Early Environmental Exposures and Childhood Respiratory Disorders

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Summary

Childhood asthma shows a moderate degree of heritability and uncovering environmental factors influencing asthma development is therefore relevant. Despite decades of epidemiologic research results remain largely inconsistent. A growing amount of research indicates that pregnancy and early childhood might be important windows of exposure. As early childhood wheezing symptoms are often closely linked to lower respiratory tract infections, further insight into common underlying environmental factors influencing both lower respiratory tract infections and asthma is valuable.

This thesis explored four environmental factors that so far have limited or inconsistent findings with regard to development of childhood respiratory disorders: maternal vitamin D status during pregnancy, maternal alcohol intake during pregnancy and lactation, early postnatal growth and the grandmother's smoking when pregnant with the mother. We used data from the Norwegian Mother and Child Cohort study, a large-scale population based pregnancy cohort which recruited pregnant women across Norway between 1999 and 2008, including more than 114,000 children.

Our results indicated that maternal vitamin D status during pregnancy was inversely associated with lower respiratory tract infections during early childhood but not associated with asthma. Furthermore, we saw no association of maternal alcohol intake during pregnancy or lactation with lower respiratory tract infections or asthma. Early postnatal weight increase was positively associated with both lower respiratory tract infections and asthma. In contrast, we saw no association between early postnatal height increase and respiratory disorders. Finally, the grandmother's smoking when pregnant with the mother was positively associated with asthma in the grandchild. This association was seemingly independent of the mother's smoking status.

The results of this thesis provide some additional information regarding early environmental exposures and childhood respiratory disorders. We offer some recommendations for future research that might be helpful in providing further insight into these research questions.

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Abbreviations

ALSPAC	Avon Longitudinal Study of Parents and Children
CI	Confidence Interval
ISAAC	International Study of Asthma and Allergies in Childhood
LRTIs	Lower respiratory tract infections
MoBa	Norwegian Mother and Child Cohort Study
MBRN	Medical Birth Registry of Norway
NorPD	Norwegian Prescription Database
OR	Odds Ratio
RR	Relative Risk
SD	Standard deviation
25(OH)D	25-hydroxyvitamin D

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1. **Introduction**

Asthma is a common chronic disease during childhood, affecting an estimated one out of eight children world-wide [1,2]. People develop asthma at different stages of their lives and some people who exhibit asthma symptoms during early childhood subsequently stop experiencing symptoms [3,4]. Current knowledge therefore indicates that asthma might very well be a spectrum of conditions. Despite a substantial amount of research exploring whether environmental factors might influence asthma development, results remain largely inconsistent [5]. Furthermore, the causes of asthma are likely multifaceted and might also be influenced by complex interactions [5]. Early childhood wheezing is often characterized by transient symptoms due to lower respiratory tract infections (LRTIs) [3,4]. Due to the close link between early life wheezing symptoms and LRTIs, further insight into common underlying risk factors seems relevant.

An increasing amount of research points towards pregnancy and early childhood as sensitive periods for environmental exposures with regard to asthma development [6,7]. Barker was one of the first to propose that the fetus and infant adapt to environmental exposures during critical developmental periods in his developmental plasticity hypothesis [8,9]. As both immune system and lung development occur largely *in utero* and during early childhood, early programming is also a plausible pathway for childhood respiratory disorders [10]. One potential explanation for the associations between environmental exposures with disease development are epigenetic changes, defined as heritable changes in gene expression without changes in DNA sequence. Increasing amounts of research is being conducted into epigenetic mechanisms with regard to asthma development [11-14]. The next few years will therefore likely provide more insight.

The purpose of this thesis was to investigate associations between a selection of early environmental exposures and childhood respiratory disorders which so far had limited or inconsistent findings. Vitamin D might influence both innate and adaptive immune functions [15,16]. Studies of maternal vitamin D status during pregnancy or cord blood vitamin D status show inconsistent results with regard to asthma development [17-20]. We therefore explored whether maternal mid-pregnancy vitamin D status might influence development of LRTIs and/or asthma in the offspring. Animal studies indicate that alcohol exposure during

pregnancy and lactation might influence both lung and immune system development in the offspring [21-23]. Data regarding whether maternal alcohol intake during pregnancy might play a role in development of childhood asthma in the offspring remains scarce [24]. We examined whether maternal alcohol intake during pregnancy or lactation might play a role in development of respiratory disorders. Growth factors also influence lung development [25,26]. Prospective studies of early childhood weight increase show positive [27-31], inverse [32] and no associations [33] with asthma development. Most previous studies report no association between length increase and asthma [27,28,30]. We therefore examined whether early postnatal growth might play a role in development of LRTIs and/or asthma. Prenatal tobacco smoke exposure is one of the most studied risk factors for asthma development [34,35]. Limited human data is available regarding whether prenatal tobacco smoke exposure might also have a trans-generational influence on asthma development [36]. We therefore examined whether the grandmother's smoking when pregnant with the mother might influence asthma in the grandchild.

This thesis used data from the Norwegian Mother and Child Cohort Study (MoBa). MoBa is a large population-based pregnancy cohort that recruited pregnant women in the middle of their pregnancy across Norway. The follow-up continued throughout pregnancy and at regular intervals following birth. The thesis combined information obtained from MoBa with information from national health registries, and biological measurements obtained by using biological samples from the MoBa biobank. The background section of this thesis includes literature available around June 2012 when the thesis was initiated. Literature that became available after this time is included in the discussion.

2. **Background**

2.1 Definition of childhood respiratory disorders

Asthma

Asthma is characterized by acute episodic deterioration, which combines with an underlying chronic airway inflammation and/or structural changes, often resulting in persistent respiratory symptoms and reduced lung function [37]. It has traditionally been considered to be an allergic airway disorder. Even though the role of T-helper-2 inflammation has provided the basis for understanding the association between atopy or IgE and the eosinophilic lung inflammation which is often seen among individuals with asthma, atopy has poor sensitivity and specificity for asthma [38]. The diagnosis of asthma is primarily based on respiratory symptoms and evidence of airway bronchodilator reversibility or airway hyper-responsiveness [37]. Because pre-school aged children have problems performing reproducible lung function tests, an asthma diagnosis in a young child is more often based on the presence of respiratory symptoms and familiar risk factors [39,40]. The most common ways to define childhood asthma in epidemiologic research include parental report through questionnaires, interview with the parents and clinical examination. Of these three methods, parental report though questionnaires are by far the most common [41].

Current information highlights that there might exist different asthma phenotypes. One way of classifying asthma phenotypes in children is based on the age and duration of wheezing symptoms: transient infant wheezing, late onset wheezing and persistent wheezing [3,4]. An estimated three out of four school age children with asthma no longer have symptoms in mid-adulthood. The risk of persistent asthma symptoms from childhood into adulthood increases with severity, sensitization and female sex [3]. The heterogeneity of childhood asthma calls for a better understanding of environmental factors that might be involved.

Lower respiratory tract infections

LRTIs are infections of the respiratory system below the larynx, including bronchitis, bronchiolitis and pneumonia [42]. Bronchitis includes viral or bacterial infections of the larger airways, while pneumonia includes infections of the small bronchioles and alveoli. LRTIs often presents with airway inflammation, increased mucus production, respiratory distress in addition to classical infectious markers. Symptoms include shortness of breath, weakness,

high fever, coughing and fatigue. The respiratory symptoms some people experience during LRTIs, especially young children, often manifests by wheezing symptoms [3,4].

Approximately 30-50% of children who experience recurrent viral induced wheezing go on to develop asthma [40]. Epidemiologic studies report mostly a positive association between LRTIs and asthma [43-45]. Respiratory syncytial virus is the most common agent causing LRTIs among infants and young children [46-48]. Early infant bronchiolitis with respiratory syncytial virus shows a consistent positive association with wheezing symptoms and asthma development [49-51]. Multiple explanations are presented for the positive association between LRTIs and asthma [40]. It has been hypothesized that viral respiratory infections might damage the airways and subsequently initiate asthma [40]. On the other hand, virus-induced wheezing might be a marker for an underlying predisposition to asthma [40]. Another possibility is a combination of the two previously mentioned mechanisms, where viral infections promote asthma in predisposed children when exposed at a critical age [40]. LRTIs are important health problems by themselves. However, the close link between LRTIs and asthma warrants further investigation into potential common environmental factors.

2.2 Distribution of childhood respiratory disorders

Asthma

Due to the heterogeneity of asthma definitions, the estimated disease burden varies greatly. An evaluation of 122 papers examining asthma between 6 and 18 years of age yielded 60 different asthma definitions [41]. The prevalence of current wheezing at 6-7 years from phase three of the International Study of Asthma and Allergies in Childhood (ISAAC) conducted between 2002 and 2003 was 11.5%, ranging from 6.8% to 21.7% , while the prevalence of current wheezing at 13-14 years was 14.1%, ranging from 5.1% to 22% [1]. Males are approximately twice as likely to develop asthma during childhood compared to females [52,53]. This pattern continues until puberty. In adulthood, asthma is more common among females and females are also more likely to experience adult onset asthma [54,55].

In Norway, the best estimated nationwide prevalence of childhood asthma is probably based on the Norwegian Prescription Database (NorPD). Based on the use of asthma medications between 2005 and 2007, the prevalence of asthma among children between 2-5 years of age was 8.5- 9.3%, while the estimated prevalence of asthma among children between 6-12 years

of age was 5.5-6.2% [56]. Similar gender differences in asthma morbidity as what has been described in other populations is seen in the Norwegian population [56].

The time trends in worldwide asthma prevalence could be evaluated by comparing the asthma prevalence in ISAAC phase one (conducted 1993-1995) and ISAAC phase three (conducted 2002-2003). The results of the time trend analysis indicated that most ISAAC centers reported an increase in asthma symptoms between these two study phases [57]. Based on the National Health Interview Survey, the U.S. Center for Disease Control reported a rapid increase in asthma prevalence among children between 1980 and the late 1990s with a subsequent plateau [2]. Even though the previously observed increase in childhood asthma prevalence might be leveling off, especially in the western countries, the disease burden remains at a high level.

Lower respiratory tract infections

The estimated disease burden due to LRTIs among children also varies by the disease classification. For example, hospital discharge registries will only include LRTIs severe enough to warrant hospitalization, whereas most LRTIs are likely diagnosed and treated by primary care physicians if they are seen by physicians at all. Pneumonia affect approximately 3% of infants during the first year of life [58]. There is no clear definition of what "recurrent" LRTIs actually means. Some authors use "recurrent" when more than one LRTI occurs [59]. Other authors define recurrent LRTIs as two episodes within one year or three episodes within any time period [60]. Similar to the gender difference seen for asthma, there is some indication that boys have a higher burden of LRTIs during early childhood [58].

The Isle of Wight birth cohort study (1989–1990) of 1,336 children followed until they were 10 years old reported that the number of children experiencing two or more LRTIs the past 12 months was 7.4% [61]. A cohort study (1999–2001) of children in Germany aged 5–7 years old found that 6.7–8.2% had a history of community-acquired pneumonia , out of which 6.9–8.2% had recurrent community-acquired pneumonia [62]. Finally, data from Toronto's Hospital found that 8% of children admitted because of community-acquired pneumonia had experienced two or more episodes with pneumonia the past year [60]. Data from the Oslo Birth Cohort, a population based cohort study, reported that 21% of children experienced at least one LRTI by 12 months of age [45]. The relatively high number of children experiencing LRTIs further highlights the need to understand contributing environmental factors.

2.3 Genetics of respiratory disorders

There is a clear familiar predisposition in development of asthma. A meta-analysis of 33 epidemiological studies indicated that children of mothers with asthma had a 3 times increased risk of asthma development, while children of fathers with asthma had a 2.4 times increased risk of asthma development [63]. A strong positive association between asthma development among sibling has also been observed [64].

Genome wide association studies have provided further insight into the genetic risk factors for asthma development [65-72]. The most consistently replicated risk locus for asthma development is the 17q12-21 locus (including the ORMDL3 and GSDMB genes). Genetic studies, mostly candidate gene studies, also indicate several genes associated with LRTIs [73-77]. Overall, the estimated heritability of asthma from twin studies is 35-80% [78,79] while the estimated heritability for respiratory syncytial virus, as classified based on the Danish hospital discharge registry, was approximately 16% [80]. Results from genetic studies have thus failed to explain a large proportion of disease variability.

A relatively new field of research is how the genome and the environment might interact through epigenetic mechanisms. A number of studies now indicate that epigenetic mechanisms, such as DNA methylation, histone modification and/or micro RNA expression, might likely play a role in development of asthma [11-14]. The field of research into how epigenetic mechanisms might underlie gene-environment interactions with regard to disease development is rapidly expanding.

Overall, the high disease burden and the relatively moderate heritability of childhood respiratory disorders highlight the need for identifying contributing environmental factors.

2.4 Environmental exposure and respiratory disorders

2.4.1 Pregnancy and early childhood as windows of exposure

Fetal lung development has five overlapping stages that begins at approximately 3 weeks' post-conception and extends at least into the second year of life [81]. Multiple studies indicate that the natural history of respiratory disorders may be traced back to the prenatal and early childhood period [6,7]. Lung function shows a strong degree of tracking from the neonatal period through childhood and up until adulthood [6,82,83]. The fetal immune system is also influenced by maternal characteristics [84,85]. The Forsdahl-Barker hypothesis highlights the importance of early environmental exposure and later disease development. Forsdahl reported in the late 1970s that the living conditions during adolescence influenced death from cardiovascular disease later in life [86]. Barker further underlined after Forsdahl published his results that nutrition during pregnancy and early childhood might influence later disease development [8,9]. Results from the Dutch famine study further showed that prenatal and early childhood exposure to this severe famine during world war 2 was positively associated with obstructive airway disease [87,88]. Pregnancy and early childhood therefore seem to be important windows of exposure with regard to respiratory disorders. Research into the origins of respiratory disorders should therefore consider early life. The most studied prenatal and early childhood environmental factors are summarized in the next two sections (Tables 1 and 2), followed by a more detailed background for the environmental factors studied in this thesis.

2.4.2 Prenatal and perinatal environmental exposures

Low maternal age at delivery [89-92] and low maternal socio-economic status [93-95] are considered risk factors for asthma development as identified in a number of studies. The socio-economic difference in asthma development is likely to differ between countries and has also most likely changed over time.

Repeatedly studied prenatal risk factor candidates for asthma development include maternal overweight/obesity [96-98], smoking [34,35,99], psychosocial stress [100-103], use of antibiotics [104-107], paracetamol use [108], folate intake [109-111], and exposure to ambient air pollution [112-114]. Whether prenatal tobacco smoke exposure might also exert trans-generational effects on development of respiratory disorders is a relatively new research question [36]. A more controversial research

question is whether maternal alcohol intake during pregnancy might constitute a risk factor for asthma development. Only one study has examined this association [24].

Environmental factors which might constitute potential protective factors for asthma include prenatal exposure to a farming environment [115,116], higher maternal antioxidant levels [117,118] and fatty acid composition [119-121]. Furthermore, maternal vitamin D intake during pregnancy is indicated as inversely associated with asthma [122-124]. In contrast, studies of maternal vitamin D status during pregnancy or cord blood vitamin D status show positive [18] and no association with asthma [17,19,20].

The most extensively studied birth outcomes that might increase the risk of asthma include low birth weight (<2500 grams) [125-130] and preterm birth (<37 completed gestational weeks) [131]. Furthermore, poor intrauterine growth is also positively associated with asthma [132,133]. Interest now exists in confirming whether large birth weight [134], late preterm birth and/or low normal gestational age [135,136] might also constitute risk factors for asthma. Finally, mode of delivery might also influence the risk of asthma development, where children delivered by caesarean section have a slightly increased risk [137,138]. Our study confirms that this increased risk is seen among children delivered by acute caesarean section and not among children delivered by elective caesarean section [139,140].

