Fetal and Infant Origins *of* Childhood Asthma

The Generation R Study

Agnes Sonnenschein-van der Voort

ACKNOWLEDGEMENTS

The general design of the Generation R Study was made possible by financial support from the Erasmus Medical Center, Rotterdam, the Erasmus University Rotterdam and the Netherlands Organization for Health Research and Development (ZonMw), the Netherlands Organisation for Scientific Research (NWO), the Ministry of Health, Welfare and Sport and the Ministry of Youth and Families.

Agnes Sonnenschein-van der Voort is the recipient of a European Respiratory Society Fellowship (STRTF 93-2012) and received a grant from the Ter Meulen Fund, Royal Netherlands Academy of Arts and Sciences (TMF2012/228).

The work presented in this thesis was conducted at the Department of Paediatrics, Division of Paediatric Respiratory Medicine, the Generation R Study Group, and the Department of Epidemiology of the Erasmus Medical Center in Rotterdam, the Netherlands, and at the ALSPAC study, School of Social and Community Medicine, University of Bristol, United Kingdom.

The printing of this thesis has been financial supported by the Erasmus University Rotterdam, the Generation R Study, Longfonds, and Stichting Astma Bestrijding. Further financial support for this dissertation was kindly provided by ALK-Abello BV, Boehringer Ingelheim BV, Chiesi Pharmaceuticals BV, Chipsoft BV, GlaxoSmithKline, Medela BV, Nutricia baby- en kindervoeding, and Thermo Fisher Scientific/IDD.

ISBN: 978-94-6169-466-9
Cover design, lay-out and printing: Optima Grafische Communicatie, Rotterdam, the Netherlands

FETAL AND INFANT ORIGINS OF CHILDHOOD ASTHMA

The Generation R Study

Foetale en vroeg postnatale oorzaken van astma op de kinderleeftijd
Het Generation R onderzoek

Proefschrift

Ter verkrijging van de graad van doctor aan de
Erasmus Universiteit Rotterdam
op gezag van de
rector magnificus

Prof. dr. H. A. P. Pols

en volgens besluit van het College voor Promoties.
De openbare verdediging zal plaatvinden op
woensdag 15 januari 2014 om 15:30 uur

door

Agnes Maria Mariamna Sonnenschein-van der Voort

geboren te Amsterdam

PROMOTIECOMMISSIE

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Chapter 3.3

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Chapter 1

1.1

Introduction and design

BACKGROUND

Asthma is a chronic inflammatory disorder of the airways. Asthma is associated with airway hyperresponsiveness and variable airflow limitation, that lead to recurrent episodes of respiratory symptoms including wheezing, shortness of breath, phlegm, and cough[1]. Symptoms in young children are nonspecific, and may also occur with viral infections. Objective tests, including spirometry or assessment of bronchial responsiveness, are not easy to conduct in young children, and have limited applicability. Therefore, a clear definition of asthma in childhood is not available[2]. In clinical practice asthma cannot be diagnosed for preschool children and usually the diagnosis of wheezing, elicited by viral infection or multiple other triggers, is used[3]. In epidemiological studies the diagnosis of asthma is based on parental- or self-reported symptoms or reported physician diagnosis[4]. These studies have shown that childhood asthma has a high prevalence across many countries worldwide[5]. The reported prevalence among school-age children is around 5-10%. In preschool children, the prevalence of asthma-related symptoms, such as wheezing and shortness of breath, is even much higher. Childhood asthma is related to a reduced quality of life, limited exercise tolerance, and higher risks of school absenteeism and hospitalization[6]. The morbidity remains high despite the availability of safe and effective treatments[7]. The lack of curative options seems to be largely due to the unknown aetiology of asthma[8].

Accumulating evidence suggest that childhood asthma has at least part of its origins in fetal life and infancy[9]. The developmental plasticity hypothesis suggests that adverse exposures in early life lead to developmental adaptations of various organ systems, including of the respiratory tract, to enhance survival in the short term. These adaptations may result in impaired airway- and lung development, which predisposes the individual to respiratory morbidity, such as asthma or chronic obstructive pulmonary disease, in later life[10]. This hypothesis is mainly based on studies showing associations of low birth with respiratory diseases in later life[11]. Not much is known about the mechanisms that explain these associations.

FETAL AND INFANT GROWTH

Low birth weight has been associated with subsequent respiratory morbidity, including asthma and respiratory tract infections[12-14]. Since low birth weight is the result of various adverse fetal exposures and growth patterns, and the starting point of infant growth, it is not per se a causal factor for respiratory morbidity in later life[15-18]. Two recent studies suggested that fetal growth characteristics in early pregnancy affect the risk of wheezing[16, 17]. Not only fetal growth, but also rapid infant growth may be associated with asthma symptoms and a reduced lung function in childhood[18-20]. Studies focussed on the association of infant growth with childhood asthma were not able to take fetal growth into account. This is a limitation because fetal and infant growth are inversely correlated[18, 19]. The associations of low birth

weight with respiratory disease in later life may also be explained by preterm birth. Preterm birth is related with impaired lung function and asthma diagnosis in childhood[21-23]. The lungs of preterm born children have not yet fully developed, which makes them more vulnerable for adverse exposures and developmental lung adaptations that may increase the risk of asthma[21-25]. The associations of gestational age, birth weight and infant growth and their interactions with the risks of wheezing and asthma are important to unravel.

FETAL EXPOSURES

The associations of low birth weight with respiratory diseases in later life may be explained by adverse fetal exposures, independent of early growth. Suggested environmental risk factors in fetal life for the development of reduced pulmonary function include psychological distress, obesity, and maternal smoking.

Maternal obesity affects birth weight and gestational age at delivery[26, 27]. Also, proinflammatory cytokine levels are higher in obese mothers. Inflammatory processes in the mother during pregnancy may lead to fetal developmental adaptations and a greater susceptibility of impaired respiratory health in childhood and atopic diseases after birth[28-31]. Maternal low-grade inflammatory status can be measured with C-reactive protein levels[32]. Also, maternal psychological distress during pregnancy may lead to developmental adaptations of the hypothalamic-pituitary-adrenal axis, the autonomic nervous system, the lung structure and function, and immune responses in the offspring[33-35]. Next to direct programming effects, a hypothesized mechanism is the intermediate role of early growth because maternal psychological distress during pregnancy may impair fetal growth[36]. Maternal smoking during pregnancy is strongly associated with fetal growth retardation and low birth weight[37]. Maternal smoking during pregnancy may also affect respiratory tract development[38-41].

EXPOSURES IN INFANCY

Potential risk factors for the development of impaired pulmonary function and risk of respiratory disease in infancy include a shorter duration of breastfeeding, and exposure to environmental tobacco smoking and air pollutants[9]. Underlying mechanisms that have been suggested to explain the associations of breastfeeding with the risks of respiratory symptoms are breast milk components, including IgA, cytokines, glycans and long-chain fatty acids that stimulate and balance the infant's innate immune system and growth[42-44]. Exposure to air pollution, including tobacco smoke, might affect the risk of respiratory symptoms via bronchial hyperreactivity, immunological changes, and direct toxic and irritant effects[45, 46]. Also, an increased vulnerability of the airways and lungs to air pollutants might be caused by tobacco smoke exposure.

HYPOTHESIS

The main hypothesis for this thesis is that early growth and adverse environmental exposures lead to adaptations in respiratory and immunological development, that increase the risk of asthma and asthma-related symptoms (Figure 1.1.1). From both an etiological and a prevention perspective, it is important to identify specific fetal and infant exposures that lead to childhood asthma in later life. The studies presented in this thesis were specifically focused on the identification of early critical periods.

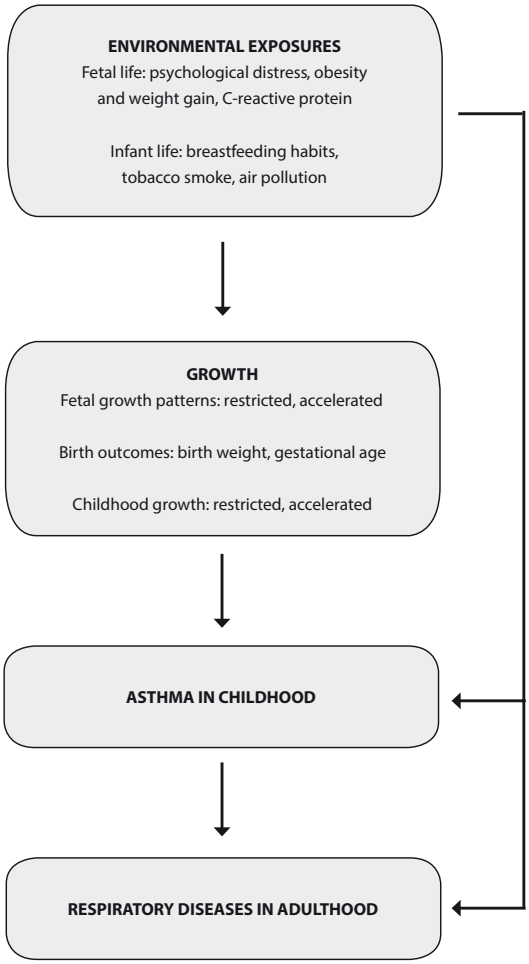

Figure 1.1.1. Overview of the origins of childhood asthma and its potential underlying early growth and environmental mechanisms studied in this thesis.

OBJECTIVES

The major aims of this thesis are:

1. To assess the associations of fetal and infant growth patterns with childhood asthma symptoms.
2. To assess the associations of fetal exposures with childhood asthma symptoms. The exposures of interest include maternal psychological distress, obesity and weight gain during pregnancy, and C-reactive protein levels.
3. To assess the associations of infant exposures with childhood asthma symptoms. The exposures of interest include breastfeeding duration and exclusiveness, air pollution and tobacco smoke exposure.

GENERAL DESIGN

The studies presented in this thesis were embedded in two population-based prospective cohort studies and a large European collaboration project.

The Generation R Study

The Generation R Study is a population-based prospective cohort study in Rotterdam, the Netherlands, following pregnant women and their children from fetal life onwards (www.generationr.nl)[47]. The study is designed to identify early environmental and genetic causes and causal pathways leading to normal and abnormal growth, development and health during fetal life, childhood and adulthood. Enrolment was aimed in first trimester, but was allowed until birth of the child. In total n=9,778 mothers with a delivery date from April 2002 until January 2006 were enrolled in the study, and response at baseline was 61%. Data collection during each trimester of pregnancy included fetal ultrasounds examinations, detailed physical examinations, biological samples, and questionnaires. Information from midwife and hospital registries was obtained and a sample of cord blood was collected at birth. During the preschool years (from birth until the age of 4 years) information was mainly obtained from postal questionnaires including questions adapted from the International Study on Asthma and Allergy in Childhood (ISAAC)[48]. Growth data was collected at community health centres. At the age of 6 years, asthma diagnosis was obtained by questionnaire. Additional detailed hands-on assessments were performed in a dedicated research centre to measure length, weight, Fraction exhaled Nitric Oxide (FeNO), as a measure of eosinophilic airway inflammation, and airway resistance (Rint) (Figure 1.1.2).

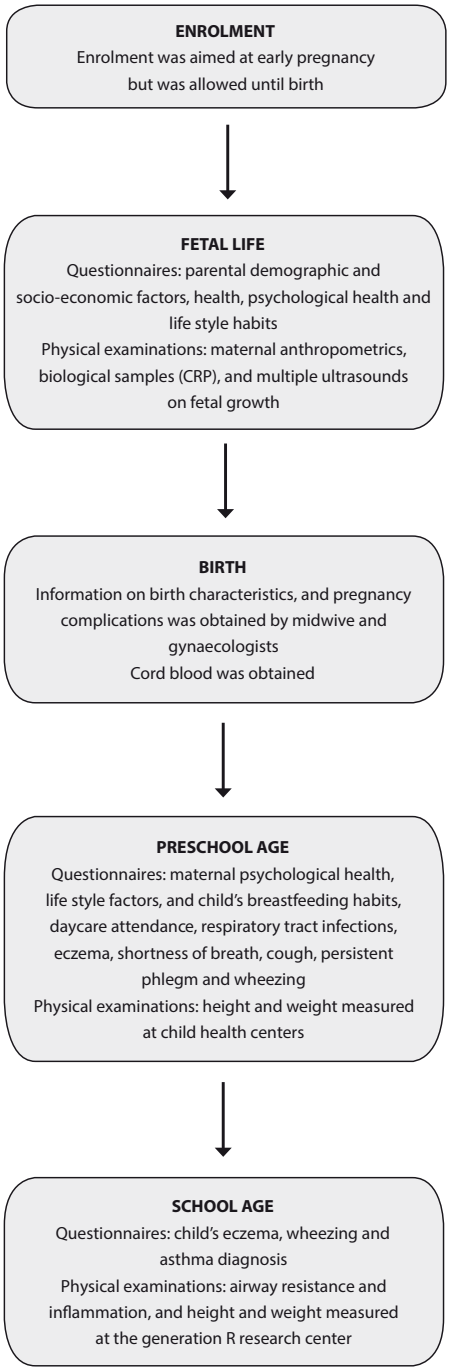

Figure 1.1.2. Overview of the data collection of the Generation R Study used in this thesis.

Avon Longitudinal Study of Parents and Children (ALSPAC)

ALSPAC is a population-based prospective cohort study, based in the United Kingdom (www.bristol.ac.uk/alspac)[49]. In brief, 14,541 pregnant women resident in one of three Bristol-based health districts with an expected delivery date between 1 April 1991 and 31 December 1992 were recruited to participate. Of these women, 14,541 were recruited and gave birth to 14,062 live born children. Detailed information about the children has been collected from questionnaires and clinic visits until the age of 17 years. In adolescence, the diagnosis of current asthma was based on questionnaires, and lung function and bronchial hyperresponsiveness were measured during clinic visits.

CHICOS Consortium

A meta-analysis was conducted within the framework of CHICOS (Child Cohort Research Strategy for Europe), a European consortium (www.chicosproject.eu). The overall aim of CHICOS is to improve child health across Europe by developing an integrated strategy for mother-child cohort research in Europe. European population-based birth- and mother-child cohorts were able to participate in the meta-analysis if they included children from 1989 onwards, had information on at least gestational age and weight at birth, and preschool wheezing or school-age asthma, and were willing and able to exchange original data. We selected European cohorts from both the CHICOS consortium and other existing collaborations.

OUTLINE OF THIS THESIS

Chapter 2 focuses on associations of early growth with childhood asthma. The results of the European meta-analysis on the associations of preterm birth, birth weight, and infant growth with preschool wheezing and school-age asthma are presented in *chapter 2.1*. The associations of fetal and infant growth with preschool asthma symptoms and school-age respiratory morbidity are presented in *chapters 2.2 and 2.3*, respectively. In *chapter 2.4*, the association of childhood growth from birth until the age of 10 year with asthma, bronchial hyperresponsiveness and lung function in adolescence is explored.

In **chapter 3,** the effect of fetal exposures on childhood asthma symptoms are described. *Chapter 3.1 and 3.2* present the influence of maternal distress and weight before and during pregnancy on preschool wheezing, respectively. The associations of C-reactive protein measured during pregnancy and in cord blood with wheezing in preschool children is presented in *chapter 3.3*.

In **chapter 4**, the effect of infant exposures on childhood asthma symptoms are described. The associations of breastfeeding duration and exclusivity, exposure to air pollution and tobacco smoke exposure with asthma symptoms until the age of 4 years are presented in *chapter 4.1 and 4.2*.

The main findings and implications described in this thesis are discussed in the general discussion in **chapter 5**, followed by a summary in **chapter 6**.

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Chapter 2

Early growth and childhood asthma

2.1

Preterm birth, early growth and the risk of childhood asthma:

A meta-analysis of 147,000 children

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Submitted

2.2

Fetal and infant growth and asthma symptoms in preschool children

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Am J Respir Crit Care Med. 2012;185(7):731-7

ABSTRACT

Background Low birth weight is associated with an increased risk of wheezing in childhood. We examined the associations of longitudinally measured fetal and infant growth patterns with the risks of asthma symptoms in preschool children.

Methods This study was embedded in a population-based prospective cohort study among 5,125 children. Second and third trimester fetal growth characteristics (head circumference, femur length, abdominal circumference, weight) were estimated by repeated ultrasounds. Infant growth (head circumference, length, weight) was measured at birth and at the ages of 3, 6, and 12 months. Parental report of asthma symptoms until the age of 4 years was yearly obtained by questionnaires.

Results Both fetal restricted and accelerated growth, defined as a negative or positive change of >0.67 standard deviation score, were not associated with asthma symptoms until the age of 4 years. Accelerated weight gain from birth to 3 months following normal fetal growth was associated with increased risks of asthma symptoms (overall odds ratio (OR) for wheezing: 1.44 (95% Confidence Interval (CI): 1.22, 1.70); shortness of breath: 1.32 (1.12, 1.56); dry cough: 1.16 (1.01, 1.34); persistent phlegm: 1.30 (1.07, 1.58)), but not with eczema: 0.95 (0.80, 1.14)). These associations were independent of other fetal growth patterns and tended to be stronger for children of atopic mothers than for children of non-atopic mothers.

Conclusions Weight gain acceleration in early infancy was associated with increased risks of asthma symptoms in preschool children, independent of fetal growth. Early infancy might be a critical period for the development of asthma.

INTRODUCTION

Low birth weight is associated with increased risks of asthma, chronic obstructive airway disease, and impaired lung function, such as lower FEV1, and FVC in adults[1]. In children, low birth weight is associated with increased risks of respiratory morbidity, including asthma and respiratory tract infections[2], but results are not consistent[3-6]. The developmental plasticity hypothesis suggests that the associations between low birth weight and common diseases in adulthood are explained by early adaptive mechanisms in response to various adverse exposures in fetal and early postnatal life[7]. These adaptive mechanisms might lead to impaired lung development, smaller airways and impaired lung function[8], and might lead to an increased susceptibility of development of respiratory diseases, including asthma and COPD[9-10]. Low birth weight per se is not likely to be the causal factor leading to asthma. The same birth weight might be the result of various growth patterns and different fetal exposures[11]. Information about fetal growth characteristics in different periods of pregnancy enables identification of critical periods for specific exposures and development of asthma in postnatal life[12-13]. Also, children with a low birth weight tend to have a postnatal catch up growth, which has also been suggested to be associated with respiratory morbidity, including childhood asthma[12, 14-15]. Studies so far focused on early growth patterns, and showed inconsistent results. This might partly be due to methodological issues including differences in definitions of fetal and infant growth patterns or asthma-related outcomes and the adjustment for gestational age and other potential confounders.

Therefore, we examined the associations of fetal and infant growth patterns with the risk of asthma symptoms in the first 4 years of life in a population-based prospective cohort study among 5,125 children who were followed up from fetal life. Some of the results of this study has been previously reported in the form of an abstract at the European Respiratory Society Conference 2011[16].

METHODS

Design and setting

This study was embedded in the Generation R Study, a population-based prospective cohort study of pregnant women and their children in Rotterdam, The Netherlands[17]. The study protocol was approved by the Medical Ethical Committee of the Erasmus Medical Centre, Rotterdam. Written informed consent was obtained from all participants. A total of 5,125 children were included for the current analyses (Figure E2.2.1 in the supplement).

Growth characteristics

Fetal growth characteristics were measured in the first trimester (crown-rump length (CRL))[18], and in the second and third trimester (head circumference (HC), abdominal circumference (AC), and femur length (FL))[19-20]. Estimated fetal weight (EFW) was calculated using the Hadlock formula[21-22]. HC, length and weight at birth were obtained from community midwife and hospital registries. Infant growth characteristics (HC, length and weight) were measured at the ages of 3, 6, and 12 months. All growth characteristics were converted into standard deviation scores (SDS) using fetal and infant reference growth charts[19, 22], Growth Analyzer 3.0, Dutch Growth Research Foundation). We calculated growth (change in SDS) between various age intervals. Growth restriction and acceleration (from 2nd trimester to birth and birth to 3 months of age) were defined as a change, either decrease or increase, of more than 0.67 SDS, representing the width of each percentile band on standard growth charts[23-24].

Asthma symptoms

Information on asthma symptoms (wheezing, shortness of breath, dry cough at night, and persistent phlegm (no, yes)) and doctor attended eczema (no, yes) was obtained by questionnaires, adapted from the International Study on Asthma and Allergy in Childhood (ISAAC)[25] at the ages of 1, 2, 3 and 4 years. Response rates for these questionnaires were 71%, 76%, 72%, 73% respectively[26].

Covariates

Maternal anthropometrics were obtained during first visit, education, history of asthma and atopy, smoking habits, parity, and children's ethnicity and pet keeping were obtained by questionnaire, completed by the mother at enrollment. Maternal gestational hypertension, diabetes and children's gestational age and sex were obtained from midwife and hospital registries at birth. Postal questionnaires at the ages of 6 and 12 months provided information about breastfeeding and daycare attendance[17].

Statistical analysis

We used adjusted generalized estimating equations (GEEs) to examine the longitudinal effects of fetal and infant growth and their interaction with each asthma symptom from the age of 1 to 4 years. With GEE analyses, repeatedly measured asthma symptoms over time were analyzed, taking correlations within the same subject into account. We calculated the overall effect (age 1 to 4 years combined) of fetal and infant growth on asthma symptoms. Missing data in covariates and outcomes were imputed using the multiple imputation

procedure[27]. All measures of association are presented as OR with 95% Confidence Intervals (CI). Statistical analyses were performed using Statistical Package of Social Sciences version 17.0 for Windows (SPSS Inc., Chicago, IL, US) and SAS 9.2 (SAS institute, Cary, NC, USA). An extensive description of the methods is provided in the supplement (Text E2.2.1).

RESULTS

Characteristics of children and their mothers are presented in Table 2.2.1. Children were born after median pregnancy duration of 40.1 weeks (range 25.3 – 43.4) with a mean birth weight of 3,440 gram (SD 551 gram) (Table 2.2.1). Wheezing was the most prevalent asthma symptom and its prevalence declined with increasing age (Table E2.2.1 in the supplement).

Table 2.2.1. Characteristics of children and their mothers

	n=5,125
Maternal characteristics	
Age (%)	
<20 years	2.1 (107)
20-25 years	12.2 (624)
25-30 years	26.4 (1,353)
30-35 years	42.4 (2,173)
≥35 years	16.9 (868)
Missing	-
Height (cm)	168.0 (7.5)
Weight (kg)	69.4 (12.8)
Body mass index	
<20 kg/m^2	8.9 (457)
20-25.0 kg/m^2	54.5 (2,791)
25-30.0 kg/m^2	24.9 (1,278)
≥30 kg/m^2	11.1 (568)
Missing	0.6 (31)
Education (%)	
Primary, or secondary	46.7 (2,394)
Higher	48.9 (2,504)
Missing	4.4 (227)
History of asthma (%)	
No	56.7 (2,906)
Yes	31.9 (1,637)
Missing	11.4 (582)

Table 2.2.1. Characteristics of children and their mothers (continued)

	n=5,125
Smoking during pregnancy (%)	
No	76.5 (3,919)
Yes	12.4 (633)
Missing	11.2 (573)
Parity (%)	
0	62.1 (3,181)
1-2	34.3 (1,756)
≥3	3.1 (161)
Missing	0.5 (27)
Gestational hypertension (%)	
No	91.8 (4,704)
Yes	4.1 (208)
Missing	4.2 (213)
Gestational diabetes (%)	
No	96.9 (4,964)
Yes	0.7 (37)
Missing	2.4 (124)
Child characteristics	
Male sex, no (%)	50.1 (2,567)
Gestational age at birth (weeks)	40.1 (37.1-42.1)
Birth weight (grams)	3,440 (551)
Ethnicity (%)	
European	66.8 (3,421)
Non-European	30.7 (1,573)
Missing	2.6 (131)
Breastfeeding (%)	
No	7.2 (370)
Yes	88.6 (4,542)
Missing	4.2 (213)
Day care attendance 1st year (%)	
No	40.1 (2,054)
Yes	43.5 (2,229)
Missing	16.4 (842)
Pet keeping (%)	
No	58.8 (3,015)
Yes	29.6 (1,519)
Missing	11.5 (591)

Values are means (SD), medians (5-95th percentile) or percentages (absolute numbers).

Birth weight and gestational age

We observed from crude analyses that birth weight was inversely associated with the risks of asthma symptoms (Table 2.2.2), but these associations attenuated and became non-significant after adjustment for gestational age (wheezing OR 0.97 (0.92, 1.02), shortness of breath OR 0.96 (0.91, 1.01), dry cough OR 1.01 (0.97, 1.06), persistent phlegm OR 0.93 (0.87, 0.99) and with eczema OR 1.01 (0.96, 1.07)). Similar changes in effect estimates were observed for children with low birth weight (<2500 grams) with and without adjustment for gestational age and the risk of asthma symptoms. As compared to term birth, preterm birth (< 36 weeks of gestational age) was positively associated with the risks of wheezing (OR 1.55 (1.30, 1.84)), shortness of breath (OR 1.54 (1.28, 1.85)) and persistent phlegm (OR 1.30 (1.03, 1.64).

Table 2.2.2. Birth characteristics and asthma symptoms

	Odds ratios (95% Confidence Interval)				
	Wheezing	**Shortness of breath**	**Dry cough**	**Persistent phlegm**	**Eczema**
Birth weight					
Weight (500 grams)	0.92 (0.89, 0.96)***	0.93 (0.89, 0.96)***	1.02 (0.99, 1.06)	0.90 (0.86, 0.95)***	1.01 (0.97, 1.06)
Gestational age adjusted weight (500 grams)	0.97 (0.92, 1.02)	0.96 (0.91, 1.01)	1.01 (0.97, 1.06)	0.93 (0.87, 0.99)*	1.01 (0.96, 1.07)
Low birth weight (<2500 grams)	1.34 (1.12, 1.62)**	1.24 (1.02, 1.52)*	0.87 (0.72, 1.05)	1.32 (1.05, 1.66)*	1.01 (0.81, 1.27)
Gestational age adjusted low birth weight (<2500 grams)	1.07 (0.85, 1.34)	0.99 (0.78, 1.27)	0.91 (0.74, 1.12)	1.05 (0.80, 1.39)	1.05 (0.81, 1.35)
Gestational age					
Gestational age (weeks)	0.94 (0.92, 0.97)***	0.95 (0.93, 0.97)***	1.02 (0.99, 1.04)	0.94 (0.92, 0.97)***	1.01 (0.98, 1.04)
Preterm birth (<37 weeks)	1.55 (1.30, 1.84)***	1.54 (1.28, 1.85)***	0.90 (0.74, 1.08)	1.30 (1.03, 1.64)*	1.00 (0.79, 1.25)

Values are odds ratios (95% Confidence Interval) and, if continuously measured, reflect the risk of asthma symptoms per 500 grams or week of gestational age increase. *P < 0.05, **p < 0.01, ***p < 0.001 using longitudinal generalized estimating equation models. Models were adjusted for maternal age, body mass index, education, history of asthma or atopy, smoking habits, parity, gestational hypertension, gestational diabetes, children's sex, ethnicity, breastfeeding status, daycare attendance and pet keeping.

Fetal and infant growth

No consistent associations of fetal length and weight growth during different trimesters with asthma symptoms were observed (Table 2.2.3). Crown-rump length in 1st trimester (data not shown) and growth of fetal abdominal and head circumference were also not associated with asthma symptoms (Table E2.2.2 in the supplement). Infant weight gain between birth and 3 months, expressed as SDS increase in weight, was positively associated with the risks of wheezing, shortness of breath and persistent phlegm (OR 1.17 (1.11, 1.23), 1.13 (1.08, 1.20), 1.15 (1.08, 1.23), respectively) in the first 4 years of life. Length growth was not associated with any asthma symptom (Table 2.2.3).

Table 2.2.3. Fetal and infant growth (change in SDS) and asthma symptoms

	Overall odds ratios (95% Confidence Interval)				
	Wheezing	**Shortness of breath**	**Dry cough**	**Persistent phlegm**	**Eczema**
Length					
2nd - 3rd trimester n=4,803	1.02 (0.98, 1.07)	1.00 (0.95, 1.05)	0.96 (0.93, 1.00)	0.99 (0.94, 1.05)	0.98 (0.93, 1.03)
3rd trimester - birth n=3,270	0.99 (0.95, 1.03)	1.01 (0.97, 1.06)	0.99 (0.95, 1.03)	0.98 (0.93, 1.03)	1.00 (0.96, 1.05)
birth - 3 months n=2,031	1.02 (0.96, 1.08)	0.99 (0.94, 1.06)	1.03 (0.98, 1.09)	0.98 (0.90, 1.06)	0.98 (0.92, 1.04)
3 - 6 months n=2,619	1.04 (0.95, 1.14)	1.08 (0.98, 1.19)	1.00 (0.92, 1.09)	0.98 (0.86, 1.11)	0.91 (0.83, 1.01)
6 - 12 months n=3,425	0.93 (0.85, 1.01)	0.97 (0.88, 1.06)	0.99 (0.91, 1.06)	1.00 (0.89, 1.12)	0.98 (0.88, 1.08)
Weight					
2nd - 3rd trimester n=4,766	1.04 (0.99, 1.08)	1.01 (0.96, 1.05)	1.00 (0.96, 1.04)	0.99 (0.93, 1.05)	1.04 (0.99, 1.10)
3rd trimester - birth n=5,023	1.00 (0.96, 1.04)	1.02 (0.98, 1.07)	0.99 (0.95, 1.03)	0.95 (0.89, 1.00)	0.99 (0.94, 1.04)
birth - 3 months n=3,558	1.17 (1.11, 1.23)***	1.13 (1.08, 1.20)***	1.04 (1.00, 1.09)	1.15 (1.07, 1.22)***	0.93 (0.88, 0.98)*
3 - 6 months n=3,391	0.97 (0.88, 1.06)	0.96 (0.87, 1.07)	1.04 (0.95, 1.13)	0.91 (0.80, 1.03)	0.88 (0.79, 0.99)*
6 - 12 months n=3,875	0.95 (0.86, 1.04)	0.95 (0.86, 1.04)	0.96 (0.89, 1.04)	0.90 (0.79, 1.02)	0.90 (0.81, 1.00)*

Values are odds ratios (95% Confidence Interval) and reflect the risk of asthma symptoms per standard deviation score (SDS) increase of length and weight. *P < 0.05, **p < 0.01, ***p<0.001 using longitudinal generalized estimating equation models. Models were adjusted for maternal age, body mass index, education, history of asthma or atopy, smoking habits, parity, gestational hypertension, gestational diabetes, children's sex, gestational age, ethnicity, breastfeeding status, daycare attendance and pet keeping.

Further exploration of fetal and infant growth patterns showed that, as compared to children with a normal fetal and infant growth pattern, those with a normal fetal, but accelerated infant growth pattern had an increased risk of wheezing (OR 1.44 (1.22, 1.70)); shortness of breath (OR 1.32 (1.12, 1.56)); dry cough (OR 1.16 (1.01, 1.34)); and persistent phlegm (OR 1.30 (1.07, 1.58)), but not of eczema (Figure 2.2.1A-E). We observed a protective effect of a restricted fetal and infant growth pattern, compared to a normal growth pattern, for wheezing and shortness of breath (Figure 2.2.1A-B). The results did not materially change when preterm born infants were excluded from the analyses or when the associations of fetal and infant growth patterns for each year separately were analyzed (Table E2.2.3 in the supplement). Analysis stratified for maternal atopy showed that the effect estimates tended to be stronger for atopic mothers than non-atopic mothers, but the p for interaction was not significant (Figure E2.2.2 in the supplement).

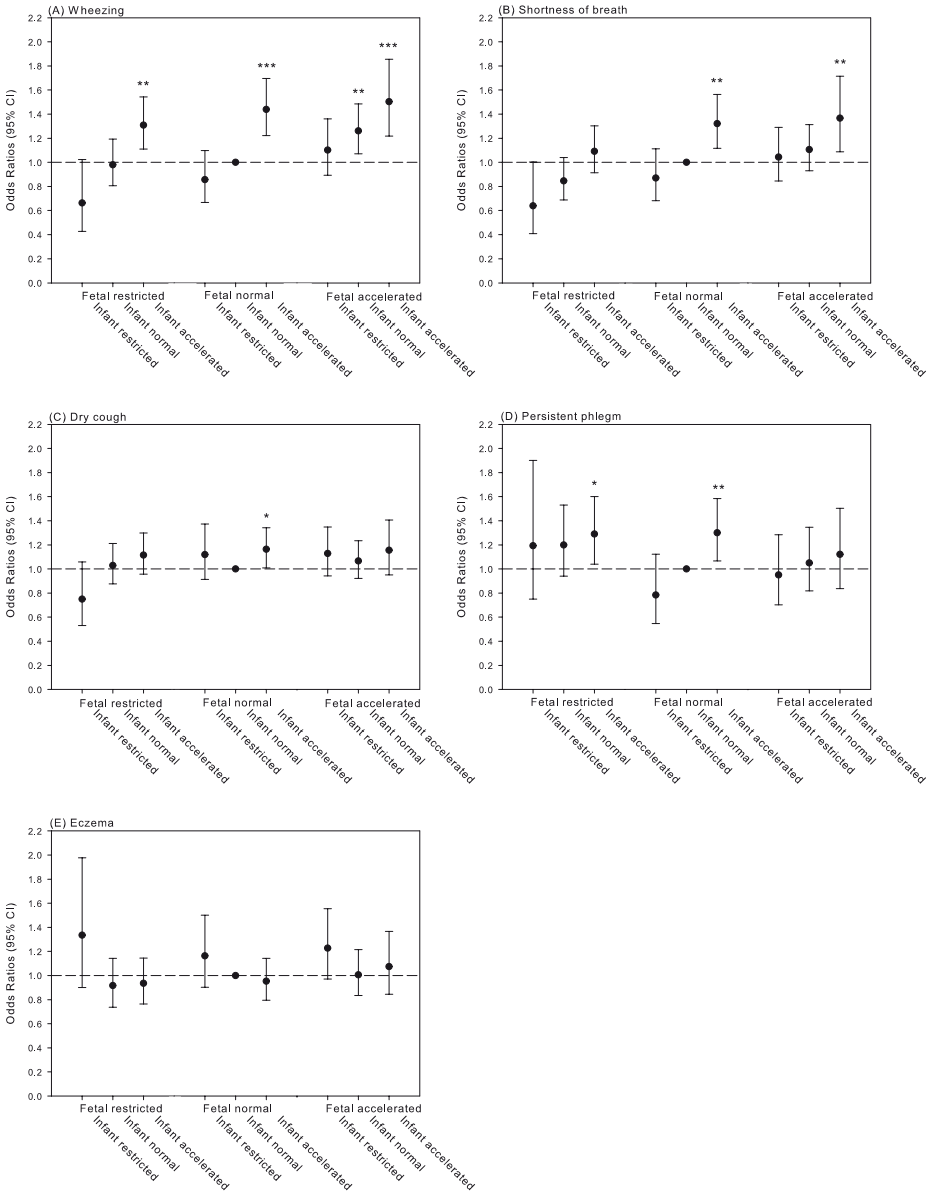

Figure 2.2.1. Weight growth patterns and asthma symptoms

Values are odds ratios (95% Confidence Interval). Normal fetal and normal infant growth pattern is used as reference category. *P < 0.05, **p < 0.01, ***p < 0.001 based on longitudinal generalized estimating equation models. Models were adjusted for maternal age, body mass index, education, history of asthma or atopy, smoking habits, parity, gestational hypertension, gestational diabetes, children's sex, gestational age, ethnicity, breastfeeding status, daycare attendance and pet keeping.

