07 (0.82–1.39)	1.13 (0.81–1.56)	1.04 (0.82–1.33)	0.96 (0.69–1.35)	0.96 (0.62–1.50)
Both parents from village or one parent from village and one from city	0.95 (0.84–1.09)	0.93 (0.78–1.11)	0.98 (0.79–1.23)	0.91 (0.78–1.06)	0.90 (0.74–1.10)	0.90 (0.71–1.16)
Both parents from city (ref)	1	1	1	1	1	1

Adj1, adjusted for centre and grandparental place of upbringing for all four grandparents.

Adj2, adjusted for centre, grandparental asthma (two grandparents), grandparental place of upbringing (four grandparents) and grandparental smoking (two grandparents).

Table 2B Hazard ratios and corresponding 95% confidence intervals for a subgroup of offspring with hay fever investigating allergic asthma according to parental place of upbringing

	All		
	$N = 2250$	$N = 1708$	$N = 1237$
Parental upbringing	Crude	Adj1	Adj2
Both parents from farm	1.14 (0.81–1.60)	1.04 (0.66–1.66)	0.96 (0.54–1.70)
Mother from farm, father from village/city	1.16 (0.89–1.51)	1.08 (0.76–1.55)	1.20 (0.76–1.87)
Father from farm, mother from village/city	0.92 (0.70–1.22)	0.85 (0.60–1.21)	0.82 (0.52–1.31)
Both parents from village or one parent from village and one from city	0.98 (0.82–1.17)	0.98 (0.78–1.24)	1.04 (0.77–1.40)
Both parents from city (ref)	1	1	1

Adj1, adjusted for centre and grandparental place of upbringing for all four grandparents.

Adj2, adjusted for centre, grandparental asthma (two grandparents), grandparental place of upbringing (four grandparents) and grandparental smoking (two grandparents).

Table 3 Quantitative bias analyses—hazard ratios with corresponding 95% confidence intervals for offspring asthma according to maternal and paternal place of upbringing, respectively, adjusted for grandparental asthma, grandparental place of upbringing and grandparental smoking

	Village vs city	Farm vs city
Mother's place of upbringing		
Own reports	1.12 (0.88–1.44)	0.83 (0.59–1.16)
Offspring reports	1.14 (0.85–1.55)	1.13 (0.77–1.67)
Father's place of upbringing		
Own reports	0.99 (0.76–1.29)	0.95 (0.67–1.34)
Offspring reports	0.90 (0.69–1.18)	1.08 (0.73–1.60)

who were born in the city themselves: maternal line (HR 1.05, 95% CI 0.67–1.65) and paternal line (HR 1.02, 95% CI 0.62–1.68) (Supplementary Table 1, available as Supplementary data at *IJE* online).

Discussion

Key results

To the best of our knowledge, this is the first study to investigate the generational effects of farm exposure on asthma. In this three-generation study, parental farm upbringing was not associated with offspring asthma among all offspring or when stratified by the offspring's own upbringing or asthma phenotype. A quantitative bias analysis showed that these estimates were similar regardless of whether the information was provided by G1 themselves or as second-hand information by their offspring (G2). Furthermore, grandparental farm upbringing was not associated with offspring asthma in either the maternal or the paternal line. Therefore, this study does not support the hypothesis suggesting generational effects from farm exposure in previous generations on offspring asthma development.

Strengths and limitations

The key strength of this study is the three-generation study design. Whereas few other studies in this field have focused on the pregnancy period or the time just before conception, this study includes information on exposures long before conception and for both mothers/grandmothers and fathers/grandfathers. However, a clear limitation of this study is that information on both exposure and outcome data is questionnaire-based and therefore may be subject to recall error. However, this error is unlikely to be differential and therefore would have skewed our estimates towards the null. In addition, some information is given on behalf of relatives. We anticipate that offspring are able to report their own place of upbringing correctly; however, a study investigating the agreement in offspring and parental reports on parental upbringing in RHINESSA showed that offspring tend to report incorrectly about their parents if their parents were born and raised in a different setting than the offspring themselves.[17] Our study did not investigate the patterns of offspring misclassification when reporting about their grandparents, but we suspect that misclassification is present in this case as well. Nevertheless, the quantitative bias analyses in the present paper showed that estimates were consistent when using offspring and parental reports on parental upbringing, respectively, suggesting that any misclassification from this source is unlikely to influence the results.

Studies have shown that exposure levels and diversity of microbes are higher on farms than in urban homes.[6,18] However, in our study, we consider the place of upbringing as a crude measure for early-life microbial exposures. Furthermore, based on results from a previous study on the urban–rural gradient in asthma, we merged farms with and without livestock in our analyses, although farms with livestock still accounted for the majority in this group.[9] In addition, the response categories in the questionnaires were

not accompanied by any objective indicators and the interpretation was left open to the participant. This may have caused some random error in the measurement of exposure. A further limitation of this study is the lack of biological material from the subjects. This makes us unable to detect any biological pathways related to the effects of farm exposure in prior generations, including epigenetic methylation patterns, and also hindered objective markers for atopic disposition. In addition, the stability of epigenetic markers in parents from early-life farm exposure is unknown. However, a recent study on prenatal smoking exposure found that the methylation markers were stable throughout childhood and into adulthood.[19] If we assume a more transient epigenetic effect, the place where the parents lived closest to conception or the place where they spend most of their life are of more importance when investigating trans- or intergenerational effects on asthma. Unfortunately, our study did not have data to further investigate this.

A study on gene–environment interactions in asthma suggested that influences on genetic susceptibility may not be sufficient to develop asthma unless an appropriate environmental stimulus is also present.[20] This is further supported by a hypothesis-generating study suggesting that a farm upbringing may be an effect modifier in the association between different toll-like receptors and early-onset asthma.[21] In our study, we could not distinguish between inter- and transgenerational effects, as defined by Krauss-Etschmann *et al.*[22] as either effects in the intrauterine environment affecting the germ line of the foetus or effects transmitted across generations that cannot be explained by direct environmental exposures.

A study in the Danish National Birth Cohort compared three methods of measuring asthma and found self-report to pose a higher prevalence when compared with the hospitalization registry (12% vs 7%) and lower prevalence when compared with the prescription registry (32%).[23] As there is no consensus about a 'gold standard' for asthma diagnosis in epidemiological studies, we cannot rule out an overestimated asthma prevalence in our study (18%). It would have been useful to have included information on asthma symptoms in the analyses to assess asthma severity but, as we did not have the time of onset for these data, they were not suitable for the Cox-regression models. However, in a post-hoc comparison of the proportion of offspring with at least three symptoms of asthma, we found the same distribution as for 'ever asthma' within the different exposure groups of upbringing. Despite the limitations in the outcome measurement, two other studies in RHINESSA have found an increased risk of offspring asthma after preconception smoking exposure.[13,24]

The dropout in the ECRHS population (G1) has been substantial (~50%) and, in addition, only a third of the invited offspring participated in RHINESSA (G2). A non-response analyses in the Nordic part of ECRHS, named the RHINE cohort, showed a similar prevalence of asthma among baseline responders (4.7%) and long-term participants in RHINE 3 (4.6%) but, as we do not have information on offspring asthma from other sources, i.e. registries, we were not able to investigate whether the prevalence of asthma varied between participants and non-participants in RHINESSA.[25] However, based on the results from the RHINE cohort, we do not expect asthma status to influence the likelihood of participation in RHINESSA.

The original ECRHS population were sampled in and around larger cities in all study centres and this may have resulted in an overrepresentation of urban-dwellers in our study population. In addition, two non-response analyses from Denmark and Belgium showed that the risk of non-participation is higher among urban residents.[26,27] Thus, we believe that dropout is related to exposure (parental upbringing) but probably not outcome (offspring asthma), and is unlikely to have skewed our results. Dropout could be associated with other asthma-relevant variables, e.g. parental smoking status, but, due to the similar prevalence of asthma among participants and non-participants in RHINE, we do not think this is of major concern.[25] Furthermore, in the relatively small group with both parents from a farm (5% of the study population), the statistical power is limited, although, as we see consistent results across a number of different analyses, we believe this is robust.

Interpretation

A few studies investigating farm exposure *in utero* suggest that it may protect against asthma in the offspring,[28,29] but there is very little evidence on the effect of parental farm exposure before conception.[28] Also, some animal studies suggest that perinatal farm exposure is positively associated with epigenetic changes, reducing the risk of asthma. In a murine study from 2011, Brand *et al.* showed that prenatal exposure to the farm-derived gram-negative bacterium *A. lwoffii F78* caused alternation in histone acetylation in specific genomic loci and prevented the development of an asthmatic phenotype in the offspring.[30] Another study assessed DNA methylation in 10 genes related to asthma and found a change in the methylation patterns in DNA from farmers' children compared with non-farmers' children.[10] These epigenetic changes clustered in genes highly associated with asthma (*ORMDL family*) and IgE regulation (*RAD50*, *IL13* and *IL4*), but not in T-regulatory genes (*FOXP3* and *RUNX3*). Both studies support the Hygiene Hypothesis by indicating that

exposure to microbes and farm environments protects against asthma development in childhood through epigenetic mechanisms and, in this cohort, we previously showed that parents' place of upbringing is negatively associated with their own asthma status.[9]

Adverse exposures such as particulate air pollution in cities or cigarette smoke are known to increase the risk of asthma development. In a three-generation study from 2018, Accordini *et al.* found that maternal smoking during pregnancy was significantly associated with a higher risk of asthma in the offspring.[24] A murine study by Gregory *et al.* showed that exposure to diesel exhaust particles and concentrated urban air particles led to the generational maternal transmission of increased risk of asthma.[29] Also, Baiz *et al.* investigated the impact of maternal exposure to air pollutants before and during pregnancy on the newborn's immune cells.[28] The relative distribution of NK cells and T-lymphocytes including CD4+CD25+ regulatory T-cells in cord blood were found to be significantly altered when exposed to ambient air pollutants. These studies all indicate that both parental and grandparental exposure to smoking or air pollutants is positively associated with an increased risk of asthma in the offspring. Compared with a farm upbringing, smoking is a more direct and often long-term exposure. Therefore, we did not expect to find effects that are of comparable magnitude to smoking in this study. However, among other covariates, we adjusted our analyses for grandparental place of upbringing and grandparental smoking (Adj2 model). The similar results of the adjusted and unadjusted HRs indicate that the bias induced through these factors is minimal (Table 2B).

Conclusion

This study does not support the hypothesis that parental or grandparental upbringing has an important effect on the risk of offspring asthma. Further human studies that address the limitations in our study and provide a more precise measurement of exposure and the means to investigate possible mechanisms, i.e. a change in gene expression due to epigenetic effects, are needed.

Supplementary data

Supplementary data are available at *IJE* online.

Author Contributions

Vivi Schünssen, Cecilie Svanes and Signe Timm had the original idea for the study and, with all co-authors, carried out the design. Christer Janson (SE), Cecilie Svanes (NO), Randi Jacobsen Bertelsen (NO), Mathias Holm (SE), Lennart Bråbäck (SE), Rain Jogi (EE), Shyamali Dharmage (AU), Jose Luis Sanchez-Ramos (ES), Jesus Martinez-Moretalla (ES) and Vivi Schlünssen (DK) were responsible for recruitment and follow-up of study participants. Signe Timm, Morten Frydenberg and Ane Johannessen were responsible for data cleaning and carried out the analyses. Torben Sigsgaard, Brittany Campbell, Marie Kjær Madsen, Nils Oskar Jogi, Linus Schiöler and Julia Dratva provided feedback during the process due to their expert knowledge in this field. Signe Timm drafted the manuscript, which was revised by all authors. All authors read and approved the final manuscript.