2.4.3 Postnatal environmental exposures

Postnatal environmental exposures might also influence development of asthma. A potential protective factor for asthma development is breastfeeding [141,142]. However, these two conducted meta-analyses report conflicting conclusions. Studies of maternal parity/number of siblings and asthma development are inconsistent, reporting positive [92,143-146], inverse [147-150] and no association [151,152]. In line with these findings, studies of day care attendance and asthma development also show inconsistent findings, with inverse [147,153-155], positive [156,157] and no association with asthma [45,149]. One potential explanation is that maternal parity/number of siblings and day care attendance might constitute risk factors for an early transient viral induced wheezing phenotype while it might be a protective factor for the late onset/persistent more allergy prone wheezing phenotype. Furthermore, meta-analyses of epidemiologic studies show inconclusive findings for an association of early childhood pet – exposure [158,159] and farm related exposures [116,160-166] with childhood asthma.

Exposure to environmental tobacco smoke [34] and indoor mold [167-170] during early childhood might constitute risk factors of asthma. Epidemiologic studies show both positive [112,171-173] and no [174-176] association of ambient air pollution exposure during childhood with asthma development. Research efforts have also attempted to evaluate whether the child's use of antibiotics and/or paracetamol might influence development of asthma. Among studies that were able to evaluate confounding by indication, three studies indicated a positive association [89,177,178] and two studies indicated no association [179,180] between antibiotic use during infancy and asthma development. Furthermore, two studies indicate a positive association between infant paracetamol use and asthma [181,182] while three studies indicate no association [183-185]. Prospective studies of early infant weight increase show positive [27-31], inverse [32] and no associations [33] with asthma development. Most of these studies report no association of early infant height increase with asthma development. In line with the studies suggesting a positive association between early childhood weight increase and asthma, early childhood overweight/obesity is also positively associated with asthma development [186-188].

Table 1 Prenatal and perinatal environmental risk factors for respiratory disorders

Category	Risk factor	Findings and strength of evidence
Parental characteristics	Maternal age at delivery	Most studies indicate that maternal age is inversely association with wheezing symptoms/asthma [89-92].
	Maternal socio-economic status	Majority of studies indicate a socio-economic gradient with regard to asthma development with the lower socio-economic classes having a higher burden of disease [93-95].
Prenatal risk factor	Maternal body mass index	A few studies indicate that maternal overweight/obesity before pregnancy is positively associated with asthma in the offspring [96-98].
	Maternal smoking	Most studies indicate a positive association between maternal smoking during pregnancy and asthma [34,35,99]. The evidence for an association between maternal second hand smoke exposure during pregnancy and asthma development is less clear [99]. One study examined whether prenatal smoke exposure might exert trans-generational effects on development of respiratory disorders [36]. This study reported independent positive associations of the grandmother's smoking when pregnant with the mother and maternal smoking during pregnancy with child asthma development.
	Maternal nutrient status	Maternal antioxidant intake [117,118] and fatty acid status during pregnancy is inversely associated [119-121] with asthma development, while maternal folic acid intake/folic acid status might be positively associated with asthma [109-111]. Studies evaluating dietary patterns during pregnancy and asthma development show no clear associations [189,190]. Maternal vitamin D intake during pregnancy [122-124] is inversely associated with wheezing symptoms/asthma. Studies of maternal vitamin D status during pregnancy or cord blood vitamin D status show positive association [18] and no association with asthma[17,19,20].
	Maternal stress	There is some evidence that exposure to prenatal stress is positively associated with wheezing symptoms and asthma [100-103].
	Maternal use of medications	Three studies report a positive association between maternal use of antibiotics during pregnancy and asthma development in the child [104-107]. A meta-analysis of six studies indicates a positive association between maternal paracetamol use during pregnancy and asthma development [108].
	Maternal exposure to air pollution	There is some indication that prenatal exposure to ambient air pollution might increase is positively associated with asthma [112-114].
	Maternal exposure to farming	There is some indication that prenatal exposure to a farm environment is inversely associated with asthma [115,116].
	Maternal alcohol intake	Only one study examined the association of prenatal alcohol exposure and asthma development, the result of which indicated no association [24]
Perinatal risk factor	Mode of delivery	Two meta-analysis indicate that delivery my caesarean section might increase the risk of asthma development [137,138]. A few studies have been able to indicate that the positive association is seen among children delivered by acute and not elective caesarean section [139,140].
	Birth weight/Small for gestational age	A number of studies indicate an inverse association of birth weight with asthma development [125-130]. Increasing interest exist in whether high birth weight might also be positively associated with asthma development, thereby indicating a non-linear association between birth weight and asthma [134]. Restricted intrauterine growth is also positively associated with asthma [132,133].
	Gestational age	A meta-analyses summarizing epidemiologic evidence indicate that preterm birth is positively associated with asthma development [131]. A positive association has also been observed in original studies published after this meta-analysis[191-193]. Increasing interest now exists in whether late preterm or low normal gestational age might also be associated with asthma [135,136].

Table 2 Early postnatal environmental risk factors for respiratory disorders

Category	Risk factor	Findings and strength of evidence
Postnatal risk factor	Maternal and paternal smoking	There is some evidence that early life exposure to maternal and/or paternal smoking is positively associated with asthma development, especially for early asthma phenotypes [34].
	Breastfeeding	Two meta-analyses summarizing epidemiologic studies indicate that breastfeeding duration is inversely associated with asthma [141,142]. One of these meta-analyses reported that the inverse association was not seen for asthma in children 5 years or over [141].
	Maternal parity/sibling	Epidemiologic studies report positive [92,143-146], inverse [147-150] and no association [151,152] between maternal parity and/or number of siblings with asthma development.
	Pets	Studies provide inconsistent evidence for an association of pet ownership during infancy with asthma development. Meta-analyses of available epidemiologic studies report no association [158] and that the association might be different for cat and dog exposure [159].
	Day care attendance	Studies of the association between early childhood day-care attendance and asthma development report inverse [147,153-155], positive [156,157] and no association [45,149].
	Growth/obesity	Prospective studies of early infant weight increase show positive [27-31], inverse [32] and no associations [33] with asthma development. Most of these studies report no association of early infant height increase with asthma development. Furthermore, studies report a positive association of overweight/obesity with asthma development [186-188].
	Ambient air pollution	Epidemiologic studies show both positive [112,171-173] and no/inconsistent [174-176] association of ambient air pollution exposure during childhood with asthma development.
	Medications	Among studies that were able to evaluate confounding by indication, three studies indicated a positive association [89,177,178] and two studies indicated no association [179,180] of antibiotic use during infancy with asthma development. Of prospective studies able to evaluate important potential sources of confounding two indicate that there might be a positive association between infant paracetamol use and asthma [181,182] while three studies indicate no association [183-185].
	Farming environment	Multiple epidemiologic studies indicate an inverse association between living on a farm, in addition to farm related exposures, and development of asthma[116,160-163]. There are some studies indicating no association [165,166] or even a positive association [164].
	Mold	Meta-analyses of observational studies indicate that the overall evidence points towards a positive association between indoor dampness/mold and a variety of asthma related respiratory outcomes [167-170].

2.5 Background for Research Topics

As indicated in the summary of epidemiologic studies in the previous section, results of prenatal and early childhood environmental factors and childhood asthma show inconsistent results. This thesis aimed at evaluating four environmental factors which so far have limited or inconclusive literature reports: maternal 25-hydroxyvitamin D level during pregnancy, maternal alcohol intake during pregnancy and lactation, early childhood growth and the grandmother's smoking when pregnant with the mother. Figure 1 is an abbreviated graphical depiction of the theoretical framework for the doctoral thesis.

2.5.1 Maternal vitamin d status during pregnancy and respiratory disorders

Vitamin D is a fat-soluble vitamin which is important in many biological processes. The most important source of vitamin D is the endogenous production through skin exposure to ultraviolet rays, as only a few foods have naturally occurring vitamin D [194]. There are two forms of vitamin D, including vitamin D2 and D3 [194]. Vitamin D3 is primarily gained through endogenous production, while both forms are obtained through diet and supplements. After absorption, vitamin D undergoes hydroxylation in the body to enable activation. The first hydroxylation occurs in the liver, producing 25-hydroxyvitamin D (25(OH)D), which is the major circulating metabolite and the most common measure of vitamin D status [194]. The second hydroxylation occurs in the kidneys, which produces 1,25-dihydroxyvitamin D, which is the biologically active form [194].

The role of vitamin D in calcium absorption and maintenance of bone health is well known. Vitamin D also has other important roles in the body, including modulation of cell growth, neuromuscular and immune function, and reduction of inflammation . Many genes encoding proteins that regulate cell proliferation, differentiation and apoptosis are modulated in part by vitamin D [195]. Both lung cells and immune cells have vitamin D receptors and can convert 25(OH)D to 1,25-dihydroxyvitamin D [15,16]. 1,25-dihydroxyvitamin D may therefore influence both innate and adaptive immune system responses with potential consequence for different immune-mediated diseases [15,16].

Maternal 25(OH)D level during pregnancy determines the fetal 25(OH)D level, which subsequently influences the fetal 1,25-dihydroxyvitamin D [196,197]. A biological plausibility

therefore exists for how maternal vitamin D levels during pregnancy may influence the developing fetus. Lower maternal vitamin D status during pregnancy is associated with several adverse pregnancy outcomes and childhood diseases [197,198].

Controversy remains about whether maternal vitamin D status during pregnancy may influence LRTIs and/or asthma in the offspring. A few previous studies evaluated the association between prenatal 25(OH)D levels (measured in either the mother during pregnancy or cord blood) and early childhood LRTIs, mostly indicating an inverse association [17-19,199]. In contrast, studies of prenatal 25(OH)D levels show positive [18] and no association with asthma [17,19,20]. Most previous studies evaluated cord blood 25(OH)D levels or maternal 25(OH)D late in pregnancy [17,18,20,199]. Only one study examined maternal 25(OH)D early in pregnancy [19]. Further insight into whether maternal vitamin D status earlier in pregnancy might influence childhood respiratory disorders is therefore useful.

2.5.2 Maternal alcohol intake and respiratory disorders

Alcohol crosses the placental barrier and passively diffuses into breast milk, with potential consequences for the fetus and breast feeding infant [200,201]. Public health advisories recommend that women who are pregnant or contemplating pregnancy avoid alcohol all together [202]. Similar guidelines have also been issued by the Norwegian Directorate of Health [203]. These recommendations followed the recognition in the 1970s that high alcohol intake during pregnancy causes fetal alcohol syndrome [204,205]. Maternal alcohol consumption during breastfeeding might also conceivably influence the breastfeeding infant [206,207]. Despite the existing public health warnings, alcohol intake among pregnant women remains common [208].

A number of animal studies examined whether prenatal alcohol exposure influenced lung and immune system development. Findings from these animal models indicate that prenatal alcohol exposure might result in smaller, underdeveloped lungs [209], inadequate response to neonatal hypoxic episodes [210,211], impaired alveolar macrophage differentiation and function [212,213], reduced surfactant protein gene expression, increased oxidative stress and altered innate immune responses, leading to increased risk of neonatal respiratory distress syndrome and respiratory infections [21-23]. Animal studies of alcohol exposure during pregnancy or lactation have not addressed specific asthma related phenotypes.

Two human studies suggest that prenatal alcohol exposure might increase the risk of severe infections leading to sepsis during the first few days of life [214,215], but these studies did not isolate LRTIs. Whether prenatal alcohol exposure might influence development of LRTIs in humans therefore remains to be examined. With respect to asthma, only one study examined the association of prenatal alcohol exposure and asthma development, the result of which indicated no association [24]. Whether maternal alcohol intake during lactation might influence LRTIs and/or asthma in the offspring has not been examined.

In order to adequately address this somewhat controversial research question it is important to have a large scale population based study, with comprehensive information concerning socio-economic factors and environmental tobacco smoke exposure, in order to evaluate whether the low-grade alcohol consumption during pregnancy that remains in the population might be a risk factor for LRTIs and/or asthma development.

2.5.3 Postnatal growth and respiratory disorders

A substantial amount of research indicates that birth weight is associated with asthma development [125-130]. Restricted intrauterine growth is also positively associated with asthma [132,133]. A few studies indicate that early childhood body mass index is positively associated with asthma development [186-188]. Despite the fact that we know that birth weight influences the rate of postnatal growth and that postnatal growth likely influences body mass index development, less research exists on the association between early postnatal growth and asthma development. Prospective studies of early childhood weight increase show positive [27-31], inverse [32] and no association [33] with asthma development. Most previous studies report no association between length increase and asthma development [27,28,30]. Previous studies of postnatal growth and wheezing/asthma did not include an evaluation of LRTIs.

Plausible mechanisms underlying an association between early postnatal growth and respiratory disorders include a direct influence of growth on lung development [25,26], shared genetic and epigenetic mechanisms [216], in addition to common environmental influences such as for example infant feeding [141,142]. Lung development is influenced by genes, transcription factors, growth factors and cytokines [25]. Neonatal lung function increases with birth weight

and birth length [217]. Neonatal lung function is also inversely associated with asthma development [6].

In contrast to birth weight and childhood growth trajectories, less research exists on growth during the immediate postnatal period and development of respiratory disorders. This immediate postnatal period is the period of the fastest growth in the entire extra uterine life span and a critical period for lung development [25]. Only one study has previously evaluated peak weight and height velocity, usually obtained within the first month of life, in relation to asthma [27]. These growth parameters seem to be associated with fetal growth [218]. Peak weight velocity is also positively associated with childhood obesity [218]. As both fetal growth and childhood obesity seems to be associated with asthma, further information on the association of peak weight and height velocity with respiratory disorders seems valuable.

2.5.4 Grandmother's smoking and respiratory disorders

Observational studies indicate that parental tobacco smoking is positively associated with asthma development in the offspring. Meta-analyses summarizing the literature find that the most consistent positive association is seen for maternal smoking during pregnancy [34,35]. In concordance with these findings, exposure to maternal smoking during pregnancy is associated with decreased lung function both in childhood and adulthood [219-222].