DISCUSSION

Our results suggest that fetal growth during different periods of pregnancy was not associated with the overall risk of asthma symptoms until the age of 4 years. However, we observed associations between early infant growth acceleration and increased risks of asthma symptoms. These associations seem to be independent of fetal growth.

Birth weight and preterm birth

Previous child cohort studies reported inconsistent associations of birth weight with wheezing or asthma in childhood[2-5]. After adjustment for gestational age, we only observed an association of birth weight with persistent phlegm, not with wheezing or other asthma symptoms. Differences with previous published studies might be due to our assessment of the outcomes at a young age at which an asthma diagnosis is not possible and asthma symptoms are common, but nonspecific and often transient[28-29]. Also, it might be that not low birth weight but preterm birth is the main risk factor for increased risks of asthma symptoms[30-31]. This is supported by our consistent associations of gestational age and preterm birth with wheezing, shortness of breath, and persistent phlegm.

Fetal and infant growth

Earlier studies used birth weight as a proxy for fetal growth[4-6, 32] and showed inconsistent associations between either low or high birth weight and the risk of asthma symptoms, asthma diagnosis or a reduced lung function. Assessing fetal and infant growth characteristics related to birth weight might help to identify specific critical periods. Two recent studies focused on the associations of fetal growth characteristics in different trimesters and the risk of childhood asthma and atopy[12-13]. Pike et al. observed no association of fetal growth characteristics and 'ever wheezing' until the age of 3 years[12]. The authors did observe an association of abdominal circumference growth between 19 and 34 weeks with atopic wheezing (relative risk (95% CI) 0.80 (0.65, 1.00)) and of head circumference growth between 11 and 19 weeks and non-atopic wheezing (relative risk 0.90 (0.81, 1.00)). They suggest that the association with atopic wheezing might be the effect of an impaired thymic development, while non-atopic wheezing might be caused by mechanical changes in growth restricted children. Turner et al. recently showed that crown-rump length in first trimester was inversely associated with 'ever wheezing' (OR 0.96 (0.93, 0.99) at the age of 5 years and diagnosed asthma (OR 0.94 (0.89, 0.99)) and lung function at the ages of 5 and 10 years[13], independent of atopy. In our study, in a larger number of children, we used ultrasound measurements in each trimester of pregnancy and observed no associations of fetal growth, including multiple growth parameters and patterns, with asthma symptoms in preschool children. We were however not able to differentiate between atopic

and non-atopic children as we had no direct measures of sensitization. When we stratified our analysis for atopic and non-atopic mothers, a proxy for atopic status of children[33], the effect estimates of the association of fetal growth characteristics and patterns with asthma symptoms tended to be stronger for children with atopic mothers than non-atopic mothers.

Previous studies in children reported a slightly increased risk of wheezing (ORs up to 1.05 (1.01, 1.09) and reduced lung function for weight gain in the first year and no associations with length growth[12, 15, 34-35]. In adulthood no effect on airway obstruction, but a modest reduction of lung volume was observed if children had either a lower or higher weight gain in the first three years of life[36]. Due to our extensive anthropometric measurements after birth, we were able to specify the critical time period in which weight gain had an effect on asthma symptoms and found that accelerated weight gain between birth and 3 months of age was associated with asthma symptoms in childhood. Furthermore, we observed that this effect was independent of fetal growth. These results are in line with Pike et al. who observed that low 3rd trimester abdominal circumference with high weight gain and adiposity in the first 6 months was associated with a higher proportion of atopic wheezing[12]. Whether their highest weight gain group in the first 6 months showed consistently increased effect estimates for wheezing, independent of fetal growth, was not presented.

Our results suggest that the effect of infant weight gain on asthma symptoms is not due to 'catch up' growth of fetal growth-restricted infants only. The underlying mechanisms are unclear. Accelerated weight growth in the first three months of life might adversely affect lung growth, including a change in alveolar numbers, lung weight, and the developing immune system[37-39]. It was suggested that early infant weight gain is associated with a higher BMI in childhood with overweight and obesity in later life[24, 40] and subsequently may have a modifying effect on asthma, asthma symptoms and lung function during childhood and on the long term[41-42]. Also, adverse changes of the immune system in early life due to increased weight gain might affect the development of childhood asthma[38-39, 43].

We observed that children with fetal and infant growth deceleration had a decreased risk of wheezing and shortness of breath up to the 4th year. A protective effect of fetal and infant growth deceleration was also observed in an earlier study on atopic wheezing, but not for non-atopic wheezing[12]. Pike et al observed that children with a normal fetal growth and a restricted infant growth tended to have a lower risk of wheezing than children with normal infant growth[12]. The underlying mechanisms for these associations were not shown. According to animal studies, it might be that fetal growth restriction lead to impaired growth of bronchial walls, affecting the airway compliance, alterations in mucus producing tissues, decrease in number of alveoli, thicker interalveolar septa and a greater volume density of lung tissue[44-46]. However, some of these adaptations resolved within weeks after birth. Hence, we speculate that at least a part of the effects on the lungs in children with a restricted fetal growth is catched up before the age of 1 to 4 years, and this might have reduced our effect estimates. If fetal growth indeed leads to respiratory symptoms via an effect on lung development, this might be of influence later in childhood.

Strengths and limitations

This study was embedded in a population-based prospective cohort study with a large number of subjects being studied from early fetal life onwards with detailed and frequently prospectively measured information about fetal and infant anthropometrics. We adjusted for a large number of confounders and the results did not differ between non-imputed and imputed analyses. Non-response would lead to biased effect estimates if the associations of fetal and infant growth with asthma symptoms would be different between those included and not included in the analyses. However, this seems unlikely because biased estimates mainly arise from loss to follow-up rather than from non-response at baseline[47]. Although we used the established Hadlock formula for calculation of the estimated fetal weight, we cannot exclude that there may be a random measurement error in this estimation, especially in late third trimester, which might have led to underestimation of the effect estimates. Although, we showed that the intra and inter observer intraclass correlations for assessing fetal growth in early pregnancy were high, measurements error is expected to be higher for fetal growth measurements than for infant growth measurements[20]. We categorized growth patterns by a change of >0.67 SD, a well-known recognized threshold value in studies on growth[23]. Other studies categorized fetal and infant growth by separating groups in tertiles[12], or used a longer time interval for the SD change which might explain some differences with our results[48]. The main outcomes in our study were self-reported symptoms. This method is widely accepted in epidemiological studies and reliably reflects the incidence of asthma symptoms in young children[49]. In preschool children a diagnosis of asthma is based on symptoms[50]. Objective tests, including spirometry or bronchial hyperresponsiveness, are difficult to perform in young children, and have limited applicability. We were not able to assign phenotypes based on patterns of wheezing including transient, late onset, persistent or other wheezing phenotypes, due to the follow-up of children until the age of 4 years only[28-29]. Follow up studies at older ages which include more detailed assessments of asthma and atopy phenotypes are needed. We did not apply Bonferroni correction since we used repeated measurements analyses and correlated outcomes of both the exposure and outcomes. However, we observed consistent associations of infant weight gain independent of fetal growth with all asthma symptoms.

In conclusion, our results suggest that not fetal growth, but accelerated growth in the first three months of life is associated with an increased risk of asthma symptoms during the first 4 years of life. The results of this study should be considered as hypothesis generating. Further studies are needed to replicate these findings and to explore underlying mechanisms of the effect of growth acceleration on respiratory health, in particular on the various phenotypes of asthma in later life.

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Supplements

TEXT E2.2.1.

Growth characteristics

Fetal growth characteristics Fetal ultrasound examinations were carried out in a dedicated research center in each trimester of pregnancy. The ultrasound examinations were performed using an Aloka® model SSD-1700 (Tokyo, Japan) or the ATL-Philips® Model HDI 5000 (Seattle, WA, USA). These examinations were used for both establishing gestational age and assessing fetal growth characteristics[1]. In the first trimester, we used crown-rump length to assess fetal growth only in mothers with a known and reliable first day of the last menstrual period and a regular menstrual cycle of 28 (range: 24–32) days and who had crown-rump length measured between a gestational age of 10 and 15 wk[2]. The first day of the last menstrual period was obtained from the referring letter from the community midwife or hospital. This date was confirmed with the subjects at the ultrasound visit, and additional information on the regularity and duration of cycle was obtained. Because using the last menstrual period has several limitations, such as the large number of mothers who do not know the exact date of their last menstrual period or have irregular menstrual cycles, gestational age was established by fetal ultrasound examination for the second- and third-trimester growth measurements. In the second and third trimesters of pregnancy, we measured head circumference (HC), abdominal circumference (AC), and femur length (FL) to the nearest millimeter using standardized ultrasound procedures[1, 3]. Estimated fetal weight was subsequently calculated by using the Hadlock formula ($\log_{10}$ EFW = 1.5662 - 0.0108 (HC) + 0.0468 (AC) + 0.171 (FL) + 0.00034 (HC)^2 - 0.003685 (AC*FL))[4-5]. Standard deviation scores (SDS) for all fetal growth characteristics were constructed[1, 5]. We calculated fetal growth (change in SDS) for HC, AC, FL and EFW between the various trimesters of pregnancy. Fetal growth (between 2nd trimester and birth) restriction and acceleration were defined as a change, either decrease or increase, of more than 0.67 SDS, which represents the width of each percentile band on a standard growth charts[6].

At birth, information on head circumference, length and weight of the infants was obtained from community midwife and hospital registries. Birth length was only available in 3,313 individuals, since this is not routinely measured in obstetric practices in The Netherlands. Gestational age adjusted standard deviation scores for length and weight at birth were constructed using reference growth standards[5].

Infant growth characteristics Infant growth was measured at the Community Health Centers according to a standard schedule and procedures by a well-trained staff at the ages of 3 months (range: 3.00-3.96 months), 6 months (range: 5.01-9.96 months), and 12 months (range: 10.00-12.97 months). Length was determined in supine position to the nearest

millimeter using a neonatometer. Weight was measured using a mechanical personal scale (SECA). Standard deviation scores for postnatal length, and weight were obtained using reference growth charts (Growth Analyzer 3.0, Dutch Growth Research Foundation). We calculated infant growth (change in SDS) from birth to 3 months, 3 to 6 months and 6 to 12 months of age. We used the same definition for infant growth restriction and acceleration (between birth and 3 months of age) as described above for fetal growth.

Covariates

Information on maternal anthropometrics, history of asthma and atopy, children's ethnicity and pet keeping were obtained by questionnaire, completed by the mother at enrollment. Socio-economical status was assessed using the highest educational level achieved by the mother. Information about active maternal smoking was obtained by postal questionnaires sent in first, second and third trimester of pregnancy and combined into smoking (no, yes)[7]. We used parity as a proxy for siblings, the correlation between those variables was good (kappa = 0.896). Maternal gestational hypertension, diabetes and gestational age and sex of the children were obtained from midwife and hospital registries at birth. Postal questionnaires at the ages of 6 and 12 months provided information about breastfeeding and daycare attendance[7].

Statistical analysis

We used generalized estimating equations (GEEs) to examine the longitudinal effects of fetal and infant growth with the risk of asthma symptoms at the ages of 1, 2, 3 and 4 years These models take into account the correlations between repeated measurements within the same subject. We used a compound symmetry matrix, as we assumed that every observation of a subject was equally correlated to any other observation of that subject. To observe if there is a specific fetal growth pattern which might explain associations in infant growth, we combined fetal and infant growth restriction, normal and accelerated growth into a new variable representing 9 different growth patterns. Fetal growth was defined from 2nd trimester to birth and infant growth was defined from birth to the age of 3 months. Thereafter, we stratified our analyses for maternal history of atopy, as a proxy for atopy in the children. The models were adjusted for potential confounders including maternal age, body mass index, education, history of asthma or atopy, smoking habits and parity, children's sex, gestational age at birth, ethnicity, breastfeeding status, daycare attendance and pet keeping. Confounders were included in our statistical models based on literature, if they were associated with both the determinant and the outcome or if they changed the effect estimates with ≥10%. The percentages of missing values within the population for analysis were lower or near to 10%, except for daycare attendance (16%). Missing data in the covariates and outcomes

were imputed with multiple imputations using chained equations, which are used to select the most likely value for a missing response. The best predictor for an asthma symptom is another asthma symptom or the same type of symptom at a different age. Therefore, at least one other asthma symptom was available in our population for analysis to predict other asthma symptoms correctly. Twenty-five new datasets were created by imputation based on all covariates and outcomes in the model plus paternal age, educational level and history of asthma or atopy[8]. All datasets were analyzed separately after which results were combined. No differences in results were observed between analyses with imputed missing data or complete cases only. We only present the results based on imputed datasets. All measures of association are presented as an overall odds ratios (OR) (effect of age 1 to 4 years combined) with their 95% Confidence Intervals (CI). Statistical analyses were performed using the Statistical Package of Social Sciences version 17.0 for Windows (SPSS Inc., Chicago, IL, US) and SAS 9.2 (SAS institute, Cary, NC, USA).

Table E2.2.1. Prevalence of asthma symptoms

	Age 1 year	Age 2 years	Age 3 years	Age 4 years
	n=4,566	***n=4,359***	***n=4,041***	***n=4,048***
Wheezing	*n=4,286*	*n=4,271*	*n=3,973*	*n=3,974*
No	70.9 (3,040)	80.0 (3,417)	87.4 (3,473)	87.1 (3,461)
Yes	29.1 (1,246)	20.0 (854)	12.6 (500)	12.9 (513)
Shortness of breath	*n=4,287*	*n=4,289*	*n=3,982*	*n=3,991*
No	78.1 (3,348)	82.4 (3,532)	88.4 (3,522)	89.5 (3,570)
Yes	21.9 (939)	17.6 (757)	11.6 (460)	10.5 (421)
Dry cough	*n=4,236*	*n=4,297*	*n=3,932*	*n=3,979*
No	77.5 (3,282)	75.9 (3,262)	76.2 (2,998)	73.3 (2,917)
Yes	22.5 (954)	24.1 (1,035)	23.8 (934)	26.7 (1,062)
Persistent phlegm	*n=4,226*	*n=4,266*	*n=4,006*	*n=4,018*
No	86.5 (3,657)	90.2 (3,846)	93.3 (3,736)	92.8 (3,729)
Yes	13.5 (569)	9.8 (420)	6.7 (270)	7.2 (289)
Eczema	*n=4,491*	*n=4,185*	*n=3,873*	*n=3,825*
No	80.9 (3,635)	85.9 (3,594)	90.7 (3,511)	92.0 (3,519)
Yes	16.7 (856)	14.1 (591)	9.3 (362)	8.0 (306)

Values are shown in % (absolute numbers).

Table E2.2.2. Fetal and infant growth (change in SDS) and asthma symptoms

	Odds Ratios of overall asthma symptoms (95% Confidence Interval)				
	Wheezing	**Shortness of breath**	**Dry cough**	**Persistent phlegm**	**Eczema**
Abdominal circumference					
2nd - 3rd trimester *n=4,794*	1.04 (1.00, 1.08)	1.01 (0.97, 1.06)	1.02 (0.99, 1.06)	1.00 (0.95, 1.05)	1.05 (1.01, 1.10)*
Head circumference					
2nd - 3rd trimester *n=4,754*	1.04 (1.00, 1.08)	1.05 (1.01, 1.10)*	1.03 (0.99, 1.06)	0.98 (0.93, 1.04)	1.01 (0.97, 1.06)
3rd trimester - birth *n=2,790*	0.98 (0.94, 1.03)	0.99 (0.94, 1.04)	0.99 (0.95, 1.03)	1.00 (0.95, 1.07)[a]	1.00 (0.95, 1.05)[a]
birth - 3 months *n=2,019*	1.07 (1.02, 1.14)**	1.06 (1.00, 1.13)	1.02 (0.97, 1.07)	1.01 (0.94, 1.08)[a]	1,00 (0.94, 1.07)[a]
3 - 6 months *n=3,261*	0.96 (0.86, 1.06)	0.95 (0.85, 1.06)	0.95 (0.87, 1.05)	0.96 (0.84, 1.09)	0.88 (0.78, 0,99)*
6 - 12 months *n=3,719*	0.98 (0.90, 1.07)	0.97 (0.88, 1.06)	1.03 (0.95, 1.11)	0.96 (0.85, 1.07)	0.96 (0.87, 1.06)

Values are odds ratios (95% Confidence Interval) and reflect the risk of asthma symptoms per standard deviation score (SDS) increase of abdominal or head circumference. *P < 0.05, **p < 0.01, ***p<0.001 using longitudinal generalized estimating equation models. Models were adjusted for maternal age, body mass index, education, history of asthma or atopy, smoking habits, parity, gestational hypertension, gestational diabetes, children's sex, gestational age, ethnicity, breastfeeding status, daycare attendance and pet keeping.

[a]not adjusted for gestational diabetes due to not enough cases in the model

Table E2.2.3. Fetal and infant growth patterns and asthma symptoms per year

		Odds ratios (95% Confidence Interval)			
		Age 1 year	**Age 2 years**	**Age 3 years**	**Age 4 years**
Growth					
			Wheezing		
Fetal restricted	Infant restricted	0.84 (0.43, 1.63)	0.39 (0.14, 1.07)	0.84 (0.34, 2.09)	0.55 (0.20, 1.47)
	Infant normal	0.91 (0.65, 1.26)	0.95 (0.66, 1.36)	1.40 (0.92, 2.13)	0.93 (0.60, 1.44)
	Infant accelerated	1.43 (1.09, 1.87)**	1.36 (0.99, 1.86)	1.27 (0.85, 1.90)	0.98 (0.68, 1.42)
Fetal normal	Infant restricted	0.99 (0.67, 1.46)	0.85 (0.53, 1.35)	0.93 (0.52, 1.64)	0.49 (0.27, 0.91)*
	Infant normal	*Reference*	*Reference*	*Reference*	*Reference*
	Infant accelerated	1.43 (1.09, 1.88)*	1.54 (1.15, 2.05)**	1.53 (1.07, 2.20)*	1.21 (0.85, 1.73)
Fetal accelated	Infant restricted	1.36 (0.97, 1.91)	0.78 (0.51, 1.21)	1.16 (0.72, 1.87)	1.03 (0.65, 1.63)
	Infant normal	1.38 (1.05, 1.81)*	1.28 (0.95, 1.74)	1.29 (0.89, 1.89)	0.91 (0.63, 1.31)
	Infant accelerated	1.49 (1.05, 2.11)*	1.66 (1.14, 2.42)**	1.46 (0.90, 2.35)	1.29 (0.83, 2.02)
			Shortness		
Fetal restricted	Infant restricted	0.74 (0.36, 1.52)	0.47 (0.20, 1.14)	0.73 (0.27, 1.99)	0.64 (0.25, 1.67)
	Infant normal	0.87 (0.61, 1.24)	0.66 (0.45, 0.98)	1.29 (0.84,. 1.99)	0.80 (0.49, 1.29)
	Infant accelerated	1.16 (0.86, 1.56)	0.96 (0.70, 1.33)	1.28 (0.84, 1.95)	0.80 (0.49. 1.29)
Fetal normal	Infant restricted	0.94 (0.62, 1.43)	0.79 (0.50, 1.24)	1.07 (0.62, 1.84)	0.70 (0.39, 1.26)
	Infant normal	*Reference*	*Reference*	*Reference*	*Reference*
	Infant accelerated	1.20 (0.90, 1.61)	1.30 (0.97, 1.74)	1.55 (1.05, 2.30)*	1.45 (1.00, 2.09)*
Fetal accelated	Infant restricted	1.12 (0.78, 1.62)	0.92 (0.62, 1.36)	1.18 (0.74, 1.91)	0.99 (0.61, 1.61)
	Infant normal	1.21 (0.90, 1.62)	0.98 (0.72, 1.34)	1.36 (0.93, 2.00)	0.89 (0.60, 1.33)
	Infant accelerated	1.56 (1.07, 2.26)*	1.24 (0.83, 1.84)	1.47 (0.87, 2.48)	1.11 (10.66, 1.86)
			Cough		
Fetal restricted	Infant restricted	0.90 (0.46, 1.76)	0.73 (0.36, 1.48)	0.62 (0.32, 1.22)	0.77 (0.40, 1.49)
	Infant normal	1.22 (0.88, 1.68)	1.06 (0.76, 1.48)	0.96 (0.69, 1.34)	0.93 (0.67, 1.27)
	Infant accelerated	1.16 (0.87, 1.56)	1.12 (0.83, 1.49)	0.97 (0.72, 1.30)	1.23 (0.93, 1.62)
Fetal normal	Infant restricted	1.22 (0.82, 1.81)	1.44 (0.97, 2.12)	1.06 (0.71, 1.56)	0.86 (0.58, 1.28)
	Infant normal	*Reference*	*Reference*	*Reference*	*Reference*
	Infant accelerated	1.11 (0.84, 1.48)	1.32 (1.02, 1.72)*	0.98 (0.74, 1.30)	1.26 (0.96, 1.65)
Fetal accelated	Infant restricted	1.31 (0.92, 1.86)	1.28 (0.90, 1.83)	0.86 (0.59, 1.24)	1.13 (0.80, 1.61)
	Infant normal	1.13 (0.84, 1.50)	1.17 (0.88, 1.55)	0.94 (0.71, 1.24)	1.06 (0.80, 1.41)
	Infant accelerated	1.20 (0.82, 1.76)	1.24 (0.86, 1.79)	1.08 (0.76, 1.55)	1.11 (0.77, 1.62)

Table E2.2.3. Fetal and infant growth patterns and asthma symptoms per year (continued)

		Odds ratios (95% Confidence Interval)			
		Age 1 year	**Age 2 years**	**Age 3 years**	**Age 4 years**
		Phlegm			
Fetal restricted	Infant restricted	1.33 (0.62, 2.86)	0.91 (0.34, 2.38)	0.92 (0.31, 2.75)	1.74 (0.70, 4.34)
	Infant normal	1.28 (0.86, 1.93)	1.22 (0.78, 1.92)	0.90 (0.49,. 1.64)	1.30 (0.75, 2.26)
	Infant accelerated	1.32 (0.93, 1.88)	1.10 (0.73, 1.66)	1.25 (0.76, 2.06)	1.60 (0.98, 2.61)
Fetal normal	Infant restricted	0.89 (0.51, 1.55)	0.89 (0.47, 1.70)	0.59 (0.23, 1.50)	0.52 (0.19, 1.42)
	Infant normal	*Reference*	*Reference*	*Reference*	*Reference*
	Infant accelerated	1.36 (0.97, 1.90)	1.18 (0.80, 1.74)	1.45 (0.91, 2.31)	1.23 (0.76, 1.99)
Fetal accelated	Infant restricted	0.86 (0.52, 1.41)	0.83 (0.46, 1.52)	0.77 (0.38, 1.56)	1.64 (0.91, 2.96)
	Infant normal	0.93 (0.62, 1.40)	1.09 (0.70, 1.70)	1.20 (0.73, 1.99)	1.10 (0.63, 1.94)
	Infant accelerated	1.13 (0.69, 1.84)	1.13 (0.66, 1.94)	1.06 (0.54, 2.06)	1.14 (0.57, 2.25)
		Eczema			
Fetal restricted	Infant restricted	2.10 (1.17, 3.74)*	1.18 (0.56, 2.48)	0.52 (0.13, 2.09)	0.63 (0.20, 2.02)
	Infant normal	0.98 (0.69, 1.38)	0.87 (0.58, 1.31)	0.84 (0.51, 1.39)	0.90 (0.54, 1.51)
	Infant accelerated	0.93 (0.68, 1.27)	0.99 (0.70, 1.39)	0.98 (0.62, 1.55)	0.79 (0.49, 1.26)
Fetal normal	Infant restricted	1.17 (0.77, 1.78)	1.11 (0.69, 1.77)	1.22 (0.67, 2.22)	1.20 (0.67, 2.15)
	Infant normal	*Reference*	*Reference*	*Reference*	*Reference*
	Infant accelerated	1.01 (0.75, 1.36)	0.92 (0.66, 1.28)	0.93 (0.62, 1.40)	0.84 (0.55, 1.28)
Fetal accelated	Infant restricted	1.76 (1.24, 2.50)**	0.98 (0.62, 1.54)	0.85 (0.47, 1.54)	0.65 (0.32, 1.32)
	Infant normal	1.00 (0.73, 1.38)	0.89 (0.63, 1.27)	1.28 (0.86, 1.90)	0.97 (0.63, 1.50)
	Infant accelerated	1.11 (0.75, 1.64)	1.11 (0.73, 1.71)	0.89 (0.50, 1.58)	1.06 (0.61, 1.85)

Values are odds ratios (95% Confidence Interval). Normal fetal and normal infant growth pattern is used as reference category.
* $p < 0.05$, ** $p < 0.01$ and ***$p < 0.001$ based on longitudinal generalized estimating equation models. Models were adjusted for maternal age, body mass index, education, history of asthma or atopy, smoking habits, parity, gestational hypertension, gestational diabetes, children's sex, gestational age, ethnicity, breastfeeding status, daycare attendance and pet keeping.

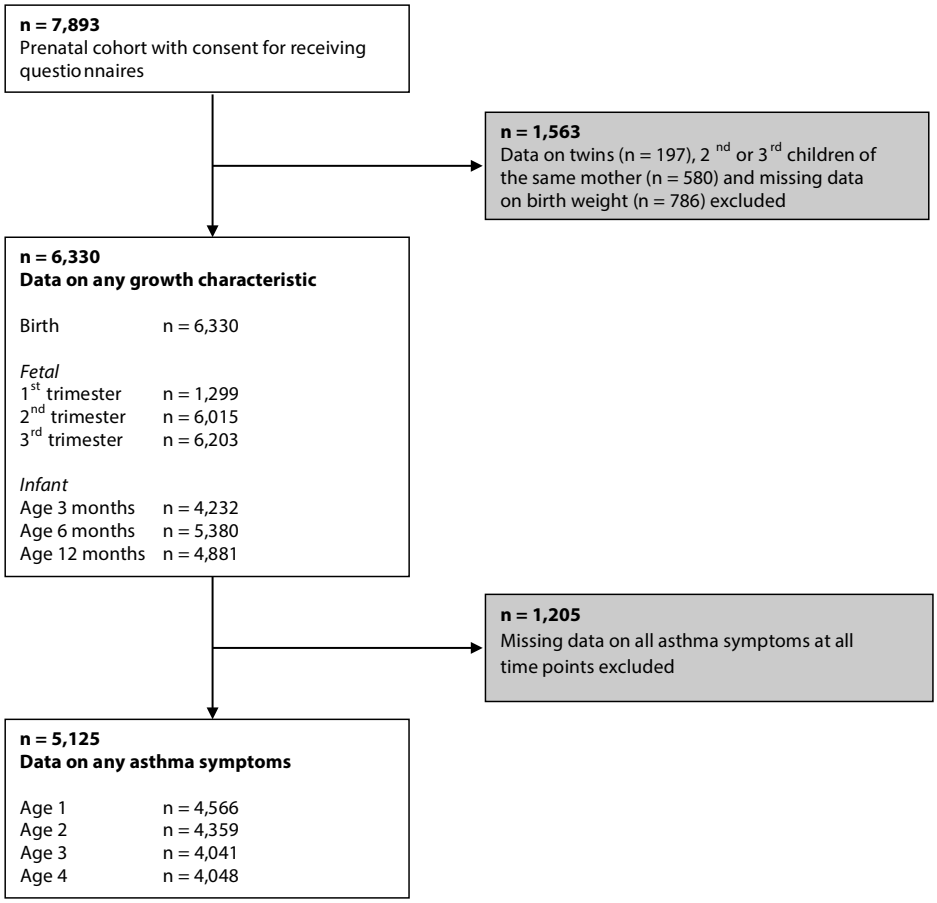
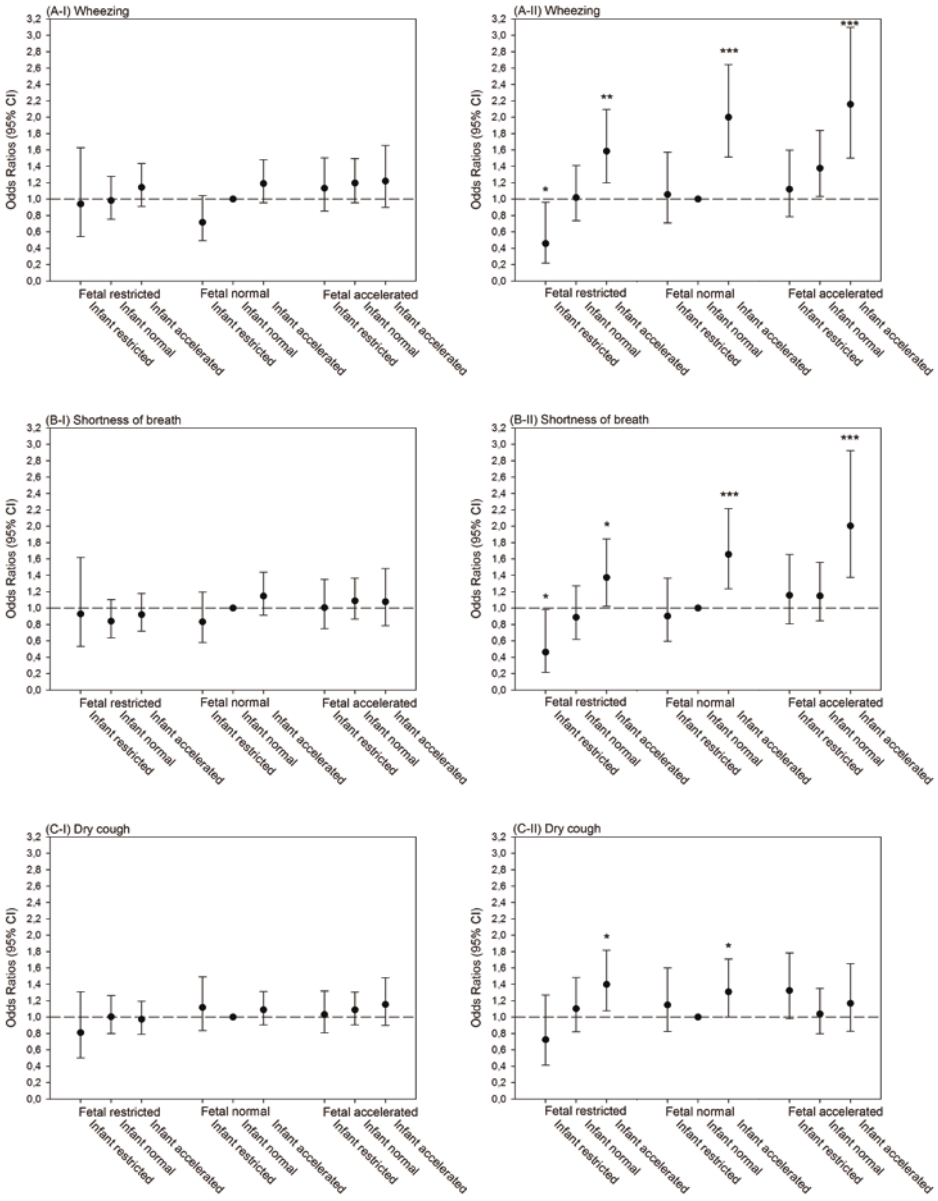


Figure E2.2.1. Flow chart of participants in study

Fetal growth characteristics include crown-rump length (1st trimester), head circumference, femur length, abdominal circumference and calculated estimated fetal weight (2nd and 3rd trimester). Birth and infant growth characteristics include head circumference, length, weight and calculated body mass index (birth, 3, 6 and 12 months).


(A-I) Wheezing
(A-II) Wheezing
(B-I) Shortness of breath
(B-II) Shortness of breath
(C-I) Dry cough
(C-II) Dry cough
Odds Ratios (95% CI)
Fetal restricted
Fetal normal
Fetal accelerated
Infant restricted
Infant normal
Infant accelerated

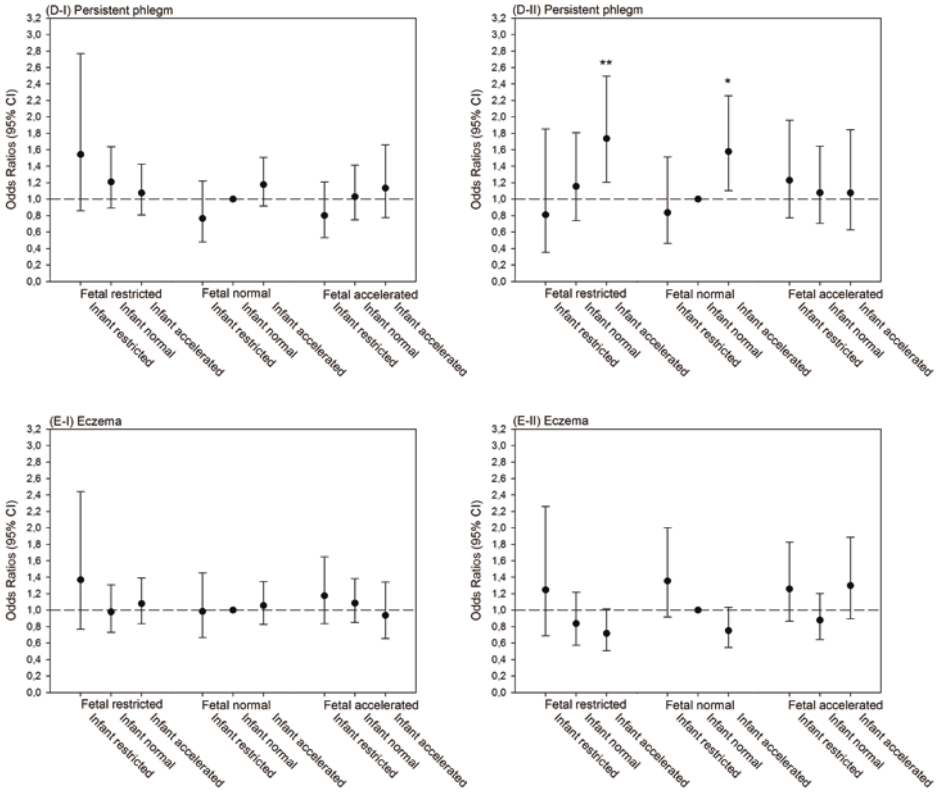


Figure E2.2.2. Weight growth patterns and asthma symptoms according to maternal atopy status.
Values are odds ratios (95% Confidence Interval). I = no history of maternal atopy, II = history of maternal atopy. Normal fetal and normal infant growth is used as reference category. *p < 0.05, *p < 0.01 and ***p<0.001 based on longitudinal generalized estimating equation models. Models were adjusted for maternal age, body mass index, education, history of asthma, smoking habits, parity, gestational hypertension, gestational diabetes, children's sex, gestational age, ethnicity, breastfeeding status, daycare attendance and pet keeping.

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2.3

Early growth patterns associated with school-age respiratory outcomes

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Submitted

2.4

Influence of childhood growth on asthma and lung function in adolescence

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Submitted

Chapter 3

Fetal exposures and childhood asthma

3.1

Parental psychological distress during pregnancy and wheezing in preschool children

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J Allergy Clin Immunol. 2013. Epub 2013/06/20 doi:10.1016/j.jaci.2013.04.044

ABSTRACT

Background Maternal psychological distress during pregnancy might affect fetal lung development, and subsequently predispose children to childhood asthma.

Objective To assess the associations of maternal psychological distress during pregnancy with early childhood wheezing.

Methods Population-based prospective cohort study among 4,848 children. We assessed maternal and paternal psychological distress at 2nd trimester of gestation and 3 years after delivery, and maternal psychological distress at 2 and 6 months after delivery by the Brief Symptom Inventory questionnaire. Wheezing of the children was annually examined by questionnaires from 1 to 4 years. Physician-diagnosed ever asthma was reported at 6 years.