Funding

The ECRHS/RHINE/RHINESSA study was supported by grants from the Faculty of Health, Aarhus University, Denmark (Project No. 240008), The Wood Dust Foundation (Project No. 444508795), the Danish Lung Association, the Swedish Heart and Lung Foundation, the Swedish Association Against Asthma and Allergy, the Swedish Association against Heart and Lung Disease, the Swedish Council for Working Life and Social Research, the Bror Hjerpstedt Foundation, the Vårdal Foundation for Health Care and Allergic Research, the Norwegian Research Council (Grant no. 214123, 230827/F20, 228174 and 135773/330), the Norwegian Asthma and Allergy Association, HelseVest Norway (Grant no. 911 631), the Icelandic Research Council, the University of Iceland Research Fund, the Icelandic GP's Research Fund, the Estonian Science Foundation (Grant No. 4350), the Estonian Research Council (Grant no. PUT562), Melbourne University, National Health & Medical Research Council of Australia, SEPAR Spain, Sociedad Española de Neumologia y Cirugía Toracica Spain and Horizon2020 PHC1 (Grant no. 633212). For further information about funding sources, please consult www.rhinessa.net. V.S. and C.S. are members of the COST BM1201 network. S.T. received a PhD scholarship from Aarhus University, Denmark.

Conflict of interest

None declared.

References

1. Ferrante G, LaGrutta S. The Burden of Pediatric Asthma. *Front Pediatr* 2018;**186**:6.
2. Hanski I, von Hertzen L, Fyhrquist N *et al.* Environmental biodiversity, human microbiota, and allergy are interrelated. *Proc Natl Acad Sci USA* 2012;**109**:8334–9.
3. Azad MB, Konya T, Maughan H *et al.* Infant gut microbiota and the hygiene hypothesis of allergic disease: impact of household pets and siblings on microbiota composition and diversity. *Allergy Asthma Clin Immunol* 2013;**9**:15.
4. Strachan DP. Hay fever, hygiene and household size. *BMJ* 1989; **299**:1259–60.
5. Portengen L, Sigsgaard T, Omland Ø, Hjort C, Heederik D, Doekes G. Low prevalence of atopy in young Danish farmers

and farming students born and raised on a farm. *Clin Exp Allergy* 2002;**32**:247–53.
6. Ege MJ, Mayer M, Normand A-C *et al*. Exposure to environmental microorganisms and childhood asthma. *N Engl J Med* 2011;**364**:701–09.
7. Campbell B, Lodge C, Lowe A, Burgess J, Matheson M, Dharmage S. Exposure to 'farming' and objective markers of atopy: a systematic review and meta-analysis. *Clin Exp Allergy* 2015;**45**:744–57.
8. Riedler J, Braun-Fahrländer C, Eder W *et al*. Exposure to farming in early life and development of asthma and allergy: a cross-sectional survey. *Lancet* 2001;**358**:1129–33.
9. Timm S, Frydenberg M, Janson C *et al*. The urban-rural gradient in asthma: a population-based study in northern Europe. *Int J Environ Res Public Health* 2015;**13**:1–14.
10. Michel S, Busato F, Genuneit J *et al*. Farm exposure and time trends in early childhood may influence DNA methylation in genes related to asthma and allergy. *Allergy* 2013;**68**: 355–64.
11. Slaats GGG, Reinius LE, Alm J, Kere J, Scheynius A, Joerink M. DNA methylation levels within the CD14 promoter region are lower in placentas of mothers living on a farm. *Allergy* 2012;**67**:895–903.
12. Li YF, Langholz B, Salam MT, Gilliland FD. Maternal and grandmaternal smoking patterns are associated with early childhood asthma. *Chest* 2005;**127**:1232–41.
13. Svanes C, Koplin J, Skulstad SM *et al*. Father's environment before conception and asthma risk in his children: a multigeneration analysis of the Respiratory Health in Northern Europe study. *Int J Epidemiol* 2017;**46**:235–45.
14. Kabesch M, Adcock IM. Epigenetics in asthma and allergy. *Curr Opin Allergy Clin Immunol* 2014;**14**:62–68.
15. Burney PGJ, Luczynska C, Chinn S, Jarvis D. The European Community Respiratory Health Survey. *Eur Respir J* 1994;**7**:954–60.
16. Lash TL, Fox MP, Maclehose RF, Maldonado G, Mccandless LC, Greenland S. Good practices for quantitative bias analysis. *Int J Epidemiol* 2014;**43**:1969–85.
17. Timm S, Schlünssen V, Benediktsdottir B *et al*. Offspring reports on parental place of upbringing: is it valid? *Epidemiology* 2019; **30**:e16–18.
18. Loss G, Apprich S, Waser M *et al*. The protective effect of farm milk consumption on childhood asthma and atopy: the GABRIELA study. *J Allergy Clin Immunol* 2011;**128**:766–73.
19. Richmond RC, Suderman M, Langdon R, Relton CL, Smith GD. DNA methylation as a marker for prenatal smoke exposure in adults. *Int J Epidemiol* 2018;**47**:1120–30.
20. Meyers DA, Postma DS, Stine OC *et al*. Genome screen for asthma and bronchial hyperresponsiveness: interactions with passive smoke exposure. *J Allergy Clin Immunol* 2005;**115**: 1169–75.
21. Lau MYZ, Dharmage SC, Burgess JA *et al*. The interaction between farming/rural environment and TLR2, TLR4, TLR6 and CD14 genetic polymorphisms in relation to early-and late-onset asthma. *Sci Rep* 2017;**7**:43681.
22. Krauss-Etschmann S, Meyer KF, Dehmel S, Hylkema MN. Inter- and transgenerational epigenetic inheritance: evidence in asthma and COPD? *Clin Epigenet* 2015;**7**:1–13.
23. Hansen S, Strøm M, Maslova E, Mortensen EL, Granström C, Olsen SF. A comparison of three methods to measure asthma in epidemiologic studies: results from the Danish National Birth Cohort. *PLoS One* 2012;**7**:e36328.
24. Accordini S, Calciano L, Johannessen A *et al*. A three-generation study on the association of tobacco smoking with asthma. *Int J Epidemiol* 2018;**47**:1106–17.
25. Johannessen A, Verlato G, Benediktsdottir B *et al*. Longterm follow-up in European respiratory health studies: patterns and implications. *BMC Pulm Med* 2014;**14**:63.
26. Kjøller M, Thoning H. Characteristics of non-response in the Danish Health Interview Surveys, 1987-1994. *Eur J Public Health* 2005;**15**:528–35.
27. Demarest S, Van Der Heyden J, Charafeddine R, Tafforeau J, Van Oyen H, Van Hal G. Socio-economic differences in participation of households in a Belgian national health survey. *Eur J Public Health* 2013;**23**:981–85.
28. Baiz N, Slama R, Béné M *et al*. Maternal exposure to air pollution before and during pregnancy can induce changes in newborn's cord blood lymphocytes involved in asthma and allergies. *Allergy* 2011;**66**:707.
29. Gregory DJ, Kobzik L, Yang Z, McGuire CC, Fedulov AV. Transgenerational transmission of asthma risk after exposure to environmental particles during pregnancy. *Am J Physiol-Lung Cell Mol Physiol* 2017; doi:10.1152/ajplung.00035.2017.
30. Brand S, Teich R, Dicke T *et al*. Epigenetic regulation in murine offspring as a novel mechanism for transmaternal asthma protection induced by microbes. *J Allergy Clin Immunol* 2011;**128**:618–25.

Supplementary material, paper 2

Table S1: Hazard ratios (HR) and corresponding 95% CI for offspring asthma according to grandparental place of upbringing

	ALL PARENTS (N=7,860)			
		Offspring from		
	All	City	Village	Farm
GRANDPARENTAL UPBRINGING (MATERNAL LINE)	crude	crude	crude	crude
Both grandparents from farm	0.89 (0.73-1.08)	0.86 (0.68-1.08)	0.87 (0.57-1.31)	1.62 (0.41-6.46)
Grandmother from farm, grandfather from village/city	1.06 (0.82-1.38)	0.98 (0.71-1.36)	1.10 (0.67-1.82)	1.73 (0.32-9.43)
Grandfather from farm, grandmother from village/city	0.93 (0.72-1.19)	0.99 (0.73-1.33)	0.72 (0.43-1.22)	2.37 (0.50-11.33)
Both grandparents from village or one grandparent from village and one from city	0.96 (0.80-1.14)	0.99 (0.82-1.21)	0.80 (0.54-1.19)	1.88 (0.45-7.84)
Both grandparents from city (ref)	1	1	1	1
		Offspring from		
	All	City	Village	Farm
GRANDPARENTAL UPBRINGING (PATERNAL LINE)	crude	crude	crude	crude
Both grandparents from farm	1.05 (0.86-1.29)	1.00 (0.79-1.28)	1.60 (0.95-2.70)	0.49 (0.20-1.20)
Grandmother from farm, grandfather from village/city	1.37 (1.04-1.79)	1.27 (0.92-1.76)	2.05 (1.12-3.78)	0.59 (0.13-2.74)
Grandfather from farm, grandmother from village/city	1.17 (0.89-1.54)	1.17 (0.84-1.62)	1.62 (0.87-3.02)	0.52 (0.13-2.02)
Both grandparents from village or one grandparent from village and one from city	1.21 (1.00-1.45)	1.15 (0.94-1.42)	1.76 (1.06-2.90)	0.69 (0.25-1.92)
Both grandparents from city (ref)	1	1	1	1

Table S1: Hazard ratios (HR) and corresponding 95% CI for offspring asthma according to grandparental place of upbringing

	ONLY PARENTS FROM CITY (N=2246)			
		Offspring from		
	All	City	Village	Farm
GRANDPARENTAL UPBRINGING (MATERNAL LINE)	crude	crude	crude	crude
Both grandparents from farm	1.15 (0.76-1.75)	1.05 (0.67-1.65)	1.77 (0.42-7.53)	7.04 (1.08-46.04)
Grandmother from farm, grandfather from village/city	1.02 (0.61-1.72)	0.97 (0.56-1.67)	1.85 (0.37-9.27)	-
Grandfather from farm, grandmother from village/city	0.93 (0.59-1.48)	1.00 (0.63-1.60)	-	-
Both grandparents from village or one grandparent from village and one from city	1.09 (0.84-1.43)	1.08 (0.82-1.42)	1.27 (0.44-3.65)	2.05 (0.18-23.54)
Both grandparents from city (ref)	1	1	1	1
		Offspring from		
	All	City	Village	Farm
GRANDPARENTAL UPBRINGING (PATERNAL LINE)	crude	crude	crude	crude
Both grandparents from farm	0.98 (0.61-1.59)	1.02 (0.62-1.68)	0.66 (0.09-4.93)	-
Grandmother from farm, grandfather from village/city	1.00 (0.58-1.73)	0.98 (0.53-1.79)	0.48 (0.06-3.61)	9.56 (1.76-51.84)
Grandfather from farm, grandmother from village/city	1.42 (0.92-2.21)	1.41 (0.89-2.21)	1.23 (0.13-11.28)	2.83 (0.19-41.65)
Both grandparents from village or one grandparent from village and one from city	1.16 (0.89-1.52)	1.18 (0.89-1.56)	0.83 (0.29-2.25)	1.86 (0.20-17.34)
Both grandparents from city (ref)	1	1	1	1