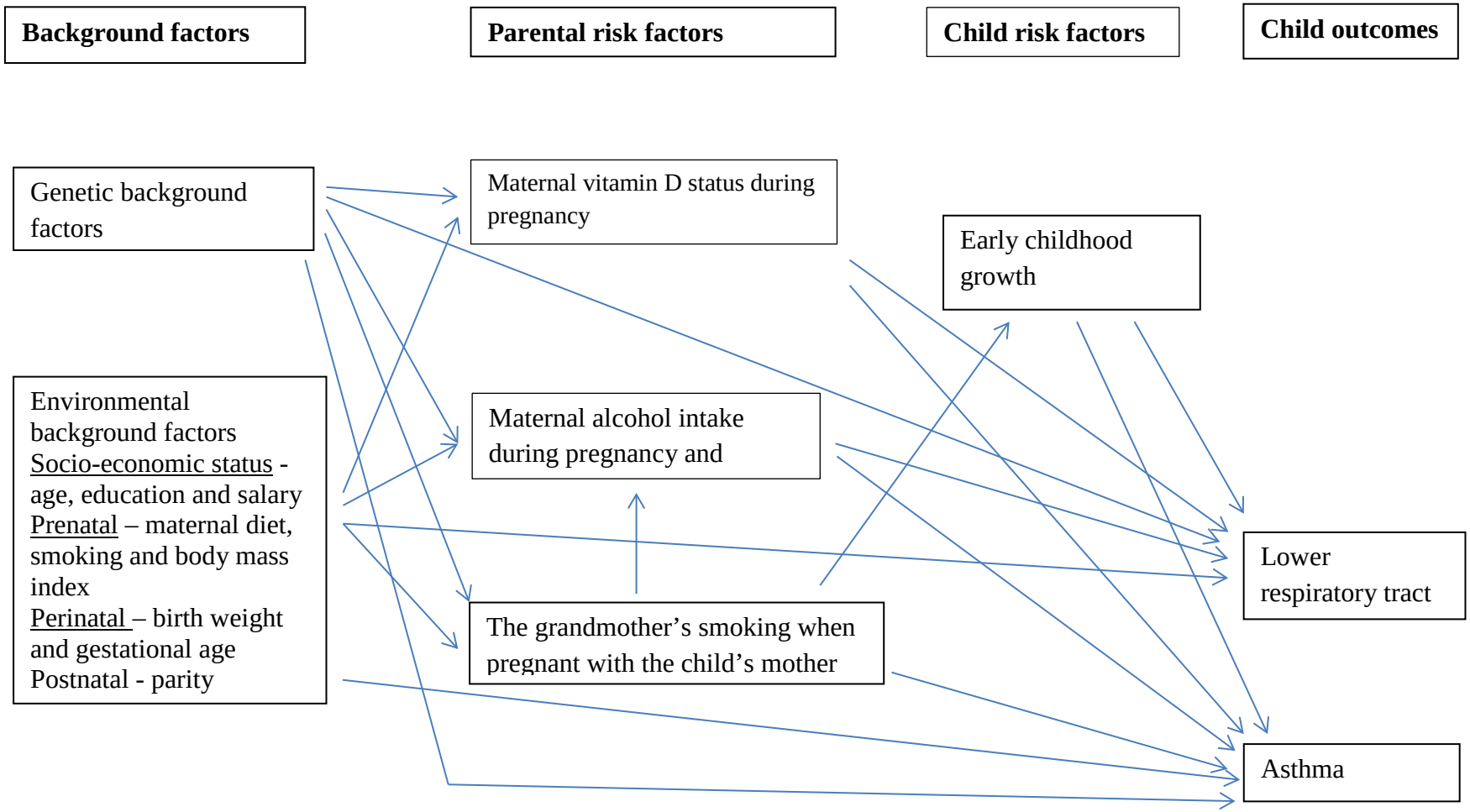
Whether there are trans-generational effects of tobacco smoke exposure is an interesting question. One previous study reported that the grandmother's smoking when pregnant with the mother might influence the grandchild's birth weight [223]. Birth weight is also a predictor for asthma [125,127,129]. Epigenetic mechanisms constitute one underlying mechanisms which might explain a trans-generational effect of tobacco smoke exposure. Differential DNA methylation, an epigenetic alternation, has been reproducibly observed in newborns whose mothers smoked during pregnancy [224]. There is some animal data that epigenetic changes due to prenatal tobacco smoke exposure might be inherited by second generation offspring with potential consequences for an asthma related phenotype [225]. Another explanation for an association between the grandmother's smoking and asthma in the grandchild is through the lower birth weight in mothers born to smokers [226,227] which is positively associated with body mass index during adulthood [228,229]. Maternal pre-pregnancy body mass index is also positively associated with asthma in the offspring [96-98].

Only one American study (the Children's Health Study in California) previously examined whether the grandmother's smoking when pregnant with the mother may influence asthma in the grandchild [36]. This study reported independent positive associations of the grandmother's smoking when pregnant with the mother and maternal smoking during pregnancy with child asthma development. Further information regarding potential trans-generational effects of prenatal tobacco smoke exposure from humans seems beneficial.

2.6 Infrastructure for epidemiologic research

The randomized controlled trial is the gold standard in establishing a causal relationship between an environmental factor and disease development [230]. However, not all environmental factors can be studied using a randomized controlled trial due to financial, ethical or logistical reasons. This thesis examined mostly environmental factors which are not possible to study using randomized controlled trials. Since randomized controlled trials are not always feasible, the prospective cohort study is the best alternative. In order to conduct prospective cohort studies of prenatal and early childhood environmental factors of high quality a proper infrastructure is crucial. Historically, most prospective cohort studies aiming at examination of prenatal and childhood environmental factors for disease development recruited participants at the time of delivery. Any information on the prenatal environment was therefore collected retrospectively. In the field of epidemiology, we know that such a retrospective method of information gathering might reduce accuracy. In order to fully understand whether prenatal environmental factors influence disease development it is an advantage to recruit women as early as possible during pregnancy. An early recruitment also becomes important in order to obtain biological samples at relevant windows of exposure. Modern technology allows biological samples to be stored, so that the samples can be used when relevant research questions appear and financial resources become available. More recent pregnancy cohorts [231-235] learned a lot from and were inspired by older birth cohorts [236-238]. Northern European countries also have comprehensive health registries that are important resources for epidemiologic research. This thesis used the infrastructure available for epidemiologic research in Norway by combining information from a large population based pregnancy cohort , with information from national health registries, in addition to biological samples stored in national bio-banks.

Figure 1 Theoretical framework for the doctoral thesis



3. Purpose and objectives

3.1 Purpose of the doctoral thesis

The purpose of the doctoral thesis was to investigate associations of early environmental exposures in relation to respiratory disorders among children.

3.2 Objectives of the individual scientific papers

Paper I: Prospective study of maternal mid-pregnancy 25-hydroxyvitamin D level and early childhood respiratory disorders.

The primary objective of this study was to examine the associations of maternal mid-pregnancy 25(OH)D level with the frequency of LRTIs by 36 months and with current asthma at 36 months in the child. We chose to study maternal 25(OH)D levels during pregnancy due to the amount of evidence indicating that vitamin D likely modulates both innate and adaptive immune system responses, with potential consequence for immune-mediated diseases. Our hypothesis was that higher maternal 25(OH)D level during pregnancy might reduce the risk of both LRTIs and asthma development in the offspring.

Paper II: Prospective study of maternal alcohol intake during pregnancy or lactation and risk of childhood asthma: the Norwegian Mother and Child Cohort Study.

The primary objective of this study was to examine maternal alcohol intake during pregnancy and lactation in relation to the risk of current asthma at 36 months and recurrent LRTIs by 36 months. Current asthma at 7 years was evaluated as a secondary outcome. The basis for this study includes a number of animal studies pointing towards a potential influence of maternal alcohol consumption during pregnancy and lactation on both lung and immune system development in the offspring. Our hypothesis was that maternal alcohol intake during pregnancy and lactation might constitute a risk factor for both LRTIs and asthma in the offspring.

Paper III: Peak weight and height velocity to age 36 months and asthma development: the Norwegian Mother and Child Cohort Study

The objective of this study was to examine the associations between the child's peak weight and height velocity to age 36 months and asthma development. Recurrent LRTIs was included as a secondary outcome. Both poor intrauterine growth and low birth weight is positively associated with asthma development. We also know that intrauterine growth influences postnatal growth and development. Epidemiologic studies indicate conflicting findings regarding an association between early infant growth and asthma development. Based on the information currently available, our hypothesis was that peak weight velocity, but not peak height velocity, is positively associated with recurrent LRTIs and asthma development.

Paper IV: Grandmother's smoking when pregnant with the mother and asthma in the grandchild: the Norwegian Mother and Child Cohort Study

The objective of this study was to examine the association between the grandmother's smoking when pregnant with the mother and asthma development in the grandchild. We know that maternal smoking during pregnancy most likely is an independent risk factor for asthma development regardless of postnatal environmental tobacco smoke exposure. The basis for this study is the potential trans-generational effects of prenatal tobacco smoke exposure. Any trans-generational associations could reflect a variety of underlying mechanisms, one of which include epigenetic mechanisms. Our hypothesis was that the grandmother's smoking when pregnant with the mother would show a positive association with asthma in the grandchild.

4. Material and methods

4.1 Study population

MoBa is a population based pregnancy cohort administered by the Norwegian Institute of Public Health [233,239]. MoBa recruited pregnant women between 1999 and 2008, at approximately 18 weeks of gestation, from all hospitals and maternity units in Norway with more than 100 births annually. A total of 50 out of the 52 hospitals and maternity units meeting the criterion of 100 annual deliveries participated. The sampling frame for the cohort was the routine ultrasound screening offered to all pregnant women in Norway around 18 gestational weeks through the public health care system. The participating health institutions sent a list with the contact information for all pregnant women registered for the routine ultrasound screening about once a week to the central data collection unit of MoBa. The eligible participants were sent an invitation, a consent form and the first questionnaire at the same time that they received the notification for their ultrasound appointment. The overall participation rate of invited pregnant women was 40.6%. Fathers were also invited to participate. All participants gave a written informed consent. The pregnancy is the unit of observation in the cohort and mothers are allowed to participate with more than one pregnancy. MoBa therefore includes approximately 95,200 mothers and 114,500 children. The last birth in the cohort was in June 2009.

Information is gathered prospectively from participants through self-administered questionnaires. Questionnaires are completed at 18, 22 and 30 gestational weeks and when the child is 6 months, 18 months, 36 months, 5 years, 7 years and 8 years [240]. The response rates for each of the MoBa follow-up questionnaires are included in Table 3. A follow-up questionnaire is also planned when the child is 13 years. Fathers were given a separate questionnaire to complete at the time of recruitment. Biological specimens (both blood samples and urine samples) are collected at the time of the ultrasound screening and at delivery. Blood samples were gathered from the mother at the ultrasound screening and at the time of delivery, from the father at the time of the ultrasound screening, and from the child (cord blood) at the time of delivery. All biological specimens were sent by the participating health institutions to the MoBa biobank at the Norwegian Institute of Public Health where they were processed and stored [241].

The information gathered through MoBa questionnaires was linked to the Medical Birth Registry of Norway (MBRN) and the NorPD using unique national personal identification numbers. The MBRN is a national registry which has registered all abortions occurring after 16 gestational weeks and all live births occurring in Norway since it was established in 1967 [242]. In this registry there is information regarding the mother's health status before and during pregnancy from hospital medical records in addition to information on the mother's health card for pregnant women which is filled out by the primary care physician responsible for the prenatal care. All this information is entered into a standardized form by either a midwife or a physician.

From January 1st 2004, all Norwegian pharmacies were obliged to send electronic data on all dispensed prescriptions to NorPD [243]. The NorPD therefore includes comprehensive information on all medications dispensed in the Norwegian population from pharmacies from 2004 onwards. Medications are classified according to the Anatomical Therapeutic Chemical classification system.

4.2 Sample selection

MoBa regularly releases new versions of the quality assured data files with increasing amounts of follow-up information. This thesis used data from version VII and VIII of the quality assured data files, which were released in 2012 and 2014 respectively. The follow-up information available therefore varies between papers. Children not successfully linked to the MBRN and children from multiple births were excluded from all studies. Paper I and II used version VII of the quality assured data files, which included 90,680 mothers and 108,859 children, out of which 104,444 were singletons with information from MBRN and therefore eligible for analysis (Table 4). Paper III and IV used version VIII of the quality assured data files, which included 95,248 mothers and 114,761 children, out of which 110,291 were singletons with information from MBRN and eligible for analysis (Table 4). The discrepancy of the number of mothers included in these two versions of the MoBa database is due to the Regional Ethics Committee of South/East Norway allowing MoBa to include passive participants, i.e. participants who gave the initial informed consent but never returned a single follow-up questionnaire, in their database.

Table 3 Response rate for individual questionnaires

Questionnaire	Time of data collection	Response rate*	Status
Q1	18 gestational weeks	90.7	Finalized
QF	Paternal questionnaire	90.2	Finalized
Q2	22 gestational weeks	90.4	Finalized
Q3	30 gestational weeks	91.0	Finalized
Q4	6 months	84.5	Finalized
Q5	18 months	72.5	Finalized
Q6	36 months	58.6	Finalized
5YQ	5 years	53.4	Ongoing
7YQ	7 years	53.9	Ongoing
8YQ	8 years	47.2	Ongoing

*Updated per 14.10.14

Table 4 Number of observations included in version VII and version VIII of the MoBa quality assured data files

Number	MoBa version VII	MoBa version VIII
Mothers	90,680	95,248
Pregnancies	106,957	112,773
Children	108,859	114,761
Groups of siblings	16,803	18,022

Paper I

Among children born between July 1st 2002 and December 31st 2003 with information from the questionnaires at 18 and 22 gestational weeks (n=17,005 eligible children) we measured maternal mid-pregnancy 25(OH)D level in a random sample of 2,473 children, out of which 1,248 singletons with information from all follow-up questionnaires up to 36 months were included in an evaluation of frequency of LRTIs by 36 months. From the random sample, 1,246 singletons had complete follow-up information to be included in the analysis of current asthma at 36 months, of which 63 had asthma. Furthermore, we measured maternal 25(OH)D level in an additional 426 singletons with current asthma at 36 months, born between July 1st 2002 and June 30th 2004, with information from all follow-up questionnaires up to 36 months. A total of 489 children with current asthma at 36 months and 1,183 non-asthmatic controls were therefore included in a case-control evaluation.

Paper II

The analysis of current asthma at 36 months included the 49,138 singletons whose mothers completed follow-up questionnaires at 18, 22 and 30 gestational weeks, and when the child was 6 and 36 months of age. The analysis of recurrent LRTIs by 36 months included the

39,791 of the 49,138 children in the analysis of current asthma at 36 months who also had follow-up information from the 18 months questionnaire. Among the 49,138 children in the analysis of current asthma at 36 months, 19,576 children had reached seven years at the time of analysis, for whom we had data from the 7 year questionnaire on 13,253 children.

Paper III

A total of 50,311 children in MoBa version VIII had information from 18 and 30 gestational weeks, in addition to the questionnaires completed when the child was 6, 18 and 36 months, and were subsequently included in the analysis of current asthma at 36 months. Out of these 50,311 children, 47,905 were asked about frequency of LRTIs at 6, 18 and 36 months, and were included in the analysis of recurrent LRTIs by 36 months. Out of the 50,311 children included in the analysis of current asthma at 36 months, 39,211 children had reached age 7 at the time of analysis, while 24,827 had follow-up information from the 7 year questionnaire and subsequently included in the analysis of current asthma at 7 years. There were 488 sibling pairs discordant for asthma at 36 months, 370 sibling pairs discordant for recurrent LRTIs and 127 sibling pairs discordant for current asthma at 7 years who were subsequently included in a sibling comparison.

Paper IV

A total of 53,169 children had follow-up information from the questionnaires completed at 18 gestational weeks, 30 gestational weeks and when the child was 6 and 36 months. These children were included in the analysis of current asthma at 36 months. Of the children in the analysis of current asthma at 36 months, 41,209 had reached 7 years, out of which 25,394 had information from the 7 year questionnaire. These children were included in the analysis of current asthma at 7 years. A total of 45,607 children with information from the MoBa questionnaires at 18 and 30 gestational weeks, in addition to the questionnaire when the child was 6 months, and who had reached 7 years by April 1st 2013 were included when evaluating dispensed asthma medications at 7 years in the NorPD.

4.3 Measures

4.3.1 Outcomes

Lower respiratory tract infections

Mothers were asked about a number of non-chronic diseases when the child was 6, 18 and 36 months of age. The question asked was as follows: has your child experienced the following health condition? LRTIs included maternal report of pneumonia, bronchitis, and/or respiratory syncytial virus when the child was 6 and 18 months, which were all queried in one question, and maternal report of pneumonia and/or bronchitis when the child was 36 months, queried as two separate questions. An open ended question further asked about the number of times the child had experienced the specific condition. Mothers reported the frequency of LRTIs during the following periods of the child's life: up to 6 months, between 6 and 18 months, and between 18 and 36 months, which were subsequently added together to obtain the total number of LRTIs the first 36 months of life. For the first paper in this thesis, we evaluated the number of LRTIs by 36 months of age, categorized as none, one or two, and three or more LRTIs. We subsequently distinguished between the number of LRTIs by 18 months and between 18 and 36 months. Recurrent LRTIs by 36 months was defined as three or more LRTIs, categorized into a binary outcome variable. This was the outcome classification used for papers II and III.