Results Mothers with psychological distress during pregnancy had increased odds of wheezing in their children from 1 to 4 years of life (OR, 1.60; 95% CI, 1.32 to 1.93 for overall distress, OR, 1.46; 95% CI, 1.20 to 1.77 for depression, and OR, 1.39; 95% CI, 1.15 to 1.67 for anxiety). We observed similar positive associations with number of wheezing episodes, wheezing patterns, and physician-diagnosed asthma at 6 years. Paternal distress during pregnancy and maternal and paternal distress after delivery did not affect these results and were not associated with childhood wheezing.

Conclusion Maternal psychological distress during pregnancy is associated with increased odds of wheezing of their child during the first 6 years of life, independent of paternal psychological distress during pregnancy and maternal and paternal psychological distress after delivery. These results suggest a possible intrauterine programming effect of maternal psychological distress leading to respiratory morbidity.

INTRODUCTION

Abnormal fetal lung- and immune development in response to adverse intra-uterine exposures may increase the risk of asthma and atopic disorders in childhood and adulthood[1,2]. Maternal psychological distress during pregnancy is one of these exposures that may specifically lead to developmental adaptations of the hypothalamic-pituitary-adrenal axis, the autonomic nervous system, the lung structure and function, and immune responses in the offspring[3-8]. However, any association between maternal psychological distress during pregnancy and childhood wheezing might also be explained by other mechanisms such as social, behavioural, or environmental factors. From both an etiological and a prevention perspective, it is important to explore the role of intrauterine mechanisms in this association. We used the information of paternal psychological distress during pregnancy to address confounding as described previously[9-11]. Stronger effect estimates for the association of maternal than for paternal psychological distress during pregnancy with childhood wheezing would indicate intrauterine mechanisms. Similar associations of maternal and paternal psychological distress during pregnancy with childhood wheezing would indicate that these associations are not driven by direct intrauterine mechanism but by residual confounding of unmeasured social, behavioural, or environmental factors within the families.

The aim of the present study was to assess the associations of maternal psychological distress during pregnancy with childhood wheezing in the first 6 years of life and to assess whether this association is independent of paternal psychological distress during pregnancy and maternal and paternal psychological distress after delivery.

METHODS

Study design and population

This study was embedded in the Generation R Study, a population-based cohort study from fetal life onwards in Rotterdam[12]. All children were born between April 2002 and January 2006. Assessments in pregnant women consisted of physical examination, fetal ultrasound, biological samples, and questionnaires. In total, 8,880 mothers were enrolled during pregnancy (Figure E3.1.1). For this study 7,490 mothers were eligible after excluding twin pregnancies, miscarriages, and mothers that lived outside the study area. Among them, 666 were excluded because of loss to follow-up or no consent for the postnatal phase of the study. In 2,095 children, no information on maternal psychological distress or on childhood wheezing was available. Finally, 4,848 (64.7%) children were included in this study. The study was approved by the Medical Ethics Committee of the Erasmus Medical Centre, Rotterdam. Written informed consent was obtained from all women.

Maternal and paternal psychological distress

Information on maternal and paternal psychological distress was obtained by postal questionnaires at 20 weeks of gestation and at 3 years after delivery using the Brief Symptom Inventory[13]. Information on maternal psychological distress was also obtained at 2 and 6 months after delivery using the same questionnaire because of the critical period for maternal distress symptoms during the first 6 months after delivery[14]. Mother and father each answered their own questionnaires. The Brief Symptom Inventory is a validated self-report questionnaire with 53 items. These items define a broad spectrum of psychological symptoms in the preceding 7 days. A global index and 2 symptom scales (depression and anxiety) were defined[13]. At 6 months and 3 years after delivery, only depression and anxiety scales were measured. The global index is a measure of current level or depth of the symptoms, and denotes overall psychological distress. Each item was rated on five-point uni-dimensional scales ranging from '0' (not at all) to '4' (extremely). Total scores for each scale were calculated by summing the items scores and dividing by the number of endorsed items. Higher scores represented an increased occurrence of overall distress, depression, or anxiety symptoms. Based on the Dutch cut-offs[15], mothers were categorized as being sensitive for clinically significant psychological distress (yes/no) when having a score above 0.71 on overall distress scale, above 0.80 on the depression scale, and above 0.71 on the anxiety scale. Fathers were categorized as being sensitive for clinically significant psychological distress (yes/no) when having a score above 0.66 on the overall distress scale, above 0.71 on the depression scale, and above 0.65 on the anxiety scale[15]. In the current study, internal consistencies (Cronbach's alpha) for the different scales of the mother and the father ranged from 0.67 to 0.99. Spearman's correlations between maternal and paternal distress scales during pregnancy and at 3 years ranged from 0.10 to 0.27, between pre- and postnatal maternal distress scales ranged from 0.22 to 0.58, and between pre- and postnatal paternal distress scales ranged from 0.14 to 0.35.

We defined patterns of maternal depression and anxiety after delivery as follows: 1) never depression or anxiety: no symptoms at any age after delivery; 2) transient depression or anxiety: symptoms at 2 or 6 months but not at 3 years after delivery; 3) late onset depression or anxiety: symptoms at 3 years after delivery but not at 2 or 6 months after delivery; 4) persistent depression or anxiety: symptoms at both 2 or 6 months and at 3 years after delivery.

Childhood wheezing

Information on wheezing in the past year was obtained by questionnaires, adapted from the International Study on Asthma and Allergy in Childhood (ISAAC)[16] at the ages of 1, 2, 3 and 4 years. Mothers answered 85.2%, 84.5%, 94.1%, and 88.3% of the questionnaires at the ages of 1, 2, 3, and 4 years respectively. Response rates for these questionnaires were 71% to 76%[17]. We defined wheezing patterns categories based on Martinez et al[18] and adapted

to preschool age[19-20]: 1) no wheezing: no recorded wheezing at any age; 2) early wheezing: at least one wheezing symptom during the first 3 years of life but no wheezing at 4 years of age; 3) late wheezing: no wheezing episodes during the first 3 years of age but wheezing at 4 years of age; 4) preschool persistent wheezing: at least one wheezing episode in the first 3 years of life and wheezing at 4 years of age. Physician-diagnosed ever asthma was obtained by questionnaire at the age of 6 years with a response rate for this questionnaire of 68%.

Covariates

Information on maternal and paternal age, smoking during pregnancy, educational level, ethnicity, history of asthma and atopy, pet keeping, and maternal parity was obtained through self-administered questionnaire at enrolment[11,21]. Maternal and paternal weight and height were measured during the first visit to the research centre. Body mass index was calculated (kg/m^2). Gestational age, sex, and birth weight of the children were obtained from midwife and hospital registries at birth. Preterm birth was defined as <37 weeks of gestational age. Postal questionnaires at the ages of 6 and 12 months, and 2 years provided information about breastfeeding, day care attendance, and childhood second hand smoke at home[21]. Information on physician-attended eczema and physician-diagnosed lower respiratory tract infections was obtained by questionnaires at the ages of 1, 2, 3, and 4 years.

Statistical analysis

Among subjects with available data on maternal psychological distress during pregnancy and childhood wheezing (n=4,848), we performed multiple imputation of missing values using chained equations where 25 completed datasets were generated and analyzed using the standard combination rules for multiple imputation[22-23]. Distributions in imputed datasets were similar to those observed (Tables E3.1.1 and E3.1.2 in the Supplemental data).

First, generalized estimating equations were performed in order to examine the associations of maternal psychological distress during pregnancy (dichotomized based on the clinical cut-offs and continuous) with the longitudinal odds of wheezing (no/yes) from the age of 1 to 4 years. These models took into account the correlations between repeated measurements of wheezing within the same subject. For optimal generalized estimating equation modelling, we selected the exchangeable correlation matrix based on the Quasilikelihood under the Independence model Criterion (QIC) and degress of freedom[24]. Models were adjusted for several potential confounder variables, selected a priori on the basis of previous studies[1-3, 17, 21, 25]. We additionally adjusted the models for maternal psychological distress 2 months, 6 months, and 3 years after delivery, and for paternal psychological distress during pregnancy and 3 years after delivery by adding them one by one to the models separately. We additionally adjusted the models for the patterns of maternal depression and anxiety after delivery. We used similar

models to assess the associations of paternal psychological distress during pregnancy with childhood wheezing adjusting for maternal psychological distress during pregnancy.

Second, we used generalized estimating equations models to examine the association of maternal and paternal psychological distress during pregnancy with the longitudinal odds of number of wheezing episodes. We performed polytomous logistic regression to explore the association of maternal and paternal psychological distress during pregnancy with preschool wheezing patterns. We used logistic regression to examine the association of maternal and paternal psychological distress during pregnancy with physician-diagnosed ever asthma at 6 years. Goodness of fit of the logistic and polytomous logistic regression models (R^2) was estimated.

Finally, we tested the interaction between maternal psychological distress during pregnancy and maternal history of asthma or atopy, as a proxy for atopy susceptibility in children, as well as the interaction between maternal psychological distress during pregnancy and maternal smoking during pregnancy, on childhood wheezing. Moreover, we performed a sensitivity analysis focused on the associations of maternal and paternal psychological distress during pregnancy with childhood wheezing, where we only included those subjects with complete data of maternal and paternal psychological distress during pregnancy and at 3 years after delivery and wheezing at 1, 2, 3, and 4 years (n=2,098). Maternal, paternal, and child characteristics of this subsample were compared to the original population for analysis (n=4,848). Statistical tests of hypotheses were two-tailed with significance level set at $p<0.05$. Statistical analyses were conducted using STATA 11.0 (Stata Corporation, College Station, Texas).

Table 3.1.1. Maternal and paternal characteristics of the study population (n = 4,848)

	Distribution (%)	
	Mother	**Father**
Age at enrolment (years)*		
<20	1.9	0.6
20-24.9	11.1	4.9
25-29.9	25.4	18.5
30-34.9	44.3	41.2
≥35	17.4	34.8
Body mass index at enrolment (kg/m^2)†		
Underweight (<20)	9.1	4.0
Normal weight (20-24.9)	56.0	47.4
Overweight (25-29.9)	24.5	41.2
Obese (≥30)	10.4	7.4

Table 3.1.1. Maternal and paternal characteristics of the study population (n = 4,848) (continued)

	Distribution (%)	
	Mother	**Father**
Smoking during pregnancy (yes *vs.* no)*	13.7	42.1
Educational level*		
Primary education	6.6	5.8
Secondary education	40.2	37.7
Higher education	53.2	56.5
Ethnicity (non-European *vs.* European)*	31.5	31.4
Parity (multiparous *vs.* nulliparous)*	40.6	—-
History of asthma and atopy (yes *vs.* no)*	35.0	29.2
Pet keeping during pregnancy (yes *vs.* no)*	32.6	—-
Overall psychological distress during pregnancy (yes *vs.* no)‡	8.1	2.6
Depression during pregnancy (yes *vs.* no)‡	8.0	2.9
Anxiety during pregnancy (yes *vs.* no)‡	9.3	6.4
Overall psychological distress at 2 months after delivery (yes *vs.* no)‡	7.1	—-
Depression symptoms at 2 months after delivery (yes *vs.* no)‡	7.3	—-
Anxiety symptoms at 2 months after delivery (yes *vs.* no)‡	7.4	—-
Depression symptoms at 6 months after delivery (yes *vs.* no)‡	7.6	—-
Anxiety symptoms at 6 months after delivery (yes *vs.* no)‡	9.0	—-
Depression symptoms at 3 years after delivery (yes *vs.* no)‡	4.2	3.2
Anxiety symptoms at 3 years after delivery (yes *vs.* no)‡	4.3	3.8
Patterns of depression symptoms after delivery§		
Never depression symptoms	87.1	—-
Transient depression symptoms	9.5	—-
Late onset depression symptoms	1.8	—-
Persistent depression symptoms	1.6	—-
Patterns of anxiety symptoms after delivery§		
Never anxiety symptoms	86.2	—-
Transient anxiety symptoms	10.2	—-
Late onset anxiety symptoms	1.9	—-
Persistent anxiety symptoms	1.7	—-

* Information obtained through self-administered questionnaire at enrolment

† Maternal weight and height was measured during the first visit to the research centre and body mass index was calculated

‡ Information obtained by postal questionnaires using the Brief Symptom Inventory; mother and father each answered their own questionnaires

§ Patterns of depression and anxiety symptoms after delivery defined, separately, according to the history of maternal depression/anxiety symptoms at 2 and 6 months and at 3 years after delivery: 1) never depression/anxiety symptoms: mothers without depression/anxiety at any age after delivery; 2) transient depression/anxiety symptoms: mothers with depression/anxiety symptoms at 2 or 6 months but not at 3 years after delivery; 3) late onset depression/anxiety symptoms at 3 years: mothers with depression/anxiety symptoms at 3 years after delivery but not at 2 or 6 months after delivery; 4) persistent depression/anxiety symptoms: mothers with depression/anxiety symptoms at 2 or 6 months and at 3 years after delivery.

Table 3.1.2. Child characteristics of the study population (n = 4,848)

	Distribution (%)
Sex (female *vs.* male)*	50.9
Preterm birth (<37 *vs.* ≥37 weeks)*	4.1
Birth weight (grams)*	
<2500	3.9
2500-3499	47.6
3500-4499	46.1
≥4500	2.5
Breastfeeding (yes *vs.* no)†	92.0
Day care attendance (yes *vs.* no)†	59.2
Second hand smoke at home (yes *vs.* no)†	17.4
Physician-attended eczema from 1 to 4 years (ever *vs.* never)‡	27.8
Physician-diagnosed lower respiratory tract infections from 1 to 4 years (ever *vs.* never)‡	20.4
Wheezing‡	
1st year	
No episodes	70.9
1-3 episodes	22.8
≥4 episodes	6.3
2nd year	
No episodes	80.5
1-3 episodes	16.3
≥4 episodes	3.2
3rd year	
No episodes	87.4
1-3 episodes	10.3
≥4 episodes	2.3
4th year	
No episodes	87.4
1-3 episodes	10.3
≥4 episodes	2.3
Wheezing patterns§	
Never wheezing	53.7
Early wheezing	33.0
Late wheezing	2.6
Persistent wheezing	10.7
Physician-diagnosed ever asthma at 6 years‖	6.0

* Information obtained from midwife and hospital registries at birth

† Information obtained by postal questionnaires at the ages of 6 and 12 months, and 2 years

‡ Information obtained by postal questionnaires at the ages of 1, 2, 3, and 4 years

§ Wheezing patterns categories based on Martinez et al.[15] and adapted to preschool age[16-17] according to the history of wheezing from the age of 1 to 4 years: 1) no wheezing: no recorded wheezing at any age; 2) early wheezing: at least one wheezing symptom during the first 3 years of life but no wheezing at 4 years of age; 3) late wheezing: no wheezing episodes during the first 3 years of age but wheezing at 4 years of age; 4) preschool persistent wheezing: at least one wheezing episode in the first 3 years of life and wheezing at 4 years of age

‖ Information obtained by postal questionnaire at the age of 6 years

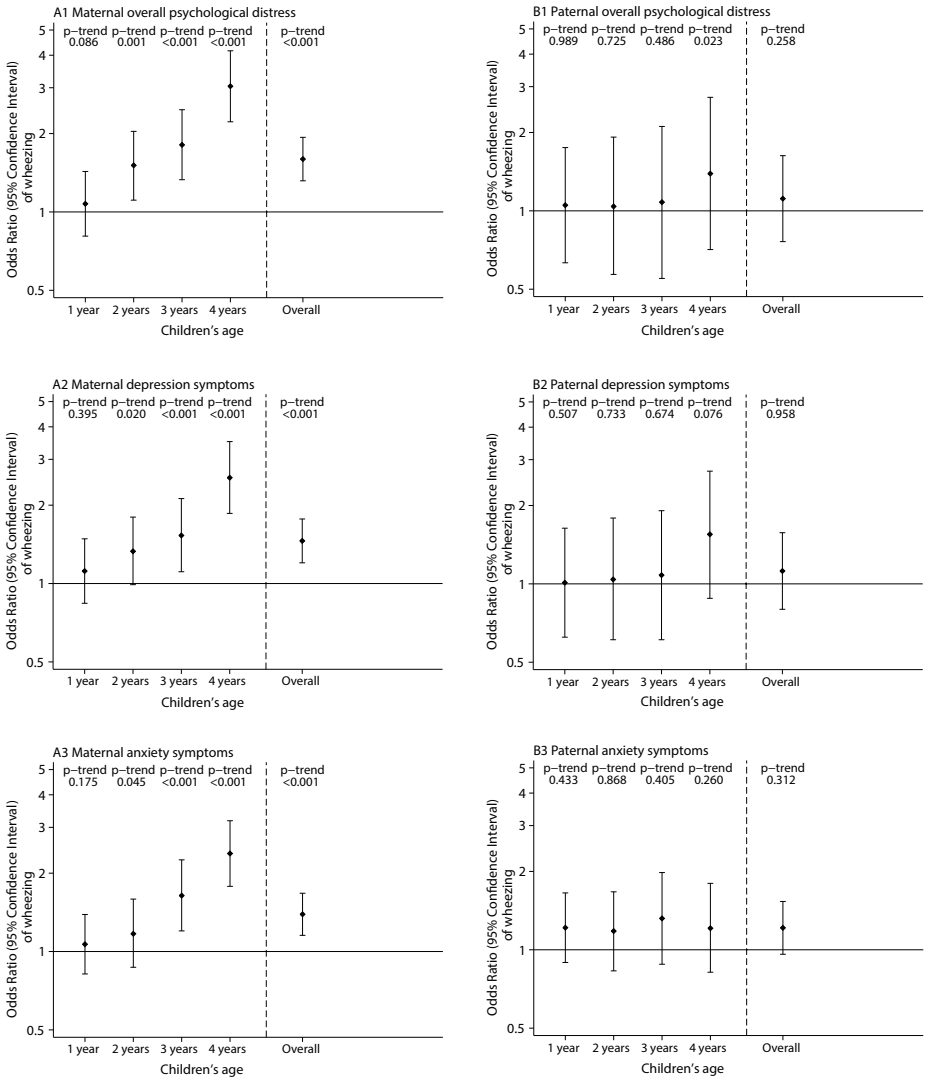

Figure 3.1.1. Associations of maternal **(A)** and paternal **(B)** psychological distress during pregnancy with wheezing from 1 to 4 years
Odd ratio (95% Confidence Interval) from generalized estimating equation models represents the odds of wheezing for the children of mothers or fathers with psychological distress (no, yes). P-trend represents the linear trend per unit increase on the psychological distress scales. Models were adjusted for maternal age, body mass index, smoking during pregnancy, educational level, ethnicity, and parity, parental history of asthma or atopy, pet keeping, and children's sex, preterm birth, birth weight, breastfeeding, day care attendance, second hand smoke at home, eczema and lower respiratory tract infections.
*Paternal models were additionally adjusted for maternal psychological distress during pregnancy.

RESULTS

Children included in the present analysis were more frequently from parents with a higher educational level, and their mothers and fathers showed less psychological distress during pregnancy (Table E3.1.3 in the Supplemental data) compared with those lost to follow-up. No differences on maternal and paternal history of asthma and atopy were found.

Of the study participants, 7.8% mothers had overall psychological distress during pregnancy (Table 3.1.1). Wheezing prevalence of the children were 29.1%, 19.5 %, 12.4%, and 12.6% at 1, 2, 3 and 4 years, respectively (Table 3.1.2). Concerning preschool wheezing patterns, 53.7% of children were classified as never wheezing, 33.0% as early wheezing, 2.6% as late wheezing, and 10.7% as persistent wheezing. Prevalence of physician-diagnosed ever asthma at 6 years was 6.0%.

As compared to mothers without psychological distress during pregnancy, mothers with overall distress, depression, or anxiety during pregnancy had increased odds of wheezing in their children overall from 1 to 4 years of life (Odds Ratio (OR), 1.60; 95% Confidence Interval (CI), 1.32 to 1.93 for overall distress, OR, 1.46; 95% CI, 1.20 to 1.77 for depression, and OR, 1.39; 95% CI, 1.15 to 1.67 for anxiety) based on generalized estimating equations models (Figure 3.1.1). Paternal overall distress, depression, and anxiety during pregnancy were not associated with increased odds of wheezing yearly from 1 to 4 years of life based on generalized estimating equations models (Figure 3.1.1). We did not observe major differences in the size of the effect estimates between the unadjusted and adjusted models (Figure E3.1.2 in the data supplement). Additional adjustment of maternal psychological distress during pregnancy in generalized estimating equations models for maternal psychological distress at 2 months, 6 months, and 3 years after delivery, for the patterns of maternal psychological distress after delivery, and for paternal psychological distress during pregnancy and at 3 years after delivery one by one separately did not materially affect the results (Tables E3.1.4 and E3.1.5 in the Supplemental data). None of the paternal psychological distress variables after delivery was associated with childhood wheezing (all P values >0.05).
As compared to children from mothers without psychological distress during pregnancy, children of mothers with overall distress had higher odds of having 1 to 3 wheezing episodes (OR, 1.56; 95% CI, 1.27 to 1.90) and 4 or more wheezing episodes (OR, 1.71; 95% CI, 1.20 to 2.43) from 1 to 4 years of life based on generalized estimating equations models (Table 3.1.3). Table 3.1.4 shows that children of mothers with overall distress during pregnancy had 1.20 (95% CI, 0.86 to 1.67) times more odds of having early wheezing, 2.46 (95% CI, 1.28 to 4.70) times more odds of late wheezing, and 2.73 (95% CI, 1.90 to 3.94) times more odds of persistent wheezing, compared to children from mothers without psychological distress during pregnancy based on polytomous logistic regression models. Similar results were observed for depression and anxiety (Table 3.1.3 and 3.1.4). Maternal overall psychological

Table 3.1.3. Associations of maternal and paternal psychological distress during pregnancy with number of wheezing episodes from 1 to 4 years

	Number of wheezing episodes			
	1-3 episodes per year		≥4 episodes per year	
	OR	(95% CI)	OR	(95% CI)
Maternal psychological distress				
Overall psychological distress				
No	Reference		Reference	
Yes	1.40	(1.15, 1.71)	1.58	(1.14, 2.20)
Per 1 unit increase	1.41	(1.20, 1.66)	1.51	(1.16, 1.95)
p-value trend		<0.001		0.002
Depression symptoms				
No	Reference		Reference	
Yes	1.28	(1.05, 1.55)	1.54	(1.11, 2.13)
Per 1 unit increase	1.20	(1.06, 1.36)	1.27	(1.04, 1.55)
p-value trend		0.004		0.018
Anxiety symptoms				
No	Reference		Reference	
Yes	1.26	(1.05, 1.50)	1.37	(1.00, 1.88)
Per 1 unit increase	1.23	(1.09, 1.40)	1.30	(1.06, 1.60)
p-value trend		0.001		0.012
Paternal psychological distress*				
Overall psychological distress				
No	Reference		Reference	
Yes	1.15	(0.79, 1.68)	0.87	(0.41, 1.84)
Per 1 unit increase	1.18	(0.86, 1.63)	1.27	(0.72, 2.23)
p-value trend		0.304		0.412
Depression symptoms				
No	Reference		Reference	
Yes	1.11	(0.79, 1.56)	1.18	(0.62, 2.24)
Per 1 unit increase	1.01	(0.80, 1.27)	1.06	(0.70, 1.61)
p-value trend		0.957		0.766
Anxiety symptoms				
No	Reference		Reference	
Yes	1.18	(0.93, 1.49)	1.41	(0.91, 2.16)
Per 1 unit increase	1.09	(0.88, 1.34)	1.22	(0.84, 1.78)
p-value trend		0.426		0.291

CI, Confidence interval; OR, Odds ratio

Odds ratio (95% Confidence Interval) from generalized estimating equation models represents the odds of wheezing episodes for the children of mothers or fathers with psychological distress during pregnancy. Maternal and paternal psychological distress were treated as dichotomized based on the clinical cut-offs (no, yes) and as continuous (per 1 unit increase). P-trend represents the linear trend per unit increase on the psychological distress scales. Models were adjusted for maternal age, body mass index, smoking during pregnancy, educational level, ethnicity, and parity, parental history of asthma or atopy, pet keeping, and children's sex, preterm birth, birth weight, breastfeeding, day care attendance, second hand smoke at home, eczema and lower respiratory tract infections.

* Models additionally adjusted for psychological distress during pregnancy.

Table 3.1.4. Associations of maternal and paternal psychological distress during pregnancy with wheezing patterns from 1 to 4 years

	Early wheezing	Late wheezing	Persistent wheezing
	OR (95% CI)	OR (95% CI)	OR (95% CI)
Maternal psychological distress			
Overall psychological distress			
No	Reference	Reference	Reference
Yes	1.23 (0.89, 1.69)	1.94 (1.04, 3.60)	2.15 (1.47, 3.13)
Per 1 unit increase on the scale	1.51 (1.16, 1.97)	2.14 (1.29, 3.54)	2.18 (1.60, 2.98)
p-value trend	0.002	0.003	<0.001
Depression symptoms			
No	Reference	Reference	Reference
Yes	1.31 (0.97, 1.76)	2.04 (1.14, 3.64)	1.84 (1.24, 2.72)
Per 1 unit increase on the scale	1.28 (1.05, 1.57)	1.69 (1.18, 2.43)	1.51 (1.18, 1.93)
p-value trend	0.015	0.004	0.001
Anxiety symptoms			
No	Reference	Reference	Reference
Yes	1.17 (0.88, 1.55)	1.81 (1.05, 3.12)	1.72 (1.22, 2.43)
Per 1 unit increase on the scale	1.27 (1.04, 1.56)	1.71 (1.18, 2.49)	1.66 (1.31, 2.10)
p-value trend	0.022	0.005	<0.001
Paternal psychological distress*			
Overall psychological distress			
No	Reference	Reference	Reference
Yes	1.29 (0.74, 2.29)	2.12 (0.79, 5.665)	1.12 (0.46, 2.70)
Per 1 unit increase on the scale	1.06 (0.68, 1.66)	1.92 (0.83, 4.48)	1.33 (0.72, 2.48)
p-value trend	0.789	0.128	0.359
Depression symptoms			
No	Reference	Reference	Reference
Yes	0.99 (0.59, 1.67)	1.72 (0.67, 4.42)	1.23 (0.60, 2.52)
Per 1 unit increase on the scale	0.86 (0.61, 1.22)	1.44 (0.81, 2.59)	1.10 (0.68, 1.78)
p-value trend	0.402	0.215	0.706
Anxiety symptoms			
No	Reference	Reference	Reference
Yes	1.24 (0.89, 1.71)	1.29 (0.58, 2.85)	1.24 (0.74, 2.09)
Per 1 unit increase on the scale	1.12 (0.84, 1.55)	1.31 (0.66, 2.63)	1.13 (0.73, 1.76)
p-value trend	0.438	0.437	0.585

CI, Confidence interval; OR, Odds ratio

Odds ratio (95% Confidence Interval) from polytomous logistic regression models. Maternal and paternal psychological distress were treated as dichotomized based on the clinical cut-offs (no, yes) and as continuous (per 1 unit increase). P-trend represents the linear trend per unit increase on the psychological distress scales. Models were adjusted for maternal age, body mass index, smoking during pregnancy, educational level, ethnicity, and parity, parental history of asthma or atopy, pet keeping, and children's sex, preterm birth, birth weight, breastfeeding, day care attendance, second hand smoke at home, eczema and lower respiratory tract infections.

Goodness of fit (R^2) was 0.10 for all models.

***** Models additionally adjusted for psychological distress during pregnancy.

Table 3.1.5. Associations of maternal and paternal psychological distress during pregnancy with physician-diagnosed ever asthma at 6 years

	Physician-diagnosed ever asthma	
	OR	**(95% CI)**
Maternal psychological distress		
Overall psychological distress		
No	Reference	
Yes	1.45	(0.91, 2.31)
Per 1 unit increase	1.27	(0.88, 1.84)
p-value trend		0.201
Depression symptoms		
No	Reference	
Yes	1.33	(0.82, 2.16)
Per 1 unit increase	1.17	(0.88, 1.57)
p-value trend		0.276
Anxiety symptoms		
No	Reference	
Yes	1.19	(0.76, 1.86)
Per 1 unit increase	1.15	(0.86, 1.55)
p-value trend		0.344
Paternal psychological distress*		
Overall psychological distress		
No	Reference	
Yes	0.72	(0.22, 2.36)
Per 1 unit increase	1.08	(0.51, 2.28)
p-value trend		0.837
Depression symptoms		
No	Reference	
Yes	1.06	(0.41, 2.72)
Per 1 unit increase	1.01	(0.53, 1.90)
p-value trend		0.982
Anxiety symptoms		
No	Reference	
Yes	0.95	(0.53, 1.68)
Per 1 unit increase	0.88	(0.49, 1.56)
p-value trend		0.651

CI, Confidence interval; OR, Odds ratio

Odds ratio (95% Confidence Interval) from logistic regression models represents the odds of physician-diagnosed asthma for the children of mothers or fathers with psychological distress during pregnancy. Maternal and paternal psychological distress were treated as dichotomized based on the clinical cut-offs (no, yes) and as continuous (per 1 unit increase). P-trend represents the linear trend per unit increase on the psychological distress scales. Models were adjusted for maternal age, body mass index, smoking during pregnancy, educational level, ethnicity, and parity, parental history of asthma or atopy, pet keeping, and children's sex, preterm birth, birth weight, breastfeeding, day care attendance, second hand smoke at home, eczema and lower respiratory tract infections.

Goodness of fit (R^2) was 0.15 for all models.

* Models additionally adjusted for maternal psychological distress during pregnancy.

distress during pregnancy was borderline associated with physician-diagnosed ever asthma at 6 years (Table 3.1.5) based on logistic regression models. We did not observe associations between paternal psychological distress pregnancy and childhood wheezing episodes and patterns or physician-diagnosed ever asthma (Tables 3.1.3, 3.1.4 and 3.1.5).

Associations of maternal psychological distress during pregnancy with wheezing from 1 to 4 years in generalized estimating equations models were similar among children of mothers with a history of asthma and atopy compared to those of mothers without, as well as among children of smokers and non-smokers mothers (P values for interaction>0.05). As compared to children in our original population for analysis, children included in the complete case analysis were more often from parents with a higher educational level, who tended to smoke less frequently, were born more frequently in The Netherlands, had a lower body mass index, reported more frequently a history of asthma and atopy, and reported less psychological distress during pregnancy (Table E3.1.6). Results from the complete case analysis (Figure E3.1.3, Table E3.1.7-E3.1.8) showed effect estimates mostly in the same direction than the previous analysis but the effect sizes differed and the associations were less often statistically significant.

DISCUSSION

Our results suggest that children exposed to maternal psychological distress during pregnancy have increased odds of childhood wheezing until the age of 6 years. The strength of the associations after adjusting for paternal psychological distress during pregnancy and maternal and paternal psychological distress after delivery, the lack of association of paternal psychological distress during pregnancy and maternal and paternal psychological distress after delivery with childhood wheezing, and the robustness of the results after adjusting for a large set of potential confounding variables support an intrauterine programming effect of maternal psychological distress during pregnancy on fetal lung development and subsequent respiratory morbidity.

The strengths of our study were its population-based prospective design, large sample size, assessment of maternal and paternal exposures with the same instrument at the same time point, assessment of maternal and paternal exposures after delivery, and repeated measures of wheezing. In addition, we adjusted for many socioeconomic and lifestyle variables known to affect maternal psychological distress and childhood wheezing. However, residual confounding cannot be completely ruled out. Therefore, we used paternal psychological distress during pregnancy as an indirect control for unmeasured variables and shared family factors.

The present study has some limitations. Information on wheezing was mainly based on maternal-reported questions[26]. Objective tests for assessing asthma are difficult to perform in young children, and have limited applicability. In preschool children a diagnosis of asthma is

based on symptoms[27]. Maternal psychological distress could have influenced the recognition and reporting of symptoms of their child. Information about maternal psychological distress at the same time as childhood wheezing questionnaires would be of interest and could have reduced potential information bias. Information about maternal psychological distress was available from repeated measurements during the preschool period. Additional adjustment for postnatal maternal psychological distress did no materially change the effect estimates of maternal psychological distress during pregnancy with childhood wheezing. Wheezing during preschool ages may be partly caused by viral infections and this phenotype is mostly not persistent and related to asthma at later ages[28]. This is in line with our observations of stronger effects for wheezing at 4 years than at 1 year, and for late-onset compared to early onset wheezing, and of a consistency of the association with physician-diagnosed ever asthma at 6 years. Also, adjustment for lower respiratory tract infections did not change the effect estimates. Follow up studies at older ages with more detailed assessments of asthma and atopy phenotypes are needed. Maternal psychological distress was measured at one time-point during pregnancy. We do not know whether maternal distress varied in intensity or persistent throughout pregnancy. Cookson et al. showed a similar effect estimate sizes between anxiety measured at week 18 and at week 32 of pregnancy[3]. Observational measurements of parental psychological distress were not feasible in this large birth cohort and we relied on self-reports. Nevertheless, all scales showed an acceptable internal validity; the Brief Symptom Inventory was validated in the Netherlands, and Dutch clinical cut-offs were available[12-13]. Finally, not all mothers and children recruited were included in this analysis and loss to follow-up was related to lower socioeconomic position. This may have affected our findings, although the inclusion in the analysis of a large set of variables related to participation may have reduced the likelihood that non-response biased the results. We observed differences between the effect estimates of our original population of analysis and the complete case analysis. These differences may be due to both a reduction of the sample size and a selected subsample which seemed biased and not representative. For that reason, we consider results based on the multiple imputation dataset more valid[22].

Only few previous studies have assessed the relation between maternal psychological distress during pregnancy and childhood wheezing[3-6]. Cookson et al. found a positive association of maternal anxiety symptoms during pregnancy with subsequent childhood physician's diagnosis asthma at the age of 7.5 years in 5,810 children[3]. Similar as in our study, they did not observe an association of paternal anxiety symptoms with childhood asthma. Moreover, when maternal anxiety symptoms both during pregnancy and after delivery were taken into account, only symptoms during pregnancy were associated with childhood asthma. Additionally to their study, we showed that maternal psychological distress affects asthma symptoms already from a young age onwards, and, due to our longitudinal design with repeatedly measured outcomes, we observed that these adverse effects became stronger with increasing age. Also, we were able to adjust for more potential confounders such as

maternal pre-pregnancy body mass index, paternal smoking, or pet keeping at home, and to examine important possible modifying effects of genetic susceptibility and second hand smoke exposure. In another population-based study of 653 mother-child pairs, while both pre- and postnatal maternal stress were independently associated with increased recurrent wheezing during the first 2 years of life, children born to mothers experiencing higher stress in both periods were particularly at risk[6]. These effects remained when adjusting for several confounders and pathways variables. These findings are not in accordance with our results where prenatal maternal psychological distress seemed to have a greater impact than postnatal maternal psychological distress. A smaller sample sized study based on 279 children observed that maternal demoralization during pregnancy predicted overall, transient, and persistent wheezing in the first 5 years of life[5]. In this study, no information on paternal demoralization during pregnancy was available, and models were not adjusted for maternal demoralization after delivery. Since maternal demoralization was a stable trait in their cohort, the authors could not separate pregnancy and early postnatal effects. A previous case-control study including 247 subjects did not observe a significant relationship between maternal depression and anxiety during pregnancy and infant's wheezing[4]. The main limitation was that mothers were asked retrospectively whether depression or anxiety constituted a problem during pregnancy. Other previous studies explored the associations of maternal stress, depression, anxiety, or cortisol levels during pregnancy with general childhood respiratory diseases and observed an association of higher maternal stress at pregnancy with an increased risk of childhood respiratory illnesses[29-30].