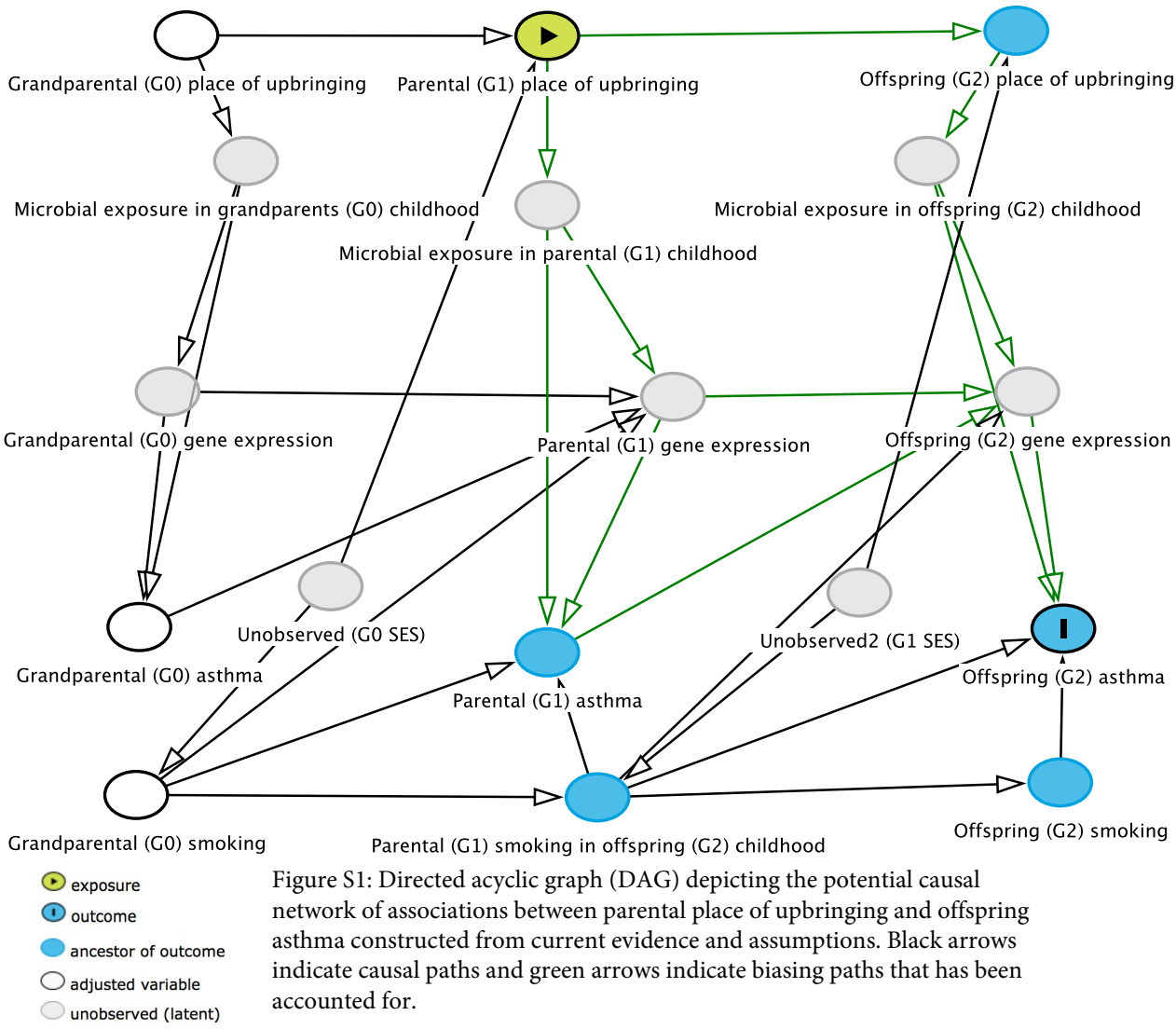


Figure S1: Directed acyclic graph (DAG) depicting the potential causal network of associations between parental place of upbringing and offspring asthma constructed from current evidence and assumptions. Black arrows indicate causal paths and green arrows indicate biasing paths that has been accounted for.

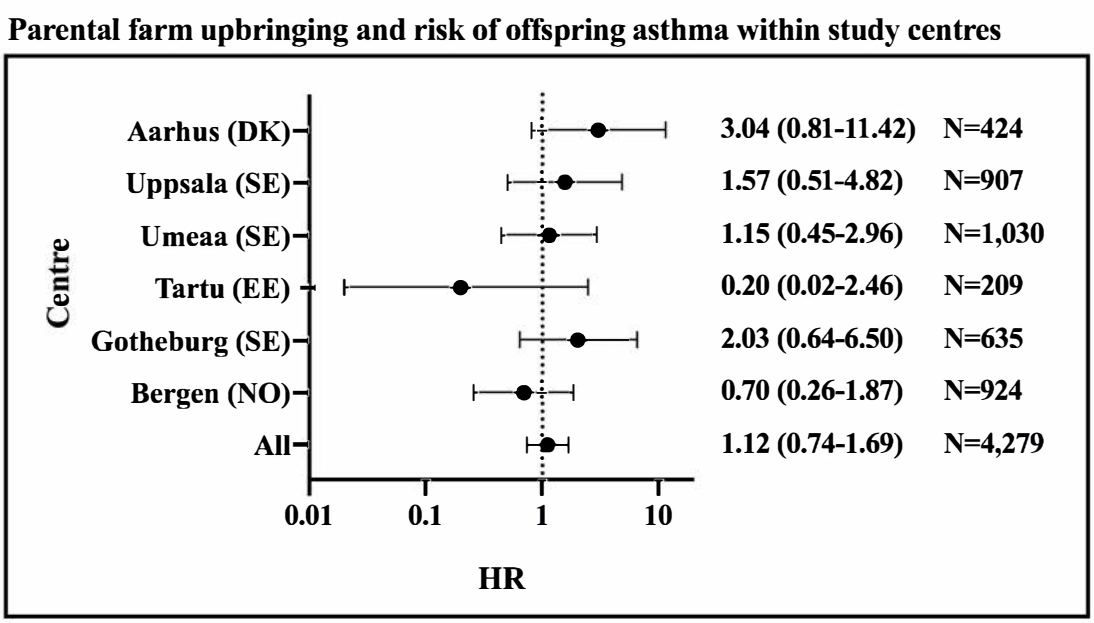


Figure S2: Hazard ratios (HR) with 95% confidence intervals (CI) for offspring asthma according to parental farm upbringing (both parents from farm VS. both parents from city) among offspring in RHINESSA (Respiratory Health in Northern Europe, Spain and Australia) adjusted for grandparental asthma, grandparental upbringing and grandparental smoking (adj2 model), for all and stratified by study centre (Table 2). Albacete (ES), Huelva (ES), Melbourne (AU) and Reykjavik (IS) were not presented due to too few data.

Paper 3: The Selection Study

Timm S, Frydenberg M, Abramson MJ, Bertelsen RJ, Bråbäck L, Benediktsdóttir B, Gislason T, Holm M, Janson C, Jõgi R, Johannessen A, Jeong-Lim K, Malinovschi A, Mishra G, Moratalla J, Sigsgaard T, Svanes C, Schlünssen V: **Asthma and selective migration from farming environments in a three-generation cohort study.** *European Journal of Epidemiology*. 2019 Jun;34(6):601-609. doi: 10.1007/s10654-019-00491-9.

Paper 3

European Journal of Epidemiology
https://doi.org/10.1007/s10654-019-00491-9

RESPIRATORY EPIDEMIOLOGY

Asthma and selective migration from farming environments in a three-generation cohort study

Signe Timm[1] · Morten Frydenberg[1] · Michael J. Abramson[2] · Randi J. Bertelsen[3] · Lennart Bråbäck[4] · Bryndis Benediktsdottir[5,6] · Thorarinn Gislason[5,7] · Mathias Holm[8] · Christer Janson[9] · Rain Jogi[10] · Ane Johannessen[11,12] · Jeong-Lim Kim[13] · Andrei Malinovschi[14] · Gita Mishra[15] · Jesús Moratalla[16] · Torben Sigsgaard[1] · Cecilie Svanes[11,12] · Vivi Schlünssen[1,17]

Received: 26 October 2018 / Accepted: 29 January 2019

Abstract
Individuals raised on a farm appear to have less asthma than individual raised elsewhere. However, selective migration might contribute to this as may also the suggested protection from farm environment. This study investigated if parents with asthma are less likely to raise their children on a farm. This study involved three generations: 6045 participants in ECRHS/RHINE cohorts (born 1945–1973, denoted G1), their 10,121 parents (denoted G0) and their 8260 offspring participating in RHINESSA (born 1963–1998, denoted G2). G2-offspring provided information on parents not participating in ECRHS/RHINE. Asthma status and place of upbringing for all three generations were reported in questionnaires by G1 in 2010–2012 and by G2 in 2013–2016. Binary regressions with farm upbringing as outcome were performed to explore associations between parental asthma and offspring farm upbringing in G0–G1 and G1–G2. Having at least one parent with asthma was not associated with offspring farm upbringing, either in G1–G2 (RR 1.11, 95% CI 0.81–1.52) or in G0–G1 (RR 0.99, 0.85–1.15). G1 parents with asthma born in a city tended to move and raise their G2 offspring on a farm (RR 2.00, 1.12–3.55), while G1 parents with asthma born on a farm were less likely to raise their G2 offspring on a farm (RR 0.34, 0.11–1.06). This pattern was not observed in analyses of G0–G1. This study suggests that the protective effect from farm upbringing on subsequent asthma development could not be explained by selective migration. Intriguingly, asthmatic parents appeared to change environment when having children.

Keywords Asthma · Farming · Selective migration · ECRHS · RHINE · RHINESSA

Background

Numerous studies suggest that being born and raised on a farm reduces the risk of asthma [1–4]. The protective effect from farm upbringing has been ascribed to a greater or more diverse microbial exposure in the farm environment, complementing the hygiene hypothesis which proposes that immunological competence is impaired after low microbial stimulation in early life [2, 5, 6]. However, findings in this field have been inconsistent [7, 8], and one important concern has been the possibility for selective migration over generations. One could therefore question if the apparently protective effect from farm upbringing is a result of asthmatic parents avoiding the farm environment rich in airway irritants such as endotoxin, allergens and organic dust [9] and raising their children in the cities. This would leave the farm effect as the mere product of selective migration rather than a biological effect of the farm environment. One thing is to claim that farm upbringing could explain less asthma in adulthood; another thing is if a healthy selection among farm dwellers contributes to curb heredity of asthma among their children.

Evidence of potential healthy selection patterns in farming is scarce, inconsistent and does not take both parents into account. One study found that 35–39-year-old men were less likely to take over the family farm if they had asthma at conscript examination, and one study found that asthmatics

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s10654-019-00491-9) contains supplementary material, which is available to authorized users.