Asthma

When the child was 36 months of age, the mother was asked the question: has your child suffered any long-term illness or health problems since the age of 18 months? One of the diseases queried was asthma, and the response alternatives included "no", "yes, has now" and "yes, had previously". An open ended question further asked about which medications the child had taken the last 12 months. We defined current asthma at 36 months based on maternal "yes, has now" in response to the question about asthma in addition to maternal report that the child had used an inhaled asthma medication in the past 12 months. The inhaled asthma medications evaluated included inhaled glucocorticoids and beta(2)-agonists. These are by far the most common asthma medications used among this young age group.

When the child was 7 years of age, mothers were asked the question: Does the child have or has he/she ever had, any of the following illnesses or health problems? One of the diseases queried was asthma. Sub-questions asked for the individual diseases included "has or has

had" and "symptoms in the past year". In addition, a closed ended question asked about whether the child had used medications for asthma the past 12 months (yes/no). Current asthma at 7 years was classified based the mother responding "yes" to "has or has had" with regard to asthma, "yes" to the child experiencing asthma "symptoms in the past year" in combination with maternal report that the child had used a medication for asthma in the past 12 months. Current asthma at 7 years was included as an outcome from paper II and onwards.

The child's dispensed asthma medications as registered in the NorPD included one dispensed prescription for asthma medication in the past 12 months at 7 years in addition to a second dispensed prescription within 12 months after the first. Medications defined as asthma medications included inhaled short- and long-acting beta(2)-agonists (R03 AC), inhaled corticosteroids (R03 BA), fixed-dose combinations of inhaled beta(2)-agonists and corticosteroids (R03 AK), and leukotriene antagonists (R03 DC). Data linked to the NorPD was only available for paper IV.

4.3.2 Exposures

Maternal vitamin D status during pregnancy

Non-fasting venous blood samples were collected into EDTA tubes from mothers during the routine ultrasound screening at approximately 18 gestational weeks. The samples were centrifuged within 30 minutes after collection and stored overnight at 4 degrees Celsius at the hospital laboratory. The blood samples were subsequently transported by regular mail to the biobank at the Norwegian Institute of Public Health. After arrival, within 1-2 days after blood sample collection, EDTA plasma was aliquoted onto polypropylene microtitre plates, sealed with heat-sealing foil, and stored at -80ºC until analysis [241]. Maternal plasma 25-hydroxyvitamin D2 and D3 levels were measured using liquid chromatography-tandem mass spectrometry at BEVITAL laboratory in Bergen, Norway. The exposure was the sum of 25-hydroxyvitamin D2 and D3, subsequently referred to as 25(OH)D. A 25(OH)D level of <=50 nmol/L has been considered deficient, 51-75 nmol/L insufficient and >75 nmol/L sufficient.

Maternal alcohol intake during pregnancy and lactation

Mothers reported frequency of drinking, average number of drinks per time and frequency of periodic binge drinking (5 or more drinks per sitting) at 18 gestational weeks, 30 gestational weeks and retrospectively when the child was six months. We created exposure variables for

frequency of alcohol intake during first, second and third trimester (never, less than once a month, and once a month or more), average number of drinks per time each trimester (none, < 1, 1-2, 3-4 and 5 + for first trimester; none, <1 and 1+ for second and third trimester) and total number of drinks consumed throughout pregnancy (none, 1-2, 3-10 and 11+). Frequency of periodic binge drinking was only evaluated for first trimester (categorized as never, less than once a month, and once a month or more). Maternal alcohol intake during lactation was categorized according to maternal frequency of drinking during the first three months following delivery (none, < once a month, 1-3 times per week, more than 3 times per week), average number of drinks per time the first three months following delivery (none, <1, 1-2, 3-4 and 5+) and total number of drinks consumed the first three months following delivery **(**none, 1-2, 3-10, 11+). We also examined alcohol intake during and after pregnancy in combination (none, during pregnancy only, after pregnancy only, during and after pregnancy).

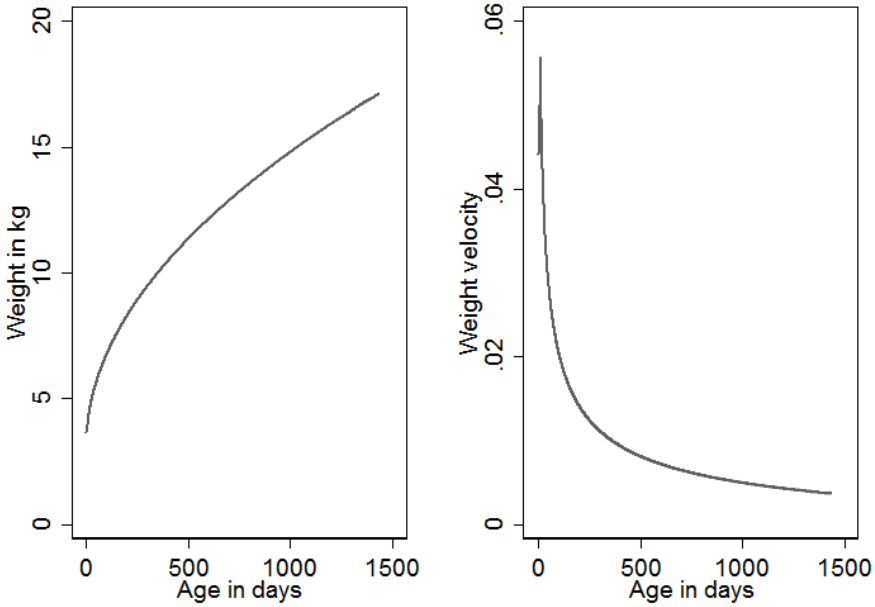
Peak weight and height velocity to age 36 months

The child's weight and length at birth was gathered from the MBRN. Mothers reported anthropometric measures through questionnaires where she was asked to enter the weight, length and age at which the measurements were taken from her child's health report cards, as recorded by public health nurses according to guidelines from the Norwegian Directorate of Health [244]. According to these guidelines, weight and length measures are obtained at age 6 weeks and 3, 6, 8, 12, 15-18, 24 and 36 months. The mother also reported the child's weight and length at 7 years. Growth curves the first 36 months of life were fitted using the modified Reed1 model for each gender separately, a random effects model with 4 parameters that allows for the inclusion of anthropometric measurements at birth: $Y=A + Bt + Cln(t+30) + D/(t+30)$ [245-247]. Of the functional parameters, A is related to the baseline height/weight at birth, B is the linear component for the growth velocity, C is related to the decrease in growth velocity over time, while D reflects the inflection point that allows growth velocity to peak after birth rather than exactly at birth. A growth curve in the form of weight in kg by age in days for the first 36 months of life is depicted in Figure 2 a. The first derivative of the Reed1 model is the growth velocity: $dy/dt=B+C/(t+30) - D/ (t+30)^2$. A growth velocity curve in the form of the rate of change in weight in kg by age in days is depicted in Figure 2 b. The individual growth velocity curves were then used to obtain biologically meaningful parameters, including peak, minimum and mean height and weight velocities. As these three parameters are strongly correlated, we decided a priori that the exposures of interest were the peak weight and height velocity. We used multilevel mixed-effects linear regression to

implement the modified Reed1 model among children who had a minimum of three postnatal anthropometric measurements. We included random effects for all four components in the Reed1 model when calculating the height velocity, whereas we included random effects for all components except for C when computing the weight velocity. The unstructured variance-covariance option was used.

Figure 2 Growth and velocity curves the first 36 months of life

a) Absolute growth curve b) Growth velocity curve



Grand maternal and maternal smoking during pregnancy

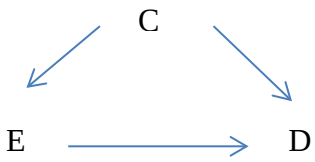
Mothers were asked “did your mother smoke when she was pregnant with you?” around 18 gestational weeks. This was used to classify the maternal grandmother’s smoking when she was pregnant with the mother. The response alternatives were “yes”, “no” and “don’t know”. Mothers further reported their own smoking habits during pregnancy at 18 gestational weeks, 30 gestational weeks and retrospectively when the child was 6 months. To evaluate whether the grandmother’s smoking had an independent association with asthma development in the grandchild from maternal smoking during pregnancy, we classified the children into four mutually exclusive exposure groups: not exposed to smoking (neither the grandmother nor the mother smoked during pregnancy), only exposed to the grandmother’s smoking (the mother did not smoke during pregnancy), only exposed to the mother’s smoking during pregnancy (the grandmother did not smoke) and both the grandmother and the mother smoked.

4.3.3 Covariates

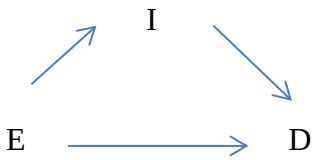
In an epidemiologic analysis covariates can be classified into confounders, effect modifiers and intermediate factors [230]. A confounder is a factor which is associated with the exposure, associated with the outcome and not on the causal pathway between the exposure and the disease Figure 3 a [230]. A covariate is an effect modifier if the magnitude or direction of the association of interest varies within levels of this covariate [230]. Finally, a covariate can be defined as an intermediate factor if it is on the causal pathway between the exposure and the disease of interest Figure 3 b [230].

Figure 3 Directed acyclic graph

a) Confounder



b) Intermediate factor



Whether you should adjust for potential intermediate factors depends on whether you are interested in the total or direct effect of the exposure of interest. The directed acyclic graph is a useful tool to distinguish between these three different categories of covariates and is increasingly being used in epidemiologic research [248]. For the purpose of this thesis, the directed acyclic graph was used as a tool to portray associations. Simplified directed acyclic graphs for each of the four papers are included in appendix I.

There were a number of background factors defined as confounders and included in all the papers written for this thesis. Maternal age, maternal parity and maternal education were included in all papers. From paper two and onwards we also included maternal salary as an additional measure of socio-economic status. Maternal asthma status was included as a confounder in all papers as a measure of genetic predisposition to asthma. Furthermore, maternal pre-pregnancy body mass index, folate intake during pregnancy and smoking during pregnancy were included as measures of health status and health behavior.

There were also additional covariates included in the specific papers. For the first paper, focusing on maternal 25(OH)D level during pregnancy as the exposure of interest, we further included maternal multivitamin use during pregnancy, region of delivery, frequency of leisure time physical activity during pregnancy, gestational age of sample collection and season of sample collection as potential confounders. For the second paper, focusing on maternal alcohol intake during pregnancy and lactation, we further included maternal smoking when the child was 6 or 36 months and breastfeeding the first 6 months as potential confounders. For the third paper examining postnatal growth, we included maternal height, paternal height, paternal body mass index, paternal asthma, child gender, child gestational age and child breast-feeding the first 6 months as potential confounders. The child's body mass index at the time of disease classification (36 months or 7 years respectively) was evaluated as a potential intermediate factor. In the fourth paper, examining the grandmother's smoking as an exposure, we evaluated the grandchild's birth weight as a potential intermediate factor.

4.4 Statistical analyses

The distribution of categorical covariates was examined used chi square tests while the distribution of continuous covariates was examined using student t-tests or analysis of variance. Due to the cohort study design of MoBa, we used log binomial regression models. Log-binomial regression models the logarithm of the probability of disease among the exposed relative to the unexposed, providing relative risks (RR) and 95% confidence intervals (CI). In order to account for sibling present in the analyses, we employed robust cluster variance estimation using the maternal ID as the cluster variable.

As mentioned, we had a case control design in Paper I when examining current asthma at 36 months, due to the sampling strategy of the sub-population of MoBa participants for whom we measured maternal 25(OH)D level during pregnancy, and we therefore used logistic regression to examine this association. The logistic regression models the logarithm of the odds of disease (probability of disease/1- probability of disease) among exposed relative to the unexposed, reporting odds ratios (OR) and 95% CI.

We used multivariable regression analyses to adjust for the covariates described in the previous section, calculating adjusted RR (adj. RR) and adjusted OR (adj. OR). The statistical

significance level was 5% for all tests and comparisons. The statistical analyses were conducted in STATA version 12 and STATA version 13 (Statacorp, Texas).

Paper I

Maternal 25(OH) D level was scaled to reflect the associations per 20 nmol/L increase, representing approximately one standard deviation (SD), and modelled continuously. The association between maternal 25(OH)D level and frequency of LRTIs by 36 months was calculated using log binomial regression. Children with 3 or more LRTIs, and children with 1-2 LRTIs, were compared to children with none. Frequency of LRTIs by 36 months was subsequently separated into frequency of LRTIs by 18 months and frequency of LRTIs between 18 and 36 months. The association between a 20 nmol/L increase in maternal 25(OH)D level and current asthma at 36 months was calculated using logistic regression. Multivariable regression analyses adjusted for all potential confounders mentioned. Sensitivity analysis excluded the asthma cases born in 2004 due the differences in time period when the children were born between the random sample and the extra asthma cases.

Paper II

First, we examined the associations of maternal alcohol intake during pregnancy with current asthma at 36 months, recurrent LRTIs by 36 months and current asthma at 7 years. Second, we evaluated associations of maternal alcohol intake the first three months following delivery among children breastfed the first three months of life. Log binomial regression models compared children of drinkers to those of non-drinkers. Multivariable regression analyses adjusted for all potential confounders. Sensitivity analyses excluded mothers who did not drink alcohol the last three months before pregnancy to evaluate the potential influence of an abstainer effect. We examined effect modification by maternal smoking and preterm delivery on the associations by including product terms in the multivariable models.

Paper III

We examined the associations of one SD increase in peak weight and height velocity with child respiratory disorders. Multivariable regression analyses adjusted for all potential confounders previously mentioned. The child's body mass index at the time of disease classification (36 months or 7 years respectively) was adjusted for in a second multivariable model as a potential intermediate factor. We further evaluated potential effect modification of preterm birth and intrauterine growth on the association of peak weight and height velocity

with childhood respiratory disorders by including product terms in the multivariable models. Intrauterine growth was classified as the birth weight being small (< 10th percentile), normal (10th-90th percentile) and large (> 90th percentile) for gestational age based on the gender specific distributions. This paper also included a sibling pair analysis comparing siblings who were discordant for the outcomes of interest using conditional logistic regression [249].

Paper IV

This paper examined the association of the grandmother's smoking when pregnant with the mother and asthma in the grandchild. In the first multivariable regression model we adjusted for all the potential confounding factors specified. A second multivariable model further adjusted for the mother's smoking status. We tested for multiplicative interaction of the mother's smoking when pregnant with the child by including product terms in the multivariable regression analysis. Furthermore, the grandmother's and the mother's smoking was evaluated in combination, classified into four mutually exclusive exposure categories. Finally, we examined the influence of adjustment for the child's birth weight as a potential intermediate factor.

Missing covariate information

Missing covariate information is a challenge in all epidemiologic research. There are different ways of dealing with missing data. One of the more sophisticated methods currently available is multiple imputation [250,251]. This method is valid if the missing covariate information has a pattern of missing completely at random or missing at random. This assumption entails that the missing covariate information is not influenced by any unmeasured characteristics. The amount of missing covariate information on individual covariates was generally low for all analyses in this thesis (<2%). In the multivariable analysis, approximately 10-15% of individuals had missing information on one or more covariates. For all papers included in this thesis, missing covariate information was imputed using multiple imputation by chained equations [250,251]. The imputation models included all covariates included in the analytical model.

Selection bias analyses

Prospective cohort studies are prone to selection bias due to loss to follow-up. A common approach is to simply conduct a lost to follow-up analysis comparing the distribution of characteristics among individuals included and not included in the analysis due to loss to

follow-up. Comprehensive lost to follow-up analysis for papers II, III and IV are included in appendix II. These analyses compared eligible individuals with information from the first follow-up questionnaires to those included in the respective analyses for each paper. There are also more sophisticated approaches which allow you to examine whether the loss to follow-up actually influences the associations of interest. To explore the possibility of selection bias due to participating mothers not responding to follow-up questionnaires, we used inverse probability weighting [252,253]. Weights were created as the probability of having the necessary follow-up information based on a number of baseline characteristics among all eligible study participants. This is conducted using a logistic regression model where you aim at including all baseline characteristics which might contribute in describing the pattern of loss to follow-up. The weighted regression analyses therefore allow you to weight the included study population to become more representative of all the eligible study participants.