The mechanisms underlying the associations of prenatal psychological distress exposure with childhood wheezing are still unclear. A possible programming effect by maternal stress during pregnancy is pointed out by studies reporting that adult mammals prenatally exposed to psychological distress have an altered hypothalamic-pituitary-adrenal axis after birth and may be predisposed to airway inflammation and hyperresponsiveness[31-32]. Stress-induced alterations in maternal cortisol may influence fetal immunomodulation and Th2 lymphocyte predominance through direct influence on cytokine production[33]. Stress was also associated with increased proportions and altered function of natural killer lymphocytes[34]. Recently, it was shown in humans that maternal stress during pregnancy was associated with altered innate and adaptive immune responses in cord blood in infants at high risk of atopic diseases[35]. Furthermore, the stress hormone adrenaline stimulates B2-adrenoreceptors that are expressed throughout the body[36-38]. Effects on the adrenergic receptors of the lungs may predispose for later respiratory problems[36-37]. Next to programming effects, a hypothesized mechanism was the intermediate role of fetal growth. Maternal psychological distress during pregnancy may impair fetal growth[39], and low birth weight children with smaller lungs and airways seem to have a higher risk of wheezing[25, 40]. However, in our study, results remained after adjusting for birth weight and gestational age at birth. The programming effect of maternal psychological distress may also operate through epigenetic programming[7]. Differ-

ential methylation patterns in the glucocorticoid receptor related to postnatal maternal care was showed recently in a rodent model and cultured cell lines[42-43]. In humans, methylation of the glucocorticoid receptor was sensitive to maternal mood in the perinatal period and the infant's hypothalamic-pituitary-adrenal axis stress reactivity[43]. Further studies are needed to identify the underlying mechanisms.

In conclusion, our results suggest intrauterine effects of maternal psychological distress during pregnancy on the presence of wheezing at early ages. Further studies are needed to explore underlying biological mechanisms and the long term consequences.

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Supplements

Table E3.1.1. Details of the imputation modelling

Software used and key setting: STATA 12.0 software (Stata Corporation, College Station, Texas) – Ice command (with 10 cycles)

Number of imputed datasets created: 25

Variables included in the imputation procedure:

Variables used in the main analyses (outcome, exposure, and potential confounders)

Child wheezing symptoms at 1st, 2nd, 3rd, and 4th year of life, child wheezing symptoms at 6 months, 1st, 2nd, 3rd, and 4th year of life, physician-diagnosed ever asthma at 6 years, maternal and paternal overall psychological distress during pregnancy, maternal and paternal depression symptoms during pregnancy, maternal and paternal anxiety symptoms during pregnancy, maternal overall psychological distress at 2 months after delivery, maternal depression symptoms at 2 months after delivery, maternal anxiety symptoms at 2 months after delivery, maternal depression symptoms at 6 months after delivery, maternal anxiety symptoms at 6 months after delivery, maternal depression symptoms at 3 years after delivery, maternal anxiety symptoms at 3 years after delivery, paternal depression symptoms at 3 years after delivery, paternal anxiety symptoms at 3 years after delivery, maternal age, maternal and paternal educational level, maternal body mass index at enrolment, parity, maternal and paternal smoking during pregnancy, family history of asthma or atopy, pet keeping during pregnancy, child sex, child ethnicity, child low birth weight, child preterm birth, child breastfeeding, child day care attendance, and child second hand smoke at home.

Variables only used for the imputation models

Child shortness of breath symptoms at 1st, 2nd, 3rd, and 4th year of life, child cough at night at 1st, 2nd, 3rd, and 4th year of life, child phlegm at 1st, 2nd, 3rd, and 4th year of life, child bronchiolitis at 6 months, 1st, and 2nd year of life, child pertussis at 6 months, 1st, 2nd and 3rd year of life, child bronchitis at 1st, 2nd, 3rd, and 4th year of life, child pneumonia at 1st, 2nd, 3rd, and 4th year of life, maternal and paternal ethnicity, maternal alcohol use during pregnancy, paternal body mass index, paternal age, maternal gestational diabetes, maternal hypertension, marital status, main caregiver of the child, family stress during pregnancy reported by the mother and the father, maternal and paternal somatisation symptoms during pregnancy, maternal and paternal obsession-compulsion symptoms during pregnancy, maternal and paternal interpersonal sensitivity symptoms during pregnancy, maternal and paternal hostility symptoms during pregnancy, maternal and paternal phobic anxiety symptoms during pregnancy, maternal and paternal paranoid ideation symptoms during pregnancy, maternal and paternal psychoticism symptoms during pregnancy, maternal somatisation symptoms at 2 months after delivery, maternal obsession-compulsion symptoms at 2 months after delivery, maternal interpersonal sensitivity symptoms at 2 and 6 months after delivery, maternal hostility symptoms at 2 and 6 months after delivery, maternal phobic anxiety symptoms at 2 months after delivery, maternal paranoid ideation symptoms at 2 months after delivery, maternal psychoticism symptoms at 2 months after delivery, maternal and paternal interpersonal sensitivity symptoms at 3 years after delivery, maternal and paternal hostility symptoms at 3 years after delivery,

Treatment of binary/categorical variables: logistic and multinomial models

Statistical interactions included in imputation models: none

Table E3.1.2. Distribution of study variables in the imputed and the observed datasets

	% data missing	Imputed dataset*	Observed dataset*
Maternal characteristics			
Age at enrolment (years)†	0.0	—-	—-
Pre-pregnancy body mass index (kg/m^2)‡	0.6		
Underweight		9.2	9.2
Normal weight		55.9	55.9
Overweight		24.5	24.5
Obese		10.4	10.4
Smoking during pregnancy (yes *vs.* no)†	10.2	13.6	13.6
Education level†	2.8		
Primary education		6.5	6.3
Secondary education		40.4	39.9
Higher education		53.1	53.8
Ethnicity (Non-European *vs.* European)	1.4	28.4	28.1
Parity (multiparous *vs.* nulliparous)†	0.3	40.4	40.4
History of asthma and atopy (yes *vs.* no)†	20.6	37.1	35.0
Pets keeping during pregnancy (yes *vs.* no)†	13.9	34.3	32.9
Overall psychological distress during pregnancy§	0.1	0.26 (0.00)	0.26 (0.01)
Depression symptoms during pregnancy§	0.2	0.20 (0.01)	0.20 (0.01)
Anxiety symptoms during pregnancy§	0.2	0.26 (0.01)	0.26 (0.01)
Overall psychological distress at 2 months after delivery§	21.2	0.24 (0.00)	0.23 (0.01)
Depression symptoms at 2 months after delivery§	21.4	0.21 (0.01)	0.20 (0.01)
Anxiety symptoms at 2 months after delivery§	21.2	0.24 (0.01)	0.22 (0.01)
Depression symptoms at 6 months after delivery§	30.7	0.23 (0.01)	0.22 (0.01)
Anxiety symptoms at 6 months after delivery§	30.7	0.27 (0.01)	0.26 (0.01)
Depression symptoms at 3 years after delivery§	22.6	0.14 (0.00)	0.13 (0.01)
Anxiety symptoms at 3 years after delivery§	22.6	0.18 (0.00)	0.17 (0.01)
Paternal characteristics			
Smoking during pregnancy (yes *vs.* no)†	9.5	41.9	41.8
Education level†	24.1		
Primary education		6.9	5.7
Secondary education		40.0	37.5
Higher education		53.1	56.8
History of asthma and aatopy (yes *vs.* no)†	33.0	32.1	29.4
Overall psychological distress during pregnancy§	26.5	0.14 (0.00)	0.13 (0.01)
Depression symptoms during pregnancy§	26.6	0.09 (0.00)	0.09 (0.01)
Anxiety symptoms during pregnancy§	26.5	0.17 (0.00)	0.16 (0.01)
Depression symptoms at 3 years after delivery§	35.4	0.11 (0.00)	0.10 (0.01)
Anxiety symptoms at 3 years after delivery§	35.3	0.17 (0.00)	0.16 (0.01)

Table E3.1.2. Distribution of study variables in the imputed and the observed datasets (continued)

	% data missing	Imputed dataset*	Observed dataset*
Maternal characteristics			
Child characteristics			
Sex (female vs. male)ll	0.0	—-	—-
Preterm (<37 *vs.* ≥37 weeks)ll	0.0	—-	—-
Birth weight (grams)ll	0.0	—-	—-
Breastfeeding (yes *vs.* no)¶	3.1	92.0	92.1
Day care attendance (yes vs. no)¶	21.6	58.1	59.2
Postnatal smoking exposure (yes *vs.* no)¶	13.9	18.6	17.4
Physician-attended eczema from 1 to 4 years (ever *vs.* never)**	3.3	35.0	27.8
Physician-diagnosedlower respiratory tract infections from 1 to 4 years (ever *vs.* never)**	3.8	26.9	20.4
Wheezing**			
1st year	13.2		
None episode		71.0	70.9
1-3 episodes		22.6	22.8
≥4 episodes		6.4	6.3
2nd year	14.6		
None episode		80.3	80.5
1-3 episodes		16.4	16.3
≥4 episodes		3.3	3.2
3rd year	20.7		
None episode		87.1	87.6
1-3 episodes		10.4	10.1
≥4 episodes		2.5	2.3
4th year	20.6		
None episode		86.8	87.4
1-3 episodes		10.7	10.3
≥4 episodes		2.5	2.3
Wheezing patterns††	33.2		
Never wheezing		56.7	53.7
Early wheezing		30.1	33.0
Late wheezing		3.1	2.6
Persistent wheezing		10.1	10.7
Physician-diagnosed asthma during first 6 years (yes *vs.* no)‡‡	31.1	6.5	6.0

* Values are percentages for categorical variables and mean (standard error) for continuous variables

† Information obtained through self-administered questionnaire at enrolment

‡ Maternal weight and height was measured during the first visit to the research centre and body mass index was calculated

§ Information obtained by postal questionnaires using the Brief Symptom Inventory; mother and father each answered the questionnaires

ll Information obtained from midwife and hospital registries at birth

¶ Information obtained by postal questionnaires at the ages of 6 and 12 months, and 2 years

** Information obtained by postal questionnaires at the ages of 1, 2, 3, and 4 years

†† Wheezing patterns categories based on Martinez et al and adapted to preschool age according to the history of wheezing from the age of 1 to 4 years: 1) no wheezing: no recorded wheezing at any age; 2) early wheezing: at least one wheezing symptom during the first 3 years of life but no wheezing at 4 years of age; 3) late wheezing: no wheezing episodes during the first 3 years of age but wheezing at 4 years of age; 4) preschool persistent wheezing: at least one wheezing episode in the first 3 years of life and wheezing at 4 years of age

Table E3.1.3. Comparison of the maternal, paternal, and child characteristics between those included and those not included in the study among the 6,824 eligible subjects*

	Included (N=4,848)	Not included (N=2,642)	*P*-value Differences
Maternal characteristics			
Age at enrolment (years)†	30.8 (4.7)	28.5 (5.7)	<0.001
Pre-pregnancy body mass index (kg/m^2)‡			<0.001
Underweight	9.1	9.4	
Normal weight	56.0	47.0	
Overweight	24.5	27.6	
Obese	10.4	16.0	
Smoking during pregnancy (yes *vs.* no)†	13.7	20.4	<0.001
Education level†			<0.001
Primary education	6.6	20.6	
Secondary education	40.2	51.2	
Higher education	53.2	28.1	
Ethnicity (Non-European *vs.* European)†	31.5	61.8	<0.001
Parity (multiparous *vs.* nulliparous)†	40.6	53.4	<0.001
History of asthma and atopy (yes *vs.* no)†	35.0	33.2	0.236
Pets keeping during pregnancy (yes *vs.* no)†	32.6	25.2	<0.001
Overall psychological distress during pregnancy§	0.26 (0.34)	0.46 (0.50)	<0.001
Depression symptoms during pregnancy§	0.20 (0.44)	0.44 (0.70)	<0.001
Anxiety symptoms during pregnancy§	0.26 (0.43)	0.45 (0.56)	<0.001
Paternal characteristics			
Smoking during pregnancy (yes *vs.* no)	42.1	47.4	<0.001
Education level			<0.001
Primary education	5.8	11.8	
Secondary education	37.7	44.4	
Higher education	56.5	43.8	
History of asthma and atopy (yes *vs.* no)	29.2	29.4	0.935
Overall psychological distress during pregnancy§	0.13 (0.21)	0.18 (0.29)	<0.001
Depression symptoms during pregnancy§	0.09 (0.27)	0.13 (0.22)	0.001
Anxiety symptoms during pregnancy§	0.16 (0.28)	0.20 (0.37)	0.003
Child characteristics			
Sex (female vs. male)‖	50.9	46.9	<0.001
Preterm (<37 *vs.* ≥37 weeks)‖	4.2	5.9	0.002
Birth weight (grams)‖	3458 (545)	3363 (556)	<0.001

*Values are percentages for categorical variables and mean (standard deviation) for continuous variables

† Information obtained through self-administered questionnaire at enrolment

‡ Maternal weight and height was measured during the first visit to the research centre and body mass index was calculated

§ Information obtained by postal questionnaires using the Brief Symptom Inventory; mother and father each answered the questionnaires

‖ Information obtained from midwife and hospital registries at birth

Table E3.1.4. Associations of maternal psychological distress during pregnancy with overall wheezing from 1 to 4 years adjusted for maternal psychological distress at 2 months, 6 months, and 3 years after delivery

	Model 1*		Model 1* + Maternal psychological distress after delivery							
			At 2 months		At 6 months		At 3 years		Patterns†	
	OR	(95% CI)	OR	(95% CI)	OR	(95% CI)	OR	(95% CI)	OR	(95% CI)
Overall psychological distress‡										
No	Reference		Reference		—-	—-	—-	—-	—-	—-
Yes	1.44	(1.20, 1.74)	1.40	(1.15, 1.71)	—-	—-	—-	—-	—-	—-
Per 1 unit increase on the scale	1.46	(1.25, 1.70)	1.44	(1.36, 1.14)	—-	—-	—-	—-	—-	—-
p-value trend		<0.001		0.001		—-		—-		—-
Depression symptoms										
No	Reference		Reference		Reference		Reference		Reference	
Yes	1.34	(1.11, 1.62)	1.31	(1.08, 1.60)	1.25	(1.02, 1.53)	1.29	(1.06, 1.57)	1.23	(1.01, 1.51)
Per 1 unit increase on the scale	1.23	(1.09, 1.38)	1.21	(1.06, 1.38)	1.17	(1.02, 1.34)	1.18	(1.03, 1.36)	1.16	(1.01, 1.33)
p-value trend		0.001		0.006		0.002		0.016		0.033
Anxiety symptoms										
No	Reference		Reference		Reference		Reference		Reference	
Yes	1.27	(1.07, 1.52)	1.24	(1.03, 1.49)	1.20	(1.00, 1.45)	1.24	(1.03, 1.49)	1.18	(0.97, 1.42)
Per 1 unit increase on the scale	1.26	(1.12, 1.42)	1.19	(1.04, 1.37)	1.21	(1.05, 1.40)	1.21	(1.06, 1.38)	1.21	(1.05, 1.39)
p-value trend		<0.001		0.014		0.009		0.006		0.009

CI, Confidence interval; OR, Odds ratio

Odds ratio (95% Confidence Interval) from generalized estimating equation models represents the odds of wheezing episodes for the children of mothers with psychological distress during pregnancy. Maternal psychological distress was treated as dichotomized based on the clinical cut-offs (no, yes) and as continuous (per 1 unit increase). P-trend represents the linear trend per unit increase on the psychological distress scales.

* Adjusted for maternal age, body mass index, smoking during pregnancy, educational level, ethnicity, and parity, parental history of asthma or atopy, pet keeping, and children's sex, preterm birth, birth weight, breastfeeding, day care attendance, second hand smoke at home, eczema, and lower respiratory tract infections.

† Patterns of maternal psychological distress after delivery (never distress, only postpartum distress, only distress at 3 years, and persistent distress)

‡ Not available at 6 months and 3years after delivery

Table E3.1.5. Associations of maternal psychological distress during pregnancy with overall wheezing from 1 to 4 years adjusted for paternal psychological distress during pregnancy and at 3 years after delivery

	Model 1*	Model 1* + Paternal psychological distress	
		During pregnancy†	At 3 years after delivery†
	OR (95% CI)	OR (95% CI)	OR (95% CI)
Overall psychological distress‡			
No	Reference	Reference	—- —-
Yes	1.44 (1.20, 1.74)	1.44 (1.19, 1.74)	—- —-
Per 1 unit increase on the scale	1.46 (1.25, 1.70)	1.44 (1.23, 1.68)	—- —-
p-value trend	<0.001	<0.001	—-
Depression symptoms			
No	Reference	Reference	Reference
Yes	1.34 (1.11, 1.62)	1.33 (1.10, 1.62)	1.35 (1.12, 1.63)
Per 1 unit increase on the scale	1.23 (1.09, 1.38)	1.23 (1.08, 1.39)	1.22 (1.08, 1.38)
p-value trend	0.001	0.002	0.001
Anxiety symptoms			
No	Reference	Reference	Reference
Yes	1.27 (1.07, 1.52)	1.26 (1.06, 1.50)	1.27 (1.06, 1.52)
Per 1 unit increase on the scale	1.26 (1.12, 1.42)	1.25 (1.10, 1.41)	1.24 (1.10, 1.41)
p-value trend	<0.001	<0.001	0.001

CI, Confidence interval; OR, Odds ratio

Odds ratio (95% Confidence Interval) from generalized estimating equation models represents the odds of wheezing episodes for the children of mothers psychological distress during pregnancy. Maternal psychological distress was treated as dichotomized based on the clinical cut-offs (no, yes) and as continuous (per 1 unit increase). P-trend represents the linear trend per unit increase on the psychological distress scales.

* Adjusted for maternal age, body mass index, smoking during pregnancy, educational level, ethnicity, and parity, parental history of asthma or atopy, pet keeping, and children's sex, preterm birth, birth weight, breastfeeding, day care attendance, second hand smoke at home, eczema, and lower respiratory tract infections.

† Models additionally adjusted by maternal psychological distress during pregnancy

‡ Not available at 3 years after delivery

Table E3.1.6. Comparison of the maternal, paternal, and child characteristics between those included in the complete-case analysis and those not included among the 4,848 subjects*

	Included (N=2,098)	Not included (N=2,750)	*P*-value Differences
Maternal characteristics			
Age at enrolment (years)†	31.7 (4.0)	30.1 (5.1)	<0.001
Pre-pregnancy body mass index (kg/m^2)‡			<0.001
Underweight	9.0	9.2	
Normal weight	60.4	52.7	
Overweight	22.7	25.9	
Obese	7.9	12.2	
Smoking during pregnancy (yes *vs.* no)†	9.5	16.9	<0.001
Education level†			<0.001
Primary education	1.8	10.4	
Secondary education	30.6	47.8	
Higher education	67.6	41.8	
Ethnicity (Non-European *vs.* European)†	16.1	43.7	<0.001
Parity (multiparous *vs.* nulliparous)†	35.6	44.4	<0.001
History of asthma and atopy (yes *vs.* no)†	36.8	33.5	0.034
Pets keeping during pregnancy (yes *vs.* no)†	35.5	30.3	<0.001
Overall psychological distress during pregnancy§	0.19 (0.24)	0.31 (0.40)	<0.001
Depression symptoms during pregnancy§	0.12 (0.29)	0.26 (0.51)	<0.001
Anxiety symptoms during pregnancy§	0.19 (0.32)	0.31 (0.50)	<0.001
Paternal characteristics			
Smoking during pregnancy (yes *vs.* no)	36.4	46.6	<0.001
Education level			<0.001
Primary education	3.3	9.2	
Secondary education	33.5	43.2	
Higher education	63.2	47.6	
History of asthma and atopy (yes *vs.* no)	29.3	29.1	0.896
Overall psychological distress during pregnancy§	0.12 (0.17)	0.16 (0.25)	<0.001
Depression symptoms during pregnancy§	0.09 (0.22)	0.12 (0.33)	<0.001
Anxiety symptoms during pregnancy§	0.15 (0.26)	0.18 (0.31)	0.017
Child characteristics			
Sex (female vs. male)‖	50.1	51.5	0.352
Preterm (<37 *vs.* ≥37 weeks)‖	3.4	4.7	0.029
Birth weight (grams)‖	3519 (526)	3412 (555)	<0.001

* Values are percentages for categorical variables and mean (standard deviation) for continuous variables

† Information obtained through self-administered questionnaire at enrolment

‡ Maternal weight and height was measured during the first visit to the research centre and body mass index was calculated

§ Information obtained by postal questionnaires using the Brief Symptom Inventory; mother and father each answered the questionnaires

‖ Information obtained from midwife and hospital registries at birth

Table E3.1.7. Complete-case analysis: associations of maternal psychological distress during pregnancy with overall wheezing from 1 to 4 years adjusted for maternal psychological distress at 3 years after delivery

	Model 1		Model 1 + Maternal psychological distress at 3 years	
	OR	**(95% CI)**	**OR**	**(95% CI)**
Overall psychological distress*				
No	Reference		—-	—-
Yes	1.23	(0.76, 1.98)	—-	—-
Per 1 unit increase on the scale	1.77	(1.20, 2.63)	—-	—-
p-value trend		0.004		—-
Depression symptoms				
No	Reference		Reference	
Yes	1.44	(0.91, 2.29)	1.38	(0.86, 2.21)
Per 1 unit increase on the scale	1.43	(1.05, 1.95)	1.36	(0.98, 1.88)
p-value trend		0.023		0.064
Anxiety symptoms				
No	Reference		Reference	
Yes	1.10	(0.72, 1.68)	1.03	(0.66, 1.60)
Per 1 unit increase on the scale	1.31	(0.98, 1.74)	1.26	(0.93, 1.71)
p-value trend		0.066		0.143

CI, Confidence interval; OR, Odds ratio

Odds ratio (95% Confidence Interval) from generalized estimating equation models represents the odds of wheezing episodes for the children of mothers with psychological distress during pregnancy. Maternal psychological distress was treated as dichotomized based on the clinical cut-offs (no, yes) and as continuous (per 1 unit increase). P-trend represents the linear trend per unit increase on the psychological distress scales. Models were adjusted for maternal age, body mass index, smoking during pregnancy, educational level, ethnicity, and parity, parental history of asthma or atopy, pet keeping, and children's sex, preterm birth, birth weight, breastfeeding, day care attendance, second hand smoke at home, eczema and lower respiratory tract infections.

* Not available at 3 years after delivery

Table E3.1.8. Complete-case analysis: associations of maternal psychological distress during pregnancy with overall wheezing from 1 to 4 years adjusted for paternal psychological distress during pregnancy and at 3 years after delivery

	Model 1		Model 1 + Paternal psychological distress			
			During pregnancy		At 3 years after delivery	
	OR	(95% CI)	OR	(95% CI)	OR	(95% CI)
Overall psychological distress*						
No	Reference		Reference		—-	—-
Yes	1.23	(0.76, 1.98)	1.26	(0.78, 2.04)	—-	—-
Per 1 unit increase on the scale	1.77	(1.20, 2.63)	1.79	(1.20, 2.67)	—-	—-
p-value trend		0.004		0.004		—-
Depression symptoms						
No	Reference		Reference		Reference	
Yes	1.44	(0.91, 2.29)	1.50	(0.95, 2.39)	1.51	(0.95, 2.40)
Per 1 unit increase on the scale	1.43	(1.05, 1.95)	1.46	(1.07, 2.00)	1.47	(1.08, 2.00)
p-value trend		0.023		0.018		0.015
Anxiety symptoms						
No	Reference		Reference		Reference	
Yes	1.10	(0.72, 1.68)	1.10	(0.72, 1.69)	1.10	(0.72, 1.68)
Per 1 unit increase on the scale	1.31	(0.98, 1.74)	1.31	(0.98, 1.75)	1.31	(0.98, 1.74)
p-value trend		0.066		0.065		0.069

CI, Confidence interval; OR, Odds ratio

Odds ratio (95% Confidence Interval) from generalized estimating equation models represents the odds of wheezing episodes for the children of mothers with psychological distress during pregnancy. Maternal psychological distress was treated as dichotomized based on the clinical cut-offs (no, yes) and as continuous (per 1 unit increase). P-trend represents the linear trend per unit increase on the psychological distress scales. Models were adjusted for maternal age, body mass index, smoking during pregnancy, educational level, ethnicity, and parity, parental history of asthma or atopy, pet keeping, and children's sex, preterm birth, birth weight, breastfeeding, day care attendance, second hand smoke at home, eczema and lower respiratory tract infections.

* Not available at 3 years after delivery

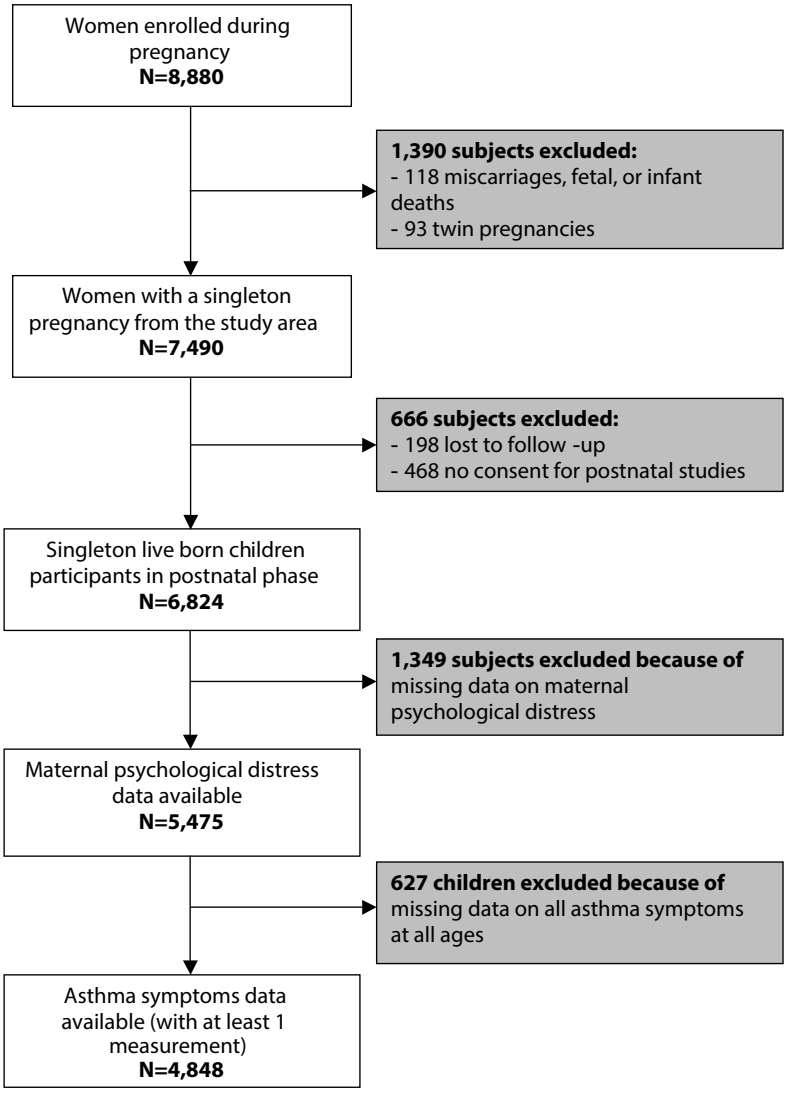

Figure E3.1.1. Flowchart of participants in the study

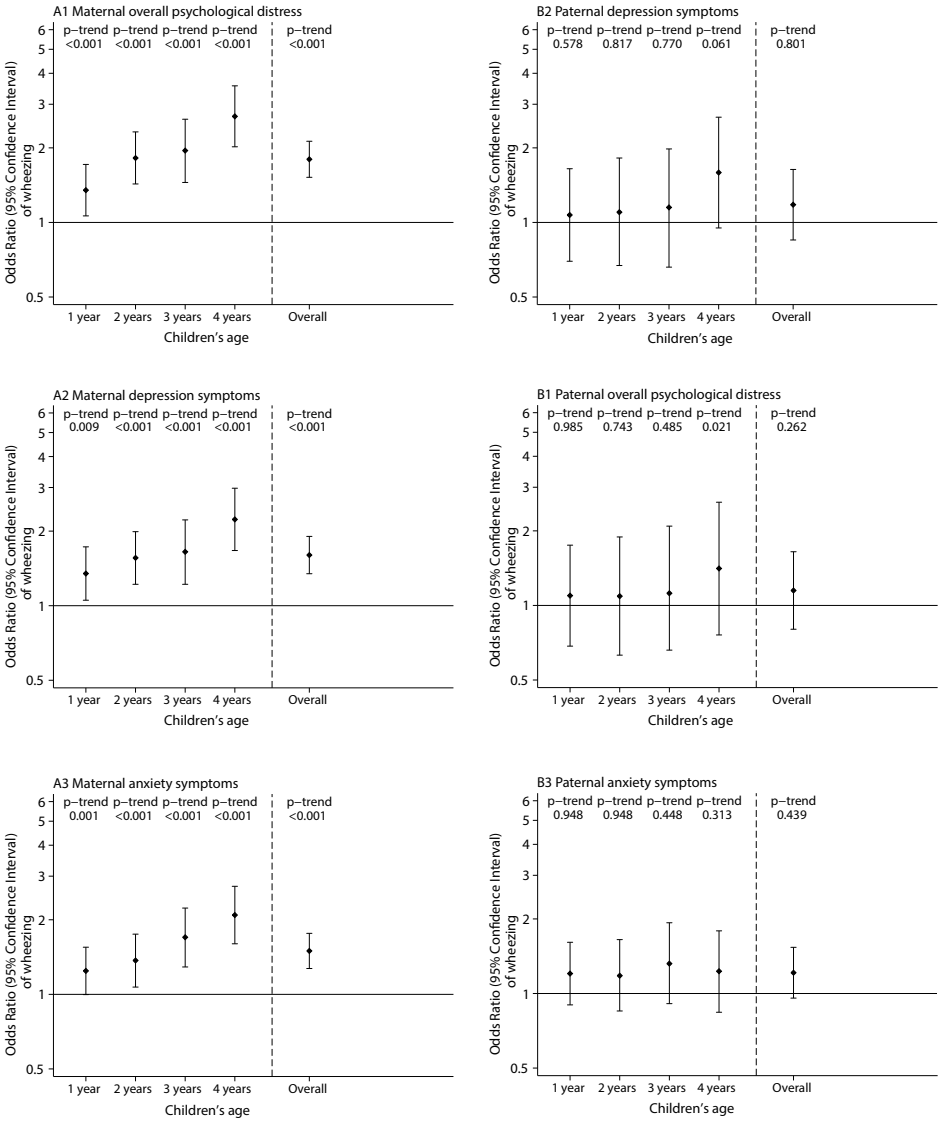

Figure E3.1.2. Unadjusted associations of maternal (**A**) and paternal (**B**) psychological distress during pregnancy with wheezing from 1 to 4 years

Odds ratio (95% Confidence Interval) from generalized estimating equation models represents the odds of wheezing for the children of mothers or fathers with psychological distress during pregnancy (no, yes). P-trend represents the linear trend per unit increase on the psychological distress scales. *Paternal models were additionally adjusted by maternal psychological distress during pregnancy.

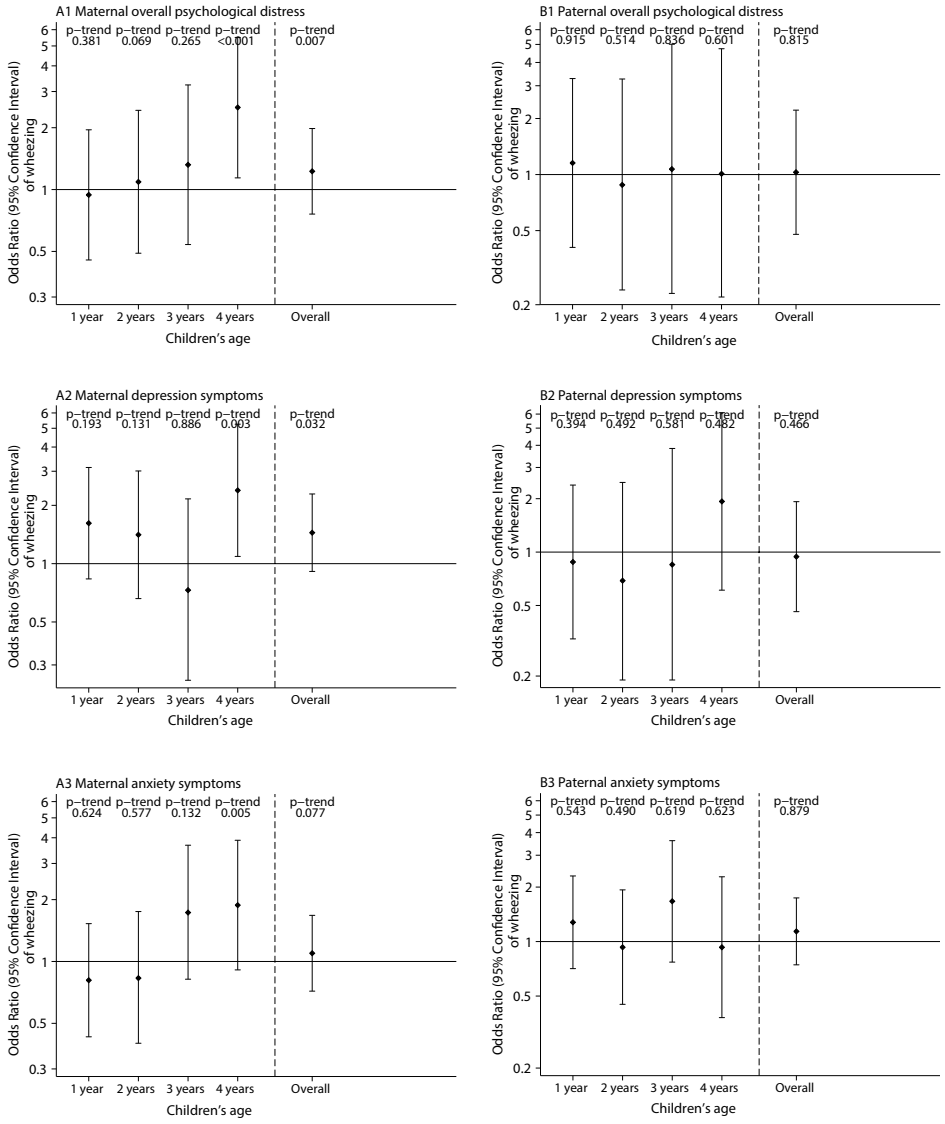

Figure E3.1.3. Complete-case analysis: adjusted associations of maternal **(A)** and paternal **(B)** psychological distress during pregnancy with wheezing from 1 to 4 years

Odds ratio (95% Confidence Interval) from generalized estimating equation models represents the odds of wheezing for the children of mothers or fathers with psychological distress during pregnancy (no, yes). P-trend represents the linear trend per unit increase on the psychological distress scales. Models were adjusted for maternal age, body mass index, smoking during pregnancy, educational level, ethnicity, and parity, parental history of asthma or atopy, pet keeping, and children's sex, preterm birth, birth weight, breastfeeding, day care attendance, second hand smoke at home, eczema and lower respiratory tract infections. *Paternal models were additionally adjusted by maternal psychological distress during pregnancy

3.2

Maternal pre-pregnancy obesity, gestational weight gain and wheezing in preschool children

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Eur Respir J. 2013 Nov;42(5):1234-43.