✉ Signe Timm
signe.timm@ph.au.dk

Extended author information available on the last page of the article

Published online: 07 February 2019

were less likely to become pig farmers [10, 11]. In contrast, another study found similar prevalence of asthma among farming students and their siblings, and one study found similar prevalence of family history of asthma among farmers' and non-farmers' children [12, 13].

It seems plausible that selective migration may persist across generations. However, the inherent challenges in investigating this among humans are evident, and to our knowledge, no studies have analysed selection with regard to farming in multiple generations. Using the unique opportunity from a multi-generation study, we aimed to investigate if asthmatic parents were less likely than non-asthmatic parents to raise their children on a farm.

Methods

Study population

The present study is embedded in the ECRHS (European Community Respiratory Health Survey), the RHINE (Respiratory Health In Northern Europe) and the RHINESSA (Respiratory Health In Northern Europe, Spain and Australia) cohort studies (see Figure S1). The ECRHS was initiated in 1988–1992 and included 1500 men and 1500 women born 1945–1973 randomly selected by each of the study centres [14]. The RHINE was a sub-study in five Northern European countries with extended questionnaires [15]. In the present study, we only consider seven countries with ten centres which constitute the RHINESSA study, comprising offspring of the ECRHS/RHINE participants: Denmark (Aarhus), Norway (Bergen), Sweden (Uppsala, Umeå, Gothenburg), Iceland (Reykjavik), Estonia (Tartu), Spain (Huelva, Albacete) and Australia (Melbourne).

This study involves three generations: Participants in the ECRHS/RHINE (G1), their parents (G0, information given by G1) and their children (G2). Furthermore, we include information via the G2 children on the parent, who was not part of ECRHS/RHINE study.

Data measurement

G1 participants provided information via the ECHRS/RHINE III questionnaires in 2010–2012 and G2 provided information via the RHINESSA questionnaires in 2013–2016, Table S1.

G0 and G1 asthma status was defined as ever asthma corresponding to an affirmative answer to "Do you/your mother/your father have or have you/they ever had asthma?". G1 participants provided this information about themselves and their parents (G0), and G2 provided this information on the G1 not part of the ECRHS/RHINE cohort. G1 and G2 place of upbringing was defined from answers to the question "What term best describes the place you/your mother/your father lived most of the time before the age of five years?" with response categories (1) farm with livestock (2) farm without livestock, (3) village in a rural area, (4) small town, (5) suburb of city and (6) inner city. The same phrasing was used when offspring reported on behalf of parents and grandparents. Data were analyzed as "farm" in which we merged farm with livestock and farm without livestock, and "city" with the remaining four response categories. A recent paper analyzing the 6 response categories separately in G1 did not detect a significantly different effect from farms with or without livestock and consequently they were merged in the present analyses [1].

A formal forward–backward translation was performed in all languages to ensure homogeneity between study centres.

Statistical analyses

Data were analysed by binary regression models with log-link estimating relative risks (RR) and corresponding 95% confidence intervals (CIs) with farm upbringing as outcome adjusted for potential confounders. Potential confounders were selected using directed acyclic graphs (DAG) depicting the causal network of interest [16] (see Figure S2). The DAG was based on current evidence and assumptions, and included the following variables: parental smoking, parental and grandparental socioeconomic status and parental place of upbringing. In the statistical model we included the minimal adjustment set for the total effect of parental asthma on offspring place of upbringing was mother's place of upbringing and father's place of upbringing. Furthermore, due to varying prevalence of farm upbringing between different study centres, it was determined a priori also to adjust for centre.

Identical analyses were conducted investigating the association between parental asthma and offspring upbringing in the two parent–offspring sets G0–G1 and G1–G2, respectively, as illustrated by the blue circles in Fig. 1. Analyses were clustered by family. In order to examine centre specific effects, the analyses were presented for each study centre separately. To investigate moving patterns, analyses were stratified by the previous generation's place of upbringing. Sensitivity analyses also included repetition of analyses with direct reporting of offspring asthma and place of upbringing from the one parent in the ECRHS/RHINE instead of the indirect report by the offspring in the RHINESSA to investigate the robustness of the results. Furthermore, sub-analyses were performed on parental hay fever and offspring farm upbringing in a clinical subsample of the ECRHS/RHINE with information on both parents' hay fever among G0–G1, and with information on only one parent's hay fever (the ECRHS/RHINE participant) among G1–G2.

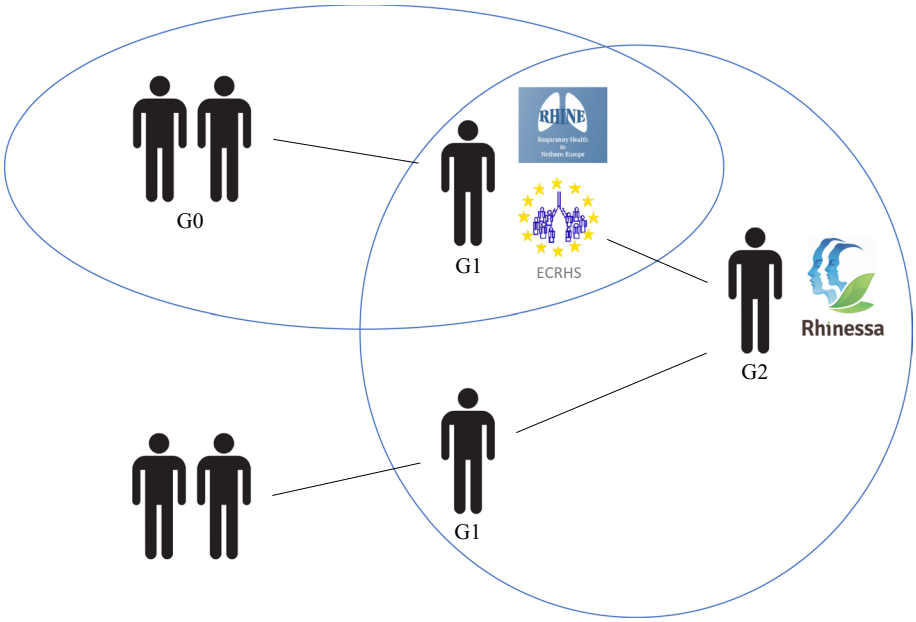


Fig. 1 Illustration of the three generations under study from the left: G0 (included in the study based on information from G1 and G2), G1 (where either mother or father was a participant in the ECRHS/RHINE cohort) and G2 (RHINESSA participants). The G1 parent who was not part of the ECRHS/RHINE cohort was included in the study based on information from G2. The blue circles illustrate the two subsets of analyses denoted G0–G1 and G1–G2, respectively

Statistical analyses were performed using Stata 15 (STATA Corp., College Station, TX, USA).

Results

Basic characteristics of the study populations ECRHS/RHINE (G1, N = 6045) and RHINESSA (G2, N = 8260) are shown in Table 1. G2 offspring born on a farm were comparable to city offspring with regard to birth year and parental asthma (Table 1). The same was observed in the G1 population. Farm-brought up offspring (G2) were more likely to have their father brought up in farms than their mothers; this difference was not observed in G1. Place of upbringing varied markedly over generations with 32% G0 participants from farms, 17% G1 participants from farms and 4% G2 participants from farms (Fig. 2).

In binary regression, parental asthma was not associated with offspring farm upbringing either among G1–G2 (RR 1.11, 95% CI 0.81–1.52) or among G0–G1 (RR 0.99, 95% CI 0.85–1.15), Tables 2 and 3. Sensitivity analyses on G1–G2 using direct reports from the ECRHS/RHINE participants instead of indirect reports from the RHINESSA participants revealed similar results (RR 1.21, 95% CI 0.80–1.82). Centre-specific estimates in general showed the same picture among both G0–G1 and G1–G2. Only Reykjavik and Melbourne stood out, showing that Icelandic G1 individuals with asthma were more likely to raise their offspring on a farm (RR 2.74, 95% CI 1.21–6.20), and the same was observed for Australian G0 individuals with asthma (RR 2.51, 95% CI 1.25–5.05), Tables 2 and 3.

When investigating moving patterns, G1 parents with asthma born in a city tended to move and raised their G2 offspring on a farm (RR 2.00, 95% CI 1.12–3.55), in contrast with G1 parents with asthma born on a farm who were less likely to raise their G2 offspring on a farm (RR 0.34, 95% CI 0.11–1.06), Table 4 and Fig. 3. This pattern was not observed in the G0–G1 analyses as G0 asthma was not associated with G1 farm upbringing when stratifying by G0 place of upbringing, Table 5 and Fig. 3.

Analyses on a clinical subsample of 1350 ECRHS participants showed that parental eczema, skin allergy, nasal allergy or hay fever were not associated with offspring farm upbringing among G0–G1 (RR 0.81, 95% CI 0.64–1.05). We revealed similar results in a sub-analysis of 4695 G2 offspring in the RHINESSA with information on only one parent's hay fever and nasal allergies among G1–G2 (RR 0.96, 95% CI 0.68–1.35).

Discussion

Key results

In this three-generation study, parental asthma was not associated with offspring farm upbringing in either of the two parent–offspring sets G0–G1 and G1–G2. Therefore, this study is not consistent with the hypothesis that the protective effect from farm upbringing on asthma may be due to selective migration from farming environments over generations. However, asthmatic G1 parents were more likely to raise their offspring in another environment than where they grew up themselves, while asthmatic G0 parents did not tend to change residential area.

Table 1 Characteristics of the study population in the RHINE/ECRHS (G1) and the RHINESSA (G2)

	G2 (RHINESSA)				G1 (RHINE/ECRHS)*			
	All	Farm upbringing	City upbringing	Missing	All	Farm upbringing	City upbringing	Missing
	N = 8260	N = 335	N = 7794	N = 131	N = 6045	N = 909	N = 4168	N = 968
Birth year, mean (min.;max.)	1984 (1963;1998)	1984 (1964;1997)	1984 (1963;1998)	1985 (1965;1998)	1956 (1945;1973)	1954 (1946;1973)	1956 (1945;1973)	1956 (1945;1972)
Sex, N (% F)	4781 (58%)	207 (62%)	4505 (58%)	69 (53%)	3311 (55%)	519 (57%)	2297 (55%)	495 (51%)
Parental asthma status, N (%)								
No parents with asthma	6389 (77%)	259 (77%)	6112 (78%)	18 (13%)	5321 (88%)	786 (86%)	3572 (86%)	963 (99%)
At least one parent with asthma	1598 (19%)	74 (22%)	1518 (20%)	6 (5%)	723 (12%)	123 (14%)	596 (14%)	4 (1%)
Missing	273 (3%)	2 (1%)	164 (2%)	107 (82%)	1 (0%)	0 (0%)	0 (0%)	1 (0%)
Maternal upbringing^, N (%)								
Farm	1222 (15%)	131 (39%)	1086 (14%)	5 (4%)	1606 (27%)	573 (63%)	758 (18%)	275 (28%)
City	6831 (83%)	202 (60%)	6616 (85%)	13 (10%)	3539 (58%)	264 (29%)	2783 (67%)	492 (51%)
Missing	207 (2%)	2 (1%)	92 (1%)	113 (86%)	900 (15%)	72 (8%)	627 (15%)	201 (21%)
Paternal upbringing^, N (%)								
Farm	1277 (16%)	205 (61%)	1069 (14%)	3 (2%)	1613 (27%)	617 (68%)	742 (18%)	254 (26%)
City	6621 (80%)	123 (37%)	6484 (83%)	14 (11%)	3367 (56%)	207 (23%)	2682 (64%)	478 (49%)
Missing	362 (4%)	7 (2%)	241 (3%)	114 (87%)	1065 (18%)	85 (9%)	744 (18%)	236 (25%)