4.5 Ethical and legal aspects

Both the mothers and the fathers participating in MoBa signed a written informed consent. The mothers also consented on behalf of their children. The MoBa participants agreed to provide information through a "broad-consent". A "broad consent" is often used in prospective cohort studies without predefined research aims as the questionnaire data and biological materials collected might be used to address a number of research questions. Relevant research questions might also first be identified a number of years after the questionnaire data and biological materials were collected. The invitation letter sent to all eligible study participants in MoBa described the main research aim and the purpose of the pregnancy cohort, in addition to the types of data that will be used in MoBa, such as information collected through questionnaires, donation of biological samples, and linkage to national health registries. Participants were given the option to reserve themselves from certain aspects of the data collection. The initial consent form specified that participants can withdraw from the study at any time, that all research projects based on data collected require permission from regional committees for medical and health research ethics, that all MoBa data will be de-identified before the use in research and that the child will be given his/her own informed consent at the age of 18 years. Participants are also informed that no individual level data, e.g. disease information, will be given back to participants.

In prospective cohorts with a broad consent it becomes important to keep all the participants informed regarding the research being conducted on the data collected from them. In MoBa, regular information is provided to participants through a yearly newsletter and through the Norwegian Institute of Public Health's website [254].

The Norwegian Data Inspectorate has approved the ongoing data collection in MoBa (01/4325-69/HTL). The individual studies included in this thesis were also approved by the Regional Ethics Committee for Medical and Health Research of South East Norway (2011/2313b). The Norwegian Data Inspectorate has further approved the linkage between MoBa and the NorPD (08/00854-2/IUR).

5. Results

5.1 Paper I

Magnus MC, Stene LC, Håberg SE, Nafstad P, Stigum H, London SJ, Nystad W. Prospective study of maternal mid-pregnancy 25-hydroxyvitamin D level and early childhood respiratory disorders.

The median gestational week of sample collection was 18 weeks (range 9, 35) and the mean 25(OH)D level was 73.7 nmol/L (SD 23.7). A total of 16.8% of mothers had 25(OH)D levels ≤ 50 nmol/L and 34.0% had 25(OH)D levels between 51 nmol/L and 75 nmol/L.

A total of 19.5% of children experienced one or two LRTIs while 5.7% experienced three or more LRTIs by 36 months. Maternal mid-pregnancy 25(OH)D level was not associated with one or two LRTIs vs. none the first 36 months of life, adj.RR 0.98 [95% CI 0.87, 1.12]. In contrast, higher maternal mid-pregnancy 25(OH)D level was associated with a modest reduced risk of three or more LRTIs vs. none by 36 months, adj.RR 0.74 [95% CI 0.58, 0.93] per 20 nmol/L increase. Multivariable adjustment caused only modest changes of the unadjusted associations. The associations of maternal mid-pregnancy 25(OH)D level with the risk of three or more LRTIs vs. none by 18 months and with the risk of three or more LRTIs vs. none between 18 and 36 months were similar.

Among children from the random sample, 5.1% had current asthma at 36 months. Maternal mid-pregnancy 25(OH)D level was not significantly associated with asthma, adj.OR 0.91 [95% CI 0.81, 1.02] per 20 nmol/L increase. Adjusting for season of sample collection attenuated the observed association towards the null. The sensitivity analyses excluding the children born in 2004 yielded a similar association.

5.2 Paper II

Magnus MC, DeRoo LA, Håberg SE, Magnus P, Nafstad P, Nystad W, London SJ. Prospective study of maternal alcohol intake during pregnancy or lactation and risk of childhood asthma: the Norwegian Mother and Child Cohort Study.

A total of 31.8% of mothers consumed alcohol during the first trimester, 9.7% during second trimester, and 15.6% during third trimester. Among mothers who consumed alcohol during the first trimester, 68.9% consumed alcohol less than once a month and 80.8% consumed an average of 2 or fewer drinks per time.

Current asthma at 36 months was reported for 5.8% of children. Examining the frequency of drinking each trimester, children of mothers who drank less than once per month and children of mothers who drank once per month or more had lower risk of asthma relative to nondrinkers. For average number of drinks per time during the first trimester, the RR of asthma was reduced in the 2 lowest consumption categories (<1 drink, adj.RR 0.90 [95% CI: 0.81, 1.00], 1 to 2 drinks adj.RR 0.82 [95% CI: 0.71, 0.94]), but not in the 2 higher categories (3 to 4 drinks adj.RR 1.13 [95% CI: 0.93, 1.36], 5+ drinks adj.RR 0.98 [95% CI:0.78, 1.22]). Frequency of periodic binge drinking during the first trimester was not associated with asthma at 36 months.

Recurrent LRTIs by 36 months were reported for 4.4% of children. Most associations of maternal alcohol intake during pregnancy with recurrent LRTIs were ~1.0, with sporadic modest increases in risk, but without any indication of a dose–response. Current asthma at 7 years was reported for 5.2% of children. The observed associations of maternal alcohol intake during pregnancy with current asthma at 7 years were similar to what was seen for current asthma at 36 months but not statistically significant.

Among children who received breast milk as the only source of milk throughout the first 3 months of life, maternal alcohol intake the first 3 months following delivery was not associated with asthma at 36 months, recurrent LRTIs by 36 months or asthma at 7 years.

Sensitivity analyses limited to children of mothers who had consumed alcohol the last 3 months before pregnancy did not change the results. There was no indication of an interaction

by maternal smoking on the associations of maternal alcohol intake during pregnancy or lactation with any of the respiratory outcomes of interest (p-values for interaction >0.1). There was no indication of an interaction by preterm delivery on the association of maternal alcohol intake during lactation with any of the respiratory outcomes of interest (p-value for interaction >0.1).

5.3 Paper III

Magnus MC, Stigum H, Håberg SE, Nafstad P, London SJ, Nystad, W. Peak weight and height velocity to age 36 months and asthma development: the Norwegian Mother and Child Cohort Study.

The mean peak weight velocity was 15.1 kg per year (SD 6.4 kg). The mean age at peak weight velocity was 14 days, where approximately 30% had peak weight velocity after birth. The mean peak height velocity was 62.0 cm per year (SD 15.2). The mean age at peak height velocity was 8 days, where approximately 15% had peak height velocity after birth.

Current asthma at 36 months was reported for 5.7% of children, while 4.3% of children experienced recurrent LRTIs by 36 months and 5.1% had current asthma at 7 years. Peak weight velocity was positively associated with current asthma at 36 months [adj. RR 1.22 (95%CI: 1.18, 1.26) per SD increase], recurrent LRTIs by 36 months [adj.RR 1.14 (1.10, 1.19) per SD increase] and current asthma at 7 years [adj.RR 1.13 (95%CI: 1.07, 1.19) per SD increase]. No association was seen between peak height velocity and any of the respiratory disorders of interest. The associations did not change after further adjustment for the child's body mass index at disease classification. There was no evidence of effect modification by preterm birth or intrauterine growth on any of the associations evaluated.

The positive association of peak weight velocity and asthma at 36 months remained in the sibling pair analysis. This was not seen for recurrent LRTIs by 36 months or asthma at 7 years.

5.4 Paper IV

Magnus MC, Håberg SE, Karlstad, Ø, Nafstad P, London SJ, Nystad W. Grandmother's smoking when pregnant with the mother and asthma in the grandchild: the Norwegian Mother and Child Cohort Study.

A total of 23.5% of mothers reported that their mother smoked when pregnant with them, 66.9% reported that their mother did not smoke when pregnant with them, while 9.6% of mothers reported "don't know". A total of 5.7% of children had current asthma at 36 months, 5.1% of children had current asthma at 7 years while 4.8% had dispensed asthma medications at 7 years in the NorPD.

The grandmother's smoking was positively associated with asthma at 36 months, adj. RR 1.15 (95% CI: 1.06, 1.24), asthma at 7 years, adj.RR 1.21 (95% CI: 1.07, 1.37) and dispensed asthma medications to the grandchild at 7 years (NorPD), adj.RR 1.15 (95% CI: 1.04, 1.26). The associations did not change after further adjustment for the mother's smoking status during pregnancy or the child's birth weight. There was no indication that this positive association was impacted by the mother's smoking status when pregnant with the child (p-value for multiplicative interaction >0.1).

We also evaluated the grandmother's smoking when pregnant with the mother and maternal smoking when pregnant with the child in combination. For all three asthma outcomes, exposure to the grandmother's smoking alone (i.e. no exposure to maternal smoking), or exposure to both the grandmother's smoking and the mother's smoking showed a tendency for a positive association. Notably, there was no independent statistically significant association of exposure to maternal smoking during pregnancy alone (i.e. no exposure to the grandmother's smoking) and asthma development.

6. Discussion

In the following, the results of the individual papers will be discussed in relation to the current literature followed by an in depth assessment of the methodological considerations. Finally, the existing public health recommendations and future research directions are addressed.

6.1 Summary and interpretation of main findings

Higher maternal mid-pregnancy 25(OH)D level was associated with a modestly reduced risk of recurrent LRTIs by 36 months while it was not associated with current asthma at 36 months. In line with our findings, four other studies found no statistically significant association between prenatal 25(OH)D levels and asthma [17,19,20], while three studies indicated an inverse association with LRTIs [17,19,199]. Since the work of this thesis was initiated, one additional study has reported no association between prenatal 25(OH)D levels with asthma [255] while another has reported an inverse association with LRTIs [256]. Our study contributed information that the previously observed inverse association of prenatal 25(OH)D levels with LRTIs may extend beyond the first 12 months of life. Our results partially support our predefined hypothesis of an inverse association between maternal 25(OH)D level and childhood respiratory disorders.

There are several potential mechanisms that might underlie the observed inverse association between maternal 25(OH)D level during pregnancy and childhood LRTIs in the offspring. Higher 1,25-dihydroxyvitamin D increases antimicrobial peptides, inhibits dendritic cell maturation, and increases Treg cells, while its influence on the Th1/Th2 balance remains unclear [15,16]. The fact that vitamin D seemingly has a more clear influence on the innate versus the adaptive immune system seems to be in line with our observed findings and those of others. Vitamin D levels also seem to be regulated by epigenetic mechanisms [257]. Whether vitamin D might also directly result in epigenetic alterations has been less studied. One animal study reported that vitamin D deficiency *in utero* resulted in changes in invariant natural killer T cells in the offspring, hypothesized to be due to epigenetic mechanisms [258]. Since we did not measure the child's vitamin D status, we could not distinguish between the relative influence of the mother's vitamin D status during pregnancy and the child's vitamin D status

during infancy/early childhood. Maternal 25(OH)D level during pregnancy may therefore simply be an indirect marker of the child's own early vitamin D status.

A few important questions therefore remain to obtain further insight into whether the observed inverse association between maternal vitamin D status during pregnancy and LRTIs in the offspring might be causal. This includes distinguishing between the relative importance of the prenatal versus early childhood vitamin D status. It also seems necessary to evaluate whether the association persists when accounting for other micronutrients. Evaluating single micronutrients in epidemiologic studies does partly disregard the interrelationship between micronutrients.

The low levels of alcohol exposure during pregnancy and lactation observed in the MoBa cohort were not associated with increased risk of LRTIs or asthma. The slight inverse associations of infrequent and low-dose prenatal alcohol exposure with asthma is likely not causal. Our findings did not support our hypothesis of a positive association between maternal alcohol intake and respiratory disorders in the offspring.

Our results are in concordance with the Danish study that was available at the time that this thesis was initiated reporting no association between prenatal alcohol exposure and asthma [24]. Since the work on this thesis was initiated, one additional Danish study has provided evidence of no association [259] while a study from the Avon Longitudinal Study of Parents and Children (ALSPAC) reported a modest inverse association similar to what was observed in our study [260]. The ALSPAC study was further able to provide substantiation to our claim that the modest inverse association of infrequent and low-dose prenatal alcohol exposure with asthma is most likely not causal by exploring a Mendelian randomization approach [260]. Our study contributed with further information that maternal alcohol intake during lactation showed no association with childhood asthma. Despite the two studies reporting a positive association between prenatal alcohol exposure and severe infections leading to sepsis [214,215], we did not find consistent evidence that prenatal alcohol exposure was associated with LRTIs. This might be because of the modest prenatal alcohol exposure within the MoBa cohort or because the increased risk of infections related to prenatal alcohol exposure might be transient [22,261].

Higher peak weight velocity, achieved during the immediate postnatal period, increased the risk of both LRTIs and asthma. Peak height velocity was not associated with any of the

respiratory disorders. Interestingly, the positive association between peak weight velocity and child respiratory disorders was not explained by intrauterine growth or the child's body mass index development, both previously associated with peak weight velocity [218]. Our results are in line with previous studies indicating a positive association between rapid early weight gain and asthma development [27-31], one of which examined peak weight velocity [27]. At the time that we were finishing our paper, a meta-analysis of multiple European birth cohorts reported a positive association between absolute weight gain between birth and 12 months and asthma development[262]. Overall, our findings supported our hypothesis of a positive association between peak weight velocity and respiratory disorders.

Plausible mechanisms underlying an association between early childhood growth and respiratory disorders include growth factors directly influencing lung development [25,26], shared genetic and epigenetic mechanisms [216,263], in addition to common environmental influences [141,142,264]. The association between the 17q12-21 loci and asthma development seems to be differential by fetal and infant smoke exposure [265]. In the MoBa cohort, both prenatal smoke exposure and birth weight is associated with cord blood DNA methylation [224,263] . Prenatal smoke exposure is a common risk factor for low birth weight, which influences postnatal growth, in addition to asthma [34,35,226]. This link could therefore plausibly underlie the observed association. A variety of environmental factors might also influence early postnatal growth and development of respiratory disorders thereby underlying the observed positive association. One example is maternal polychlorinated biphenyl level during pregnancy, which has been associated with both birth weight and asthma development [266,267].

There are knowledge gaps that still need to be filled regarding the observed positive association between early childhood weight increase with respiratory disorders. Since our study indicated that this positive association was seemingly independent of the intrauterine growth and of the child's body mass index development, we still remain largely ignorant regarding what underlying biological mechanisms this associations reflects. The observed association between early weight increase and asthma development therefore warrants further studies from basic research looking into potential underlying mechanisms.

The grandmother's smoking when pregnant with the mother increased the risk of asthma in the grandchild independent of the mother's smoking status. Given limited information on the

grandmother's socio-economic status, asthma status and other factors, unmeasured confounding may be present. Our findings supported the Children's Health study in California indicating a positive association between the grandmother's smoking when pregnant with the mother and asthma in the grandchild [36] During the time that this thesis was conducted, ALSPAC published a study indicating no association between the grandmother's smoking when pregnant with the mother and asthma in the grandchild [268]. The ALSPAC study did find a gender-specific association between the grandmother's smoking when pregnant with the father and asthma in the female grandchild [268]. Our findings supported our predefined hypothesis of a positive association between the grandmother's smoking when pregnant with the mother and asthma in the grandchild.

Results from one human study support a link between prenatal tobacco smoke exposure, DNA methylation changes and asthma related lung function [269]. Furthermore, there is some animal data that epigenetic changes due to prenatal tobacco smoke exposure might be inherited by second generation offspring with potential consequences for an asthma related phenotype [225]. In MoBa, it has previously been shown that maternal smoking during pregnancy influenced cord blood DNA methylation in the offspring [224]. They did not find that the grandmother's smoking when pregnant with the mother was associated with DNA methylation in the grandchild at loci previously associated with the mother's smoking during pregnancy [270]. However, this does not exclude the possibility that the grandmother's smoking is associated with the grandchild's DNA methylation in other areas of the genome. A total of three studies have now reported that the grandmother's smoking when pregnant with the mother is associated with the grandchild's birth weight [223,271,272], two of which were published while this thesis was being conducted. The grandchild's birth weight did not explain the observed positive association of the grandmother's smoking and asthma in the grandchild in our study.