3.3

Influence of maternal and cord blood C-reactive protein on childhood respiratory symptoms and eczema

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Pediatr Allergy Immunol. 2013;24(5):469-75.

ABSTRACT

Background Inflammatory processes during pregnancy might affect fetal lung development and immune responses. We examined the associations of maternal and cord blood C-reactive protein levels with respiratory symptoms, and eczema in preschool children.

Methods This study was embedded in a population-based prospective cohort study of 4,984 children. Generalized Estimating Equations were used to assess the effect of C-reactive protein levels on respiratory symptoms or eczema. C-reactive protein levels were measured during early pregnancy and at birth. Wheezing, lower respiratory tract infections, and eczema until the age of 4 years were annually obtained by questionnaires.

Results Maternal C-reactive protein was not associated with the risks of wheezing and lower respiratory tract infections. Compared to children with maternal C-reactive protein in the lowest quarter, children in the highest quarter had increased risks of eczema OR 1.20 (1.03, 1.40). Compared to children with cord blood C-reactive protein lower than 0.20 mg/l, those with levels higher than 0.20 mg/l had increased risks of wheezing, OR 1.21 (1.07, 1.36), and lower respiratory tract infections, OR 1.21 (1.05, 1.39), but not of eczema.

Conclusions Our results suggest that elevated maternal C-reactive protein in pregnancy is associated with a higher risk of eczema, and C-reactive protein in cord blood with a higher risk of wheezing and lower respiratory tract infections in the first 4 years.

INTRODUCTION

C-reactive protein is an acute phase protein that increases in response to infectious and non-infectious stimuli, and is generally used as a marker for systemic inflammation[1]. Previous studies have shown that elevated C-reactive protein levels are associated with a reduced lung function, COPD, and asthma in adults[2-4] and children[5]. Elevated maternal C-reactive protein levels during pregnancy lead to fetal growth restriction[6], and are associated with endothelial dysfunction, vascular dysfunction and suboptimal placental development[7-9]. Recently, a prospective cohort study among 504 mothers and children showed that maternal C-reactive protein levels in pregnancy are associated with increased risks of wheezing and lower respiratory tract infections in the offspring until the age of 14 months[10]. These findings suggest that inflammatory processes in the mother during pregnancy lead to fetal developmental adaptations and a greater susceptibility of impaired respiratory health in childhood. Elevated levels of maternal C-reactive protein probably have an indirect effect on the developing fetus because the protein does not pass the placenta[11]. The underlying pathways might include fetal growth restriction and smaller lungs and airways[12-14], a pro-inflammatory fetal or newborn status leading to cytokine dysregulation, or other adaptations of the infant's immune system subsequently influencing the development of asthma[15]. Cord blood C-reactive protein levels do reflect fetal levels and can have both direct effects, such as a T_H2 skewed immune system, and indirect effects, as described for maternal C-reactive protein, on the fetus. Therefore, the timing of elevated C-reactive protein levels may have different effects on respiratory health of the child. Thus far, the roles of maternal and cord blood C-reactive protein levels in the development of childhood asthma remain unclear.

Therefore, we examined in a population-based prospective cohort study, among 4,984 children followed up from early fetal life, the associations between maternal and cord blood C-reactive protein levels with wheezing, lower respiratory tract infections, and eczema in the first four years of life.

METHODS

Design and setting

This study was embedded in the Generation R Study, a population-based prospective cohort study of pregnant women and their children from fetal life onwards in Rotterdam, The Netherlands[16]. The study protocol was approved by the Medical Ethical Committee of the Erasmus Medical Centre, Rotterdam. Written informed consent was obtained from all participants.

C-reactive protein levels

Maternal venous blood samples were collected in early pregnancy (median gestational age 13.1, 95% range 9.5 to 17.5 weeks) and fetal umbilical cord blood samples were collected by midwives and obstetricians immediately after delivery. High-sensitivity C-reactive protein levels were analyzed using an immunoturbidimetric assay on the Architect System[9].

Respiratory symptoms and eczema

Information on wheezing (no; yes) and physician-diagnosed lower respiratory tract infections (no; yes) was obtained by questionnaires at the ages of 1, 2, 3 and 4 years. Wheezing questions were adapted from the International Study on Asthma and Allergy in Childhood (ISAAC)[17]. We defined preschool age wheezing patterns as no wheezing, early wheezing, late wheezing or persistent wheezing (supporting information). Physician-diagnosed eczema was annually assessed from 1 to 4 years (no, yes). Response rates for the questionnaires were 71%, 76%, 72%, 73%, respectively[18].

Statistical Analysis

The associations of maternal and cord blood C-reactive protein levels with repeatedly measured wheezing, lower respiratory tract infections, and eczema at the ages of 1, 2, 3 and 4 years were analyzed using generalized estimating equations (GEEs) adjusted for potential confounders (supporting information). With GEE analyses, repeatedly measured wheezing over time can be analyzed, taking into account that these repeated measurements within the same subject are correlated. We used an unstructured correlation matrix, allowing a distinct correlation between every pair of measurements of a subject. We used the lowest quarter of maternal C-reactive protein as the reference group. Maternal body mass index, gestational hypertensive problems, smoking during pregnancy, birth weight, gestational age at birth, and cord blood C-reactive protein levels were also added as interactions (product terms) in the GEE models to explore potential effect modification on the associations of maternal C-reactive protein with respiratory symptoms and eczema. Birth weight and gestational age at birth were added as interactions to explore potential effect modification on the associations of cord blood C-reactive protein levels with respiratory symptoms and eczema. Missing data in the covariates and outcomes were imputed with multiple imputations[19]. Imputations were based on all determinants, covariates and outcomes in the model plus paternal age, educational level and history of asthma or atopy and other childhood asthma symptoms including shortness of breath, dry cough at night and persistent phlegm[20]. No major change in effect estimates was observed when we used non-imputed data. All measures of association are presented as odds ratios (OR) with their 95% Confidence Intervals (CI). For data preparation

the Statistical Package of Social Sciences version 20.0 for Windows (SPSS Inc., Chicago, IL, US) was used and statistical analyses were performed using SAS 9.2 (SAS institute, Cary, NC, USA). (An extensive description of the methods is given in the supporting information, Text E3.3.1).

RESULTS

Of the singleton live births (n=7,696), data on both maternal and cord blood C-reactive protein levels were not available for n=1,678 subjects (Supporting information, Figure E3.3.1). Subjects without information on any outcome were excluded (n=1,034), giving the following three study populations per outcome: wheezing (n=4,949), lower respiratory tract infections (n=4,880), and eczema (n=4,806) out of the final population of n=4,984 subjects with data on at least one C-reactive protein level and one outcome. As compared to mothers with information on C-reactive protein levels, those with missing data more often had a higher body mass index, were lower educated, more frequently multiparous, and less often had gestational hypertensive problems. Compared to children with information on cord blood C-reactive protein levels, those with missing data more often were from mothers with gestational hypertensive problems, had a lower birth weight and gestational age, and attended daycare more often (supporting information, Table E3.3.1, E3.3.2).

The total precision (inter-assay variation) for hs-CRP was 0.9% at 12.9mg/L and 1.3% at 39.9 mg/L. The limit of quantification is the analyte concentration at which the coefficient of variation was 20%, the lowest level of detection was 0.20 mg/L[6]. We categorized maternal C-reactive protein levels into quartiles (<2.29 mg/L; 2.30-4.29 mg/L; 4.30-7.69 mg/L; >7.70 mg/L). Maternal C-reactive protein levels were under the detection limit (0.15% (n=6)) were included in the lowest quarter of the distribution. Cord blood C-reactive protein levels were dichotomized (<0.20 mg/L; ≥0.20 mg/L) due to small variation of the C-reactive protein level values (range: <0.20-43.10). The prevalence of wheezing declined from the age of 1 to 4 years (age 1: 29.8%, age 4: 14.0%). Similarly, the prevalence of lower respiratory tract infections (age 1: 15.8%, age 4: 6.2%) and eczema (age 1: 23.0%, age 4: 8.5%) declined.

Maternal and child characteristics are presented in Table 3.3.1.

Maternal C-reactive protein levels were not consistently associated with wheezing, lower respiratory tract infections and eczema in the child at the ages of 1, 2, 3 and 4 years separately nor longitudinally (Figure 3.3.1). As compared to children from mothers with C-reactive protein levels in the lowest quarter, children from mothers in the highest quarter had an increased risk of eczema OR 1.20 (1.03, 1.40) until the age of 4 years. The overall test for trend was not significant. No effect modification was observed for maternal C-reactive protein levels with maternal body mass index, gestational hypertensive complications, gestational age at birth, birth weight, and cord blood C-reactive protein levels (p-values for interaction

Table 3.3.1. Maternal and child baseline characteristics

	n=4,984	
	Observed	**After Multiple Imputations**
Maternal characteristics		
Age (years)	30.7 (4.8)	30.7 (4.8)
Body mass index (kg/m^2)		
<20	9.5 (472)	9.5 (473)
20-25.0	56.1 (2,782)	56.1 (2,797)
25-30.0	24.0 (1,190)	24.1 (1,201)
≥30	10.3 (513)	10.3 (513)
Missing	*0.5 (27)*	-
Education (%)		
Primary, or secondary	52.4 (2,513)	48.4 (2,411)
Higher	47.6 (2,279)	51.6 (2,573)
Missing	*3.9 (192)*	-
History of asthma or atopy (%)		
No	61.8 (2,554)	63.2 (3,141)
Yes	38.2 (1,582)	36.8 (1,843)
Missing	*17.0 (848)*	-
Smoking during pregnancy (%)		
No	86.2 (3,806)	85.9 (4,283)
Yes	13.8 (609)	14.1 (701)
Missing	*11.4 (569)*	-
Parity (%)		
0	58.1 (2,880)	58.0 (2,892)
≥1	41.9 (2,081)	42.0 (2,092)
Missing	*0.5 (23)*	-
Gestational hypertensive problems (%)		
No	94.2 (4,638)	93.8 (4,675)
Yes	5.8 (286)	6.2 (309)
Missing	*1.2 (60)*	-
Maternal C-reactive protein levels (mg/l)*	4.2 (0.6 – 24.9)	4.2 (0.6 – 24.9)
Gestational age at blood sampling (weeks)	13.1 (9.5, 17.5)	13.1 (9.5, 17.5)
Child characteristics		
Female sex, no (%)	50.0 (2,491)	50.1 (2,491)
Gestational age at birth (weeks)	40.1 (36.1 - 42.3)	40.1 (36.1 - 42.3)
Birth weight (grams)	3,459 (544)	3,460 (544)
Ethnicity (%)		
European	70.2 (3,422)	69.6 (3,471)
Non-European	29.8 (1,450)	30.4 (1,513)
Missing	*2.2 (112)*	-

Table 3.3.1. Maternal and child baseline characteristics (continued)

	n=4,984	
	Observed	**After Multiple Imputations**
Breastfeeding (%)		
No	7.7 (372)	7.8 (390)
Yes	92.3 (4,431)	92.2 (4,594)
Missing	*3.6 (181)*	-
Day care attendance 1st year (%)		
No	41.7 (1,581)	44.7 (2,228)
Yes	58.3 (2,210)	55.3 (2,756)
Missing	*23.9 (1,193)*	-
Pet keeping (%)		
No	66.0 (2,863)	66.4 (3,311)
Yes	34.0 (1,474)	33.6 (1,673)
Missing	*13.0 (647)*	-
Cord blood C-reactive protein levels (mg/l)*		
< 0.20	78.4 (2,671)	78.4 (2,671)
≥ 0.20	21.6 (738)	21.6 (738)
Missing	*31.6 (1,575)*	*31.6 (1,575)*

Values are means (SD), medians (95% range) or percentages (absolute numbers).
Missing percentages are given for the total population of analysis n=4,984. Other percentages are valid percentages. *Maternal and cord blood C-reactive protein levels were not imputed (mg/l), Maternal C-reactive protein levels were missing for 17.1%.

>0.05). We observed effect modification of C-reactive protein levels by maternal smoking on eczema (p for interaction <0.05), but not on respiratory symptoms. Stratified analyses for maternal atopy, as a proxy for atopic susceptibility of the children, showed that the effect estimates for wheezing and lower respiratory tract infections were higher, but still not significant in the group of atopic mothers (Supporting information, Table E3.3.3). With eczema as the outcome, no differences were observed between mothers with and without atopy. P-values for interaction of CRP with maternal atopy were 0.35 for the outcome wheezing, 0.57 for lower respiratory tract infections, and 0.78 for eczema. We observed no association of maternal C-reactive protein levels with preschool wheezing patterns (Supporting information, Table E3.3.4).

Cord blood C-reactive protein levels were not consistently associated with wheezing, lower respiratory tract infections and eczema at the ages of 1, 2, 3 and 4 years (Figure 3.3.2). Longitudinal analyses showed that as compared to children with cord blood C-reactive protein levels lower than 0.20 mg/L, those with higher C-reactive protein levels had increased risks of wheezing OR 1.21 (1.07, 1.36), of lower respiratory tract infections OR 1.21 (1.05, 1.39), but not of eczema in the first 4 years of life. No effect modification was observed for cord

blood C-reactive protein levels with birth weight. We observed a significant modifying effect of C-reactive protein levels with gestational age at birth (p-value for interaction <0.01). In stratified analyses on gestational age, we observed that preterm born children with increased C-reactive protein levels had higher overall effect estimates for wheezing, OR 4.58 (2.03, 10.31) vs. 1.16 (1.03, 1.31), compared to term born children with increased C-reactive protein levels (Table 3.3.2). These higher effect estimates were also observed in each year separately (not shown). The interaction terms for lower respiratory tract infections and eczema with gestational age were not significant (Table 3.3.2). After stratification for maternal atopic status, we observed that children with non-atopic mothers had higher overall effect estimates for wheezing (OR 1.28 (1.11, 1.48) vs. 1.07 (0.87, 1.33)), lower respiratory tract infections (OR 1.26 (1.05, 1.51) vs. 1.08 (0.83, 1.39)), and eczema (OR 1.13 (0.92, 1.37) vs. 0.83 (0.64, 1.07)), as compared to children from atopic mothers (p for interaction all >0.05) (Supporting information, Table E3.3.5). An increased cord blood C-reactive protein was associated with in increased risk of an early wheezing pattern (OR 1.25 (1.02, 1.53)) (Supporting information, Table E3.3.6). After additional adjustment for lower respiratory tract infections the estimates for the association of cord blood C-reactive protein levels with wheezing attenuated into a non-significant effect (not shown).

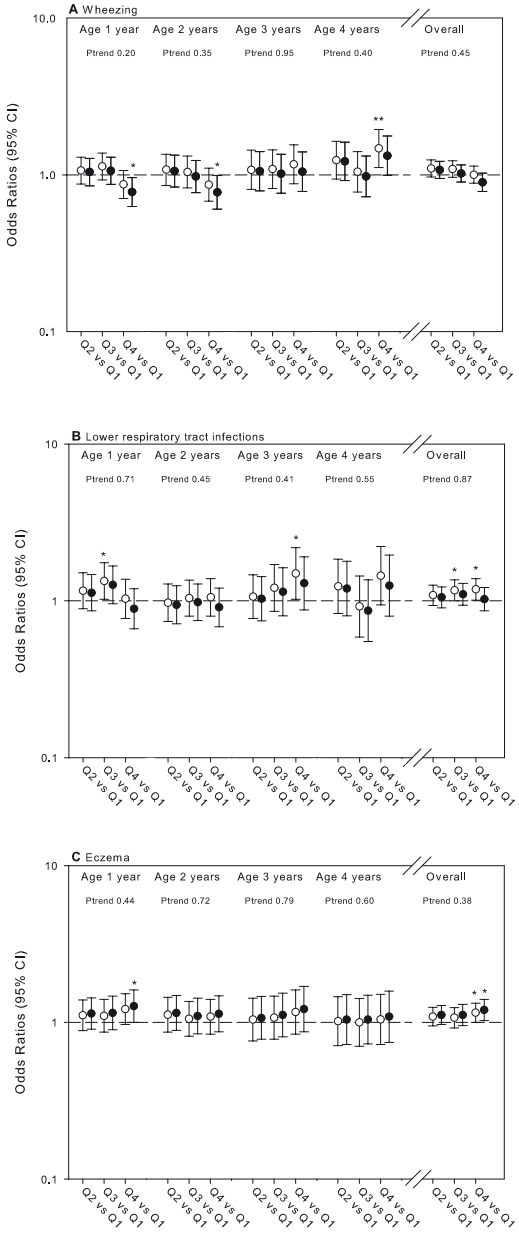

Figure 3.3.1. Maternal C-reactive protein levels and risks of wheezing, lower respiratory tract infections and eczema until the age of 4 years. Values are Odds Ratios (95% Confidence Interval) and reflect the risks of (**A**) wheezing, (**B**) lower respiratory tract infections, or (**C**) eczema of children in a specific quarter group compared to the lowest quarter (Q1). *P < 0.05 using generalized estimating equation models. White bullets represent crude odds ratios, black bullets represent adjusted odds ratios in which models were adjusted for maternal age, body mass index, education, history of asthma or atopy, smoking habits, parity, gestational hypertensive problems, and pregnancy duration at blood sampling, and children's sex, gestational age, birth weight, ethnicity, breastfeeding status, daycare attendance and pet keeping.

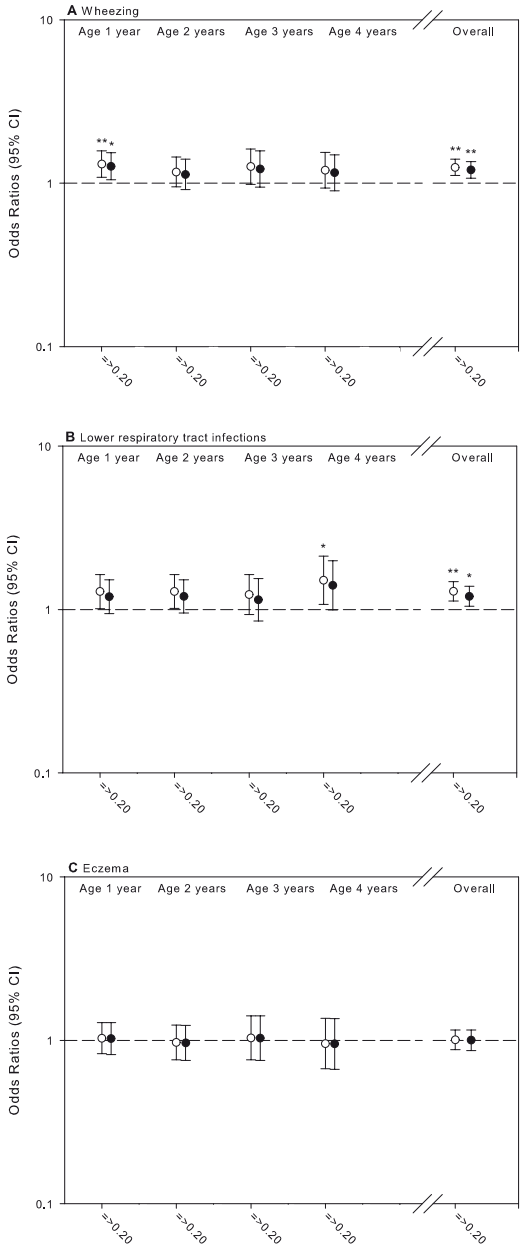

Figure 3.3.2. Cord blood C-reactive protein levels and risks of wheezing, lower respiratory tract infections and eczema until the age of 4 years. Values are Odds Ratios (95% Confidence Interval) and reflect the risks of (**A**) wheezing, (**B**) lower respiratory tract infections, or (**C**) eczema. *P < 0.05 and **p < 0.01 using generalized equating estimates models. White bullets represent the crude odds ratios, black bullets represent adjusted odds ratios in which models were adjusted for maternal age, body mass index, education, history of asthma or atopy, smoking habits, gestational hypertensive problems, parity, children's sex, gestational age, birth weight, ethnicity, breastfeeding status, daycare attendance and pet keeping.

Table 3.3.2. Cord blood C-reactive protein levels (mg/l) and wheezing until the age of 4 years stratified for preterm birth

	Odds Ratios (95% Confidence Intervals) of overall		
	Wheezing	**LRTI**	**Eczema**
Cord blood CRP			
<37 weeks			
< 0.20	*Reference*	*Reference*	*Reference*
n=73			
≥ 0.20-43.10	4.58 (2.03, 10.31)**	2.94 (1.04, 8.30)*	0.48 (0.19, 1.24)[a]
n=20			
≥37 weeks			
< 0.20	*Reference*	*Reference*	*Reference*
n=2,598			
≥ 0.20-43.10	1.16 (1.03, 1.31)*	1.16 (1.01, 1.34)*	1.01 (0.88, 1.16)[a]
n=718			

*P < 0.05 using generalized equating estimates models. Models were adjusted for maternal age, body mass index, education, history of asthma or atopy, smoking habits, gestational hypertensive problems, parity, children's sex, birth weight, ethnicity, breastfeeding status, daycare attendance and pet keeping. P-value for interaction CRP * gestational age at birth with: wheezing < 0.01, lower respiratory tract infections = 0.31, eczema = 0.06. [a] Not adjusted for breastfeeding due to lack of power.

DISCUSSION

Our results suggest that elevated maternal C-reactive protein levels in early pregnancy are associated with a lower risk of wheezing in the first two years and an overall higher risk of eczema, whereas cord blood C-reactive protein levels are associated with a higher overall risk of wheezing and lower respiratory tract infections.

A previous study suggested that children have a threefold increased risk of recurrent wheezing and a more than twofold increased risk of recurrent lower respiratory tract infections at the age of 14 months among children in the highest tertile compared to the lowest tertile of maternal C-reactive protein levels during pregnancy[10]. We observed a lower risk of wheezing in the first year for the highest maternal C-reactive protein levels group and no association of maternal C-reactive protein levels with lower respiratory tract infections. The C-reactive protein levels between the studies were measured during similar weeks of pregnancy and the 25%-75% ranges were comparable (2.0-7.0 mg/L vs. 2.3-7.7 mg/L for Morales et al. and our study, respectively). Differences in the observed effects are unlikely to be the result of different laboratory methods (regular C-reactive protein levels vs. high sensitivity C-reactive protein levels) with different detection limits (2.0 mg/L vs. 0.2 mg/L, respectively) because both the lowest tertile and quartile reference group that were used included corresponding low C-reactive protein levels. A more likely explanation is that we assessed our outcomes annually and in a larger number of subjects, and were able to assess the influence of many potential effect modifiers. Pregnancy can be seen as an inflammatory

stressor and elevated C-reactive protein levels with values of >10 mg/l are within the normal range for pregnant women throughout gestation[21]. The highest quarter might have included mothers with an acute systemic inflammation and might have affected the strength of the associations. However, a sensitivity analysis excluding mothers with C-reactive protein levels >100 mg/L showed similar effect estimates. As we performed multiple tests, we cannot exclude that some results might be a chance finding. However, because of the correlation in outcomes we did not apply adjustment for multiple testing.

The mechanisms explaining the relation between maternal C-reactive protein levels and a reduced risk of wheezing in the first year, and an increased risk of eczema until the age of 4 years are not clear. The different direction of effect estimates between maternal and cord blood C-reactive protein levels may suggest that the timing of increased C-reactive protein levels is critical for the association with lung and airway development. Early adverse exposures might trigger developmental adaptations in the child, as suggested by the developmental origins hypothesis. This could lead to an adapted risk of respiratory symptoms and eczema in early childhood. C-reactive protein cannot pass the placenta, thus the suggested association of maternal C-reactive protein levels and wheezing and eczema is not likely to be direct or causal. C-reactive protein is produced in the liver under IL-6 stimulation, and IL-6 may change the T_H1/T_H2 cell balance by inhibiting T_H1 differentiation as well as promotion of T_H2 differentiation[22]. A late exposure will not result in preventive adaptations, but we suggest that exposure to infections in late pregnancy makes the child more responsive to infections. The observed association between cord blood C-reactive protein and an early preschool wheezing pattern (supporting information) support the observed associations between cord blood C-reactive protein and wheezing and lower respiratory tract infections. Thus, increased cord blood C-reactive protein levels increase the risk of infections in the first four years of life. Also, after additional adjustment for lower respiratory tract infections the estimates attenuated into a non-significant effect. This suggests that the association between cord blood C-reactive protein and wheezing is, at least partly, explained by infectious mechanisms.

Elevated C-reactive protein levels are suggested to be partially driven by an increased body mass index[23]. Also, they are suggested to be associated with preeclampsia, subsequently leading to increased risk of wheezing via an impaired placental functioning and its adverse effect on lung development[13, 24, 25]. However, in our study we did not observe these modifying effects.

An elevated C-reactive protein level in cord blood might be the result of placental problems like inflammatory lesions[26], a pro-inflammatory fetal or newborn status leading to cytokine dysregulation, or other adaptations of the infant's immune system subsequently influencing the development of infections and asthma[15]. We observed a modifying effect of gestational age at birth. The effect of elevated C-reactive protein levels on wheezing and lower respiratory tract infections were stronger in preterm than in term born children. This might be explained by a combined effect of an immature lung development, an immature immune system and

thereby an increased susceptibility to infections, and the effect of C-reactive protein and other cytokines as IL-6 which changed the immune system towards being more vulnerable[22].

Strengths and limitations

This study was embedded in a population-based prospective cohort study with a large number of subjects being studied from early life onwards with detailed prospectively measured information about C-reactive protein levels, a large number of confounders and data on wheezing, physician-diagnosed lower respiratory tract infections, and eczema. In our population for analysis 17.1% did not have data on maternal C-reactive protein levels and 31.6% of the subjects did not have data on cord-blood C-reactive protein levels. This non-response would lead to biased effect estimates if the associations of maternal and cord blood C-reactive protein levels with respiratory symptoms or eczema would be different between those included and not included in the analyses. Based on those included and not include in the analyses, we speculate that our observed effect estimates would be underestimated if those not included would have had higher cord blood C-reactive protein levels and would have reported respiratory symptoms more often. Results also would be underestimated if those subjects not included would have lower maternal C-reactive protein levels and would have reported less eczema. A limitation of our study is that we were not able to assess inflammation throughout pregnancy. C-reactive protein has a short half-life and we only measured C-reactive protein levels once during first trimester of pregnancy (median gestational age 13.1, 95% range 9.5 to 17.5 weeks). However, previous studies observed that C-reactive protein levels in early pregnancy correlated with those later in pregnancy[21, 27], and with pregnancy outcomes as gestational hypertensive complications, preterm birth, and birth weight[6, 9, 12]. A small part of the cord blood C-reactive protein levels (+/- 20% of 0.20 mg/L) could have been in the measurement error range, which could have either over- or underestimated our results. The main outcomes were self-reported. This is a widely accepted method in epidemiological studies and reliably reflects the incidence of respiratory symptoms and eczema in young children[17, 28]. In preschool children, a diagnosis of asthma is often difficult, and based on symptoms. Objective tests, including lung function or bronchial hyperresponsiveness, are difficult to perform in young children or are not informative, and not recommended by current guidelines.

In conclusion, our results suggest that elevated maternal C-reactive protein levels are associated with a higher risk of eczema while elevated cord blood C-reactive protein levels are associated with an increased risk of wheezing and respiratory tract infections in the first 4 years. These effects suggest different underlying pathways leading to different adaptive mechanisms and susceptibility of respiratory diseases and eczema. Cord blood C-reactive protein levels can have both a direct and indirect effect on the fetus. Therefore the timing of

elevated C-reactive protein levels may have different effects on respiratory health of the child. Further studies are needed to explore the specific underlying mechanisms and the effect of maternal and cord blood C-reactive levels on various phenotypes of respiratory diseases and eczema in later life.

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Supplements

TEXT E3.3.1.

Design and setting

This study was embedded in the Generation R Study, a population-based prospective cohort study of pregnant women and their children from fetal life onwards in Rotterdam, The Netherlands, and has previously been described in detail[1]. The study protocol was approved by the Medical Ethical Committee of the Erasmus Medical Centre, Rotterdam. Written informed consent was obtained from all participants.

C-reactive protein levels

Maternal venous blood samples were collected in early pregnancy (median gestational age 13.1, 95% range 9.5 to 17.5 weeks) and fetal umbilical cord blood samples were collected by midwives and obstetricians immediately after delivery. High-sensitivity C-reactive protein levels were analyzed using an immunoturbidimetric assay on the Architect System (Abbot Diagnostics B.V., Hoofddorp, The Netherlands) as described previously in detail[2]. The lowest level of detection was 0.20 mg/L[3].

Respiratory symptoms and eczema

Information on wheezing and physician-diagnosed lower respiratory tract infections was obtained by questionnaires at the ages of 1, 2, 3 and 4 years. Wheezing questions were adapted from the International Study on Asthma and Allergy in Childhood (ISAAC)[4]. We defined preschool age wheezing patterns as: 1) no wheezing: no recorded wheezing at any age; 2) early wheezing: at least one wheezing symptom during the first 3 years of life but no wheezing at 4 years of age; 3) late wheezing: no wheezing episodes during the first 3 years of age but wheezing at 4 years of age; 4) preschool persistent wheezing: at least one wheezing episode in the first 3 years of life and wheezing at 4 years of age, based on Martinez et al[5]. Physician-diagnosed lower respiratory tract infections were reported as pertussis, bronchitis, bronchiolitis, or pneumonia. Physician-diagnosed eczema was annually assessed from 1 to 4 years (no, yes). Response rates for the questionnaires were 71%, 76%, 72%, 73%, respectively[6].

Covariates

Information on maternal history of asthma or atopy, socio-economic status, parity, children's ethnicity and pet keeping was obtained by questionnaires, completed by the mother at enrolment. Maternal history of asthma was defined as having a history of asthma, and maternal atopy was defined as having a history of hay fever or eczema or being allergic to house dust mite. Maternal body mass index was measured as height and weight at enrolment in the study. Information on active maternal smoking was obtained by postal questionnaires sent in first, second and third trimester of pregnancy and combined into smoking (no, yes)[1, 7]. Information on gestational hypertensive complications (gestational hypertension, preeclampsia, eclampsia, and HELLP-syndrome (Hemolysis Elevated Liver enzymes and Low Platelets)), birth weight, gestational age and sex of the children was obtained from midwife and hospital registries at birth. Postal questionnaires at the ages of 6 and 12 months provided information about breastfeeding and of 12 months of daycare attendance[1, 6].

Statistical Analysis

The associations of maternal and cord blood C-reactive protein levels with repeatedly measured wheezing, lower respiratory tract infections, and eczema at the ages of 1, 2, 3 and 4 years were analyzed using generalized estimating equations (GEEs). With GEE analyses, repeatedly measured wheezing over time can be analyzed, taking into account that these repeated measurements within the same subject are correlated. We used the lowest quartile of maternal C-reactive protein as the reference group. All models were adjusted for potential confounders which were included in the model based on literature or a change in effect estimate of >10%. We tested the interaction of C-reactive protein levels with maternal body mass index, gestational hypertensive problems, atopic status, smoking during pregnancy, birth weight, gestational age at birth and cord blood C-reactive protein levels (product terms) in the GEE models to explore potential effect modification on the associations with respiratory symptoms and eczema. Maternal atopic status, birth weight and gestational age at birth were added as product terms with cord blood C-reactive protein levels to explore potential effect modification on the associations with respiratory symptoms and eczema. The percentages of missing values were lower than 10%, except for maternal history of asthma or atopy (17.0%), smoking during pregnancy (11.4%), attending day care (23.9%) and pet keeping (13.0%). Missing data in the covariates and outcomes were imputed with multiple imputations[8]. Twenty-five new datasets were created by imputation based on all determinants, covariates and outcomes in the model plus paternal age, educational level and history of asthma or atopy and other asthma symptoms including shortness of breath, dry cough at night and persistent phlegm[9]. Information on paternal characteristics and the other asthma symptoms were available from the same questionnaires as maternal characteristics

and wheezing, respectively, were obtained. All datasets were analyzed separately after which results were combined. No major change in effect estimates was observed when we used non-imputed data. All measures of association are presented as odds ratios (OR) with their 95% Confidence Intervals (CI). For data preparation the Statistical Package of Social Sciences version 20.0 for Windows (SPSS Inc., Chicago, IL, US) was used and statistical analyses were performed using SAS 9.2 (SAS institute, Cary, NC, USA).

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Table E3.3.1. Differences in characteristics of mothers and their children between groups with or without information on maternal C-reactive protein (n=4,984)

	Maternal C-reactive protein available n= 4,133	Maternal C-reactive protein *not* available n=851	P-value for difference
Maternal characteristics			
Age (years)	30.7 (4.6)	30.7 (5.4)	n.s.
Body mass index (kg/m^2)			
<20	10.0 (415)	6.8 (58)	<0.01
20-25.0	57.8 (2,387)	48.2 (410)	
25-30.0	22.7 (939)	30.8 (262)	
≥30	9.5 (392)	14.2 (121)	
Education (%)			
Primary, or secondary	47.1 (1,948)	54.4 (463)	<0.01
Higher	52.9 (2,185)	45.6 (388)	
History of asthma or atopy (%)			
No	62.6 (2,588)	65.0 (553)	n.s.
Yes	37.4 (1,545)	35.0 (298)	
Smoking during pregnancy (%)			
No	86.0 (3,553)	85.9 (731)	n.s.
Yes	14.0 (580)	14.1 (120)	
Parity (%)			
0	59.5 (2,461)	50.6 (431)	<0.01
≥1	40.5 (1,672)	49.4 (420)	
Gestational hypertensive problems (%)			
No	93.3 (3,855)	96.2 (819)	<0.01
Yes	6.7 (278)	3.8 (32)	
Child characteristics			
Female sex, no (%)	50.0 (2,065)	50.1 (426)	n.s.
Gestational age at birth (weeks)	40.3 (37.1, 42.1)	40.0 (37.3, 42.0)	<0.01
Birth weight (grams)	3,458 (549)	3,466 (518)	n.s.
Ethnicity (%)			
European	71.2 (2,944)	61.9 (527)	<0.01
Non-European	28.8 (1,189)	38.1 (324)	
Breastfeeding (%)			
No	7.8 (321)	8.1 (69)	n.s.
Yes	92.2 (3,812)	91.9 (782)	
Day care attendance 1st year (%)			
No	43.4 (1,795)	50.9 (433)	<0.01
Yes	56.6 (2,338)	49.1 (418)	

Table E3.3.1. Differences in characteristics of mothers and their children between groups with or without information on maternal C-reactive protein (n=4,984) (continued)

	Maternal C-reactive protein available n= 4,133	Maternal C-reactive protein *not* available n=851	P-value for difference
Pet keeping (%)			
No	65.6 (2,712)	70.4 (599)	<0.01
Yes	34.4 (1,421)	29.6 (252)	
Cord blood C-reactive protein levels (mg/l)			
< 0.20	49.0 (2,027)	75.7 (644)	<0.05
≥ 0.20	12.8 (531)	24.3 (207)	
missing	38.2 (1,575)	-	
Ever wheezing (%)			
No	54.7 (2,260)	54.8 (466)	n.s.
Yes	45.3 (1,873)	45.2 (385)	
Ever lower respiratory tract infections (%)			
No	67.7 (2,799)	60.6 (516)	<0.01
Yes	32.3 (1,334)	39.4 (335)	
Ever eczema (%)			
No	62.8 (2,597)	65.5 (557)	n.s.
Yes	37.2 (1,536)	34.5 (294)	

P for difference was calculated using chi-square tests for categorical variables, student's t-test for continues variables and Mann-Whitney for continues not normal distributed variables.