^As reported by G2 in RHINESSA, *For the RHINE/ECHRS cohort i.e. "parental asthma" refers to asthma of their parents meaning G0

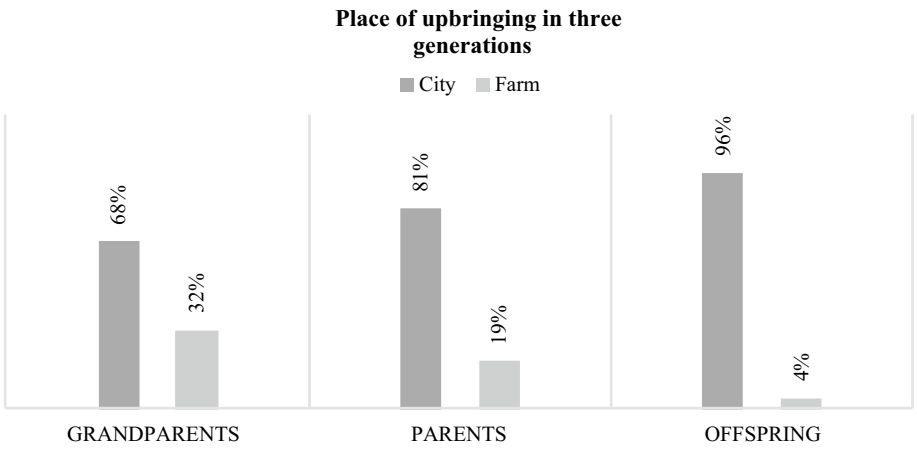


Fig. 2 Bar chart depicting the prevalence of farm and city upbringing in three generations in RHINESSA

Strengths and limitations

The multi-generation design is the most important strength of this study. To our knowledge, this is also the first study to investigate asthma and selective migration over generations taking both parents' asthma status into account.

However, all variables were measured by questionnaires, which poses a risk of recall bias. Furthermore, several variables were reported on behalf of others. We believe that both offspring and parents were able to report their own place of upbringing correctly; however, offspring's report on behalf of their parents may be subject to misclassification. We envisage that this misclassification may be influenced by the offspring's own place of upbringing, and it may have introduced bias to the results. However, we do not believe that the misclassification is related to the parents' asthma status and would therefore expect any bias from this to be non-differential. Reports of asthma (both self-reported and

Table 2 Risk ratios (RR) for offspring farm upbringing (outcome) according to parental asthma (exposure) in G1–G2 adjusted for G1 place of upbringing and stratified by study centre

	At least one parent with asthma		Crude RR	RR (95% CI)
	Yes (% offspring farm upb.)	No (% offspring farm upb.)		
Aarhus (DK)	169 (5.9%)	707 (4.2%)	1.39	1.55 (0.78; 3.06)
Albacete (ES)	36 (0.0%)	38 (0.0%)		
Bergen (NO)	318 (2.5%)	1330 (2.8%)	0.90	0.95 (0.46; 1.99)
Gothenburg (SE)	141 (1.4%)	792 (0.9%)	1.60	1.49 (0.32; 7.03)
Huelva (ES)	14 (0.0%)	55 (0.0%)		
Melbourne (AU)	87 (0.0%)	4 (0.0%)		
Reykjavik (IS)	231 (3.9%)	928 (1.4%)	2.78	2.74 (1.21; 6.20)
Tartu (EE)	37 (0.0%)	488 (5.5%)		
Umeå (SE)	287 (11.8%)	1009 (8.8%)	1.34	1.37 (0.96; 1.95)
Uppsala (SE)	272 (4.0%)	1020 (5.5%)	0.74	0.77 (0.42; 1.43)
All	1592 (4.6%)	6371 (4.1%)	1.10	1.11 (0.81; 1.52)

Table 3 Risk ratios (RR) for offspring farm upbringing (outcome) according to parental asthma (exposure) in G0–G1 adjusted for G0 place of upbringing and stratified by study centre

	At least one parent with asthma		Crude RR	RR (95% CI)
	Yes (% offspring farm upb.)	No (% offspring farm upb.)		
Aarhus (DK)	82 (14.6%)	492 (17.9%)	0.82	0.79 (0.48; 1.29)
Albacete (ES)	12 (8.3%)	31 (6.5%)	1.29	2.28 (0.25; 21.11)
Bergen (NO)	123 (19.5%)	758 (21.6%)	0.90	0.90 (0.61; 1.34)
Gothenburg (SE)	88 (8.0%)	609 (8.9%)	0.90	0.92 (0.45; 1.87)
Huelva (ES)	6 (0.0%)	32 (6.3%)		
Melbourne (AU)	23 (21.7%)	81 (7.4%)	2.93	2.51 (1.25; 5.05)
Reykjavik (IS)	113 (9.7%)	590 (11.0%)	0.88	0.81 (0.43; 1.50)
Tartu (EE)	31 (25.8%)	254 (28.3%)	0.91	1.07 (0.56; 2.07)
Umeå (SE)	122 (35.2%)	751 (29.0%)	1.21	1.10 (0.89; 1.35)
Uppsala (SE)	119 (10.1%)	760 (15.1%)	0.67	0.80 (0.49; 1.33)
All	719 (17.1%)	4358 (18.0%)	0.98	0.99 (0.85; 1.15)

Table 4 Risk ratios (RR) for offspring farm upbringing (outcome) according to parental asthma (exposure) in G1–G2 adjusted for study centre and the opposite parent's place of upbringing, and stratified by G1 place of upbringing

	At least one parent with asthma		Crude RR	RR (95% CI)
	Yes (% G2 offspring farm upb.)	No (% G2 offspring farm upb.)		
G1 father born in a city	940 (2.7%)	3798 (1.8%)	1.49	1.54 (0.98; 2.41)
G1 mother born in a city	928 (3.8%)	3828 (2.5%)	1.49	1.54 (1.07; 2.22)
Both born in a city	824 (2.1%)	3339 (1.0%)	1.97	2.00 (1.12; 3.55)
G1 father born on a farm	152 (13.8%)	724 (14.8%)	0.94	0.92 (0.60; 1.42)
G1 mother born on a farm	164 (6.7%)	694 (11.2%)	0.60	0.63 (0.34; 1.16)
Both born on a farm	48 (6.3%)	235 (19.1%)	0.33	0.34 (0.11; 1.06)

offspring-reported parental asthma) usually have a high specificity and a moderate sensitivity [17, 18]. If cases with asthma were incorrectly reported as healthy, this might have attenuated the results in our analyses.

Drop-out from the parent population was substantial (approx. 50%), and selection bias cannot be ruled out [15]. Furthermore, the response rate among offspring in the RHINESSA was modest (34%). This may have biased our results if non-response and dropout were related both to asthma and place of upbringing. A recent non-response analysis in the RHINE cohort concluded that asthma prevalence was similar among the baseline study population and long-term responders (4.7% at baseline and 4.6% in RHINE 3) [15]. However, the original ECRHS/RHINE populations

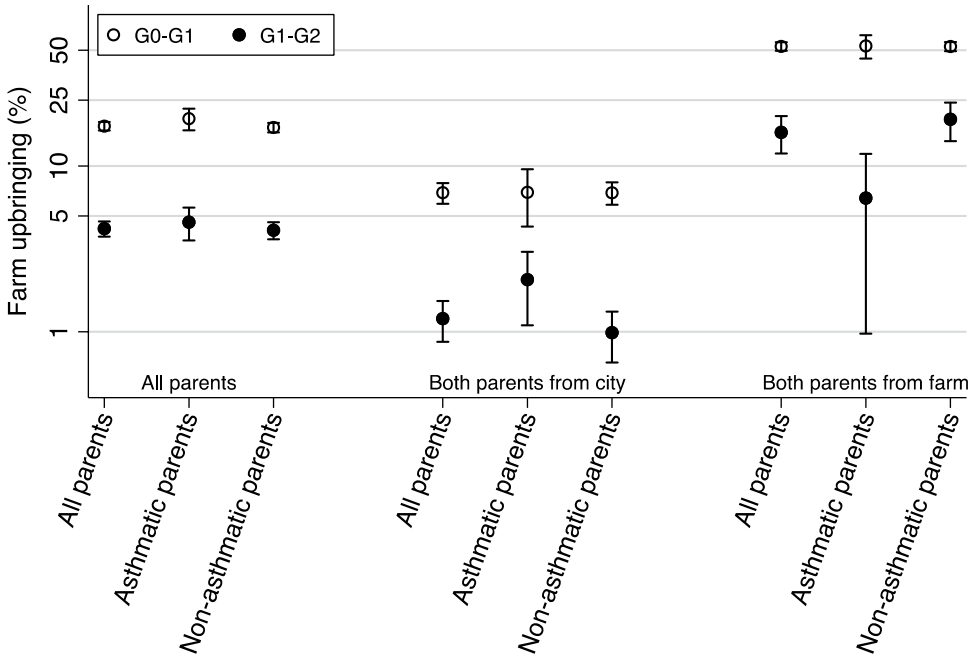


Fig. 3 Offspring farm upbringing (outcome) according to parental asthma status (exposure) and parental place of upbringing

Table 5 Risk Ratios (RR) for offspring farm upbringing (outcome) according to parental asthma (exposure) in G0–G1 adjusted for study centre and the opposite parent's place of upbringing, and stratified by G0 place of upbringing

	At least one grandparent with asthma		Crude RR	RR (95% CI)
	Yes (% G1 offspring farm upb.)	No (% G1 offspring farm upb.)		
G0 father born in a city	402 (7.7%)	2407 (7.2%)	1.05	1.10 (0.76; 1.58)
G0 mother born in a city	420 (8.3%)	2442 (8.8%)	0.93	0.88 (0.63; 1.23)
Both born in a city	360 (6.9%)	2174 (6.9%)	1.03	1.07 (0.74; 1.54)
G0 father born on a farm	190 (41.6%)	1141 (46.0%)	0.89	0.98 (0.83; 1.15)
G0 mother born on a farm	172 (43.6%)	1160 (41.7%)	1.02	1.02 (0.88; 1.20)
Both born on a farm	130 (53.1%)	873 (52.7%)	1.01	1.01 (0.85; 1.19)

were sampled in and around larger cities, and urban dwellers are over represented. Place of upbringing or residence may have influenced the likelihood of non-response in both the RHINE/ECRHS and the RHINESSA. A recent non-response study from Denmark suggested that participation rates among inhabitants in Copenhagen were significantly lower than in the rest of the country, and a Belgian study similarly found that odds ratio for non-participation rose with increasing level of urbanization [19, 20]. As non-response is only related to outcome (residency) and not exposure (asthma status) of interest, the potential bias from this issue would be non-differential.

Another limitation is the lack of analyses on selective migration due to allergies as the farm effect is most evident for allergic disorders such as hay fever [8, 21, 22]. However, the results of our sub-analyses showed the same picture as for asthma, although the information on parental diseases for both G0–G1 and G1–G2 analyses was incomplete. In contrast, Bråbäck et al. observed that Swedish middle-aged men with hay fever at conscript examination were less likely to take over the family farm [10].