We found no independent association of maternal smoking during pregnancy with asthma in the offspring. This was somewhat surprising based on the previously reported positive association between maternal smoking during pregnancy and LRTIs in the offspring from the MoBa cohort [273]. Our finding might partly be explained by the lower proportion of mothers who smoked during pregnancy in MoBa as compared to other cohorts which have reported a positive association between maternal smoking during pregnancy and asthma development [34,35]. The Norwegian government banned smoking from all public buildings in 2004. Furthermore, the Norwegian Health Directorate has conducted multiple health

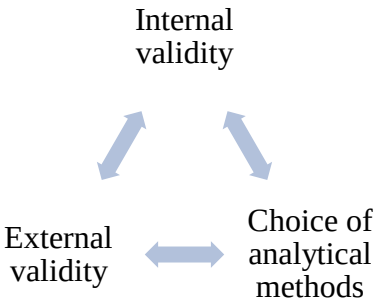
awareness campaigns during the MoBa enrollment period targeted at getting pregnant mothers to stop smoking. The proportion of women who smoke during pregnancy has gone down during the MoBa enrollment period, similar to what has happened in the general Norwegian population.

In order to disentangle whether the observed positive association between the grandmother's smoking and asthma in the grandchild is causal further studies are needed. First and foremost, future studies need more comprehensive information regarding the grandmother's characteristics. In addition, only a limited amount of human data is currently available regarding whether prenatal tobacco smoke exposure might conceivably exert a trans-generational influence on epigenetic mechanisms. We propose this as a potential underlying mechanism of our observed association but more data from humans is necessary to further substantiate this assertion.

6.2 Methodological Considerations

The main strength of the studies included in this thesis is the size and prospective data collection of the MoBa cohort. In addition, we were able to use information from two national health registries with comprehensive health information. The extensive amount of information available enabled us to gain new insight into the research hypotheses and to address some gaps in knowledge highlighted by previous studies. There are multiple methodological considerations which must be discussed with regard to epidemiologic studies. Here we will discuss three important methodological considerations, including the choice of analytical methods, internal validity and external validity as illustrated by Figure 4. All of these methodological considerations have potential consequences for the interpretation of our results.

Figure 4 Methodological considerations in epidemiologic studies



6.2.1 Choice of analytical methods

Regression analysis

This thesis used multivariable regression models to obtain relevant measures of association after adjusting for potential confounding. We primarily used log linear regression analysis to obtain RRs. Since we used current asthma as the case definition throughout this thesis, the asthma cases are prevalent cases. The measures of association obtained by using log linear models could therefore be interpreted as prevalence ratios. One might argue that it would have been better to report ORs, which could have been interpreted as a prevalence OR. When the disease of interest is relatively rare, the measures of association obtained by evaluating RRs or ORs should yield similar results [230]. This was verified by running logistic regression models for the main associations evaluated in this thesis.

There are assumptions related to the regression models used that should be addressed. One assumption is that the association between a continuous exposure and the binary outcome follows a linear trend [230]. Paper I and paper III in this thesis evaluated continuous exposures (maternal 25(OH)D level and peak weight and height velocity). We evaluated deviations from a linear trend of the association by fractional polynomial plots and by including polynomials (second order terms) in the multivariable regression models. Neither of these methods pointed to any non-linear associations. Another assumption for the regression models used in this thesis is the independence of the observations/study subjects [230]. The MoBa cohort includes a number of siblings and we accounted for this by using cluster variance estimations.

Evaluation of effect modification

Another assumption in regression modelling is the absence of effect modification. It is important to emphasize that effect modification is scale dependent, i.e. whether you are analyzing your data on an absolute or a relative scale [230]. We are therefore clear that what we are evaluating in this thesis is the presence or absence of effect modification on the multiplicative/relative scale. The approach used in this thesis was not to explore all two or three way effect modifications, but rather to evaluate a limited number of pre-defined potential effect modifiers based on knowledge of the literature. The primary method used to evaluate effect modification was stratified analysis and to test the significance of product terms in the multivariable regression models. The evaluation of effect modification as it is used in this thesis can largely be looked upon as an evaluation of statistical interaction as opposed to an evaluation of biological interaction.

Rothman argues against the use of statistical tests to evaluate effect modification [230]. In paper IV, we also evaluated effect modification by generating a categorical variable with four mutually exclusive exposure categories based on a binary exposure and a binary potential effect modifier. The reason that Rothman recommends this second approach is because he states that biological interaction should be evaluated as the absence of additivity of effects. Using such a categorization as what was conducted in paper IV therefore allows for an evaluation of departure of additivity of effects when working on a multiplicative scale and the possibility to say something about biological interaction as opposed to a statistical interaction.

Handling missing data

The amount of missing information on individual covariates was generally low (<2%). The chosen method to evaluate the influence of missing data in this thesis was multiple imputation by chained equations [250,251]. Using chained equations allowed us to specify the model to be used for the imputation of the individual covariates, which is to be preferred when compared to multiple imputation using multivariate normal regression which assumes an underlying multivariate normal distribution. Any multiple imputation method assumes that the pattern of missing data only depends on the observed covariates and not on anything unobserved [250,251]. The measures of association obtained by complete case analysis and multiple imputation analysis were very similar in the analyses included in this thesis. However, the imputed datasets are only as good as your imputation model. Therefore, we cannot exclude the

possibility that both the complete case analysis and the multiple imputation analysis might be equally biased by unmeasured factors.

In paper III we used mixed linear regression to generate the exposure variable, i.e. the peak weight and height velocity. This method is more generous with regard to missing data [274]. All of the anthropometric measurements available for each child were included in the mixed model with only the age of measurement as a predictor. The growth curves could have been generated using linear regression but an important advantage of mixed linear regression is the precision. A mixed linear regression models the within and between person variation and subsequently reduces the uncertainty of the estimated parameters [274]. A more mathematically sophisticated approach to the growth modelling might be to use splines in the regression analysis [230]. The Reed1 growth model fitted the growth data in MoBa very well when compared to a number of other available growth models [275].

6.2.2 Internal validity

Multiple sources of bias might influence the internal validity of epidemiologic studies. These include selection bias, information bias and confounding. These three sources of bias will now briefly be discussed with regard to their potential influence on the associations reported in this thesis.

6.2.2.1 Selection bias

A bias in epidemiologic studies due to selection of study participants might occur if the selection process is associated with the exposure and the disease of interest [230]. The consequence of selection bias might be a bias of the measures of association towards the null or away from the null. There are two potential sources of selection bias in the scientific papers included in this thesis. The first is the proportion of invited pregnant women who agreed to participate in MoBa. The second is the mother's failure to return completed follow-up questionnaires. Interestingly, results from ALSPAC and the Danish National Birth Cohort found no strong indication that failure to respond to follow-up questionnaires influenced exposure-outcome associations [276-278].

The participation rate in MoBa among invited pregnant women was 40.6%. An evaluation of selection bias with regard to selection into the MoBa cohort was conducted by comparing MoBa participants to all women who gave birth in Norway during the MoBa inclusion period using information from the MBRN [239]. MoBa showed a strong under-representation of the youngest women (<25 years), those living alone, mothers with more than two previous births and mothers with previous stillbirths (relative deviation 30-45%) [239]. In addition, smokers, women with stillbirths and neonatal deaths were markedly under-represented in the cohort (relative deviation 22-43%), while multivitamin and folic acid supplement users were over-represented (relative deviation 31-43%) [239]. However, these differences had seemingly little influence on a number of evaluated associations [239]. This selection bias evaluation suggests that the burden of exposures and outcomes, but not necessarily estimates of exposure-outcome associations, might be biased due to self-selection into the MoBa cohort.

The second source of selection bias is the failure to return the necessary follow-up questionnaires to be included in the study sample for the individual scientific papers in this thesis. We evaluated this by two different approaches. First, we compared characteristics of included study participants to all eligible MoBa participants (i.e. singletons linked to the MBRN) who had information from the first follow-up questionnaire. We therefore used information from the MBRN and the first MoBa follow-up questionnaire to compare the individuals with and without the necessary follow-up information to be included in the respective analyses. Lost to follow-up analyses for papers II, III and IV are included in appendix II. These analyses indicated that included study participants were older, had longer education, had a lower parity, were less likely to be overweight/obese and were less likely to smoke during pregnancy compared to individuals without the necessary follow-up information. Overall, these results indicate the presence of a selection of more health conscious mothers. The more relevant question is whether these differences influenced the observed associations. One method available to evaluate the potential impact of selection bias on observed measures of association is inverse probability weighting, where you use weighting to make the included study participants representative of all eligible participants [252,253]. We explored inverse probability weighting to evaluate selection bias in paper II, III and IV. The findings from the inverse probability weighing showed similar associations as the main/un-weighted analyses. The variation between the weighted and un-weighted analyses were therefore minimal.

Overall, we do not think that selection bias strongly influenced the observed associations reported in this thesis. However, it will be interesting to re-examine some of the associations with regard to asthma at age 7 when more follow-up information becomes available.

6.2.2.2 Information bias

Another important source of bias in epidemiologic research is information bias. This type of bias is related to how relevant information is obtained. Information bias occurs when gathering of information and classification of the exposure and/or outcome varies between groups. Information bias may arise from recall bias, interviewer/detection bias or other sources of misclassification [230]. All these different sources of information bias present their own challenge when it comes to epidemiologic research. Recall bias occurs when study participants remember and therefore report information differently [230]. In cohort studies, where information is gathered prospectively at regular intervals, recall bias is likely to constitute less of a problem. Interviewer/detection bias occurs when the gathering of information from individuals/study participants depend on their exposure and/or disease status. Similarly to recall bias, this is not likely to pose a large problem in cohort studies because of the uniform prospective gathering of information.

In conclusion, misclassification of the exposure and/or the outcome is therefore the most likely source of information bias in the scientific papers included in this thesis and is therefore discussed in more detail. Misclassification can either be differential or nondifferential [230]. In prospective cohort studies, misclassification is most likely nondifferential. Nondifferential misclassification of a binary exposure or outcome will most often bias the associations towards the null [230]. However, this is not always the case. When exposures or outcomes have more than two categories, the consequences of nondifferential misclassification is more unpredictable [230].

<u>Exposure misclassification</u>

The information on maternal vitamin D level during pregnancy in paper I was obtained using a biomarker. There are multiple sources of misclassification when using a biomarker: the time point at which the biological samples are collected, the procedures for sample collection, treatment and storage of samples, in addition to the choice of assay.

The plasma samples used to measure the mother's vitamin D status during pregnancy were collected at approximately the 18th gestational week. The metabolite used, 25(OH)D, has a half-life of about 20 days [194]. The half-life of the metabolite therefore indicates the time period of exposure that our measurement might reflect. After the blood samples were collected from the participants, they were stored overnight in the hospitals where the mother was receiving her prenatal care at approximately 4 degrees Celsius. The samples were subsequently mailed to the MoBa biobank in room temperature. Upon arrival at the biobank, the samples were processed and subsequently stored at -80 degrees Celsius. Some degradation of the samples might therefore have occurred.

The 25(OH)D level was measured at BEVITAL laboratory in Bergen, Norway. This laboratory is approved by the Vitamin D External Quality Assessment Scheme. BEVITAL has published the reproducibility of their assay which measures fat soluble vitamins [279]. The within and between day coefficients of variance for 25-hydroxyvitamin D2 in this assay are 4.3%-4.5% and 4.6%-7.7%, respectively [279]. The within and between day coefficients of variance for 25-hydroxyvitamin D3 are 4.4%-5.3% and 7.3%-8.2%, respectively [279]. Ideally, we would have included duplicates when we conducted our measurements, in order to check the variation more specifically to our own data.

In conclusion, maternal 25(OH)D level is a good measure of vitamin D status during pregnancy. However, it is important to bear in mind the time point at which the biological sample was obtained, in addition to the half-life of the metabolite, which decides what window of exposure the 25(OH)D level might reflect.

Using maternal self-report of alcohol consumption through questionnaires might be a potential source of exposure misclassification in Study II. One would expect an underreporting due to the social stigmatism surrounding alcohol intake during pregnancy. A validation study including 441 pregnant women residing in Aarhus, Denmark found that the average alcohol consumption during pregnancy could be obtained by self-report through questionnaires with reasonable validity [280]. The researchers compared three methods of maternal report of alcohol consumption during pregnancy: interview about alcohol consumption the past week, a questionnaire with one item of current alcohol consumption and a two week diary. The percent agreement ranged between 73 and 82% [280].

In MoBa, we also had the opportunity to examine the consistency of maternal report of alcohol intake across follow-up questionnaires. For example, comparing maternal report of alcohol intake during the third trimester, which was reported both at 30 gestational weeks and when the child was 6 months, we saw that 78% reported the same amount of alcohol intake in consecutive questionnaires. This consistency of maternal report is reassuring. Our ability to evaluate maternal alcohol intake during breastfeeding might be weakened, as women might time their alcohol intake when they know it will be several hours until the next feeding. We had no information on the timing of alcohol intake relative to breastfeeding.

In conclusion, maternal self-report of alcohol intake during pregnancy and lactation through questionnaires is an adequate measure of exposure. However, we cannot exclude the possibility of underreporting.

Information on the child's anthropometric measurements was obtained by maternal report though questionnaires in Study III. By asking the mother to refer to the child's health report card, we attempted to minimize misclassification. A validation study of the mother's report of the child's anthropometric measurements was conducted by comparing maternal report of height and weight values through MoBa questionnaires to height and weight measurements obtained by trained study staff members in the Bergen Growth Study [281]. In the validation study, a total of 77 children were available for comparison with measurements taken with a mean of 32 days apart in the two studies (SD 33). The child's body mass index calculated in the two studies had a correlation coefficient of 0.86 (95% CI: 0.81, 0.90) [282].

In conclusion, this validation study indicated a reasonable validity of maternal report of anthropometric measurements through MoBa questionnaires.

Using maternal self-report to classify her prenatal tobacco smoke exposure in addition to her own smoking status might be a source of exposure misclassification in Paper IV. A large validation study from the U.S. Nurses' Health Study indicated that the adult daughter's report of her mother's smoking both prenatally and during childhood had a high validity when compared to the mother's own report [283]. The sensitivity of the daughters' report of her own prenatal tobacco smoke exposure ranged from 74% to 85%, while the specificity was between 90% and 95% (kappa = 0.72-0.81) [283].

Maternal self-report of her own smoking status during pregnancy in MoBa has been validated against cotinine levels measured around 18 gestational weeks indicating a reasonably validity [284]. The sensitivity and specificity for self-reported daily smoking, using a cotinine level of 30 nmol/l as the cut-off level to indicate active smoking, were 82 and 99%, respectively [284]. Furthermore, the reproducibility of the mother's report of her own prenatal tobacco smoke exposure has been evaluated among mothers who participated with more than one pregnancy in MoBa, yielding a kappa statistic of 0.80 [285].

In conclusion, both maternal report of her own prenatal tobacco smoke exposure in addition to her own smoking status when pregnant with her child are adequate measures of exposure. However, we cannot exclude the possibility of underreporting.

Outcome misclassification

An important challenge with regard to classification of asthma in epidemiologic studies is the lack of an agreed-upon gold standard. Bronchial hyper-responsiveness is widely looked upon as an important feature of asthma. Since bronchial hyper-responsiveness is not consistently found among asthmatics and may exists in the absence of clinical symptoms, validation studies show that this clinical test has poor sensitivity (50%) and specificity (75%) for clinically diagnosed asthma [286]. Other measures of lung function have also been examined as clinical tests for asthma, where the FEV_1/FVC ratio has showed a 35% sensitivity and 79% specificity for clinically diagnosed asthma [286]. The current opinion is therefore that asthma is a clinical diagnosis and an accurate diagnosis of asthma is largely based on the expert clinicians' evaluation of symptom history.