Table E3.3.2. Differences in characteristics of mothers and their children between groups with or without information on cord blood C-reactive protein (n=4,984)

	Cord blood C-reactive protein available n=3,409	Cord blood C-reactive protein *not* available n=1,575	P-value for difference
Maternal characteristics			
Age (years)	30.6 (4.8)	30.8 (4.7)	n.s.
Body mass index (kg/m^2)			
<20	9.2 (313)	10.2 (160)	n.s.
20-25.0	56.3 (1,919)	55.7 (878)	
25-30.0	24.4 (833)	23.4 (368)	
≥30	10.1 (344)	10.7 (169)	
Education (%)			
Primary, or secondary	48.1 (1,640)	49.0 (771)	n.s.
Higher	51.9 (1,769)	51.0 (804)	
History of asthma or atopy (%)			
No	63.6 (2,167)	61.9 (975)	n.s.
Yes	36.4 (1,242)	38.1 (600)	
Smoking during pregnancy (%)			
No	85.7 (2,920)	86.6 (1,364)	n.s.
Yes	14.3 (489)	13.4 (211)	
Parity (%)			
0	57.6 (1,963)	59.0 (929)	n.s.
≥1	42.4 (1,446)	41.0 (646)	
Gestational hypertensive problems (%)			
No	95.2 (3,244)	90.9 (1,431)	<0.01
Yes	4.8 (165)	9.1 (144)	
Maternal C-reactive protein levels (mg/l)*	4.2 (0.6 - 25.8)	4.1 (0.6 - 23.0)	n.s.
Child characteristics			
Female sex, no (%)	49.4 (1,684)	51.2 (807)	n.s.
Gestational age at birth (weeks)	40.3 (37.4, 42.1)	40.1 (36.1, 42.1)	<0.01
Birth weight (grams)	3,494 (502)	3,386 (618)	<0.01
Ethnicity (%)			
European	70.0 (2,386)	68.9 (1,085)	n.s.
Non-European	30.0 (1,023)	31.1 (490)	
Breastfeeding (%)			
No	7.9 (270)	7.6 (120)	n.s.
Yes	92.1 (3,139)	92.4 (1,455)	
Day care attendance 1st year (%)			
No	45.8 (1,560)	42.5 (669)	<0.05
Yes	54.2 (1,849)	57.5 (906)	

Table E3.3.2. Differences in characteristics of mothers and their children between groups with or without information on cord blood C-reactive protein (n=4,984) (continued)

	Cord blood C-reactive protein available n=3,409	Cord blood C-reactive protein *not* available n=1,575	P-value for difference
Pet keeping (%)			
No	67.0 (2,284)	65.2 (1,027)	n.s.
Yes	33.0 (1,125)	34.8 (548)	
Ever wheezing (%)			
No	55.6 (1,897)	52.6 (829)	n.s.
Yes	44.4 (1,512)	47.4 (746)	
Ever lower respiratory tract infections (%)			
No	64.7 (2,205)	70.5 (1,110)	<0.01
Yes	35.3 (1,204)	29.5 (465)	
Ever eczema (%)			
No	64.0 (2,181)	61.7 (972)	n.s.
Yes	36.0 (1,228)	38.3 (603)	

P for difference was calculated using chi-square tests for categorical variables, student's t-test for continues variables and Mann-Whitney for continues not normal distributed variables.

Table E3.3.3. Maternal C-reactive protein levels (mg/l) and wheezing of their children until the age of 4 years stratified for maternal atopy

	Age 1 year	Age 2 years	Age 3 years	Age 4 years	Overall
	Odds Ratios (95% Confidence Intervals) of wheezing				
No maternal atopy					
≤0.20-2.29	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*
n=661					
2.30-4.29	0.92 (0.71, 1.19)	1.01 (0.75, 1.36)	0.93 (0.64, 1.37)	1.17 (0.82, 1.66)	0.98 (0.84, 1.16)
n=673					
4.30-7.69	0.87 (0.68, 1.11)	0.88 (0.65, 1.21)	0.96 (0.65, 1.41)	1.01 (0.70, 1.46)	0.91 (0.77, 1.07)
n=661					
7.70-343.0	0.67 (0.51, 0.88)**	0.70 (0.51, 0.98)*	1.05 (0.72, 1.52)	1.23 (0.85, 1.79)	0.81 (0.68, 0.97)*
n=673					
p for trend	*0.25*	*0.20*	*0.88*	*0.75*	*0.36*
Maternal atopy					
≤0.20-2.29	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*
n=359					
2.30-4.29	1.30 (0.93, 1.81)	1.14 (0.79, 1.66)	1.26 (0.80, 2.01)	1.33 (0.84, 2.09)	1.26 (1.03, 1.53)*
n=394					
4.30-7.69	1.49 (1.06, 2.09)*	1.13 (0.77, 1.67)	1.10 (0.68, 1.76)	0.90 (0.53, 1.50)	1.23 (0.99, 1.52)
n=346					
7.70-343.0	0.97 (0.67, 1.40)	0.88 (0.59, 1.31)	1.02 (0.61, 1.70)	1.46 (0.90, 2.37)	1.04 (0.83, 1.31)
n=366					
p for trend	*0.44*	*0.77*	*0.88*	*0.40*	*0.98*
	Odds Ratios (95% Confidence Intervals) of lower respiratory tract infections				
No maternal atopy					
≤0.20-2.29	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*
n=661					
2.30-4.29	1.09 (0.78, 1.53)	0.90 (0.63, 1.27)	1.15 (0.74, 1.78)	1.15 (0.67, 1.97)	1.05 (0.87, 1.27)
n=673					
4.30-7.69	1.00 (0.71, 1.42)	0.93 (0.65, 1.34)	1.16 (0.72, 1.85)	0.83 (0.48, 1.46)	1.00 (0.81, 1.22)
n=661					
7.70-343.0	0.88 (0.60, 1.28)	0.93 (0.64, 1.34)	1.28 (0.78, 2.12)	1.17 (0.66, 2.06)	1.01 (0.82, 1.26)
n=673					
p for trend	*0.80*	*0.54*	*0.60*	*0.57*	*0.66*
Maternal atopy					
≤0.20-2.29	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*
n=359					
2.30-4.29	1.23 (0.77, 1.95)	1.06 (0.66, 1.69)	0.88 (0.51, 1.53)	1.32 (0.67, 2.63)	1.10 (0.83, 1.45)
n=394					
4.30-7.69	1.86 (1.21, 2.88)**	1.07 (0.68, 1.69)	1.13 (0.66, 1.94)	0.92 (0.39, 2.15)	1.32 (1.00, 1.73)
n=346					
7.70-343.0	0.88 (0.54, 1.44)	0.85 (0.52, 1.40)	1.29 (0.74, 2.26)	1.37 (0.64, 2.92)	1.02 (0.74, 1.39)
n=366					
p for trend	*0.22*	*0.51*	*0.55*	*0.90*	*0.53*

Table E3.3.3. Maternal C-reactive protein levels (mg/l) and wheezing of their children until the age of 4 years stratified for maternal atopy (continued)

	Age 1 year	Age 2 years	Age 3 years	Age 4 years	Overall
	Odds Ratios (95% Confidence Intervals) of eczema				
No maternal atopy					
≤0.20-2.29	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*
n=661					
2.30-4.29	1.21 (0.89, 1.66)	0.95 (0.68, 1.33)	1.11 (0.74, 1.67)	1.00 (0.64, 1.56)	1.10 (0.91, 1.33)
n=673					
4.30-7.69	1.16 (0.83, 1.63)	0.95 (0.67, 1.36)	1.04 (0.68, 1.59)	0.87 (0.55, 1.38)	1.04 (0.84, 1.29)
n=661					
7.70-343.0	1.37 (1.01, 1.88)	1.09 (0.77, 1.53)	1.25 (0.80, 1.95)	0.95 (0.59, 1.53)	1.21 (0.99, 1.49)
n=673					
p for trend	*0.34*	*1.00*	*0.60*	*0.93*	*0.27*
Maternal atopy					
≤0.20-2.29	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*
n=359					
2.30-4.29	1.02 (0.71, 1.46)	1.51 (0.98, 2.33)	0.99 (0.59, 1.65)	1.10 (0.61, 2.00)	1.13 (0.90, 1.42)
n=394					
4.30-7.69	1.12 (0.77, 1.62)	1.36 (0.87, 2.13)	1.21 (0.74, 1.98)	1.34 (0.76, 2.36)	1.22 (0.96, 1.56)
n=346					
7.70-343.0	1.09 (0.74, 1.60)	1.19 (0.76, 1.88)	1.13 (0.69, 1.87)	1.30 (0.71, 2.36)	1.16 (0.90, 1.50)
n=366					
p for trend	*0.94*	*0.46*	*0.75*	*0.56*	*0.86*

Values are Odds Ratios (95% Confidence Interval) and reflect the risks of wheezing, lower respiratory tract infections, or eczema of children in a specific quarter group compared to the lowest quarter. *P < 0.05 ** P>0.01, using generalized estimating equation models. Models were adjusted for maternal age, body mass index, education, history of asthma, smoking habits, parity, gestational hypertensive problems, and pregnancy duration at blood sampling, and children's sex, gestational age, birth weight, ethnicity, breastfeeding status, daycare attendance and pet keeping. For P for trend we included maternal C-reactive protein levels as a continuous variable in the model. P-value for interaction CRP * maternal atopy with: wheezing = 0.35, lower respiratory tract infections = 0.57, and eczema = 0.78.

Table E3.3.4. Maternal C-reactive protein levels and pre-school wheezing phenotypes

	Never	Early	Late	Persistent
C-reactive protein				
≤0.20-2.29	*Reference*	*Reference*	*Reference*	*Reference*
n=1,020				
2.30-4.29	*Reference*	1.14 (0.91, 1.43)	1.05 (0.57, 1.92)	1.26 (0.90, 1.76)
n=1,031				
4.30-7.69	*Reference*	1.14 (0.92, 1.41)	1.06 (0.59, 1.89)	0.90 (0.63, 1.29)
n=1,007				
7.70-343.0	*Reference*	0.87 (0.68, 1.11)	0.97 (0.52, 1.83)	1.06 (0.74, 1.50)
n=1,003				
p for trend	*Reference*	*0.52*	*0.64*	*0.92*

Values are Odds Ratios (95% Confidence Interval) and reflect the risks of wheezing, lower respiratory tract infections, or eczema of children in a specific quartile group compared to the lowest quartile. *P < 0.05 ** P>0.01, using generalized estimating equation models. Models were adjusted for maternal age, body mass index, education, history of asthma or atopy, smoking habits, parity, gestational hypertension, children's sex, gestational age, birth weight, ethnicity, breastfeeding status, daycare attendance and pet keeping. For P for trend we included maternal C-reactive protein levels as a continuous variable in the model.

Table E3.3.5. Cord blood C-reactive protein levels (mg/l) and wheezing, lower respiratory tract infections, and eczema until the age of 4 years stratified for maternal atopy

	Age 1 year	Age 2 years	Age 3 years	Age 4 years	Overall
	Odds Ratios (95% Confidence Intervals) of wheezing				
No maternal atopy					
< 0.20	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*
n=1,720					
≥ 0.20-43.10	1.31 (1.02, 1.67)*	1.27 (0.98, 1.64)	1.29 (0.93, 1.78)	1.21 (0.89, 1.64)	1.28 (1.11, 1.48)**
n=505					
Maternal atopy					
< 0.20	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*
n=951					
≥ 0.20-43.10	1.20 (0.85, 1.69)	0.91 (0.61, 1.34)	1.12 (0.72, 1.72)	1.06 (0.69, 1.65)	1.07 (0.87, 1.33)
n=233					
	Odds Ratios (95% Confidence Intervals) of lower respiratory tract infections				
No maternal atopy					
< 0.20	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*
n=1,720					
≥ 0.20-43.10	1.24 (0.91, 1.69)	1.28 (0.95, 1.71)	1.20 (0.82, 1.77)	1.53 (0.97, 2.41)	1.26 (1.05, 1.51)*
n=505					
Maternal atopy					
< 0.20	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*
n=951					
≥ 0.20-43.10	1.10 (0.73, 1.68)	1.03 (0.67, 1.58)	1.04 (0.62, 1.74)	1.22 (0.68, 2.18)	1.08 (0.83, 1.39)
n=233					
	Odds Ratios (95% Confidence Intervals) of eczema				
No maternal atopy					
< 0.20	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*
n=1,720					
≥ 0.20-43.10	1.10 (0.82, 1.48)	1.12 (0.82, 1.53)	1.17 (0.79, 1.73)	1.16 (0.74, 1.83)	1.13 (0.92, 1.37)
n=505					
Maternal atopy					
< 0.20	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*
n=951					
≥ 0.20-43.10	0.93 (0.63, 1.35)	0.72 (0.45, 1.17)	0.85 (0.49, 1.49)	0.69 (0.38, 1.25)	0.83 (0.64, 1.07)
n=233					

*P < 0.05 and **p < 0.01 using generalized equating estimates models. Models were adjusted for maternal age, body mass index, education, history of asthma, smoking habits, gestational hypertensive problems, parity, children's sex, gestational age, birth weight, ethnicity, breastfeeding status, daycare attendance and pet keeping. P-value for interaction CRP * maternal atopy with: wheezing = 0.36, lower respiratory tract infections = 0.49, eczema = 0.12.

Table E3.3.6. Cord blood C-reactive protein with pre-school wheezing patterns

	Never	Early	Late	Persistent
C-reactive protein				
< 0.20	*Reference*	*Reference*	*Reference*	*Reference*
n=2,671				
≥ 0.20-43.10	*Reference*	1.25 (1.02, 1.53)*	1.02 (0.61, 1.71)	1.21 (0.89, 1.63)
n=738				

*P < 0.05 and **p < 0.01 using generalized equating estimates models. Models were adjusted for maternal age, body mass index, education, history of asthma, smoking habits, pregnancy induced hypertension, parity, children's sex, gestational age, birth weight, ethnicity, breastfeeding status, daycare attendance and pet keeping.

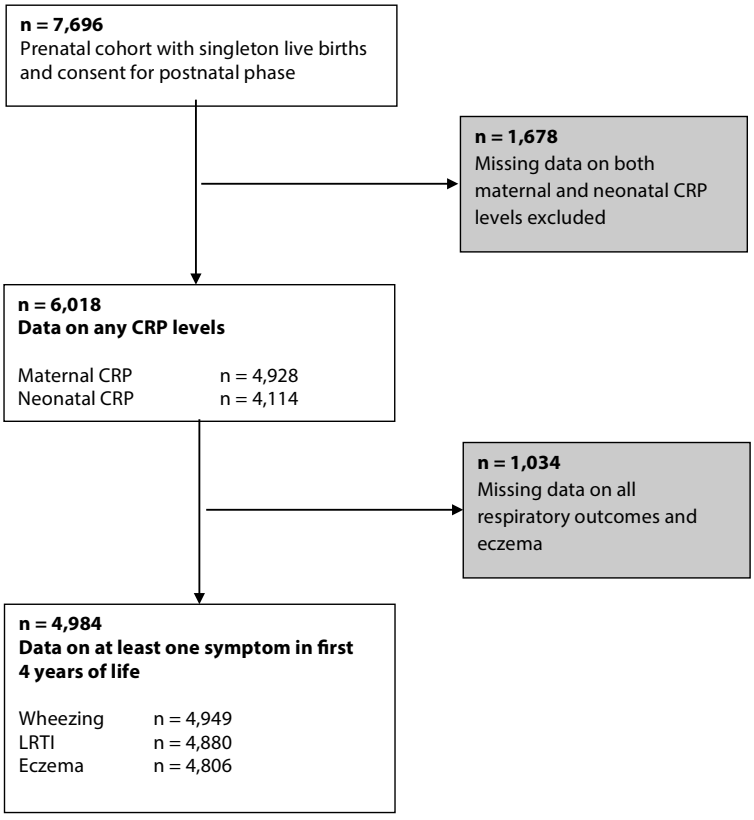

Figure E3.3.1. Flowchart

Chapter 4

Infant exposures and childhood asthma

4.1

Duration and exclusiveness of breastfeeding and childhood asthma symptoms

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Eur Respir J. 2012;39(1):81-9.

4.2

Air pollution, fetal and infant tobacco smoke exposure, and wheezing in preschool children

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Environmental health. 2012;11:91

ABSTRACT

Background Air pollution is associated with asthma exacerbations. We examined the associations of exposure to ambient particulate matter (PM_{10}) and nitrogen dioxide (NO_2) with the risk of wheezing in preschool children, and assessed whether these associations were modified by tobacco smoke exposure.

Methods This study was embedded in the Generation R Study, a population-based prospective cohort study among 4,634 children. PM_{10} and NO_2 levels were estimated for the home addresses using dispersion modeling. Annual parental reports of wheezing until the age of 3 years and fetal and infant tobacco smoke exposure was obtained by questionnaires.

Results Average annual PM_{10} or NO_2 exposure levels per year were not associated with wheezing in the same year. Longitudinal analyses revealed non-significant tendencies towards positive associations of PM_{10} or NO_2 exposure levels with wheezing during the first 3 years of life (overall odds ratios (95% Confidence Interval): 1.21 (0.79, 1.87) and 1.06 (0.92, 1.22)) per 10 $\mu g/m^3$ increase PM_{10} and NO_2, respectively). Stratified analyses showed that the associations were stronger and only significant among children who were exposed to both fetal and infant tobacco smoke (overall odds ratios 4.54 (1.17, 17.65) and 1.85 (1.15, 2.96)) per 10 $\mu g/m^3$ increase PM_{10} and NO_2, respectively (p-value for interactions <0.05).

Conclusions Our results suggest that long term exposure to traffic-related air pollutants is associated with increased risks of wheezing in children exposed to tobacco smoke in fetal life and infancy. Smoke exposure in early life might lead to increased vulnerability of the lungs to air pollution.

BACKGROUND

Higher exposure levels to air pollutants have been associated with increased risks of asthma exacerbations in adults and children aged older than 5 years[1-5]. The influence of air pollution on asthma and wheezing in younger children is less clear[6-9]. The effects of air pollutants on airway symptoms may differ between children and adults. Children older than 6 months of age may breathe more through the mouth than adults, and benefit less from the filtering, humidifying and temperature raising effect of the nose and might therefore inhale higher air pollutants levels[10]. Also, children spend more time outdoors than adults, and have a larger ratio of lung surface area to body weight[7, 10, 11], leading to a potential stronger effect of air pollution on airway symptoms, including wheezing[12]. A limited number of prospective birth cohort studies suggested associations of exposure to traffic-related air pollution, including particulate matter (PM_{10}) and nitrogen dioxide (NO_2), and the risk of wheezing and asthma in children up to the age of 8 years[8, 9, 13, 14]. Thus far, results seem inconsistent[6]. This might be due to differences in study design, exposure and outcome assessment or confounding due to socio-demographic variables or a family history of asthma. Like some other environmental exposures, fetal and infant tobacco smoke exposure negatively influence the risk of asthma symptoms in early childhood, and might increase the susceptibility for the adverse effects of air pollution[15]. Therefore the associations between air pollution and asthma symptoms may be modified by tobacco smoke exposure[3].

We examined the associations of exposure to traffic-related air pollutants PM_{10} and $NO_{2,}$ during different exposure windows, with the risk of wheezing in preschool children in a prospective birth cohort study among 4,634 children living in the city of Rotterdam, The Netherlands. In addition, we assessed whether fetal or infant tobacco smoke exposure modified these associations.

METHODS

Design and setting

This study was embedded in the Generation R Study, a prospective cohort study from early fetal life to young adulthood in Rotterdam in the Netherlands[16]. The study protocol was approved by the Medical Ethical Committee of the Erasmus Medical Center, Rotterdam. Written informed consent was obtained from all participants. In total 7,295 children born between 2002 and 2006 and their parents participated in the postnatal phase of the study. Of all eligible children in the study area, 61% participated in the present study. We excluded twins (n=179), 2nd and 3rd pregnancies in the study (n=539) and children of whom we did not receive any questionnaire (n=996). Of the remaining children (n=5,581) valid air pollution

data were available for 4,937 children (Figure 4.2.1). Air pollution exposure could not be assessed for 644 children, due to incomplete address history, moving outside the study area or invalid measurements. We excluded children without any information about wheezing (n=303 subjects). The final study population for analysis consisted of 4,634 children.

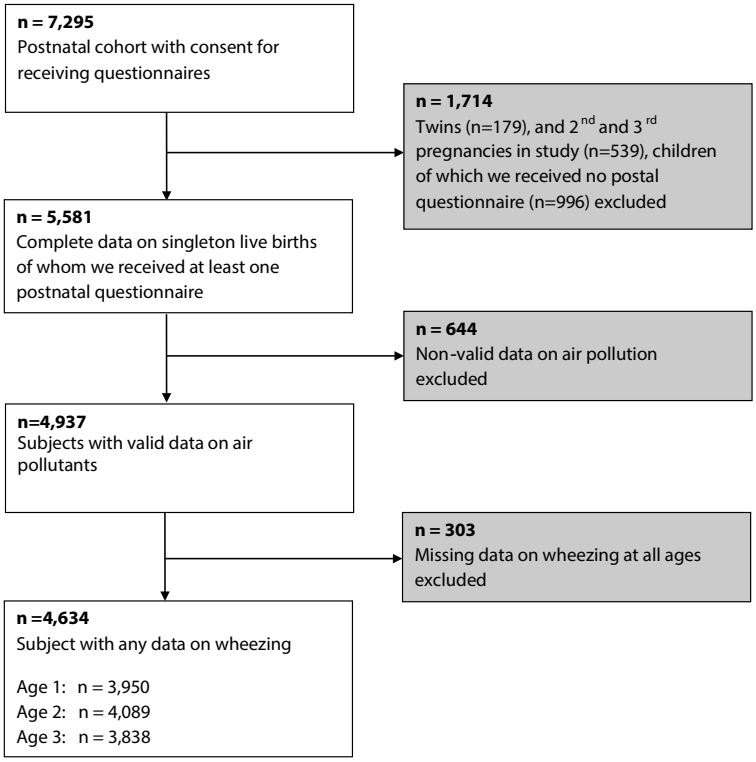

Figure 4.2.1. Flow chart of participants in study

Traffic-related air pollution exposure

Individual child exposures levels to particulate matter (PM_{10}) and nitrogen dioxide (NO_2) were assessed at the home address, using a combination of continuous monitoring and dispersion modeling, taking into account both the spatial and temporal variation in air pollution. The exposure assessment has been described in detail previously[17]. Briefly, annual average concentrations of PM_{10} and NO_2 for the years 2002-2008 were assessed for all addresses in the study area. This was done using the 3 Dutch national standard methods for air quality modeling, designated to calculate the contribution of different air pollution sources[18]. Subsequently, hourly concentrations of PM_{10} and NO_2 were derived, using air pollution measurements from

3 continuous monitoring stations (hourly calibration), taking into account wind conditions and fixed temporal patterns in source contributions. Based on participants' home addresses, we derived individual exposure estimates for different periods during the first 3 years of life, including average exposure to air pollutants annually and overall. Average exposures were calculated for periods with <20% of the concentrations missing. For the other periods, air pollution exposures were set to missing. The performance of this model has been evaluated by two studies in the same study area which show a good agreement between predicted annual average PM_{10} and NO_2 concentrations, and concentrations measured at monitoring stations[19, 20].

Respiratory symptoms

Information on wheezing ("Has your child had problems with a wheezing chest during the last year ?" no; yes) was obtained by questionnaires at the ages of 1, 2 and 3 years. Questions were adapted from the International Study on Asthma and Allergy in Childhood (ISAAC)[21]. Response rates for these questionnaires were 71%, 76% and 72%, respectively[22].

Covariates

Information on maternal educational level, parity, smoking habits, smoking habits of the partner, history of asthma or atopy, children's ethnicity and pet keeping were obtained by a questionnaire at enrolment. We used parity as a proxy for siblings (correlation: kappa = 0.894). Fetal smoke exposure was defined using data of maternal smoking habits during first, second and third trimester of pregnancy collected by questionnaires. We categorised groups as those children who were never exposed to tobacco smoke or in first trimester only (no fetal smoke exposure) and those who were continuously exposed to tobacco smoke in trimesters thereafter (fetal smoke exposure)[15]. Infant smoke exposure was defined as exposure to household tobacco smoke by anyone at the age of 2 years of the child (no; yes, data collected by questionnaires). Sex, gestational age at birth and birth weight of the children were obtained from midwife and hospital registries at birth. Postal questionnaires sent at the ages of 6 and 12 months provided information about breastfeeding. A questionnaire sent at the age of 12 months provided information on daycare attendance. Questionnaires filled in by the parents at the ages of 1, 2 and 3 years provided information about doctor attended lower respiratory tract infections (Has your child had pertussis, bronchitis, bronchiolitis or pneumonia in the past year for which a doctor or hospital was attended? no; yes)[16, 22].

Statistical analysis

We used multiple logistic regression models to analyze the associations of exposure to air pollution in the previous year with the risks of wheezing at the ages of 1, 2 and 3 years. With Generalized Estimating Equation (GEE) analyses, we were able to take the correlation between repeated measurements in the same subject into account, and to calculate the overall effect (average air pollution levels in the first 3 years of life with wheezing at age 1 to 3 years combined). We used a compound symmetry correlation matrix in these models. All models were adjusted for potential confounders including maternal age, education, parity, smoking habits during pregnancy, smoking habits of the partner, history of asthma or atopy, and children's sex, gestational age at birth, birth weight, ethnicity, breastfeeding status, daycare attendance, pet keeping and lower respiratory tract infections. Average exposures to PM_{10} and NO_2, annually and overall, were analyzed as continuous variables and as quartiles (lowest quartile as the reference group). Tests for trend were performed by including average air pollutant concentration levels as continuous variables into the fully adjusted logistic regression model and we calculated the risk per 10 $\mu g/m^3$ increase. Next, we stratified our models for tobacco smoke exposure to assess whether any observed association of air pollution with childhood wheezing was modified by environmental tobacco smoke exposure. For this analysis we also tested the interaction between air pollution and environmental tobacco smoke exposure. The tobacco smoke variables were combined into a new variable with 4 early smoke exposure categories: never; only fetal; only infant; and fetal and infant, using the variables about maternal smoking habits during pregnancy (fetal smoke exposure) and exposure to household tobacco smoke at the age of 2 years (infant smoke exposure). We performed multiple imputations to handle missing values of the covariates and outcomes by generating 25 independent datasets[23]. We imputed both covariates and outcomes, as missing values may introduce bias in GEE models[24]. Imputations were based on the relationships between all covariates and outcomes included in this study plus paternal age, educational level, history of asthma or atopy and information about shortness of breath in the past year of the children at the age of 1, 2 and 3 years. All datasets were analysed separately after which results were combined. No differences in results were observed between analyses with imputed missing data or complete cases only. We only present results based on imputed datasets. All measures of association are presented with their 95% Confidence Intervals (CI). Statistical analyses were performed using the Statistical Package of Social Sciences version 17.0 for Windows (SPSS Inc., Chicago, IL, USA) and SAS 9.2 (SAS institute, Cary, NC, USA).

RESULTS

Subject characteristics

Children were born at a median gestational age of 39.9 (2.5-97.5% range: 37.0-42.1) weeks with a mean birth weight of 3,439 (SD 556) grams (Table 4.2.1). Of all children who were exposed to tobacco smoke during fetal life, 59.3% was exposed to household tobacco smoke in infancy, whereas of all children who were not exposed to tobacco smoke during fetal life, 12.2% was exposed to household tobacco smoke in infancy. (Table E4.2.1 in the data supplement). The wheezing prevalence declined with increasing age. Mean annual PM_{10} levels were 28.9, 28.3 and 27.9 $\mu g/m^3$ and mean annual NO_2 levels were 38.7, 37.5 and 36.2 $\mu g/m^3$ at the ages of 1, 2 and 3 years, respectively (Table E4.2.2 in the data supplement).

Air pollution and risk of wheezing

We observed no associations of average PM_{10} and NO_2 concentrations during the previous year with the risks of wheezing at the ages of 1, 2 or 3 years separately or in the overall longitudinal model (Table 4.2.2). Additional analyses showed that children exposed to the highest 25% PM_{10} and NO_2 levels did not have an increased risk of wheezing in the first 3 years compared to those exposed to the lowest 25% air pollutants levels (results not shown). At the age of 1 year only, information about the average exposure to air pollutants and wheezing during the last month was available. As compared to the average per year exposure we observed a larger variation in exposure levels of air pollutants measured in the previous month at the age 1 year (Table E4.2.2). Furthermore, exposure to increased levels of PM_{10} during the previous month tended to be associated with an elevated risk of wheezing but the effect estimate did not reach statistical significance (OR 1.25 (0.98, 1.58) per 10 $\mu g/m^3$). Increased levels of NO_2 during the previous month were associated with wheezing (OR 1.32 (1.11, 1.55) per 10 $\mu g/m^3$) (Table 4.2.3). We observed no time-dependent effect of air pollutants on wheezing in the first 3 years (p-values for interaction time*air pollutant: >0.05). We explored the confounding and modifying effect of lower respiratory tract infections and did not observe changes in our effect estimates after adjusting the analyses for lower respiratory tract infections. Also, the interaction between air pollution and lower respiratory tract infections was not significant, and we observed no associations between air pollutants and lower respiratory tract infections (data not shown).

Air pollution, tobacco smoke exposure and risk of wheezing

We found no associations of air pollutants levels with the annual risks of wheezing stratified for fetal and infant smoke exposure (Table E4.2.3). Stratified longitudinal analyses showed

Table 4.2.1. Maternal and child characteristics

	n=4,634	
	Observed	**After multiple imputations**
Maternal characteristics		
Age (years)*	31.1 (4.9)	31.1 (4.9)
Highest completed education (%)		
Non-completed, primary or secondary	47.1 (2,050)	48.2 (2,234)
Higher	52.9 (2,299)	51.8 (2,400)
Missing	*6.2 (285)*	-
Parity (%)		
Nulliparity	61.6 (2,762)	61.4 (2,844)
Multiparity	38.4 (1,722)	38.6 (1,790)
Missing	*3.2 (150)*	-
History of asthma or atopy (%)		
No	61.9 (2,369)	59.0 (3,734)
Yes	38.1 (1,460)	41.0 (1,900)
Missing	*17.4 (805)*	-
Fetal and Child characteristics		
Male sex (%)	49.9 (2,313)	49.9 (2,313)
Gestational age at birth (weeks)[$]	39.9 (37.0-42.1)	39.9 (37.0-42.1)
Birth weight (grams)*	3,439 (556)	3,439 (556)
Ethnicity (%)		
European	70.4 (3,144)	69.9 (3,240)
Non-European	29.6 (1,320)	30.1 (1,394)
Missing	*3.7 (170)*	-
Breastfed (%)		
No	7.7 (339)	8.0 (371)
Yes	92.3 (4,089)	92.0 (4,263)
Missing	*4.4 (206)*	-
Day care attendance (%)		
No	48.0 (1,894)	50.0 (2,316)
Yes	52.0 (2,050)	50.0 (2,318)
Missing	*14.9 (690)*	-
Pet keeping (%)		
No	65.5 (2,399)	64.6 (2,993)
Yes	34.5 (1,263)	35.4 (1,641)
Missing	*21.0 (972)*	-
Lower respiratory tract infections 1 year		
No	86.4 (3,165)	85.4 (3,957)
Yes	13.6 (498)	14.6 (677)
Missing	*21.0 (971)*	-

Table 4.2.1. Maternal and child characteristics (continued)

	n=4,634	
	Observed	**After multiple imputations**
Lower respiratory tract infections 2 years		
No	87.9 (3,494)	87.4 (4,052)
Yes	12.1 (484)	12.6 (582)
Missing	*14.2 (659)*	-
Lower respiratory tract infections 3 years		
No	93.3 (3,453)	92.7 (4,294)
Yes	6.7 (247)	7.3 (340)
Missing	*20.2 (934)*	-
Smoking of father (%)		
No	57.4 (2,153)	57.4 (2,658)
Yes	42.6 (1,599)	42.6 (1,976)
Missing	*19.0 (882)*	-
Fetal smoke exposure (%)		
No	86.9 (3,246)	86.4 (4003)
Yes	13.1 (489)	13.6 (631)
Missing	*19.4 (899)*	-
Infant smoke exposure (%)		
No	82.3 (3,391)	81.4 (3,770)
Yes	17.7 (728)	18.6 (864)
Missing	*11.1 (515)*	-
Wheezing age 1 year (%)		
No	74.0 (2,922)	74.1 (3,433)
Yes	26.0 (1,028)	25.9 (1,201)
Missing	*14.8 (684)*	-
Wheezing age 2 years (%)		
No	82.1 (3,358)	82.6 (3,827)
Yes	17.9 (731)	17.4 (807)
Missing	*11.8 (545)*	-
Wheezing age 3 years (%)		
No	89.0 (3,417)	89.4 (4,143)
Yes	11.0 (421)	10.6 (491)
Missing	*17.2 (796)*	-

Values are percentages (absolute values), means (SD)* or medians (5-95th percentile)[$].

Missing percentages are given for the total population of analysis n=4634. Other percentages are valid percentages.

Table 4.2.2. Exposure to air pollutants (previous year, overall) and risks of wheezing

	Odds ratio of wheezing (95% Confidence Interval)			
	Age 1 year	**Age 2 years**	**Age 3 years**	**Overall**
PM_{10}				
Crude	1.07 (0.77, 1.50)	1.54 (0.90, 2.61)	1.00 (0.51, 1.95)	1.28 (0.85, 1.91)
Adjusted	1.21 (0.84, 1.74)	1.49 (0.83, 2.66)	0.90 (0.43, 1.91)	1.28 (0.83, 1.98)
NO_2				
Crude	1.01 (0.85, 1.20)	1.04 (0.85, 1.27)	1.03 (0.79, 1.33)	1.05 (0.92, 1.19)
Adjusted	1.07 (0.89, 1.29)	1.04 (0.83, 1.29)	0.97 (0.72, 1.30)	1.07 (0.93, 1.23)

Values are odds ratios (95% Confidence Interval) from logistic regression models representing the risks of wheezing per 10 μg/m^3 increase in PM_{10} or NO_2. The overall effect is from generalized estimating equation models, based on average air pollution levels from birth until the age of 3 years with wheezing at the ages of 1, 2 and 3 years combined.

Models are adjusted for maternal age, education, parity, smoking, smoking of the partner, history of asthma or atopy and children's sex, gestational age, birth weight, ethnicity, breastfeeding, daycare attendance, pet keeping and lower respiratory tract infections at the corresponding ages.