Interpretation

Study designs and conclusions drawn from the current evidence on selective migration due to asthma are inconsistent. In a recent Swedish study, Bråbäck et al. investigated selective migration from the farming environment among 43,234 young men from farmparents [10]. They found that at the age of 35–39 years, farm living was significantly less likely if the men had asthma at the conscript examination, and they concluded that selective migration possibly could contribute to explain the observed lower prevalence of asthma among farmers' children. In line with this finding, Vogelzang et al. observed that asthmatic adolescents were less likely to become pig farmers than non-asthmatics [11]. Conversely, Eduard et al. found a similar prevalence of asthma among Danish farm students and their

non-farming siblings, suggesting no healthy worker selection into farming [12]. Likewise, a Swedish study among 707 children on the island of Gotland found no difference in frequency of family history of asthma between farmers' and non-farmers' children [13]. Interestingly, Bråbäck et al. and Vogelzang et al. both suggested selective migration in populations comparable in calendar time to G1 in our study, while Eduard et al. and Klintberg et al. both suggested no selective migration in populations comparable to G2 in our study. Thus, the literature suggests different pictures for different generations; however, we observed the same patterns for both G0–G1 and G1–G2.

As far as we know, we are the first to report moving patterns among asthmatics independently of their residence. We interpret the differences in moving patterns between G0 and G1 as a result of the different periods in history influencing the ability to move. Student's *T* test revealed no association between asthma status and socioeconomic status in G1 ($p = 0.27$), and we do therefore not believe that socioeconomic status can explain this finding. Mobility may also have varied markedly between study centres. The huge structural changes of the farms have occurred during the past five or six decades which may also have influenced the likelihood of moving. In addition, the G0 were not enlightened about the environmental influences on asthma, while we speculate that asthmatic G1 parents may suspect that their childhood exposures caused or worsened asthma and therefore tended to move. Furthermore, farmers with asthma may realize that they cannot continue as farmers because of their work-related worsening of symptoms and thus seek avoidance. A similar tendency seems to have affected pet keeping, as several studies have observed a "healthy pet-keeping effect" [23, 24].

Conclusion

This three-generation cohort study suggests that selective migration is not an important explanatory factor for the protective effect from farm upbringing on asthma, since parental asthma was not associated with offspring farm upbringing, either in analyses of the younger generations G1–G2 or analyses of the older generations G0–G1. Interestingly, individuals with asthma in G1 tended to move and raise their children in a different environment from where they grew up themselves, whereas individuals with asthma in G0 tended to stay in the same environment when raising children, probably due to differences in mobility patterns over generations.

This study contributes to the understanding of potential selection across generations for the farming effect on asthma. Further studies are needed to confirm these observations.

Funding The ECRHS/RHINE/RHINESSA study was supported by grants from The Faculty of Health, Aarhus University, Denmark (Project No. 240008), The Wood Dust Foundation (Project No. 444508795), The Danish Lung Association, the Swedish Heart and Lung Foundation, the Swedish Association Against Asthma and Allergy, the Swedish Association against Heart and Lung Disease, the Swedish Council for Working Life and Social Research, The Bror Hjerpstedt Foundation, The Vårdal Foundation for Health Care and Allergic Research, The Norwegian Research Council (Grant Nos. 214123, 230827/F20, 228174 and 135773/330), The Norwegian Asthma and Allergy Association, HelseVest Norway (Grant No. 911 631), The Icelandic Research Council, The University of Iceland Research Fund, The Icelandic GP's Research Fund, The Estonian Science Foundation (Grant No. 4350), The Estonian Research Council (Grant No. PUT562), Melbourne University, National Health & Medical Research Council of Australia, SEPAR Spain, Sociedad Española de Neumologia y Cirugía Toracica Spain and Horizon2020 PHC1 (Grant No. 633212). For further information about funding sources, please consult www.rhinessa.net. Vivi Schlünssen and Cecilie Svanes are members of the COST BM1201 network. Signe Timm received a PhD scholarship from Aarhus University, Denmark.

Compliance with ethical standards

Conflict of interest Michael Abramson has received investigator-initiated grants from Pfizer and Boehringer-Ingelheim for unrelated research. The other authors declare no conflict of interest.

References

1. Timm S, Frydenberg M, Janson C, Campbell B, Forsberg B, Gislason T, Holm M, Jogi R, Omenaas E, Sigsgaard T, Svanes C, Schlünssen V. The urban-rural gradient in asthma: a population-based study in Northern Europe. Int J Environ Res Public Health. 2015;13:1–14. https://doi.org/10.3390/ijerph13010093.
2. Ege M, Mayer M. Exposure to environmental microorganisms and childhood asthma. N Engl J Med. 2011;364:701–9.
3. Von Ehrenstein OS, Von Mutius E, Illi S, Baumann L, Böhm O, Von Kries R. Reduced risk of hay fever and asthma among children of farmers. Clin Exp Allergy. 2000;30:187–93. https://doi.org/10.1046/j.1365-2222.2000.00801.x.
4. Bråbäck L, Hjern A, Rasmussen F. Trends in asthma, allergic rhinitis and eczema among Swedish conscripts from farming and non-farming environments. A nationwide study over three decades. Clin Exp Allergy. 2004;34:38–43.
5. Heederik D, Von Mutius E. Does diversity of environmental microbial exposure matter for the occurrence of allergy and asthma? J Allergy Clin Immunol. 2012;130:44–50. https://doi.org/10.1016/j.jaci.2012.01.067.
6. Strachan DP. Hay fever, hygiene and household size. BMJ. 1989;299:1259–60.
7. Ege MJ, Frei R, Bieli C, Schram-Bijkerk D, Waser M, Benz MR, Weiss G, Nyberg F, van Hage M, Pershagen G, Brunekreef B, Riedler J, Lauener R, Braun-Fahrländer C, von Mutius E. Not all farming environments protect against the development of asthma and wheeze in children. J Allergy Clin Immunol. 2007;119:1140–7. https://doi.org/10.1016/j.jaci.2007.01.037.
8. Wlasiuk G, Vercelli D. The farm effect, or: when, what and how a farming environment protects from asthma and allergic disease. Curr Opin Allergy Clin Immunol. 2012;12:461–6. https://doi.org/10.1097/ACI.0b013e328357a3bc.
9. Basinas I, Sigsgaard T, Kromhout H, Heederik D, Wouters IM, Schlünssen V. A comprehensive review of levels and determinants

of personal exposure to dust and endotoxin in livestock farming. J Expo Sci Environ Epidemiol. 2013. https://doi.org/10.1038/jes.2013.83.
10. Bråbäck L, Hjern A, Rasmussen F, Van Schayck CP, Knottnerus JA. Selective migration contributes to a healthy worker effect in the farming population. J Clin Epidemiol. 2006;59:102–3. https://doi.org/10.1016/j.jclinepi.2005.08.003.
11. Vogelzang PFJ, van der Gulden JWJ, Tielen MJ, Folgering H, van Schayck CP. Health based selection for asthma, but not for chronic bronchitis, in pig farmers: an evidence based hypothesis. Eur Respir J. 1999;13:187. https://doi.org/10.1034/j.1399-3003.1999.13a34.x.
12. Eduard W, Schlünssen V, Sigsgaard T, Omland Ø, Pearce N, Douwes J. Can selection explain the protective effects of farming on asthma? Ann Agric Environ Med. 2015;22:467–9. https://doi.org/10.5604/12321966.1167715.
13. Klintberg B, Berglund N, Lilja G, Wickman M, van Hage-Hamsten M. Fewer allergic respiratory disorders among farmers' children in a closed birth cohort from Sweden. Eur Respir J. 2001;17:1151–7. https://doi.org/10.1183/09031936.01.00027301.
14. Burney PGJ, Luczynska C, Chinn S, Jarvis D. The European community respiratory health survey. Eur Respir J. 1994;7:954–60. https://doi.org/10.1183/09031936.94.07050954.
15. Johannessen A, Verlato G, Benediktsdottir B, Forsberg B, Franklin K, Gislason T, Holm M, Janson C, Jögi R, Lindberg E, Macsali F, Omenaas E, Real FG, Saure EW, Schlünssen V, Sigsgaard T, Skorge TD, Svanes C, Torén K, Waatevik M, Nilsen RM, de Marco R. Longterm follow-up in European respiratory health studies—patterns and implications. BMC Pulm Med. 2014;14:63. https://doi.org/10.1186/1471-2466-14-63.
16. Glymour MM, Greenland S. Causal diagrams. In: Rothmann K, Greenland S, Lash T, editors. Modern Epidemiology. Lippincott Williams & Wilkins; 2012. p. 183–212.
17. Pekkanen J, Pearce N. Defining asthma in epidemiological studies. Eur Respir J. 1999;14:951–7. https://doi.org/10.1034/j.1399-3003.1999.14d37.x.
18. Kuiper IN, Svanes C, Benediktsdottir B, Bertelsen RJ, Bråbäck L, Dharmage SC, Holm M, Jögi R. Agreement in reporting of asthma by parents or offspring—the RHINESSA generation study. BMC Pulm Med. 2018. https://doi.org/10.1186/s12890-018-0687-4.
19. Kjøller M, Thoning H. Characteristics of non-response in the Danish Health Interview Surveys, 1987–1994. Eur J Public Health. 2005;15:528–35. https://doi.org/10.1093/eurpub/cki023.
20. Demarest S, Van Der Heyden J, Charafeddine R, Tafforeau J, Van Oyen H, Van Hal G. Socio-economic differences in participation of households in a Belgian national health survey. Eur J Public Health. 2013;23:981–5. https://doi.org/10.1093/eurpub/cks158.
21. Christensen S, Timm S, Janson C, Benediktsdottir B, Forsberg B, Holm M, Jogi R, Johannessen A, Omenaas E, Sigsgaard T, Svanes C, Schlünssen V. A clear urban-rural gradient of allergic rhinitis in a population-based study in Northern Europe. Eur Clin Respir J. 2016;3:33463.
22. Campbell B, Lodge C, Lowe A, Burgess J, Matheson M, Dharmage S. Exposure to 'farming' and objective markers of atopy: a systematic review and meta-analysis. Clin Exp Allergy. 2015;45:744–57. https://doi.org/10.1111/cea.12429.
23. Svanes C, Zock J-P, Antó J, Dharmage S, Norbäck D, Jarvis D, De Marco R, Plana E, Raherison C, Sunyer J. Do asthma and allergy influence subsequent pet keeping? An analysis of childhood and adulthood. J Allergy Clin Immunol. 2006. https://doi.org/10.1016/j.jaci.2006.06.017.
24. Bornehag CG, Sundell J, Hagerhed L, Janson S. Pet-keeping in early childhood and airway, nose and skin symptoms later in life. Allergy Eur J Allergy Clin Immunol. 2003;58:939–44. https://doi.org/10.1034/j.1398-9995.2003.00050.x.