The majority of epidemiologic studies use information gathered through questionnaires to classify asthma. The ISAAC symptom questions yielded a high validity of parental report of their child's asthma status when validated against clinical examinations [287,288]. ALSPAC conducted a validation study of one of the core ISAAC questions regarding doctor diagnosed asthma against registered physician confirmed asthma in the British General Practice Research Database, reporting a 82% positive predictive value and a 97% negative predictive value [286]. Another ISAAC question with high validity include wheezing symptoms the past year [286,288].

Another factor which is often incorporated into the asthma classification in epidemiologic studies is treatment. In the current thesis, maternal report that the child had used an asthma medication the past year was included in the classification of current asthma. For current asthma at 36 months, the two groups of commonly used medication for asthma included in the definition were glucocorticoids and beta(2)-agonists. These are by far the most commonly used medications for asthma in this age group. Notably, incorporating the child use of leukotriene antagonists into the definition of current asthma at 36 months did not change the asthma prevalence (5.73% versus 5.71% in MoBa version VIII). Maternal report that the child had used medications for asthma the past year in the MoBa 7 year questionnaire was validated against dispensed asthma medications in the NorPD and showed a reasonable validity of maternal report [289]. The sensitivity of maternal report was estimated to be 85.0% while the specificity was 96.8% [289]. This validation study therefore indicated a reasonable validity of maternal report that the child had used medications for asthma.

In conclusion, using parental report of asthma and use of asthma medication in the offspring is a reasonable measure of the child's asthma status. The MoBa questionnaires completed when the child was 6, 18 and 36 months would have benefitted from more comprehensive questions regarding asthma related symptoms taken from the ISAAC questionnaires. The asthma definition in the current thesis is likely fairly stringent.

Using parental report of LRTIs through questionnaires might also have caused misclassification. Comparing different methods of obtaining information about LRTIs, the current gold standard is hospital records. In Norway, the Hospital Discharge Registry became available for linkage on an individual basis from 2008 and includes information about all discharges from Norwegian hospitals. As the largest group of children experiencing LRTIs in Norway will be treated by their primary care physician, if they see a physician at all, this registry is not ideal for case ascertainment of common LRTIs. In MoBa, mothers were not asked about doctor diagnosis of LRTIs. They were asked about hospital admission for LRTIs, but we could not determine how many infections resulted in the child being admitted to a hospital across the various questionnaires because of the possibility of double counting. Thus we could not use this information to identify recurrent LRTIs. In addition, by limiting the outcome to hospitalized LRTIs, we would have missed episodes which were less severe.

One important question is whether parents are able to adequately distinguish between upper respiratory tract infections and LRTIs in the offspring. Based on maternal report in MoBa questionnaires, approximately 98% of children had 3 or more colds in the first 36 months, approximately 10% of children had 3 or more throat infections the first 36 months, while around 16% had 3 or more ear infections the first 36 months. As only 4% of children had 3 or more LRTIs, this suggests that maternal report of LRTIs is capturing something more severe/rare than common upper respiratory tract infections. We did not have information about the use of antibiotics for LRTIs. However, use of antibiotics would only cover bacterial infections, and because many LRTIs are viral in origin, it might not be advisable to use this restriction in the definition of LRTIs [290].

In conclusion, parental report is a reasonable method to classify LRTIs. It might be difficult for mothers to remember the number of LRTIs within the specific age periods. This might have resulted in an under-reporting and thereby and underestimation of the number of LRTIs the first 36 months of life.

6.2.2.3 Confounding

The third major potential source of bias in all epidemiologic studies is confounding. The definition of a confounder was described in the methods section of this thesis. Confounding might bias the observed associations towards or away from the null [230].

The approach used to identify potential confounders for the scientific papers in this thesis was directed acyclic graphs [248]. We included all potential confounders identified through the directed acyclic graphs in all the multivariable analyses. Our approach therefore did not attempt to distinguish a set of minimally sufficient variables to adjust for through the analysis of the directed acyclic graph. The approach used in this thesis might therefore be considered relatively conservative. The impact of adjusting for potential confounding factors had only a moderate influence on the observed associations in this thesis when comparing crude and adjusted associations. Two maternal characteristics that seemed to attenuate several of the examined associations towards the null were maternal education and maternal pre-pregnancy body-mass index. The possibility of unmeasured confounding will also be discussed.

Impact of adjustment for socio-economic status

Epidemiologic studies indicate socio-economic differences in asthma [93-95]. Some of the exposures, in particular the grandmother's smoking and maternal alcohol intake during pregnancy and lactation, have very strong socio-economic gradients. The main indicator of socio-economic status included in this thesis was maternal education. Paper II, III and IV also adjusted for the mother's salary. For most of the multivariable analyses, adjustment for maternal education attenuated the observed associations towards the null. Further adjustment for the mother's salary did not change the results. The fact that adjustment for salary did not change the observed associations might be due to the universal health care access in Norway, where access to health care does not seem to be influenced by income. In contrast, education might influence a wide range of health behavior with consequence for health outcomes. In MoBa, mothers with a higher educational attainment had a lower proportion of smoking during pregnancy, lower proportion of overweight/obesity, higher vitamin D status during pregnancy and higher folate intake during pregnancy. This indicates a general health conscious behavior among the women with a longer educational attainment.

Impact of adjustment for maternal pre-pregnancy body-mass index

Maternal pre-pregnancy body-mass index is positively associated with asthma development in the offspring. Multivariable adjustment of the associations examined in this thesis for maternal pre-pregnancy body-mass index attenuated the observed associations towards the null. In MoBa, maternal pre-pregnancy body mass index was higher among older mothers, mother with higher parity, mothers with asthma, mothers who did not take a folic acid supplement during pregnancy and among mothers who reported that they did not drink during pregnancy. We found a seemingly non-linear variation in maternal vitamin D status during pregnancy by maternal pre-pregnancy body-mass index. These characteristics seem to indicate that maternal pre-pregnancy body-mass index is likely reflecting a wide range of health related behavior which might explain the attenuation of the observed associations.

Unmeasured confounding

The modest inverse association of infrequent/low-dose prenatal alcohol exposure and asthma development reported in paper II might be influenced by unmeasured confounding. An excellent study from ALSPAC used Mendelian randomization to examine this issue [260]. In accordance with our findings, they reported a modest inverse association between maternal alcohol intake during pregnancy and asthma development. A Mendelian randomization

analysis based on alcohol dehydrogenase (ADH)1B genotype supports our assertion that the slight inverse association observed in that study, similar to ours, is unlikely to be causal. In paper III we also incorporated a sibling pair comparison when examining the association of early childhood growth with respiratory disorders. A comparison between discordant sibling pairs adjusts for unmeasured confounding by shared genetic and environmental factors. Differences between a standard and a sibling pair analysis may therefore indicate confounding by factors that are shared by siblings [249]. Interestingly, the sibling pair analysis yielded similar association between peak weight velocity and asthma at 36 months. This was not seen for the other two respiratory disorders evaluated. However, we are cautious in our interpretation of the sibling pair analysis due to the low number of discordant sibling pairs available for evaluation. The association between the grandmother's smoking when pregnant with the mother and asthma development in the offspring might also be subject to unmeasured confounding. We were not able to adjust for the grandmother's socio-economic status or the grandmother's asthma status. This could account for the modest positive association observed between the grandmother's smoking and asthma in the grandchild. Notably, adjustment for maternal education, maternal salary and maternal asthma status did not greatly influence the observed positive association. The study of the grandmother's smoking when pregnant with the mother and asthma in the grandchild from ALSPAC adjusted for a wide range of characteristics related to the grandparents [268]. Notably, this study reported no significant association.

6.2.3 External validity

Participants in MoBa showed a strong under-representation of a set of high risk groups as identified in other studies, such as for example smokers, younger mothers and women with previous spontaneous abortions/stillbirths, when compared to all live births registered in the MBRN during the MoBa inclusion period [239]. Generalization of the associations identified in MoBa to such high risk groups should therefore be done with caution. However, a number of exposure associations with pregnancy outcomes were found to be of similar magnitude among MoBa participants as when examined in the general Norwegian population using the MBRN [239]. However, this might not be the case with regard to associations between early environmental exposures and later health outcomes. Furthermore, several minority groups are likely underrepresented in MoBa because the participants were required to have a good

understanding of the Norwegian language. Whether associations identified among MoBa participants are generalizable to other ethnic groups therefore remains to be seen.

The Norwegian population also has a number of characteristics that is important to bear in mind when evaluating whether associations found in MoBa are generalizable to other populations. For example, our universal health care access might influence some associations. Associations found in MoBa might therefore not be generalizable to populations with a larger degree of differential access to health care. Norway also has a relatively high educational level. The number of women with completed higher education (college/university degree) in 2013 was 33% according to Statistics Norway [291]. Some of the exposure-outcome associations identified in MoBa will therefore have to be replicated in other population based cohort studies in order to confirm generalizability.

6.3 Public health recommendations

This section will briefly discuss current public health recommendations with regard to the four environmental factors evaluated in this thesis. We are not advocating any changes to existing national public health recommendations.

In the first paper, we reported an inverse association of maternal vitamin D status during pregnancy with LRTIs in the offspring. The Norwegian Health Directorate recommends that pregnant women take one spoon of cod liver oil per day which includes 10 µg of vitamin D [292]. Interestingly, the mean vitamin D status among the MoBa mothers was fairly high. Only 16.8% of mothers had 25(OH)D levels ≤ 50 nmol/L. We could not distinguish between the relative importance of the mother's vitamin D status during pregnancy and the child's vitamin D status. Norwegian mothers who breastfeed are recommended to give their child vitamin D drops due to the low vitamin D status of breastfed infants [293]. Our results may lend some support to the ongoing debate regarding what vitamin D levels should be considered sufficient with regard to non-bone related health outcomes.

In the second paper we reported no association between maternal alcohol intake during pregnancy and respiratory disorders in the offspring. As stated, we do not believe that the modest inverse association between infrequent/low-dose prenatal alcohol exposure and asthma at 36 months is causal. The Norwegian Health Directorate recommends that pregnant

women abstain from alcohol since low/moderate alcohol consumption is associated with several adverse birth and child health outcomes [203]. However, it is reassuring that the moderate prenatal alcohol consumption that persists in the Norwegian population, especially before women become aware that they are pregnant, does not confer an increased risk of respiratory disorders in the offspring.

In the third paper we reported a positive association of peak weight velocity the first 36 months of life with respiratory disorders. The growth of Norwegian children are continuously monitored through regular check-ups at health stations by public health nurses according to recommendations by the Norwegian Health Directorate [244]. Our findings do not advocate any public health interventions with regard to the rate of growth among children younger than three years of age. At this young age it is not ethical to discuss any interventions to influence the growth of the children unless they are experiencing growth restriction.

In the fourth paper, we report a positive association of the grandmother's smoking when pregnant with the mother and asthma in the grandchild. We found no independent association of maternal smoking during pregnancy with asthma in the offspring. Due to the association of maternal smoking during pregnancy with several pregnancy and child health outcomes, the Norwegian Health Directorate currently recommends that women who are pregnant stop smoking [292]. It is interesting to speculate that this public health recommendation might conceivably confer a benefit for more than one generation.

6.4 Future research directions

Findings from a small number of epidemiologic studies are insufficient to establish the causality of an association. The European countries have several excellent pregnancy and birth cohorts which might be able to replicate studies of certain associations and/or provide data for pooled analyses. Another important aspect of replication of associations in other populations is to evaluate the generalizability of the findings. Based on an initiative by the European Union, a large number of European pregnancy/birth cohorts have established a collaboration: Developing a Child Cohort Research Strategy for Europe [294]. It will be interesting to see the fruits of this collaboration over the next years.

In addition, we have some concrete thoughts regarding research hypotheses visited in this thesis that could be further evaluated and how this can be done. First of all, further studies on vitamin D status and development of respiratory disorders should ideally include repeated measurements of maternal vitamin D status during pregnancy and the child's vitamin D status during early childhood in order to properly distinguish between the role of prenatal and early childhood vitamin D status. It would also be valuable with information on the association between maternal alcohol intake during pregnancy and respiratory disorders in the offspring from populations where higher alcohol intake during pregnancy persists in the population. With regard to the hypothesis of early childhood growth and development of respiratory disorders, a number of studies now point in the same direction. Future studies should therefore attempt to evaluate potential underlying biological mechanisms for the positive association between early childhood weight increase and respiratory disorders. Finally, the association of the grandmother's smoking when pregnant with the mother and asthma in the grandchild should be revisited in cohorts with more comprehensive information regarding the grandmother's socio-economic and health related factors. In addition, more information on the association of the grandmother's smoking when pregnant with the father and asthma in the grandchild is of value, as only one study has examined this.

Both genetic and epigenetic studies are of value to further explore the research hypotheses visited in this thesis. With regard to genetic studies, one example is the use of genetic data to conduct Mendelian randomization in order to evaluate the potential causality of the identified associations. This method was used in an excellent manner by ALSPAC in order to evaluate the causality of the inverse association between maternal alcohol intake during pregnancy and asthma in the offspring [260]. However, Mendelian randomization requires that you have a well-defined genetic polymorphism that can be evaluated which might not always be the case. Epigenetic studies can be used to examine the potential underlying mechanisms between early environmental exposures and health outcomes. In MoBa, it has been demonstrated that maternal smoking during pregnancy is associated with cord blood DNA methylation [224]. In addition, cord blood DNA methylation is differential by the child's birth weight [263]. We are planning to evaluate other early environmental exposures and their impact on cord blood DNA methylation. The rapid growth in the field of epigenetics will likely provide valuable information over the next years.

6.5 Conclusion

The main findings of this thesis is that higher maternal vitamin D status during pregnancy might reduce the risk of LRTIs in the offspring, that children who have a rapid weight increase the first 36 months of life might have increased risk of both LRTIs and asthma, and that the grandmother's smoking when pregnant with the mother might increase the risk of asthma in the grandchild. Our findings therefore support the notion that early environmental exposures might indeed influence development of respiratory disorders. In addition, we provide some indication that environmental exposures during pregnancy might also have trans-generational influences. This thesis is an example of results that can be obtained when combining information from a large population based cohort study, national health registries and a large national biobank. Hopefully, it will provide inspiration to future research hypotheses that may be evaluated in a similar manner.

7. References

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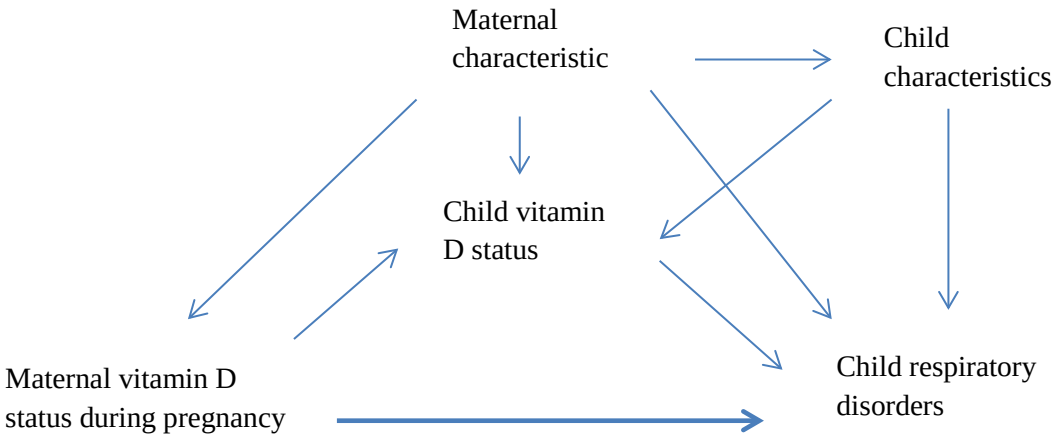
Appendix I

Paper I Directed acyclic graph

Prospective study of maternal mid-pregnancy 25-hydroxyvitamin D level and early childhood respiratory disorders.