Table 4.2.3. Exposure to air pollutants in the previous month and wheezing in the same month

	Odds ratio of wheezing in previous month age 1 year (95% Confidence Interval)	
	PM_{10}	**NO_2**
	n=373	n=373
Quartile 1	Reference n=83	Reference n=72
Quartile 2	1.24 (0.90, 1.71) n=97	1.28 (0.91, 1.79) n=87
Quartile 3	1.08 (0.77, 1.49) n=82	1.54 (1.11, 2.13)* n=103
Quartile 4	1.38 (1.01, 1.88)* n=111	1.62 (1.17, 2.24)** n=111
Trend	1.25 (0.98, 1.58) p=0.07	1.32 (1.11, 1.55) p<0.01

Values are odds ratios (95% Confidence Interval) for wheezing from logistic regression models. *P < 0.05 and **p < 0.01. Models are adjusted for maternal age, education, parity, smoking, smoking of the partner, history of asthma or atopy and children's sex, gestational age, birth weight, ethnicity, breastfeeding, daycare attendance, pet keeping and lower respiratory tract infections at age 1 year. Trend represents the risk of wheezing per 10μg/m^3 increase in PM_{10} or NO_2.

that the associations of average PM_{10} and NO_2 exposure levels with the overall longitudinal risks of wheezing during the first 3 years of life were stronger and significant among children who were exposed to tobacco smoke both during fetal and infant life (overall odds ratios 4.54 (1.17, 17.65) and 1.85 (1.15, 2.96) per 10 μg/m^3 increase in PM_{10} and NO_2, respectively) (Figure 4.2.2). We did not observe associations of traffic-related air pollutants with wheezing among children who were exposed to smoke during fetal life only or during infancy only. However, we observed elevated odds ratios for infant smoke exposure, but these effect estimates were not significant. We additionally assessed whether tobacco smoke exposure modified the as-

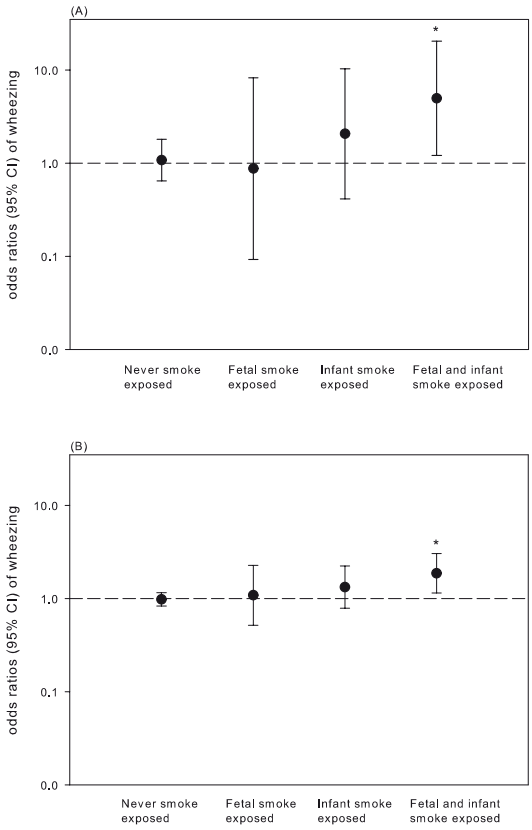

Figure 4.2.2. Exposure to air pollutants PM_{10} (**A**), NO_2 (**B**), tobacco smoke and wheezing.
Values are overall odds ratios (95% Confidence Interval) from generalized estimating equation models based on average air pollution levels from birth until the age of 3 years with wheezing at the ages of 1, 2 andx 3 years combined, representing the risks of wheezing per 10µg/m^3 increase in PM_{10} or NO_2 stratified for tobacco smoke exposure. Models are adjusted for maternal age, education, parity, history of atopy or asthma and children's ethnicity, sex, gestational age, birth weight, breastfeeding, daycare attendance, pet keeping and lower respiratory tract infections at 1, 2 and 3 years of age. P-values for interaction: tobacco smoke exposure * average level $PM_{10,}$ p-value <0.05; tobacco smoke exposure * average level $NO_{2,}$ p-value <0.01.

sociation of air pollution with risks of wheezing by using interaction terms. These interaction terms were statistically significant for the associations of air pollutants with longitudinally measured wheezing (P-values for interaction: PM10*smoking: p-value <0.05; NO2*smoking: p-value <0.01). However, per year analyses showed that the association of air pollutants with wheezing was modified by tobacco smoke exposure only at the age of 3 years (P-values for interaction per year: PM_{10}*smoking: p-value = 0.35 (age 1), p-value = 0.20 (age 2), and p-value <0.05 (age 3). P-values for interaction NO_2*smoking are: p-value = 0.23 (age 1), p-value = 0.14 (age 2), and p-value <0.05 (age 3)).

DISCUSSION

Our study suggests that long term exposure to higher levels of traffic-related air pollutants PM_{10} and NO_2 are associated with increased risks of wheezing in the first 3 years of life among children who are exposed to tobacco smoke during fetal and infant life. We did not observe associations of traffic-related air pollutants with wheezing among children who were not exposed to tobacco smoke.

Previous studies reported inconsistent findings for the associations of traffic-related air pollution with asthma symptoms and doctor diagnosed asthma[6, 7]. Associations of NO_2 and $PM_{2.5}$ with overall wheezing until the age of 8 years were observed in another study in the Netherlands[14]. A Swedish cohort study observed associations of air pollution in the first year of life with persistent wheezing until 4 years of age[25]. A study in Germany observed no associations of long term exposure to $PM_{2.5}$ or NO_2 with the risks of parental reports of asthma symptoms, but observed an association of $PM_{2.5}$ exposure levels with doctor diagnosed asthma at the age of 6 years[26]. Finally, a large Canadian study reported inconsistent results for the associations of air pollutant levels with the risk of asthma until the age of 4 years, depending on the exposure assessment. The authors reported no association of traffic-related air pollution based on land use regression modeling with the risks of asthma, but reported associations of distance to industrial point sources with an increased risk of asthma[27]. Differences between our study and previous published studies include our detailed method to assess air pollution exposure levels in a large city, the availability of many potential confounders and the interaction with smoke exposure. Also, earlier studies did not use individual exposure levels[27], took only the birth addresses into account or were not able to adjust for home movement[9, 14, 25]. Children in our study were exposed to a smaller range of NO_2 exposure (range 28.8-56.1 $\mu g/m^3$) as compared with another Dutch study (NO_2 range 12.6-58.4 $\mu g/m^3$) which might have led to smaller effect estimates[14]. By using long term exposure averages, the potential short term high risk exposure levels may be missed. At the age of 1 year only, we obtained information about wheezing in the last month and the average exposure to air pollutants during that month. Increased levels of air pollutants exposure during the previous 1 month were associated with increased risks of wheezing. We were not able to asses this short time interval at older ages.

We observed an interaction between air pollution and tobacco smoke exposure for the association with longitudinally measured wheezing. However, in our per year analyses we observed that this interaction was only significant at the age of 3 years. This might be explained by the idea that from the age of 3 years onwards wheezing represents another phenotype than earlier wheezing in which other factors such as atopic susceptibility in the origins of wheezing become more important. Also, infant smoke exposure was assessed after respiratory outcomes at age 1 year. This might be a reason for observing no significant interaction between exposure to air pollutants, tobacco smoke and wheezing before the age of 3 years.

Our results suggest that tobacco smoke exposure increases the vulnerability of the lungs to air pollutants. The interaction between particulate matter and tobacco smoke exposure was previously explored by Rabinovitch et al[3]. They observed that environmental tobacco smoke exposure modifies the acute effects of low-level ambient $PM_{2.5}$ exposure on childhood asthma. Albuterol usage and leukotriene E_4 were only related to $PM_{2.5}$ concentrations on days when urine cotinine levels were low, which suggest that only when children were not or to a small amount exposed of tobacco smoke, exposure to air pollution was positively associated with asthma. Their results were in the opposite direction as compared to our results. This difference might be explained by differences in study design and methods. We assessed reported tobacco smoke exposure both in fetal and infant life, wheezing at younger ages, and long term exposure to tobacco smoke and air pollution. Rabinovitch et al assessed biological markers of smoke exposure in childhood, used albuterol usage as a proxy for asthma, at an older age, and assessed the short term effects of air pollutants. Previous studies suggested that both short term and long term exposure to air pollutants are important for the development of asthma exacerbations or respiratory symptoms[25, 28-34]. Our results suggest that short term exposure to air pollutants might be important for developing respiratory symptoms, whereas long term exposure to air pollutants might be important in the presence of tobacco smoke exposure. However our results should be considered as hypothesis generating. More studies are needed to explore the combined effects of air pollution and tobacco smoke exposure on the development of respiratory symptoms. Previously, we have reported that children from mothers who smoked continuously during pregnancy and during the first years after pregnancy had increased risks of wheezing in the first years of life[15]. Fetal smoke exposure has been suggested to have a different underlying mechanism in the pathway to wheezing than infant smoke exposure. Fetal smoke exposure may lead to impaired lung development and immunological changes while for infant smoke exposure it includes bronchial hyperreactivity, immunological changes, and direct toxic and irritant effects (35-37). Increased vulnerability of the airways and lungs to air pollutants might be caused by both fetal and infant smoke exposure via their pathophysiological mechanisms. Among children with infant smoke exposure, we observed a non-significant elevated odds ratio for the associations of air pollution with wheezing. This tendency was not observed in children with only fetal smoke exposure. This might be due to the direct toxic effects of both infant smoke exposure and exposure to air pollutants, which are absent in fetal smoke exposure only[38]. The mechanisms underlying the association of air pollution exposure with wheezing or asthma might also include the induction of airway inflammation and oxidative stress, modification of enzyme functions, disruption of immune responses and increased reactivity to allergens[26, 38-40]. Also, respiratory infectious diseases might play a role. However, we did not observe a confounding or modifying effect of respiratory tract infections or associations between air pollutants and respiratory tract infections. Therefore, the associations of air pollution with wheezing in

our study are probably not explained by infectious mechanisms. Further studies exploring potential underlying causal mechanisms are needed.

This study was embedded in a population-based prospective design with a large number of subjects being studied from early life onwards with detailed and frequently prospectively measured information about air pollution levels at the corresponding home-addresses. We adjusted for a large number of confounders and the results did not differ between non-imputed and imputed analysis. Non-response at enrolment and lost to follow-up would lead to biased effect estimates if the associations of air pollutants with wheezing would be different between those included and not included in the analyses. Selection bias due to non-participation at enrolment in the prenatal phase might have occurred because our study population tends to have a selection towards more affluent and healthy mothers[16] who might have reported less wheezing symptoms and tobacco smoke exposure in their children and have been exposed to lower air pollutant levels[41]. If so, our observed effect estimates would be underestimated. Mothers and children lost to follow-up during the postnatal phase were lower educated (67% vs. 47%) and smoked more frequently during pregnancy (21% vs. 13%). If children who were lost to follow up would have had more wheezing episodes, this could have led to an underestimation of the observed effect of air pollution and tobacco smoke exposure on wheezing as well. One of the limitations of our study is that we might reflect a selection towards a more healthy population, as the prevalence of preterm birth is lower than average in The Netherlands, 4.7% versus 7.7%. A homogeneous population would not affect the observed association of air pollution with wheezing among children exposed and not exposed to tobacco smoke. However such a population might affect the generalizability. The observed effects might be different in a population with more preterm born children. Also, preterm birth could modify the effect between air pollution and wheezing, because airways and lungs of preterm born children might be less developed and therefore might be even more vulnerable to air pollution. Previous studies were limited in their ability to consider the intraurban gradients and temporal variations in air pollutants. However, some had obtained more subject-specific exposure levels[6, 7]. A strength of our study is that we were able to consider detailed spatial and temporal contrasts in exposure, in which we were able to take home movements into account. In the first 3 years of life 39.9% of the children moved at least once. Still there might be misclassification of air pollution assessment. We only calculated exposure levels at home addresses and not at the day care centers or other places where the child may spend days and nights. We assumed that most of the time children until the age of 3 years are near or at their home addresses. Furthermore, other types of indoor or commuting exposure were not taken into account. If any, we expect that this misclassification is non-differential and may have led to an underestimation of the associations[42]. We had no information on smaller particle sizes than 10 µm. Smaller particles sizes such as $PM_{2.5}$ might more adversely affect respiratory morbidity than PM_{10} due to deeper peripheral lung deposition. However, previous studies which measured both PM_{10} and $PM_{2.5}$ observed strong

correlations between exposure to PM_{10} and $PM_{2.5}$ and similar effect sizes of these exposures on childhood asthma or wheezing[32, 43]. Although assessing smoking habits by questionnaires is valid in epidemiological studies, misclassification may occur due to underreporting[44]. However, the use of biomarkers of tobacco smoke exposure in urine, saliva or blood, or nicotine in indoor air seems not superior to self-report[44-47]. First trimester adverse exposures might be important for fetal lung development[48]. Using data from the same study population, we have previously shown that children do not have an increased risk of preschool wheezing when mothers quitted smoking as soon as they knew they were pregnant[15]. Based on results of our previous study, we categorized no fetal smoke exposure as children who were never exposed to tobacco smoke or were exposed to tobacco smoke until first trimester of pregnancy only[15]. We performed a sensitivity analysis without including fetal smoke exposure during first trimester only, and observed that the effect sizes did not materially change. Still, it might be that our categorization led to some misclassification, with an underestimation of the effect estimates when first trimester only smoking would have comparable effects as continued smoking during pregnancy. The main outcome in our study was self-reported wheezing. This method is widely accepted in epidemiological studies and reliably reflects the prevalence of wheezing in young children[49]. In preschool children a diagnosis of asthma is based on symptoms[50], and objective tests, including lung function or bronchial responsiveness, are difficult to perform in young children and have a very limited if any diagnostic value. Follow up studies at older ages will include more detailed asthma and atopy measurements.

CONCLUSIONS

In conclusion, our results suggest that higher long term exposure levels to traffic-related air pollution lead to higher risks of wheezing in preschool children who were exposed to fetal and infant tobacco smoke. Further studies are needed to explore underlying mechanisms of exposure to air pollutants with and without interaction with tobacco smoke exposure and various types of wheezing and asthma in later life.

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Supplements

Table E4.2.1. Cross table of fetal smoke exposure with infant smoke exposure

	No infant smoke exposure (%)	Infant smoke exposure (%)	Total
No fetal smoke exposure (%)	3,513 (87.8)	490 (12.2)	4,003 (100)
Fetal smoke exposure (%)	257 (40.7)	374 (59.3)	631 (100)
Total	3,770	864	4,634

Values are numbers (percentages)

Table E4.2.2. Levels of air pollutant

	Overall	Previous month	Previous year		
	Age 1 - 3 years	**Age 1 year**	**Age 1 year**	**Age 2 years**	**Age 3 years**
PM_{10} ($\mu g/m^3$)	n=3,295	n=3,898	n=3,963	n=3,771	n=3,166
Mean (SD)	28.36 (1.29)	28.29 (4.61)	28.86 (2.11)	28.27 (1.57)	27.92 (1.67)
Min	25.84	20.04	24.47	24.19	23.96
25%	27.49	24.77	27.49	27.29	26.73
50%	28.18	27.51	28.60	28.25	27.91
75%	28.89	31.59	29.78	29.13	28.91
Max	36.01	44.28	39.81	35.82	35.76
NO_2 ($\mu g/m^3$)	n=3,295	n=3,897	n=3,963	n=3,772	n=3,166
Mean (SD)	37.39 (4.01)	38.14 (6.81)	38.66 (4.20)	37.46 (4.17)	36.22 (4.28)
Min	28.81	18.20	29.66	27.10	27.02
25%	34.61	33.73	35.72	34.54	33.35
50%	37.10	39.07	38.34	37.33	35.69
75%	39.32	42.95	40.68	39.49	38.58
Max	56.05	58.27	59.60	55.87	55.68

Table E4.2.3. Exposure to air pollutants in previous year, tobacco smoke and wheezing

	Odds ratio of wheezing (95% CI)									
	PM_{10}					NO_2				
	Tobacco smoke exposure					Tobacco smoke exposure				
	Total	Never	Fetal	Infant	Fetal- and infant	Total	Never	Fetal	Infant	Fetal- and infant
Age 1 year	1.21 (0.84, 1.74)	1.09 (0.71, 1.68)	1.38 (0.24, 7.97)	2.22 (0.65, 7.59)	1.96 (0.50, 7.64)	1.07 (0.89, 1.29)	1.00 (0.81, 1.24)	1.35 (0.53, 3.45)	1.32 (0.67, 2.60)	1.49 (0.75, 2.97)
Age 2 years	1.49 (0.83, 2.66)	1.29 (0.65, 2.54)	0.57 (0.04, 9.39)	3.98 (0.54, 29.59)	4.40 (0.56, 34.40)	1.04 (0.83, 1.29)	0.97 (0.75, 1.26)	0.73 (0.25, 2.13)	1.32 (0.60, 2.88)	1.76 (0.84, 3.71)
Age 3 years	0.90 (0.43, 1.91)	0.59 (0.24, 1.43)	0.39 (0.01, 19.83)	4.07 (0.27, 60.76)	3.80 (0.36, 40.54)	0.97 (0.72, 1.30)	0.86 (0.60, 1.21)	0.40 (0.07, 2.20)	0.88 (0.30, 2.60)	2.34 (0.96, 5.67)

Values are odds ratios (95% Confidence Interval) for wheezing at the ages of 1, 2 and 3 years per 10 µg/m^3 increase of PM_{10} or NO_2 in the total group and stratified for fetal and infant tobacco smoke exposure. *P < 0.05. Models are adjusted for maternal age, education, parity, history of asthma or atopy and children's sex, gestational age, birth weight, ethnicity, breastfeeding, daycare attendance, pet keeping and lower respiratory tract infections at the corresponding ages. Total analyses were additionally adjusted for maternal smoking and smoking of the partner. P-values for interaction PM_{10} * smoking: p-value = 0.35 (age 1), p-value = 0.20 (age 2), and p-value <0.05 (age 3). P-values for interaction NO_2 * smoking: p-value = 0.23 (age 1), p-value = 0.14 (age 2), and p-value <0.05 (age 3).

Chapter 5

General discussion

5.1

General discussion

INTRODUCTION

Low birth weight has been associated with a wide range of adult diseases[1-4]. These observations have resulted in the developmental origins of health and disease hypothesis[1]. This hypothesis proposes that organ systems may develop in different ways, depending on the environment it is exposed to. Adverse exposures may result in specific adaptations, which improve survival and development on short term, but eventually might lead to health problems in later life[1-5]. Low birth weight has been associated with subsequent respiratory morbidity, including asthma and chronic obstructive pulmonary disease (COPD)[1, 3, 6-9]. Since low birth weight is the result of various adverse fetal exposures and growth patterns, and the starting point of infant growth, it is not per se a causal factor for respiratory morbidity in later life[10-13].

The aim of this thesis was to identify specific fetal and infant growth patterns, their specific exposures and their interactions leading to asthma symptoms or diagnosis in childhood. The main results, merits and shortcomings of these studies have been discussed in the previous chapters. This chapter provides a more general discussion of the main findings of the studies in this thesis, considers general methodological issues, and gives suggestions for further research.

MAIN FINDINGS

Early growth and childhood asthma

Low birth weight and preterm birth are associated with increased risks of asthma symptoms. Not much is known about specific fetal and infant growth patterns versus the risk for development of asthma in childhood.

First, we performed an individual participant data meta-analysis for 147,252 children of 31 birth-cohort studies to determine the associations of birth and infant growth characteristics with the risks of preschool wheezing and school-age asthma. Results from this large-scale meta-analysis of individual participant data suggested that younger gestational age at birth and higher infant weight gain were associated with a 3.27-fold and 4.47-fold increased risk of preschool wheezing and school-age asthma, respectively (Table 5.1.1). The associations of low birth weight with childhood asthma outcomes were largely explained by gestational age at birth. The highest risk for childhood asthma outcomes was observed among children born before a gestational age of 32 weeks followed with a high infant weight gain.

Second, we examined the associations of fetal and infant growth patterns with the risks of asthma symptoms in the first 4 years of life. We demonstrated in a Dutch population-based cohort study among 5,125 children that neither fetal restricted nor accelerated weight and length growth, defined as a negative or positive change of more than 0.67 standard deviation

Table 5.1.1. Overview of results of studies presented in this thesis on early growth and childhood lung function and disease

	Lung function					Symptoms and disease	
	Rint	Bronchial responsiveness or reversibility	Spirometry			Wheezing	Asthma
			FVC	FEV_1	FEF_{25-75}		
Preterm birth	=	n.s.	n.s.	n.s.	n.s.	↑	↑
Low birth weight	=	n.s.	n.s.	n.s.	n.s.	↑	↑
Gestational age	=	n.s.	n.s.	n.s.	n.s.	↓	↓
Birth weight	↓	=	↑	↑	=	↓/=	↓/=
Birth Length	↓	=	↓	↓	=	=	=
Fetal length gain	↓	n.s.	n.s.	n.s.	n.s.	=	=
Fetal weight gain	↓	n.s.	n.s.	n.s.	n.s.	=	=
Infant weight gain	=	↑	↑	↑	↓	↑	↑/=
Infant length gain	=	=	=	=	=	=	=

Lung function was measured at 6 (Rint), 8 (bronchial responsiveness, spirometry) or 15 years (bronchial reversibility, spirometry), and lung disease until 4 years (wheezing) and from 6 to 18 (asthma) years. Arrows represent directions of associations. Upper going arrows represent a positive association, lower going arrows represent a negative association. The equal sign represents that there is no association observed. n.s. means not studied.

score, respectively, were associated with the risks of asthma symptoms until the age of 4 years (Table 5.1.1). However, we did observe associations of infant growth acceleration from birth until 3 months with an up to 1.44-fold increased risk of asthma symptoms. These associations seemed to be independent of fetal growth patterns. The association between a low birth weight and asthma symptoms was explained by gestational age at birth.

Third, in the same Dutch population-based cohort study we examined the associations of birth characteristics, and fetal and infant growth with airway resistance, physician-diagnosed asthma, and wheezing among 6,259 children aged 6 years. Our results showed that a lower gestational age adjusted birth weight was associated with an increased airway resistance in childhood (Table 5.1.1). Preterm birth was associated with a 1.95-fold increased risk of wheezing and a 2.14-fold risk of physician-diagnosed asthma but not with airway resistance. School-age children with an increased airway resistance had a lower fetal length and weight growth and lower infant length growth. Children with persistent wheezing and physician-diagnosed asthma had increased airway resistance. The pathways from preterm birth to asthma outcomes may include other mechanisms than differences in airway resistance.

Fourth, we assessed the effects of growth after birth on lung function and asthma diagnosis in adolescence in a population-based cohort among 9,723 children in the United Kingdom. We demonstrated that a more rapid weight gain, adjusted for length gain, during different periods of childhood was positively associated with asthma, bronchial responsiveness or reversibility and FVC and FEV_1, but negatively with FEF_{25-75}, and FEV_1/FVC and FEF_{25-75}/FVC ratios (Table 5.1.1). In conclusion, more rapid weight gain in early childhood is associated with increased risk of asthma, bronchial responsiveness or reversibility and measures of airway

obstruction in late childhood and adolescence. Increased height gain in mid childhood was associated with a decreased risk of asthma only.

Potential underlying pathways for the associations of preterm birth, and fetal and childhood growth with asthma related symptoms might include a disrupted fetal and infant lung growth and development, a distortion of the T-helper type 1 (T_H1)/T_H2 balance, both due to adverse exposures or epigenetic mechanisms[14-18], or differences in adipose tissue, leading to increased leptin levels which stimulates the production of proinflammatory cytokines and a chronic systemic inflammation status, or indirectly through mechanical effects on lung function[19-22].

In summary, the results of the studies on early growth and childhood asthma suggest that, at birth, younger gestational age is an important risk factor for the development of asthma symptoms. Fetal growth seems to have an influence on lung structure growth, whereas infant growth seems to influence the development of asthma symptoms. The mechanisms underlying these associations need to be explored in detail in future studies.

Fetal exposures and childhood asthma

Abnormal fetal lung- and immune development in response to adverse intra-uterine exposures may increase the risk of asthma and atopic disorders in childhood and adulthood. We have studied three growth, immunomodulatory, and inflammatory related environmental exposures in fetal life.

First, maternal psychological distress during pregnancy may lead to an increased risk of childhood asthma via developmental adaptations of the hypothalamic-pituitary-adrenal axis, the autonomic nervous system, lung structure and function, and immune responses in the offspring. In a Dutch population-based prospective cohort study among 4,848 mothers and children, we observed that maternal psychological distress during pregnancy was associated with a 1.6-fold increased risk of wheezing in preschool children (Table 5.1.2). This association was independent of paternal psychological distress or maternal postnatal psychological distress, and many other confounders such as smoking during pregnancy, maternal educational level, and ethnicity. Furthermore, the results remained after adjusting for birth weight and gestational age at birth. These results suggest a possible intrauterine programming effect such as immunomodulation or epigenetics of maternal psychological distress on respiratory morbidity.

Second, overweight and obesity are associated with a continuous low-grade inflammatory status, which might influence growth and immune development of the fetus and subsequent increased risk of respiratory morbidity. Maternal pre-pregnancy obesity is suggested to be associated with childhood asthma symptoms[23-26]. The possible intermediating role of gestational weight gain is not clear. Among mothers with a history of asthma or atopy, maternal pre-pregnancy obesity was associated with a 1.47-fold overall increased risk of preschool

Table 5.1.2. Overview of results of studies presented in this thesis on fetal exposures and pre-school asthma symptoms

	Asthma symptom	
	Wheezing	**Eczema**
Maternal psychological distress	↑	n.s.
Maternal pre-pregnancy obesity	↑/=	n.s.
Maternal gestational weight gain	↑	n.s.
Maternal C-reactive protein 1st trimester	↓/=	↑
Fetal C-reactive protein in cord blood at birth	↑	=

Preschool asthma symptoms were annually obtained until the age of 4 years. Arrows represent directions of associations. Upper going arrows represent a positive association, lower going arrows represent a negative association. The equal sign represents that there is no association observed. n.s. means not studied.

wheezing. We observed that gestational weight gain was associated with a 1.09-fold increased risk of wheezing of the child (Table 5.1.2). This was studied among 4,656 mothers and their children. The effect of maternal pre-pregnancy body mass index and gestational weight gain on preschool wheezing could not be explained by child's growth, infectious or atopic mechanisms. Similar as for the associations of infant growth patterns and asthma symptoms, a potential underlying mechanism could be the role of pro-inflammatory leptin[27].

Third, C-reactive protein and its role on childhood respiratory symptoms was examined among 4,984 mothers and their children. C-reactive protein is associated with an increased inflammatory status and therefore suggested to be associated with the development of the immune system of the child and subsequent increased risk of respiratory diseases. The results of this study showed that elevated maternal C-reactive protein levels in early pregnancy were associated with a 0.77-fold lower risk of wheezing in the first two years and an overall 1.20-fold higher risk of eczema (Table 5.1.2). Cord blood C-reactive protein levels were associated with a higher overall risk of wheezing and lower respiratory tract infections. C-reactive protein is produced in the liver under IL-6 stimulation, which may change the T_H1/T_H2 cell balance leading to respiratory morbidity[28].

The results of the associations of maternal psychological distress, obesity and gestational weight gain, and C-reactive protein with childhood asthma symptoms suggest that fetal environmental exposures influence the risk of developing childhood asthma in which immunomodulatory and inflammatory factors seem to play an important role.

Infant exposures and childhood asthma

Breastfeeding and air pollution are two major exposures in early childhood that are suggested to affect childhood asthma.

A substantial body of evidence suggests that breastfeeding is associated with a reduced risk of childhood asthma and asthma symptoms[29, 30] but the effect of duration and exclusiveness of breastfeeding is less clear. We observed that no breastfeeding compared to prolonged

Table 5.1.3. Overview of results of studies presented in this thesis on infant exposures and pre-school asthma symptoms

	Asthma symptom	
	Wheezing	**Eczema**
Breastfeeding duration	↓	n.s.
Breastfeeding exclusiveness	↓	n.s.
Exposure to air pollutant PM_{10}	=	n.s.
Exposure to air pollutant NO_2	=	n.s.

Preschool asthma symptoms were annually obtained until the age of 4 years. Arrows represent directions of associations. Upper going arrows represent a positive association, lower going arrows represent a negative association. The equal sign represents that there is no association observed. n.s. means not studied.

and exclusive breastfeeding was associated with an up to 1.44-fold increased risk of asthma symptoms in preschool children (Table 5.1.3). These associations seemed at least partly explained by infectious but not by atopic mechanisms. The protective effect of breastfeeding on the various types of asthma and lung function in later life needs to be examined in the future.

Higher exposure levels to air pollutants have been associated with increased risks of asthma exacerbations in adults and children[31-33]. The influence of air pollution and its interaction with tobacco smoke exposure on wheezing in early childhood is less clear[34-36]. No associations between long term exposure to air pollutants and wheezing were observed (Table 5.1.3). The exposure to higher air pollutant levels in addition to fetal and infant tobacco smoke exposure was associated with an up to 4.54-fold increased risks of wheezing. The pathway may include more vulnerable lung tissue in children exposed to tobacco smoke, thru which air pollutants can irritate the lungs.

The results of infant exposures with childhood asthma symptoms suggest that breastfeeding duration and exclusiveness or exposure to air pollution affects the development of asthma symptoms, potentially as a result of infectious mechanisms or irritative agents such as tobacco smoke ingredients. However, long term effects of these infant exposures on asthma or lung function at older ages need to be further elucidated.

METHODOLOGICAL CONSIDERATIONS

Most of the studies presented in this thesis were based in the Generation R study, a prospective population-based cohort study with a follow up from fetal life onwards in Rotterdam, The Netherlands[37]. A meta-analysis was performed using individual data from 31 birth cohort studies in Europe. One study was performed with data of older children, and had been based in the Avon Longitudinal Study of Parents And Children (also known as children of the 90's), which is a population-based prospective cohort study with follow up from birth onwards in Bristol, United Kingdom[38]. Specific methodological considerations of the presented studies

have been discussed in the separate chapters of this thesis. In the following paragraphs, some general methodological issues regarding the internal validity of epidemiological studies are discussed including selection bias, information bias, and confounding. Briefly, the external validity will be discussed.

Selection bias

If the association between the determinant and the outcome of interest is different between subjects who participate and those who do not participate in the study, but were eligible, selection bias may occur[39]. In the Generation R cohort it is estimated that 61% (n = 9,778) of all eligible pregnant mothers participated in the study. This non-response at baseline is not likely to be random. Participants more often had a higher socio-economic status and were from a Dutch ethnicity more often, compared to non-participants[40]. This might have resulted in biased effects. However, this seems less likely because it is suggested that biased estimates in cohort studies mainly arise from loss to follow-up rather than from non-response at baseline[41]. Selective loss to follow-up may result in selection bias when the association between the determinant and the outcome of interest is different between those who continued participation in the study and those who are lost to follow-up. Of all children included in the Generation R study, 85.2% (n = 8,305) participated in the follow up studies at the age of 6 years and 69.6% (n = 6,899) had information on any respiratory outcome at the age of 6 years. Overall, mothers and children lost to follow-up more often had a lower socio-economic status and unhealthy life style habits. This selection might have biased our effect estimates, but this bias is difficult to quantify.

For the study performed in the ALSPAC cohort, all pregnant women residents in the old administrative county of Avon were eligible to participate if their estimated delivery date fell between 1 April 1991 and 31 December 1992. Any resulting child from these pregnancies was considered eligible. From these eligible pregnancies, 71.8% (n = 14,541) participated in the ALSPAC cohort. A comparison study suggested that children participating in the ALSPAC cohorts were more likely to be white and of higher socio-economical status. Those lost to follow up were more likely man and from deprived background[42]. Similarly as for the Generation R Study, this selection might have biased the observed effect estimates, but quantification of this bias is difficult.

Information bias

A systematic error in a study can arise when the information about the participants of the study is incorrect (misclassified) and this error is called information bias[39]. Misclassification of the exposure can be differential (non-random), if the misclassification is different for those with and without the outcome of interest, or non-differential (random), if it is unrelated to

the occurrence or the presence of the outcome of the study. Similarly, misclassification of the outcome can be differential or non-differential. Differential misclassification may lead to biased effect estimates, either over- or underestimated. Non-differential misclassification usually leads to an underestimation or a dilution of the effect estimates.

Exposure data used in our studies including maternal pre-pregnancy weight and gestational weight gain, childhood weight and height, C-reactive protein levels, and air pollution levels, were collected longitudinally and before assessment of the outcome. Both the data collectors and the parents were unaware of the research questions under study, which makes differential misclassification of the exposure less likely. However, fetal growth and gestational age at birth were based on crown rump length of the fetus in early pregnancy. The use of last menstrual period has several limitations, such as the large number of mothers who do not know the exact date of their last menstrual period or have irregular menstrual cycles. Embryos and fetuses have virtually identical growth velocities during early gestation. Although, differences in size might be observed between fetuses[43], hence using crown rump length is reducing the variation in early growth to zero. Therefore, we cannot exclude that there may be a random measurement error in the estimation of pregnancy duration. We suggest that this error is non-differential and therefore might have lead to an underestimation of the effect estimates[44, 45]. Also, mothers with psychological distress might have been more aware or anxious of their child's health and might therefore have reported more often asthma symptoms. This could have resulted in an overestimation of the effect estimates. Finally, breastfeeding habits might be influenced by a family history of asthma or atopy because affected parents might have been aware of a possible association between breastfeeding and childhood asthma or atopy. Therefore, mothers with a positive family history of asthma or atopy more often breastfed their child for more than 6 months, and these mothers might have been more aware of asthma symptoms and subsequently more reported such symptoms. This might have resulted in an overestimation of the observed effects, or, if children had less symptoms, an underestimation of the observed effect. Lifestyle factors such as tobacco smoking and low socio-economical status, are known to be underreported. This might have led to an underestimation of the effect estimates because the difference in the risk of the outcome between those who for example smoke and those who do not smoke becomes smaller due to underreporting.

Confounding

A confounder is an extraneous variable that is associated with both the determinant and the outcome of interest and is not an intermediate step in the causal pathway between the exposure and outcome[39]. Our studies are adjusted for many potential confounders. However, we cannot exclude that the effect estimates might be biased due to residual confounders such as atopic status of the child, and intermediates such as body mass index in later life.

Unfortunately, we were not able to take these confounders into account because they were not yet measured in our study, or not known at the time of analyses and writing.

External validity

External validity is the extent to which results of a study can be applied to other populations. The Generation R study is based on the general population in Rotterdam, the Netherlands. The largest ethnic groups are the Dutch, Surinamese, Turkish and Moroccan groups. Both household income and highest followed educational level in mothers and fathers in the study cohort suggest a selection towards a higher socioeconomic status than in the whole study area[46]. This pattern is similar in our follow-up assessments until the age of 6 years and in other large scale cohort studies[47]. Specifically, the population that was under study for the projects presented in this thesis, seemed a reasonable representative subgroup of the general population, with rather good representation of different ethnic backgrounds, educational levels and socioeconomic status. Although, there is a selection towards a more western background and a higher educated population. The results of this thesis could therefore presumably be applied to a western mixed ethnicity population.

The meta-analysis was based on individual participant data of 31 cohort studies from countries throughout Europe. However, countries from the Eastern part of Europe did participate but in quantity were relatively underrepresented. Still, we assume that the overall population of analysis was a good representation of the average European population and we suggest that these results can be applied to all general populations in Europe.

For the study embedded in the ALSPAC study it was previously shown that the study represents the whole of Britain in terms of ethnicity, socioeconomic status and income[42]. Therefore, the results may be applied to other general Western European populations.

CAUSALITY

In our observational studies we were unable to assess causal effects of exposures, but associations only. However, taking the Hill's criteria for causation of our population-based prospective studies into account, we observed strong effect estimates (ORs up to 2 for the main results), consistency with previous studies, adjusted for a large number of confounders, temporality between exposures and outcomes, dose response effects, and plausible underlying mechanisms and coherency from animal studies. The experimental and analogous criteria could not be fulfilled. Additionally, in twin-studies an inverse association between birth weight or body mass index and childhood asthma has been observed, which suggest an association independent of genetic or environmental factors[48-50]. Another approach to explore causality is a Mendelian randomization approach. The Mendelian randomization, the

random assortment of genes from parents to offspring that occurs during gamete formation and conception, provides an opportunity for assessing the causal nature of environmental exposures[51]. A recent study that applied such an approach suggested a causal association between body mass index and asthma in mid childhood[52]. Specifically, the authors observed that both fat and lean mass were associated with increased risks of childhood asthma[52]. This would imply that at least a part of childhood asthma is the result of obesity in childhood, which is consistent with the observed associations of rapid infant weight gain, which often precedes overweight or obesity, and childhood asthma in this thesis.