Affiliations

Signe Timm[1] · Morten Frydenberg[1] · Michael J. Abramson[2] · Randi J. Bertelsen[3] · Lennart Bråbäck[4] · Bryndis Benediktsdottir[5,6] · Thorarinn Gislason[5,7] · Mathias Holm[8] · Christer Janson[9] · Rain Jogi[10] · Ane Johannessen[11,12] · Jeong-Lim Kim[13] · Andrei Malinovschi[14] · Gita Mishra[15] · Jesús Moratalla[16] · Torben Sigsgaard[1] · Cecilie Svanes[11,12] · Vivi Schlünssen[1,17]

1 Department of Public Health, Danish Ramazzini Centre, Aarhus University, Bartholins Alle 2, Building 1260, 8000 Aarhus C, Denmark

2 School of Public Health and Preventive Medicine, Monash University, Melbourne, Australia

3 Institute of Clinical Science, University of Bergen, Bergen, Norway

4 Section of Sustainable Health, Department of Public Health and Clinical Medicine, Umeå University, Umeå, Sweden

5 Medical Faculty, University of Iceland, Reykjavík, Iceland

6 Primary Health Care Center, Gardabaer, Iceland

7 Department of Sleep, Landspitali University Hospital, Reykjavík, Iceland

8 Section of Occupational and Environmental Medicine, Sahlgrenska University Hospital, Gothenburg, Sweden

9 Department of Medical Sciences: Respiratory, Allergy and Sleep Research, Uppsala University, Uppsala, Sweden

10 Department of Pulmonology (ARKS), University of Tartu, Tartu, Estonia

11 Department of Global Public Health and Primary Care, Centre for International Health, University of Bergen, Bergen, Norway

12 Department of Occupational Medicine, Haukeland University Hospital, Bergen, Norway

13 Section of Occupational and Environmental Medicine, The Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden

14 Department of Medical Sciences, Clinical Physiology, Uppsala University, Uppsala, Sweden

15 School of Public Health, The University of Queensland, Brisbane, QLD 4006, Australia

[16] Department of Internal Medicine, Albacete University Hospital, Albacete, Spain

[17] National Research Centre for The Working Environment, Copenhagen, Denmark

Supplementary material, paper 3

Table S1: Data sources and measurements. G1 provided information in the RHINE III/ECRHS 3 questionnaire in 2010-2012 and G2 provided information in the RHINESSA questionnaire in 2013-2016.

Covariate	**Data source**	**Measurement**
Place of upbringing		*"What term best describes the place you/your mother/your father lived most of the time before the age of five years?"* Response categories: (1) farm with livestock, (2) farm without livestock, (3) village in rural area, (4) small town, (5) suburb of town and (6) inner city
G2	G2	
G1	G1+G2	
G0	G2	
Asthma		*"Do you/your mother/your father have or have you/she/he ever had asthma?"*
G2	G2	
G1	G1+G2	
G0	G1	

Figure S1:

Flow chart depicting the participants in the ECRHS/RHINE and the RHINESSA analysed in this study. Participants from the ECRHS/RHINE with children were included only if their children participated in the RHINESSA.

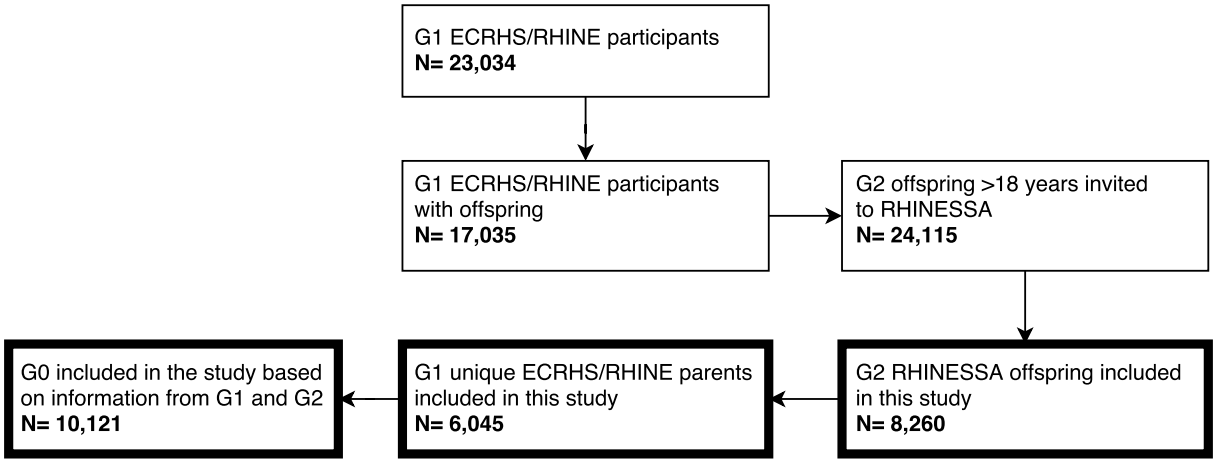
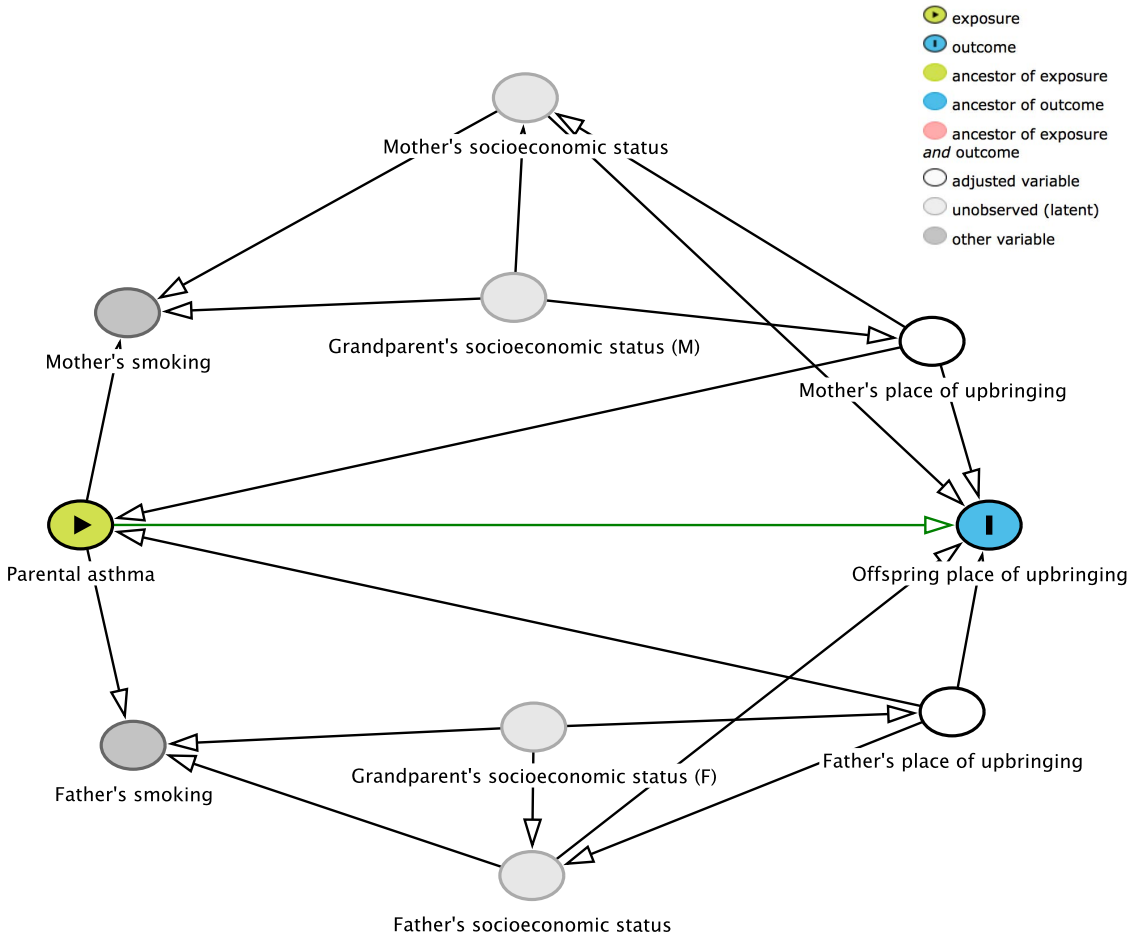


Figure S2:

Directed acyclic graph (DAG) depicting the potential causal network of associations between parental asthma and offspring place of upbringing constructed from current evidence and assumptions.



Paper 4: The Agreement Study

Timm S, Schlünssen V, Benediktsdóttir B, Bertelsen RJ, Bråbäck L, Holm M, Jogi R, Malinovschi A, Svanes C, Frydenberg M: **Offspring reports on parental place of upbringing: is it valid?** *Epidemiology*. 2019 May;30(3):e16-e18. doi: 10.1097/EDE.0000000000000988

Paper 4

Offspring Reports on Parental Place of Upbringing

Is It Valid?

To the Editor:

Exposure and disease across generations gain increasing interest, and information is often reported by family members, particularly offspring reporting about their parents. It is crucial to know the validity of secondhand information, but this is poorly investigated. A study on agreement in reported reproductive outcomes showed that husbands' misclassification of their wives' reproductive history was substantial and undermined the validity of the study.[1] In contrast, two studies on maternal/paternal smoking concluded that offspring's report of mother's smoking prenatally and in childhood are good proxy measures for parent's own report of smoking.[2,3]

Many studies have associated upbringing on a farm with a lower risk of subsequent asthma and hay fever, hypothesizing that the microbial richness on farms might play a key role.[4–6] Farm living in early life seems to influence methylation of asthma-related genes, which suggests that the effect of farm upbringing might potentially be passed on to the next generation.[7] However, it is not known if parental place of upbringing confers an effect on offspring's asthma and hay fever via intergenerational or transgenerational pathways.

The present study includes questionnaire information on parental place of upbringing from two sources: the parents themselves and their offspring. We aimed to investigate the agreement between offspring's and parent's reporting of parental place of upbringing, which to our knowledge has not been investigated before.

We analyzed 4215 parent–offspring pairs. The parents were RHINE III participants (2010) born between 1945 and 1973 from Denmark, Norway, Sweden, Iceland, and Estonia, and their adult offspring (born between 1963 and 1998) who participated in the Respiratory Health In Northern Europe, Switzerland, Spain, and Australia (RHINESSA) study in 2012–2016. The cohorts are described in details elsewhere.[8]

Place of upbringing was based on the question "What term best describes the place you/your mother/your father lived most of the time before the age of 5 years?" with response categories: (1) *farm with livestock*, (2) *farm without livestock*, (3) *village in a rural area*, (4) *small town*, (5) *suburb of city*, and (6) *inner city*. We present to which degree the offspring misclassified their parent on whether or not the parent was brought up on a farm, by combining responses (1) and (2) into "farm," and (3) to (6) into "not farm." A parent was defined as misclassified if the offspring reported the opposite parental place of upbringing to the parent's report. We have used the term "misclassification" although we are aware that it can be questioned whether parental own reports are correct.

Risk of misclassification is presented for all and stratified by parental-reported farm upbringing or not, and by offspring place of upbringing, sex, asthma, and hay fever (the two main outcomes of interest in the RHINESSA study).