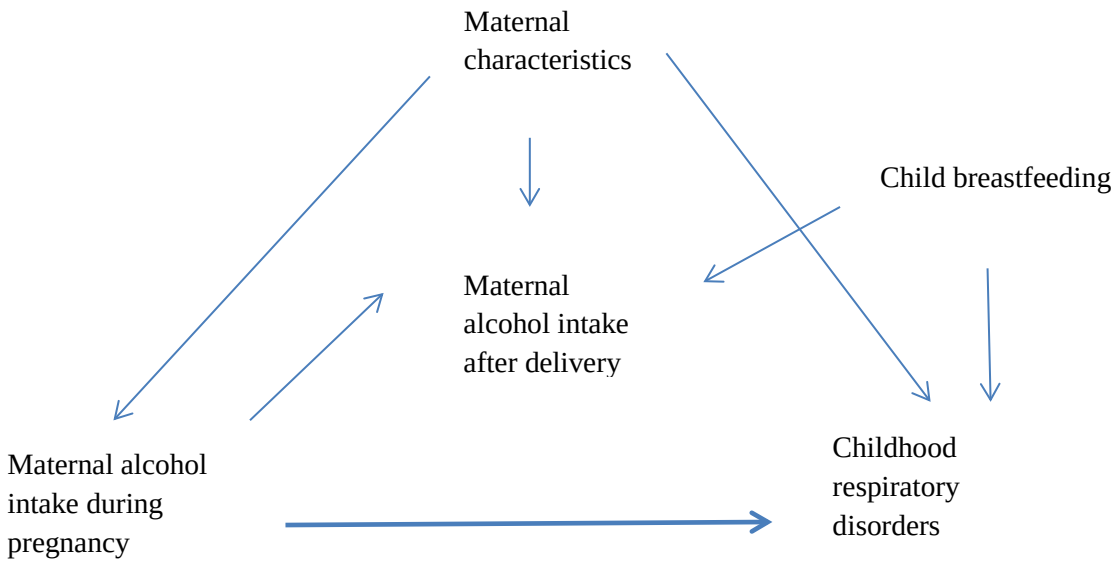


Maternal Characteristics: maternal age, education, parity, region of delivery, pre-pregnancy body mass index, smoking during pregnancy, folate level during pregnancy, history of asthma, multivitamin use during pregnancy, frequency of leisure time physical activity during pregnancy, gestational week of sample collection and season of sample collection

Child characteristics: Child gender, infant feeding and child supplement use during infancy

Paper II Directed acyclic graph

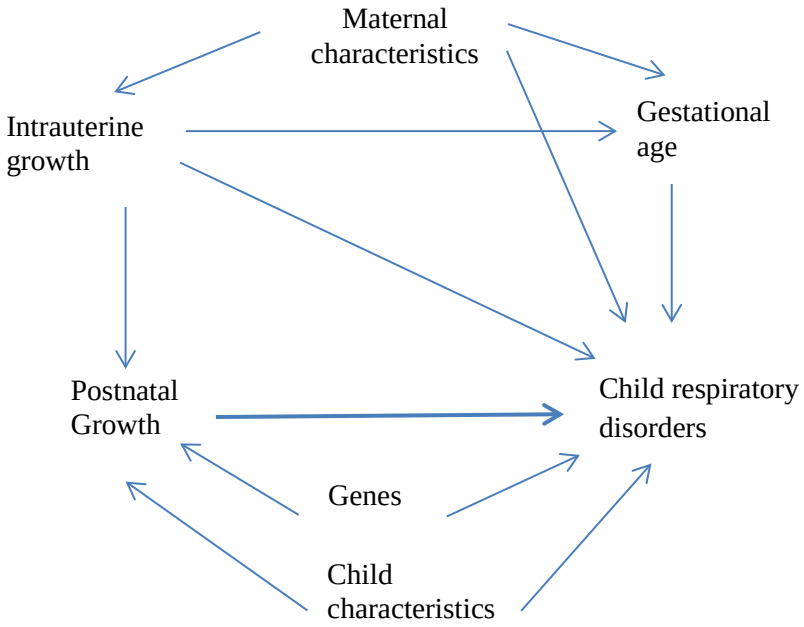
Prospective study of maternal alcohol intake during pregnancy or lactation and risk of childhood asthma: the Norwegian Mother and Child Cohort Study.



Maternal characteristics: maternal age, education, parity, pre-pregnancy body mass index, salary, smoking during pregnancy, folate intake during pregnancy, smoking when the child is 6 and/or 36 months and history of asthma

Paper III Directed acyclic graph

Peak weight and height velocity to age 36 months and asthma development: the Norwegian Mother and Child Cohort Study.



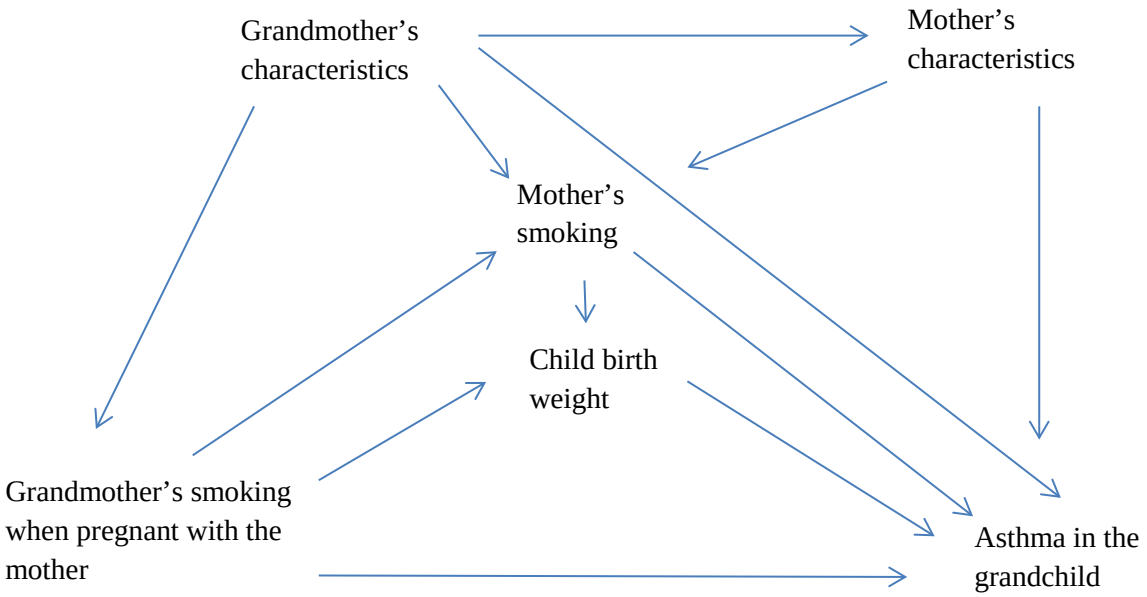
Child Characteristics: child gender and breastfeeding the first 6 months

Maternal Characteristics: maternal age, education, salary, parity, smoking during pregnancy and folate intake during pregnancy.

Genes: Genetic predisposition for growth and asthma. Measured by maternal height, maternal body mass index, paternal height and paternal body mass index, in addition to maternal and paternal history of asthma.

Paper IV Directed acyclic graph

Grandmother's smoking when pregnant with the mother and asthma in the grandchild: the Norwegian Mother and Child Cohort Study.



Grandmother's characteristics: grandmother's socio-economic status (education and income), the grandmother's health related behavior and the grandmother's asthma status

Mother's characteristics: maternal age, parity, education, salary, pre-pregnancy body mass index, and asthma status

Appendix II

Appendix II, Table 1 : Lost to follow-up analysis for the analysis of current asthma at 36 months and current asthma at 7 years for paper II using information from the medical birth registry and the baseline follow-up questionnaire

Characteristics	Eligible for analysis of asthma at 36 months without necessary follow-up information n=50,077 (%)	Sample used for analysis of asthma at 36 months n=49,138 (%)	Eligible for analysis of asthma at 7 years without necessary follow-up information n= 6,323 (%)	Sample used for analysis of asthma at 7 years n= 13,253 (%)
Maternal age				
< 25	13.4	8.8	13.1	9.0
25-29	32.6	33.3	36.3	33.9
30-34	37.1	39.9	36.0	40.0
35 and older	16.8	18.0	14.6	17.2
Maternal parity				
Primiparous	41.4	48.4	47.8	43.4
1	37.1	34.1	33.2	35.7
2	16.6	13.8	14.9	16.2
3 or more	4.9	3.7	4.2	4.6
Maternal education				
Less than high school	10.8	5.3	8.9	6.3
High school	32.6	26.4	34.4	30.0
Up to 4 years of college	36.9	43.4	39.9	44.4
More than 4 years of college	19.6	25.0	16.8	19.3
Maternal salary				
<200,000 NOK	34.3	25.3	34.5	30.8
200,000 – 400,000 NOK	55.2	61.9	57.1	61.6
>400,000 NOK	10.5	12.7	8.5	7.7
Maternal pre-pregnancy BMI				
Underweight (<18.5)	3.4	2.9	3.0	2.7
Normal weight (18.5-24.9)	64.3	66.5	62.9	65.5
Overweight (25-29.9)	22.0	21.7	22.9	22.7
Obese (>=30)	10.3	8.9	11.2	9.1
Maternal asthma				
No	92.2	92.7	92.7	93.1
Yes	7.8	7.3	7.3	6.9
Maternal smoking during pregnancy *				
No	89.2	93.8	88.7	92.5
Yes	10.8	6.2	11.3	7.5
Frequency of drinking before pregnancy				
Never	8.7	7.8	8.2	8.5
Less than once a month	26.7	23.8	26.8	25.7
1-3 times per month	36.0	36.3	38.8	38.7
Once a week	19.1	20.7	17.3	18.6
More than once a week	9.5	11.5	8.9	8.6

Number of drinks per time pre-pregnancy				
None	8.7	7.7	8.2	8.5
Less than 1	3.7	3.2	3.2	3.5
1-2	30.3	32.0	27.4	31.3
3-4	29.9	31.3	30.4	31.1
5 or 6	18.6	17.8	20.8	17.7
More than 6	8.8	8.0	10.1	7.9
Frequency of drinking first trimester				
Never	70.8	68.2	67.1	65.0
Less than once a month	20.0	21.9	23.1	24.4
Once a month or more	9.3	9.9	9.8	10.6
Number of drinks per time first trimester				
None	70.6	68.4	67.0	65.1
Less than 1	16.2	16.5	16.9	18.3
1-2	8.1	9.0	9.6	10.7
3-4	2.8	3.3	3.4	3.3
5 or more	2.4	2.7	3.1	2.6
Frequency of binge drinking first trimester				
Never	86.5	85.5	85.7	86.1
Less than once a month	10.2	11.1	10.9	11.1
Once a month or more	3.3	3.4	3.4	2.8

* Maternal smoking during pregnancy classified solely based on the baseline questionnaire administered at approximately 18 gestational weeks.

Appendix II, Table 2 : Lost to follow-up analysis for the analysis of current asthma at 36 months and current asthma at 7 years for paper III using information from the medical birth registry and the baseline follow-up questionnaire

Characteristics	Eligible for analysis of asthma at 36 months without necessary follow-up information (N= 49,653)	Sample used for analysis of asthma at 36 months (N=50,311)	Eligible for analysis of asthma at 7 years without necessary follow-up information (N= 14,384)	Sample used for analysis of asthma at 7 years (N=24,827)
Maternal age (Mean (SD))	29.9 (4.8)	30.4 (4.4)	30.1 (4.5)	30.5 (4.3)
Maternal parity (%)				
Primiparous	41.4	48.2	49.3	45.8
1	37.3	33.9	33.4	34.9
2	16.5	14.1	13.7	15.1
3 or more	4.8	3.8	3.7	4.2
Maternal education (%)				
Less than high school	11.1	5.3	6.9	5.4
High school	32.6	26.6	29.2	27.7
Up to 4 years of college	36.7	43.3	41.1	44.9
More than 4 years of college	19.6	24.8	22.9	22.0
Maternal salary (%)				
<200,000 NOK	34.2	25.8	28.6	27.9
200,000 – 400,000 NOK	55.1	61.8	60.7	62.6
>400,000 NOK	10.7	12.5	10.7	9.5
Maternal height, cm (Mean (SD))	167.9 (6.1)	168.3 (5.9)	168.2 (5.9)	168.3 (5.9)
Maternal pre-pregnancy BMI (%)				
Underweight (<18.5)	3.5	2.9	3.2	2.7
Normal weight (18.5-24.9)	64.3	66.9	65.7	66.7
Overweight (25-29.9)	22.2	21.6	21.8	22.1
Obese (>=30)	10.0	8.6	9.3	8.5
Maternal smoking during pregnancy (%)*				
No	89.2	93.7	91.9	93.6
Yes	10.9	6.3	8.1	6.4
Maternal asthma (%)				
No	92.0	92.9	92.7	93.0
Yes	8.0	7.1	7.3	7.0
Child gender				
Male	51.5	51.1	51.2	51.2
Female	48.5	48.9	48.8	48.8
Child gestational age (Mean SD))	39.3 (2.5)	39.5 (1.7)	39.5 (1.7)	39.5 (1.7)

* Maternal smoking during pregnancy classified solely based on the baseline questionnaire administered at approximately 18 gestational weeks.

Appendix II, Table 3: Lost to follow-up analysis for the analysis of current asthma at 36 months and current asthma at 7 years for paper IV using information from the medical birth registry and the baseline follow-up questionnaire

Characteristics	Eligible for analysis of asthma at 36 months without necessary follow-up information n=46,795	Sample used for analysis of asthma at 36 months n=53,169	Eligible for analysis of asthma at 7 years without necessary follow-up information n= 15,815	Sample used for analysis of asthma at 7 years n=25,394
Maternal age (Mean(std.dev.))	29.9 (4.8)	30.4 (4.4)	30.0 (4.5)	30.5 (4.4)
Maternal parity (%)				
Primiparous	41.3	47.9	48.8	45.6
1	37.2	34.2	33.7	35.2
2	16.6	14.1	13.8	15.0
3 or more	4.9	3.8	3.8	4.2
Maternal education (%)				
Less than high school	11.2	5.5	7.3	5.5
High school	32.8	26.8	29.7	27.6
Up to 4 years of college	36.5	43.1	40.7	44.9
More than 4 years of college	19.5	24.6	22.4	21.9
Maternal salary (%)				
<200,000 NOK	34.4	26.1	29.3	28.0
200,000 – 400,000 NOK	55.1	61.4	59.9	62.5
>400,000 NOK	10.5	12.5	10.8	9.5
Maternal pre-pregnancy BMI (%)				
Underweight (<18.5)	3.5	2.9	3.2	2.7
Normal weight (18.5-24.9)	64.1	66.6	65.4	66.3
Overweight (25-29.9)	22.1	21.6	21.9	22.2
Obese (>=30)	10.4	8.9	9.6	8.9
Maternal asthma (%)				
No	92.0	92.8	92.6	93.0
Yes	8.0	7.2	7.4	7.0
Child birth weight, grams (Mean(std.dev.)	3,575 (625)	3,606 (540)	3,610 (542)	3,615 (546)
Grandmother smoking when pregnant with the mother (%)				
No	63.2	66.9	65.1	67.1
Yes	26.3	23.5	24.9	23.6
Don’t know	10.6	9.6	10.0	9.4
Maternal smoking during pregnancy (%)*				
No	89.1	93.6	91.5	93.5
Yes	10.9	6.5	8.5	6.5

* Maternal smoking during pregnancy classified solely based on the baseline questionnaire administered at approximately 18 gestational weeks.