Depending on the exposure under study, our observational studies provide moderate to good evidence for causal relationships of fetal and infant growth patterns and exposures with childhood asthma symptoms based on the Bradford Hill criteria and previous twin and Mendelian randomisation studies.

CLINICAL IMPLICATIONS AND DIRECTIONS FOR FUTURE RESEARCH

Previously, several prediction models[53, 54], of which one recently has been validated in the Generation R Study[55], have identified risk scores that predict the probability of having asthma at school age among preschool children with suggestive symptoms. In future prediction studies, it should be assessed whether the risk factors observed in this thesis are of additional value in the prediction models. Thereafter, the newly obtained prediction models should be tested in specific clinical settings such as (pediatric) hospitals, general practitioner practices and child health centers. Randomized controlled trials to assess the effect of prevention strategies for the risk factors studied in this thesis are difficult to perform. For example, breastfeeding habits cannot be randomized due to ethical limitations. Alternatively, a design to assess the preventive effects of reducing adverse risk factors or stimulating beneficial factors might be an intervention trial in which one arm receives an intervention, such as promotion of breastfeeding or counseling for quitting smoking, and the other arm usual care[56, 57]. This design might also be applicable to other risk factors for asthma development examined in this thesis.

The potential risk factors observed in this thesis might have clinical implications. Many children experience respiratory morbidity during early childhood but only 30% continue to develop asthma in childhood[58]. If a young child has one or more risk factors that are known to be strongly associated with persistent wheezing, physician diagnosed asthma, or restricted lung function in later life, clinicians would have a better target for secondary prevention strategies and treatment. Also, clinicians could be more restrictive in treatment for those who probably have transient respiratory morbidity.

The largest part of this thesis was focussed on children of a pre-school age. Because asthma is difficult to diagnose in young children and non-invasive objective tests are not

available, the first aspect of future studies will be to study the associations of early growth and fetal and infant environmental exposures with asthma diagnosis, atopic status and lung function measurements in school-age children, adolescence, and up to adulthood. Secondly, asthma is a heterogeneous disease with several identified phenotypes[59]. These phenotypes are suggested to have different specific underlying mechanisms and prognosis and therefore it would be a valuable addition to this thesis and other previously published work to disentangle specific risk factors and their association with various phenotypes. Third, recent studies in small and selected populations have demonstrated that adverse fetal exposures such as maternal smoking, suboptimal diet and folic acid supplements lead to persistent epigenetic modifications[14, 60-62]. Epigenetic modifications, such as DNA methylation in promoter regions of specific genes, may affect expression of specific genes altering lung development and the susceptibility for development of lung disease. Therefore, the epigenetic origins of childhood asthma should be explored[1, 3, 6-9]. Last, the complex microbial and immunological interactions that possibly influence the development of childhood asthma need to be examined[63].

CONCLUSION

Asthma symptoms are common in childhood and are responsible for a large proportion of the morbidity in childhood. We identified fetal and infant growth patterns and environmental exposures that influence the risk of childhood asthma. More research is needed to evaluate the associations of the identified risk factors on asthma in later life, and the possible epigenetic mechanisms. Ultimately, by identification of early life exposures related to the development of asthma throughout childhood, we hope to develop preventive strategies focused on pregnant women and young children to improve respiratory health during childhood.

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Chapter 6

6.1

Summary

In this thesis we examined the fetal and infant origins of childhood asthma. Early growth and adverse environmental exposures lead to an adapted respiratory and immunological development, which subsequently increase the risk of asthma and asthma symptoms. From both an etiological and a prevention perspective, it is important to identify specific fetal and infant exposures that lead to childhood asthma in later life. The studies presented in this thesis were specifically focused on the identification of early critical periods.

Chapter 1 is a general introduction and provides the hypothesis on which this thesis was based. It also provides the aims of the performed studies and describes the outline of the thesis.

Chapter 2 describes the associations of fetal and infant growth with the development of asthma outcomes in childhood. In **Chapter 2.1** we observed that younger gestational age at birth and higher infant weight gain were associated with increased risks of childhood asthma. The association of lower birth weight with childhood asthma was largely explained by gestational age at birth. From **Chapter 2.2** we concluded that weight gain acceleration in early infancy was associated with increased risks of asthma symptoms in preschool children, independent of fetal growth. Therefore, early infancy might be a critical period for the development of asthma. **Chapter 2.3** shows that airway resistance in school-age children is influenced by fetal growth restriction, but not by preterm birth, and is associated with asthma outcomes. The pathways from preterm birth to asthma outcomes may include other mechanisms than differences in airway resistance. In **Chapter 2.4** we observed that rapid weight gain in early childhood is associated with bronchial responsiveness, and a decreased lung function in adolescence. Furthermore, rapid height gain seems to be associated with smaller lungs.

In conclusion, early growth, and especially weight gain, seems an important factor in the development of childhood asthma.

Chapter 3 describes the associations of fetal exposures with the development of childhood asthma. In **Chapter 3.1** we observed that maternal psychological distress during pregnancy is associated with increased odds of wheezing of their child during the first 6 years of life, independent of paternal psychological distress during pregnancy and maternal and paternal psychological distress after delivery. **Chapter 3.2** shows that mothers with pre-pregnancy obesity and a history of asthma or atopy, and higher gestational weight gain showed higher risks of wheezing in their offspring. These associations could not be explained by growth, infectious or atopic mechanisms. **Chapter 3.3** suggest that elevated maternal C-reactive protein in pregnancy is associated with a higher risk of eczema, and C-reactive protein in cord blood with a higher risk of wheezing and lower respiratory tract infections in the first 4 years.

In conclusion, immunomodulatory and inflammatory related environmental exposures in fetal life are associated with the development of childhood asthma.

Chapter 4 describes the associations of infant exposures with the development of childhood asthma. **Chapter 4.1** suggest that shorter duration and non-exclusivity of breastfeeding were associated with increased risks of asthma-related symptoms in preschool children. These associations seemed at least partly explained by infectious but not by atopic mechanisms. In **Chapter 4.2** we suggest that long term exposure to traffic-related air pollutants is associated with increased risks of wheezing in children exposed to tobacco smoke in fetal life and infancy. Smoke exposure in early life might lead to increased vulnerability of the lungs to air pollution.

In conclusion, breastfeeding and air pollution, two major exposures in early childhood, are suggested to affect the risks of childhood asthma.

Finally, in **Chapter 5** we discuss the results of the studies in this thesis in a general discussion and place our findings in a broader perspective. Furthermore, methodological issues of the studies, causality of the observed associations and directions for future research are described**.**

6.2

Samenvatting

In dit proefschrift hebben we onderzocht welke foetale en vroeg postnatale factoren geassocieerd zijn met de ontwikkeling van astma op de kinderleeftijd. Vroege groei en nadelige omgevingsfactoren kunnen leiden tot een aangepaste ontwikkeling van de longen en luchtwegen, welke vervolgens het risico op astma en astma symptomen kunnen vergroten. Vanuit zowel een etiologisch als een preventief perspectief is het belangrijk om specifieke foetale en vroeg postnatale omgevingsfactoren die kunnen leiden tot astma te identificeren. De studies in dit proefschrift richten zich in het bijzonder op de identificatie van belangrijke periodes voor het ontstaan van astma.

Hoofdstuk 1 is een algemene introductie en beschrijft de hypothese waarop dit proefschrift is gebaseerd. Ook worden de doelen van de uitgevoerde studies en de verdere opzet van het proefschrift beschreven.

Hoofdstuk 2 beschrijft de associatie van foetale en vroeg postnatale groei met de ontwikkeling van astma op de kinderleeftijd. In **Hoofdstuk 2.1** laten we zien dat een kortere zwangerschapsduur en een grotere gewichtstoename in de vroeg postnatale periode geassocieerd is met een verhoogd risico op het ontstaan van astma klachten. De associatie van een laag geboortegewicht met astma wordt voornamelijk verklaard door een kortere zwangerschapsduur. Uit **Hoofdstuk 2.2** kunnen we concluderen dat een grotere gewichtstoename in de vroeg postnatale periode geassocieerd is met meer astma klachten op de kleuterleeftijd. Deze associatie is onafhankelijk van de foetale groei. Daarom lijkt de vroege postnatale fase een belangrijke periode voor het ontstaan van astma. **Hoofdstuk 2.3** laat zien dat luchtwegweerstand in schoolgaande kinderen beïnvloed wordt door foetale groei restrictie, maar niet door vroeggeboorte, en geassocieerd is met astma uitkomsten. De relatie tussen vroeggeboorte en astma uitkomsten wordt mogelijk verklaard door andere mechanismen dan luchtwegweerstand. In **Hoofdstuk 2.4** laat zien dat een grotere gewichtstoename in de vroege postnatale periode geassocieerd is met toegenomen bronchiale hyperreactiviteit en verminderde longfunctie in jongvolwassenen. Ook laten we zien dat snelle lengtegroei geassocieerd is met kleinere longen.

Uit de studies van hoofdstuk 2 concluderen we dat vroege groei, en met name snelle gewichtstoename in de vroege postnatale periode, een belangrijke factor is in de ontwikkeling van astma op de kinderleeftijd.

Hoofdstuk 3 beschrijft de associatie tussen blootstelling aan omgevingsfactoren in de foetale periode en de ontwikkeling van astma op de kinderleeftijd. **Hoofdstuk 3.1** beschrijft dat maternale psychologische stress gedurende de zwangerschap geassocieerd is met een verhoogd risico op wheezing van het kind tijdens de eerste zes levensjaren. Dit is onafhankelijk van paternale psychologische stress gedurende de zwangerschap en maternale en paternale psychologische stress na de geboorte van het kind. **Hoofdstuk 3.2** laat zien dat moeders

die obees zijn voor de zwangerschap en ook atopie of astma hebben, en dat moeders met een verhoogde toename van gewicht tijdens de zwangerschap, geassocieerd zijn met een verhoogd risico op wheezing van hun kind. Deze associaties kunnen niet worden verklaard door groei, infectieuze of atopische mechanismen. **Hoofdstuk 3.3** toont dat een verhoogd maternaal C-reactief proteïne in de zwangerschap geassocieerd is met een verhoogd risico op eczeem bij het kind. Ook toont dit hoofdstuk aan dat een verhoogd C-reactief proteïne in navelstrengbloed geassocieerd is met een hoger risico op het ontstaan van wheezing en lage luchtweg infecties in de eerste vier levensjaren.

Uit de studies van hoofdstuk 3 concluderen we dat immunomodulatoire en inflammatoire gerelateerde blootstellingen in het foetale leven zijn geassocieerd met het risico op het ontstaan van astma op de kinderleeftijd.

Hoofdstuk 4 beschrijft de associatie tussen blootstelling aan omgevingsfactoren in de vroeg postnatale periode en het ontstaan van astma op de kinderleeftijd. **Hoofdstuk 4.1** suggereert dat een kortere duur en het niet exclusief geven van borstvoeding geassocieerd is met een verhoogd risico op het ontstaan van astma klachten bij jonge kinderen. Deze associatie kan gedeeltelijk verklaard worden door infectieuze mechanismen, maar niet door atopische mechanismen. In **Hoofdstuk 4.2** laten we zien dat een langdurige blootstelling aan luchtvervuiling geassocieerd is met een verhoogd risico op wheezing in kinderen die ook blootgesteld zijn aan foetale en vroeg postnatale tabaksrook. De blootstelling aan tabaksrook zou kunnen leiden tot een verhoogde kwetsbaarheid van de longen voor luchtvervuiling.

Uit de studies van hoofdstuk 4 concluderen we dat borstvoeding en luchtvervuiling, twee belangrijke vroeg postnatale blootstellingen, zijn geassocieerd met het risico op het ontstaan van astma op de kinderleeftijd.

Ten slotte, in **Hoofdstuk 5,** bediscussiëren we de resultaten uit de studies in dit proefschrift in een algemene discussie en plaatsen we onze bevindingen in een breder perspectief. Ook beschrijven we de methodologische beperkingen van deze studies, de causaliteit van de geobserveerde associaties, en geven we suggesties voor toekomstig onderzoek.

Chapter 7

7.1

Publication list

1. **Sonnenschein-van der Voort AM**, Jaddoe VW, Moll HA, Hofman A, van der Valk RJ, de Jongste JC, Duijts L. Influence of maternal and cord blood C-reactive protein on childhood respiratory symptoms and eczema. *Pediatr Allergy Immunol*. 2013;24(5):469-75. Epub 2013/06/19 DOI 10.1111/pai.12094

2. **Sonnenschein-van der Voort AM**, Duijts L. Breastfeeding is protective against early childhood asthma. *Evid Based Med.* 2013;18(4):156-7. Epub 2012/11/06 DOI 10.1136/eb-2012-100910

3. **Sonnenschein-van der Voort AM**, Jaddoe VW, van der Valk RJ, Willemsen SP, Hofman A, Moll HA, de Jongste JC, Duijts L. Duration and exclusiveness of breastfeeding and childhood asthma-related symptoms. *Eur Respir J.* 2012;39(1):81-9. Epub 2011/07/23 DOI 10.1183/09031936.00178110

4. **Sonnenschein-van der Voort AM**, Jaddoe VW, Raat H, Moll HA, Hofman A, de Jongste JC, Duijts L. Fetal and infant growth and asthma symptoms in preschool children: the Generation R Study. *Am J Respir Crit Care Med.* 2012;185(7):731-7. Epub 2012/01/24 DOI 10.1164/rccm.201107-1266OC

5. **Sonnenschein-van der Voort AM**, de Kluizenaar Y, Jaddoe VW, Gabriele C, Raat H, Moll HA, Hofman A, Pierik FH, Miedema HM, de Jongste, JC, Duijts L. Air pollution, fetal and infant tobacco smoke exposure, and wheezing in preschool children: a population-based prospective birth cohort. *Environ health*. 2012;11:91. Epub 2012/12/13 DOI 10.1186/1476-069X-11-91

6. Guxens M, **Sonnenschein-van der Voort AM**, Tiemeier H, Hofman A, Sunyer J, de Jongste JC, Jaddoe VW, Duijts L. Parental psychological distress during pregnancy and wheezing in preschool children: The Generation R Study. *J Allergy Clin Immunol.* 2013. Epub 2013/06/20 DOI 10.1016/j.jaci.2013.04.044

7. Leermakers ET, **Sonnenschein-van der Voort AM**, Heppe DH, de Jongste JC, Moll HA, Franco OH, Hofman A, Jaddoe VW, Duijts L. Maternal fish consumption during pregnancy and risks of wheezing and eczema in childhood: the Generation R Study. *Eur J Clin Nutr.* 2013;67(4):353-9. Epub 2013/02/28 DOI 10.1038/ejcn.2013.36

8. Leermakers ET, **Sonnenschein-van der Voort AM**, Gaillard R, Hofman A, de Jongste JC, Jaddoe VW, Duijts L. Maternal weight, gestational weight gain and preschool wheezing. The Generation R Study. *Eur Respir J.* 2013;42(5):1234-43. Epub 2013/03/09. DOI 10.1183/09031936.00148212

9. Hafkamp-de Groen E, **Sonnenschein-van der Voort AM**, Mackenbach JP, Duijts L, Jaddoe VW, Moll HA, Hofman A, de Jongste JC, Raat H. Socioeconomic and sociodemographic factors associated with asthma related outcomes in early childhood: The Generation R Study. *Plos One* 2013;8(11). DOI: 10.1371/journal.pone.0078266.

10. van der Valk RJ, Kiefte-de Jong JC, **Sonnenschein-van der Voort AM**, Duijts L, Hafkamp-de Groen E, Moll HA, Tiemeier H, Steegers EA, Hofman A, Jaddoe VW, de Jongste JC. Neonatal folate, homocysteine, vitamin B12 levels and methylenetetrahydrofolate reductase variants in childhood asthma and eczema. *Allergy*. 2013;68(6):788-95. Epub 2013/05/23 DOI 10.1111/all.12146

11. **Sonnenschein-van der Voort AM**, Gaillard R, de Jongste JC, Hofman A, Jaddoe VW, Duijts L. Fetal and infant growth patterns, airway resistance and school-age asthma. The Generation R Study. Submitted

12. **Sonnenschein-van der Voort AM**, Arends LR, de Jongste JC, Annesi-Maesano I, Arshad SH, Barros H, Basterrechea M, Bisgaard H, Chatzi L, Corpeleijn E, Correia S, Craig LC, Devereux G, Dogaru C, Dostal M, Duchen K, Eggesbø M, van der Ent CK, Fantini MP, Forastiere F, Frey U, Gehring U, Gori D, van der Gugten AC, Hanke W, Henderson AJ, Heude B, Iñiguez C, Inskip H, Keil T, Kelleher CC, Kogevinas M, Kreiner-Møller E, Kuehni CE, Küpers LK, Lancz K, Larsen PS, Lau S, Ludvigsson J, Mommers M, Nybo Andersen AM, Palkovicova L, Pike KC, Pizzi C, Polanska K, Porta D, Richiardi L, Roberts G, Schmidt A, Sram RJ, Sunyer J, Thijs C, Torrent M, Viljoen K, Wijga AH, Vrijheid M, Jaddoe VWV, Duijts L, Preterm birth, early growth and the risk of childhood asthma: A meta-analysis of 147,000 children. Submitted

13. **Sonnenschein-van der Voort AM,** Howe LD, Granell R, Duijts L, Sterne J.A.C, Tilling K, Henderson A.J. Influence of childhood growth on asthma and lung function in adolescence. Submitted

14. Zugna D, Galassi C, Maesano IA, Baïz N, Barros H, Basterrechea M, Correia S, Duijts L, Esplugues A, Fantini MP, Forastiere F, Gascon M, Gori D, Inskip H, Larsen PS, Mommers M, Nybo Andersen AM, Penders J, Petersen MS, Pike K, Porta D, **Sonnenschein-van der Voort AM**, Steuerwald U, Sunyer J, Torrent M, Vrijheid M, Richiardi L, Rusconi F. Maternal complications in pregnancy and infant wheezing: a study in fourteen birth cohorts. Submitted

7.2

About the author

Agnes Maria Mariamna Sonnenschein-van der Voort was born on the 2nd of March 1985 in Amsterdam, The Netherlands. In 2003 she completed secondary school at the Etty Hillesum Lyceum in Deventer. In the same year she started studying Earth Sciences at Utrecht University. After finishing her Bachelor's degree in 2006, she got admitted to study Medicine at the Erasmus Medical Center, Rotterdam. In 2008 she started the master Clinical Research at the Netherlands Institute for Health Sciences on top of the regular medical curriculum. As a part of the Master of Science programme she attended a summer programme at the Johns Hopkins Bloomberg School of Public Health, at the Johns Hopkins University in Baltimore, United States of America. She obtained a "doctoral" degree in medicine in 2010 and in 2011 she obtained her Master of Science in Clinical Research degree after which she could extend her research project into the current PhD traject on fetal and infant origins of childhood asthma at the Generation R Study, at the departments of Paediatrics (promotor: Prof J.C. de Jongste, co-promotor: Dr L. Duijts), and Epidemiology (promotor: Prof V.W.V Jaddoe). During her PhD traject she spent 6 months at the Avon Longitudinal Study of Parents and Children (ALSPAC) and worked on the association of early growth with asthma in adolescence under supervision of Prof AJ. Henderson, Prof J.A.C. Sterne and Prof K. Tilling. At this moment she is doing her clinical rotations and hopes to graduate as a medical doctor in 2015. Agnes lives in The Hague, together with her husband Anne.

7.3

PhD Portfolio

Summary of PhD training and teaching

Name PhD student:	Agnes Sonnenschein-van der Voort
Erasmus MC Department:	Paediatrics, Respiratory Medicine; Epidemiology
Research School:	Nihes
PhD period:	01 June 2011 – 31 March 2013
Promotors:	prof. dr. J.C. de Jongste, prof. dr. V.W.V. Jaddoe
Co-promotor:	dr. L. Duijts

1. PhD training

	Year	Workload (ECTS)
GENERAL COURSES		
Specific courses		
- Master of Science in Clinical Research at the Netherlands Institute of Health Sciences, NIHES, Rotterdam Including a Summer Programme at Johns Hopkins Bloomberg School of Public Health, at the Johns Hopkins University in Baltimore, United States of America	2008-2011	
Seminars and workshops		
- Dag voor de jonge onderzoekers, NVK, Veldhoven	2011	0.5
- Young investigators day, NRS, Amsterdam	2011	0.5
- Networking workshop, VENA	2012	0.2
- Generation R Research meetings	2011-2012	1.0
- Seminars at the department of Epidemiology, Erasmus MC	2011-2012	1.0
- Seminars at the School of Social and Community Medicine, University of Bristol, United Kingdom	2012-2013	1.0
PRESENTATIONS		
Invited speaker		
- VLOV symposium (Vlaamse Organisatie van Vroedvrouwen). Waregem, Belgium. *Duration and exclusiveness of breastfeeding and childhood asthma.*	2011	1.0
Other		
- Research meeting children's respiratory medicine, department of paedicatrics, division of Respiratory Medicine, Erasmus MC-Sophia. *Duration and exclusiveness of breastfeeding and childhood asthma.*	2011	1.0
- Paediatrics Research meeting – Erasmus MC-Sophia. *Fetal and infant growth and asthma symptoms in preschool children.*	2011	1.0
- Generation R Research meeting. *Fetal flow, placental function, growth and asthma symptoms in preschool children.*	2012	1.0

-	Research meeting children's respiratory medicine, department of paedicatrics, division of Respiratory Medicine, Erasmus MC-Sophia. *Fetal flow, placental function, growth and asthma symptoms in preschool children.*	2012	1.0
-	Research meeting children's respiratory medicine, department of paedicatrics, division of Respiratory Medicine, Erasmus MC-Sophia. *Early growth and childhood asthma: a meta-analysis on 147,000 children.*	2012	1.0
-	Sophia Onderzoekersdag, Erasmus MC - Sophia. *Vroege groei en astma op de kinderleeftijd.*	2013	1.4

Presentations on international conferences

-	5th Conference of Epidemiological Longitudinal Studies in Europe, Paphos, Cyprus (oral presentation). *Duration and exclusiveness of breastfeeding and childhood asthma.*	2010	1.4
-	21th European Respiratory Society, Amsterdam, the Netherlands (poster discussion). *Fetal and infant growth and asthma symptoms in preschool children.*	2011	1.4
-	American Thorax Society conference, San Francisco, USA (poster presentation). *Maternal distress and asthma symptoms in preschool children.*	2012	0.7
-	American Thorax Society conference, San Francisco, USA (poster presentation). *Air pollution, tobacco smoke exposure and wheezing in preschool children.*	2012	0.7
-	DOHAD satellite meeting, Rotterdam, the Netherlands (oral presentation). *Early growth and childhood asthma: a meta-analysis on 147,000 children.*	2012	1.4
-	23th European Respiratory Society, Barcelona, the Netherlands (poster discussion). *Growth in childhood with lung function in adolescence.*	2013	0.7
-	23th European Respiratory Society, Barcelona, the Netherlands (oral presentation). *Early growth and childhood asthma: a meta-analysis on 147,000 children.*	2013	1.4

OTHER

Scholarships, grants and prizes

-	European Respiratory Society (ERS) short term research and training fellowship (nr. STRTF 93-2012), € 7700.	2012
-	Koninklijke Nederlandse Academie voor de Wetenschap (KNAW) Ter Meulen Fonds research fellowship (nr. TMF2012/228), € 9150.	2012
-	Pfizer Nutrition Young Investigator Award, Developmental Origins of Health And Disease (DOHAD) satellite meeting, Rotterdam, the Netherlands, 2012, € 500.	2012
-	ERS Grant for best abstract in Paediatric Respiratory Epidemiology, Barcelona, European Respiratory Society (ERS) – Paediatric Assembly, 2013, € 1000.	2013
-	Vereniging Trustfonds Erasmus Universiteit Rotterdam, several travel grants including: Johns Hopkins Summer Programme, CELSE 2010, ATS 2012, and ERS 2013.	2010-2013

2. Teaching

	Year	Workload (ECTS)
SUPERVISING PRACTICALS		
- NIHES ESP01: Principles of Research and Medicine and Epidemiology.	2011	1
SUPERVISING MASTER'S THESES		
Epidemiology		
- Elisabeth T.M. Leermakers, *Maternal pre-pregnancy obesity, gestational weight gain and wheezing in children. The Generation R Study.*	2011	1.5
Medicine		
- Varsha P.S. Doelam. *Fetal exposure to Maternal and paternal Smoking and respiratory morbidity at the age of 6 years. The Generation R Study.*	2012	1.5
- Anouk E. Muntz. *Duration and exclusivity of breastfeeding with respiratory morbidity at the age of 6 years. The Generation R Study.*	2013	1.5
Supervising Bachelor's thesis		
Medicine		
- Nathalie S. Bale, *Maternal C-reactive protein levels and wheezing in preschool children. The Generation R Study*	2011	1.0
Other		
- Reviewed articles for *Allergy, Asthma & Clinical Immunology; Expert Review of Respiratory Medicine; International Journal of Hygiene and Environmental Health; Journal of Evaluation and Program Planning, Paediatrics and International Child Health*	2012-2013	2.0

7.4

Dankwoord

DANKWOORD

Ineens ben ik nu op het eindstation van de sneltrein waar ik in 2011 ben ingestapt. Het was een leerzame reis waarin er hard gewerkt is, maar er ook veel plezier is gemaakt. Zonder de steun van velen had dit promotietraject niet zo voorspoedig kunnen verlopen. Graag maak ik daarom gebruik van deze gelegenheid om jullie allemaal te bedanken.

Dit proefschrift had hier niet nu al gelegen als ik niet begeleid was door een geweldig team van twee mannen en één vrouw sterk. Het eerste onderdeel van dit team zijn mijn promotoren: Prof.dr. de Jongste en Prof.dr. Jaddoe. Beste Johan, ik ben u zeer dankbaar voor de gelegenheid die u mij heeft gegeven om mijn masteronderzoek uit te breiden naar een volwaardig proefschrift. U wist altijd kritisch te kijken naar mijn werk en regelmatig kreeg ik het terug met geweldige ideeën, en niet alleen wetenschappelijk, maar u wist ook altijd het juiste woord te vinden zodat het hele manuscript ineens veel duidelijker werd. U zei ook eerlijk wat u ergens van vond, zowel over verbeterpunten als over behaalde successen. Beste Vincent, in 2010 kwam ik bij je langs om te vragen of je nog een leuk onderzoek had voor een masterstudent. Gelukkig was er voldoende te doen en kwam ik een jaartje onderzoek doen. Tijdens dat jaar had je mij enthousiast gekregen om door te gaan met onderzoek doen en is het logisch dat jij onderdeel werd van mijn powerteam. Bedankt voor alles wat je me geleerd hebt, en dat is niet weinig! Nu ben je officieel hoogleraar en ben ik trots dat ik je eerste promovenda mag zijn.

Evenzo belangrijk in het team was mijn co-promotor: Dr. L Duijts. Beste Liesbeth, tijdens onze eerste kennismaking zat jij in Bristol en ik in Rotterdam. Overleggen deden we via Skype en e-mail en niet een keer per week, maar zo nodig meerdere keren per dag. Toen hadden we nog geen idee dat het niet veel later omgekeerd zou zijn: ik in Bristol en jij in Rotterdam. Je was een hele fijne begeleider: gedurende menig deadline hebben we nachtelijk mailcontact gehad, tot in de kleine uurtjes bleef ook jij scherp totdat we tevreden waren en we vlak voor het sluiten van de deadline ons werk konden indienen en niet zonder resultaat! Bedankt voor al je support, je oppeppende mailtjes en alle gezellige overleggen waarin we waar mogelijk wat extra tijd inplanden voor een bezoekje aan Doppio mét muffins. Gelukkig lopen er nog wat projectjes dus voorlopig kunnen we vrolijk verder samen.

Beste Prof.dr. Reiss, bedankt dat u zitting wilde nemen in de kleine commissie en tevens de taak van secretaris op u wilde nemen. Uw presentaties zorgden de afgelopen jaren altijd voor een nieuwe dosis inspiratie voor dit proefschrift. Beste Prof.dr. Smit, de afgelopen jaren heb ik u leren kennen tijdens verschillende congressen waar u altijd even langs kwam bij mijn poster of presentatie. Ik ben erg blij dat u deel wilde uitmaken van mijn kleine commissie. Dear Prof.dr. Henderson, a special word of thanks to you, for your willingness to be a part of

the small committee, for you being here today, and for giving me the opportunity to join you at the ALSPAC cohort. It was great to have so many coffees and cakes with you, which is essential for performing good research… Beste Prof.dr. Franco, Prof.dr. Hofman en Prof.dr. Rings hartelijk dank voor uw deelname aan de grote commissie en uw aanwezigheid bij de verdediging.

Marieke en Romy, mijn paranimfen, wat ben ik blij dat jullie vandaag naast mij staan. Marieke: toen wij 10 jaar geleden samen in een roeiploeg zaten studeerde ik nog aardwetenschappen en jij verpleegkunde. We hadden destijds niet kunnen voorspellen dat we in 2013 samen naar het congres van de ERS zouden gaan. Bedankt voor onze jaren trouwe vriendschap van lange avonden op Triton en in het Neutje, via springend in de regenplas in Londen: stupid cows!, tot làààrge bullets in Barcelona, en niet te vergeten onze spontane relax avondjes. Romy, mijn onderzoeksmaatje van het eerste uur. In ons masterjaar bij Generation R zaten we samen aan een bureau, samen de eerste syntaxen schrijven en voor het eerst spreken op een congres. En nu zijn we alweer allebei aan het eind van ons promotietraject. Succes met het samenvoegen van al je mooie papers, je bent er bijna! En je weet me te vinden als je weer eens ruzie hebt met je figuren…

Beste co-auteurs bedankt voor alle input en het fijne samenwerken. Succes bereik je nooit alleen. Een aantal wil ik er graag bij naam noemen. Lidia (Prof.dr. Arends): bedankt voor het nachten doorwerken voor de meta-analyse. Lisan: na je onderzoek als student ben je nu bezig met je eigen promotietraject: toi toi toi! Monica: het is gelukt, we hebben de tiramisu!!! (alleen nog even een datum prikken). Ralf: bedankt voor je hulp als ik weer eens in de clinch lag met SAS. TNO co-auteurs: bedankt voor alle supersnelle reacties. Dear collaborators of the CHICOS-EGWA meta-analysis, thank you for the great collaboration, especially for your prompt responses and valuable comments. Let's continue and hope we also will be successful with our ELF-project.

Natuurlijk ook hartelijk dank aan alle Generation R deelnemers en hun ouders. Zonder jullie inzet en doorzettingsvermogen om alle vragenlijsten in te vullen had ik dit proefschrift niet kunnen schrijven. Veel dank aan alle onderzoeksmedewerkers op het onderzoekscentrum van Generation R, dankzij jullie hebben de deelnemers een leuke dag en ook zonder jullie inzet had ik dit proefschrift niet kunnen schrijven. Ook veel dank aan alle andere medewerkers: Patricia (bedankt voor het plannen van afspraken bij Vincent, Johan en Liesbeth tegelijk, en voor alles wat je nu voor mijn promotie geregeld hebt), Rose (bedankt voor het doorsturen van mijn post), Karin (altijd in voor een grap), Karien (treinmaatje naar Den Haag), Ronald (wanneer gaan we borrelen?), Alwin (dankzij jou kon ik nog harder werken) en Natalia (even een kopje thee erbij). Claudia en Marjolein: zonder jullie geen data en zonder data geen proefschrift, bedankt voor jullie precisie en altijd snelle levering van data!

Lieve collega promovendi bij Generation R, wat is het een gezellige boel geweest in het AE-gebouw en daarna in het penthouse in het NA-gebouw. Een bijzonder woord van dank aan de vele kamergenoten die ik de afgelopen jaren heb gehad: Ilse en Akghar mijn eerste roomies: veel kletsen, maar ook hard werken; Romy en Denise: bedankt dat ik meerdere malen bij jullie op de kamer mocht bivakkeren; en natuurlijk Ralf en Esther: wat een toptijd hebben we gehad op onze schimmelkamer, bedankt voor alle feedback en thee mét koekjes. Zoe en Martijn bedankt voor jullie gastvrijheid, en jullie gezelligheid als ik weer eens kwam aanwaaien (Gerard, bedankt voor het lenen van je bureau!). Rob, Denise, Layla, Romy en Edith, bedankt voor jullie support bij mijn eerste congres op het heel relaxte Cyprus.

Lieve collega promovendi van de pulmogroep, ook jullie bedankt voor de gezellige en leerzame tijd: na de researchmeeting nog even koffiedrinken en een borrel her en der. Esther en Sandra bedankt voor de leuke tijd op de ATS in San Francisco, nogmaals bedankt voor de bekertjes water! Sandra en Marjolein bedankt voor het succesvolle tripje naar de ERS in Barcelona: borrel hier, etentje daar… Ook de rest van het pulmo-team bedankt voor de gezellige research-meetings en etentjes. Irma, naast het inplannen van afspraken met Johan kon ik voor van alles bij je terecht. Bedankt voor het zoeken van de juiste stempels, het printen van alle officiële brieven op mooi papier (dat waren er stiekem best veel) en bedankt voor alles wat je nu voor mijn promotie geregeld hebt.

Dear Raquel, Laura, Alison, Oliver, Anu and Lexine thank you for your hospitality, the great pub quizzes, tea with cakes, movies, and clubbing we did in Bristol. Dear Zina en Bernard, thanks for everything you did during my stay in Bristol.

Lieve Barry, Stephanie, Nathalie en Mirjam, mede master-bikkels, bedankt voor onze leuke tijd in de collegebanken. Bedankt voor alle koffie momenten tijdens de afgelopen jaren. Heel veel succes met jullie promoties, eerste baan als dokter, en/of het lopen van jullie coschappen.

Channe, Marieke, Roelfke, Jolien, Janneke, Laurien, Ron, Robert, Joost, Reinoud, Wouter en William, eindelijk eens een moment om stil te staan bij onze vriendschappen. Lief en leed hebben we gedeeld en zullen we nog jaren met elkaar delen. Maria: knijp! (Meer hoef ik toch niet te zeggen…) Janneke: bedankt voor alle gezellige etentjes, wanneer gaan we weer? Julia: ik heb weer tijd voor een strandwandeling. Desiree: ik kom nu echt binnenkort naar Bergen. Vincent en Maarten: het is borreltijd! Lieve OJN, OJB en OZK vrienden: ik ben weer beschikbaar, wanneer gaan we skypen, wanneer is ons weekend?

Paul, Hiltje, en Femke, bedankt voor alle gezellige etentjes in Deventer, al waren jullie laatste tijd iets vaker in Den Haag... Vanaf nu komen we weer wat vaker jullie kant op hoor!

Lieve oma's bedankt voor jullie vele telefoontjes en jullie steun en vertrouwen.

Theo, grote broer, ik ben trots op je! Bedankt voor je vele berichtjes. Pap en mam, ik had hier vandaag niet kunnen staan zonder jullie voortdurende steun en vertrouwen. De basis die jullie me hebben meegegeven heeft zeker bijgedragen aan dat ik hier vandaag kan staan. Dank jullie wel!

Anne, bedankt voor het soms noodzakelijke chocolade ijs en bedankt voor het samen vieren van onze successen met bubbels. Bedankt voor het zetje dat je me gaf om zes maanden naar Bristol te gaan. Als doctor en dokter-in-spe kan ik me geen betere man voorstellen om mijn leven mee te delen.

Mijn boek is klaar, iedereen nogmaals bedankt.

Nu is het tijd voor een feestje!