Overall, 10% of offspring misclassified their parent's place of upbringing (Figure). However, offspring misclassification was much higher regarding parents who reported being raised on farms (30%) than regarding parents not from farms (5%). Note that, if we were to consider parental farm upbringing as the target, this corresponds to a sensitivity of 70% and a specificity of 95%. In general,

The RHINE/RHINESSA study (Respiratory Health in Northern Europe/Respiratory Health in Northern Europe, Spain, and Australia) was supported by grants from the Faculty of Health, Aarhus University, Denmark (Project No. 240008); the Wood Dust Foundation (Project No. 444508795); the Danish Lung Association; the Swedish Heart and Lung Foundation; the Swedish Association Against Asthma and Allergy; the Swedish Association against Heart and Lung Disease; the Swedish Council for Working Life and Social Research; the Bror Hjerpstedt Foundation; the Vårdal Foundation for Health Care and Allergic Research; the Norwegian Research Council (Grant No. 214123, 230827/F20, 228174, and 135773/330); the Norwegian Asthma and Allergy Association, Helse Vest, Norway (Grant No. 911 631); the Icelandic Research Council; the University of Iceland Research Fund; the Icelandic GP's Research Fund; the Estonian Science Foundation (Grant No. 4350); and the Estonian Research Council (Grant No. PUT562). For further information about funding sources, please consult www.rhinessa.net.

V.S. and C.S. are members of the COST BM1201 network. S.T. received a PhD scholarship from Aarhus University, Denmark. The other authors have no conflicts to report.

SDC Supplemental digital content is available through direct URL citations in the HTML and PDF versions of this article (www.epidem.com).

Availability of data and material: The dataset is held and managed by the Department of Global Public Health and Primary Care, University of Bergen, Bergen, Norway. Data cannot be made freely available as they are subject to secrecy in accordance with the Norwegian Public Access to Information and Secrecy Act, but can be made available to researchers on request (subject to a review of secrecy). Requests for data can be sent to the principal investigator of the Respiratory Health In Northern Europe, Switzerland, Spain, and Australia (RHINESSA): Cecilie Svanes, e-mail: cecilie.svanes@med.uib.no.

ISSN: 1044-3983/2019/303-e16
DOI: 10.1097/EDE.0000000000000988

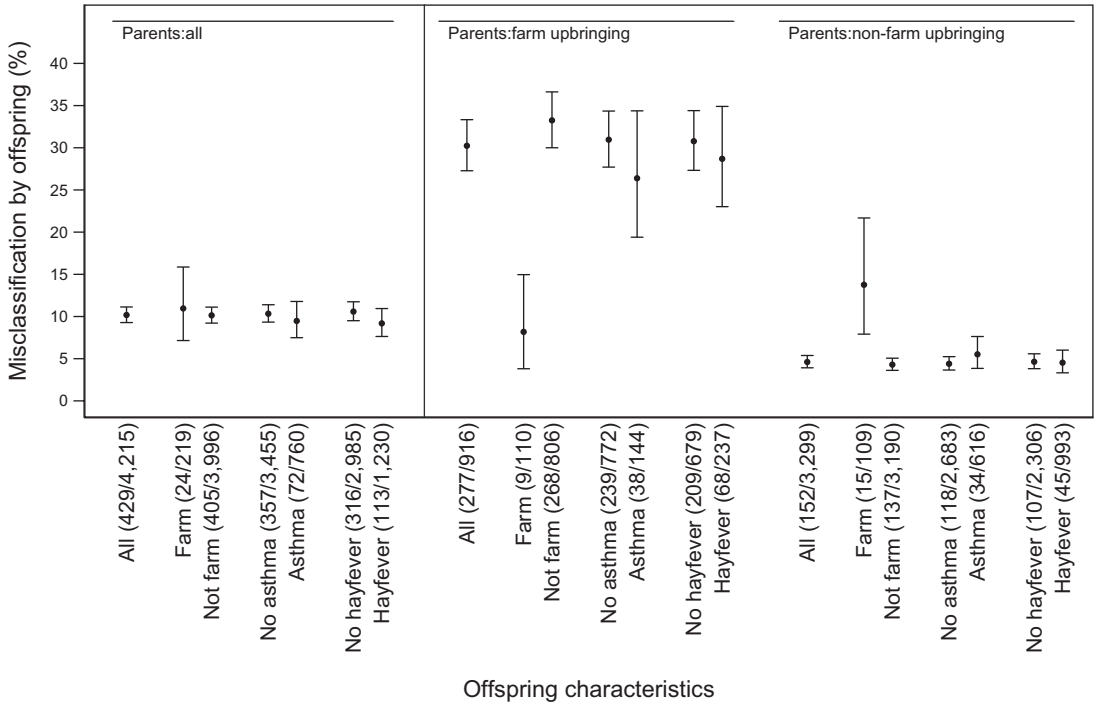


FIGURE. Percentage of offspring misclassifying their parent's upbringing (farm or not), subdivided on parent's report and offspring characteristics. Based on 4,215 parent–offspring pairs (offspring aged >18 when reporting). Numbers in each group are in brackets (misclassified/total) and vertical bars indicate 95 % confidence intervals.

misclassification was not related to offspring asthma or hay fever status neither overall nor for subgroups (eFigure; http://links.lww.com/EDE/B467). However, misclassification was highly dependent on the combination of where a parent and an offspring were raised: the risk of misclassification among nonfarm-raised offspring with farm-raised parents was 33% compared with 8% among farm-raised offspring with farm-raised parents. Likewise, farm-raised offspring with nonfarm-raised parents misclassified 14% compared with 4% among nonfarm-raised offspring with nonfarm-raised parents. Results were similar across age groups, for males and females, and regarding mothers or fathers (data not shown).

Our main findings are that an apparently low rate of misclassification overall was masking patterns of higher misclassification within subgroups, and that offspring misclassification of parental place of upbringing was highly influenced by their own place of upbringing. One might speculate whether the understood definition of a "farm" might differ between generations. Another limitation of our study may be our inability to detect misclassification within the broad nonfarm category.

According to these results, secondhand information should be used with caution, as systematic misclassification may be encased and follow central characteristics of the responder or the concerned family member as seen in this case, where offspring tend to report the same for their parent as for themselves. If these patterns exist in other studies using secondhand information, this will in general challenge the validity of such data sources in epidemiologic research. In future studies, the data presented in this paper may facilitate quantitative bias analyses on comparable variables to look into the magnitude of bias from secondhand misclassification.

Signe Timm
Department of Public Health
Danish Ramazzini Centre
Aarhus University
Aarhus, Denmark
signe.timm@ph.au.dk

Vivi Schlünssen
Department of Public Health
Danish Ramazzini Centre
Aarhus University
Aarhus, Denmark
National Research Centre for Working Environment
Copenhagen, Denmark

Bryndis Benediktsdottir
Faculty of Medicine
University of Iceland
Reykjavík, Iceland
Primary Health Care Center
Gardabaer, Iceland

Randi Jacobsen Bertelsen
Institute of Clinical Science
University of Bergen
Bergen, Norway

Lennart Bråbäck
Department of Public Health and Clinical Medicine
Umeå University
Umeå, Sweden

Mathias Holm
Section of Occupational and Environmental Medicine
Sahlgrenska University Hospital
Gothenburg, Sweden

Rain Jogi
Department of Pulmonology (ARKS)
University of Tartu
Tartu, Estonia

Andrei Malinovschi
Clinical Physiology, Department of Medical Sciences
Uppsala University
Uppsala, Sweden

Cecilie Svanes
Centre for International Health
Department of Global Public Health and Primary Care
University of Bergen
Bergen, Norway
Department of Occupational Medicine
Haukeland University Hospital
Bergen, Norway

Morten Frydenberg
Department of Public Health
Danish Ramazzini Centre
Aarhus University
Aarhus, Denmark

REFERENCES

1. Fikree FF, Gray RH, Shah F. Can men be trusted? A comparison of pregnancy histories reported by husbands and wives. *Am J Epidemiol.* 1993;138:237–242.
2. Pape K, Svanes C, Malinovschi A, et al. Agreement of offspring-reported parental smoking status: the RHINESSA generation study. *BMC Public Health.* 2019;19:94.
3. Simard JF, Rosner BA, Michels KB. Exposure to cigarette smoke in utero: comparison of reports from mother and daughter. *Epidemiology.* 2008;19:628–633.
4. Ege M, Mayer M. Exposure to environmental microorganisms and childhood asthma. *N Engl J Med*. 2011;364:701–709. Available at: http://www.nejm.org/doi/full/10.1056/nejmoa1007302. Accessed 8 December 2013.
5. Von Ehrenstein OS, Von Mutius E, Illi S, Baumann L, Böhm O, von Kries R. Reduced risk of hay fever and asthma among children of farmers. *Clin Exp Allergy.* 2000;30:187–193.
6. Timm S, Frydenberg M, Janson C, et al. The urban-rural gradient in asthma: a population-based study in Northern Europe. *Int J Environ Res Public Health*. 2015;13:1–14.
7. Michel S, Busato F, Genuneit J, et al.; PASTURE Study Group. Farm exposure and time trends in early childhood may influence DNA methylation in genes related to asthma and allergy. *Allergy.* 2013;68:355–364.
8. Kuiper IN, Svanes C, Benediktsdottir B, et al. Agreement in reporting of asthma by parents or offspring - the RHINESSA generation study. *BMC Pulm Med.* 2018;18:122.

Supplementary material, paper 4

eFigure

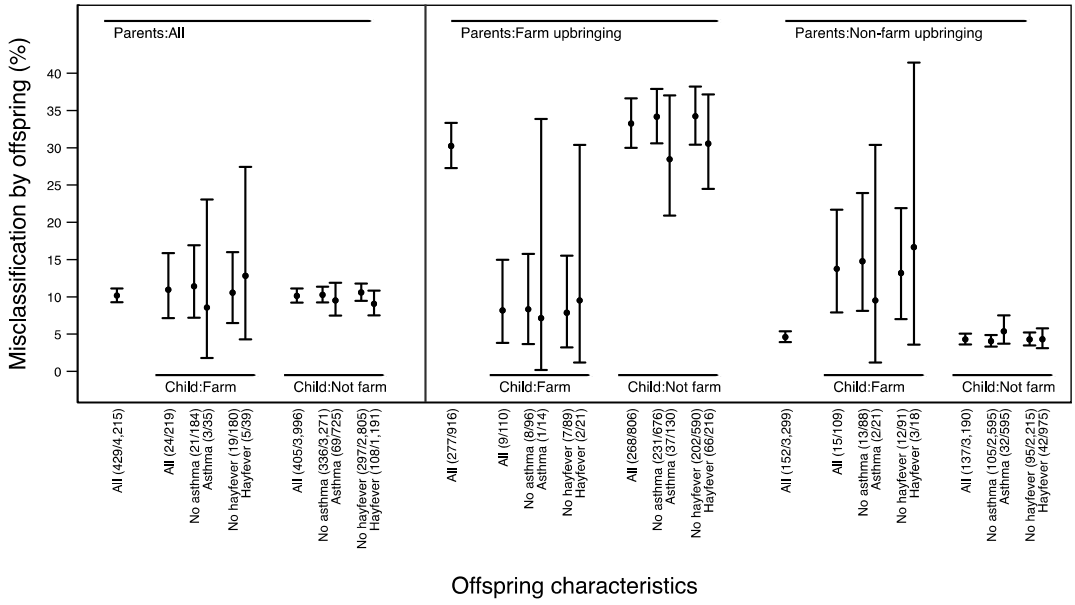


FIGURE. Percentage of offspring misclassifying their parent's upbringing (farm or not), subdivided on parent's report and offspring characteristics. Based on 4,215 parent-offspring pairs (offspring aged >18 when reporting). Numbers in each group are in brackets (misclassified/total) and vertical bars indicate 95 % confidence intervals.