Asthma and wheezing in childhood

perinatal risk factors and early detection

Daan Caudri

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Asthma and Wheezing in Childhood
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Astma en piepende ademhaling op de kinderleeftijd
perinatale risicofactoren en vroege detectie

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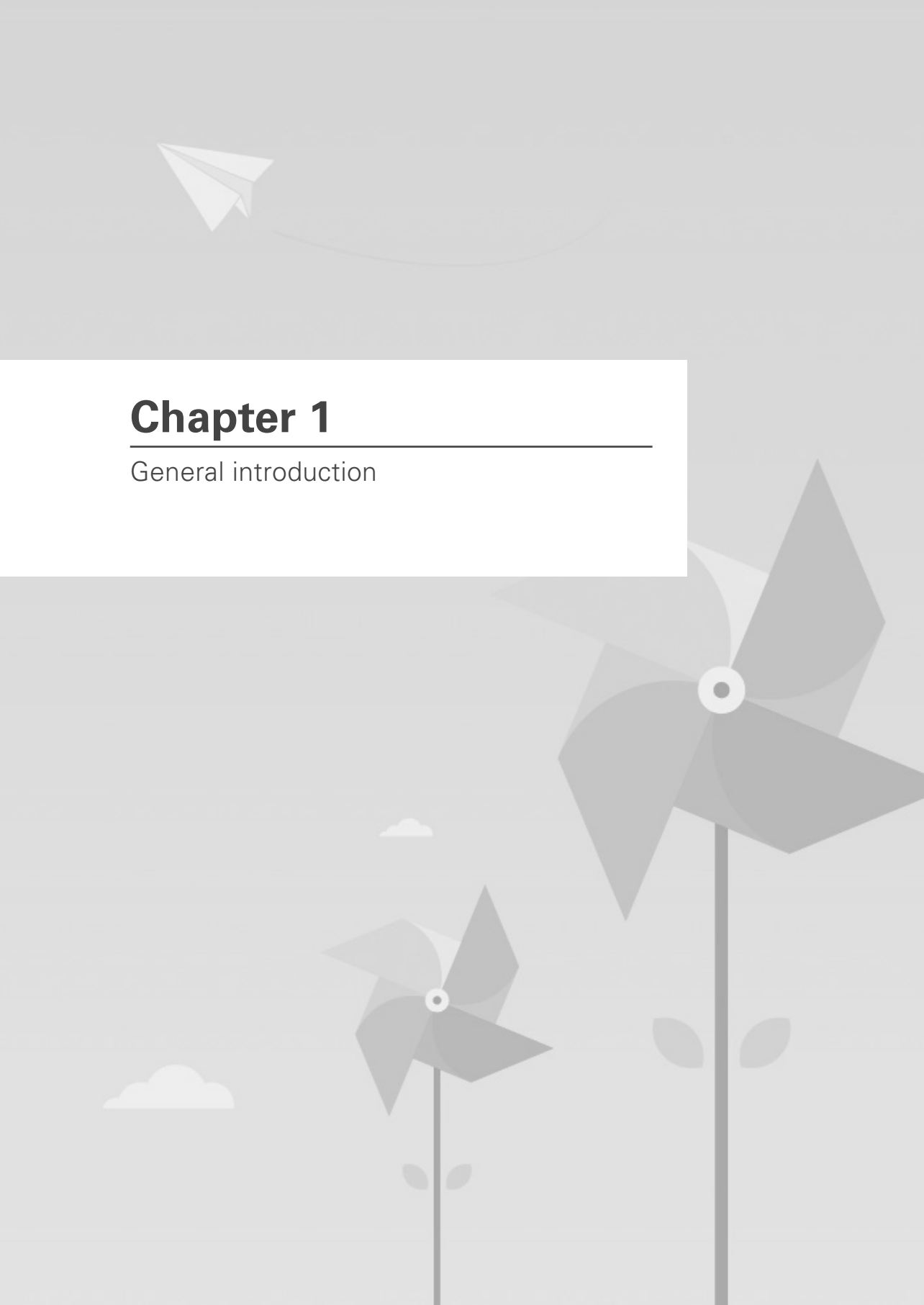

GENERAL INTRODUCTION

The prevalence of childhood asthma and atopic disease have increased dramatically during the end of the last century, especially in Western countries.[1] Presently, asthma is the most frequent chronic disorder in childhood, with a high burden in terms of morbidity, health care costs, absenteeism from school, and reduced quality of life, despite the availability of effective and safe treatment.[2] Two major challenges in the field of childhood asthma, have still been insufficiently addressed. In this thesis we focused on both these issues.

The first challenge is the search for new ways to prevent asthma development in children using interventions early in life. Understanding which factors are responsible for the rising prevalence in asthma and allergies is essential to find targets for future prevention. The sudden increase in prevalence of childhood asthma and atopic disease during the end of the last century suggests, that environmental factors play an important role in the development of allergic disease.[3] Previous research has shown evidence that a crucial window for the effect of environmental factors may exist in prenatal and early postnatal life.[4] Events occurring during fetal and early postnatal life may impair lung development in later life, and lead to development of respiratory disorders including asthma.[5] This is supported by the finding that lung function measured directly after birth is a strong indicator of lung function in adolescence.[6] In **part I** of this thesis we investigated the association between several perinatal factors and the longitudinal development of asthma symptoms during childhood, to assess which perinatal factors may be promising targets for preventive interventions early in life.

A second challenge will be the clinical problem of early identification of children who will develop asthma, and those that have only transient symptoms at a young age. It should be realized that even if successful prevention programs are developed, asthma symptoms such as wheeze and cough will remain highly prevalent in children, especially at preschool age. Data from the PIAMA study have shown that up to a quarter of all children in the Netherlands will experience wheezing symptoms in the first year of life.[7] Consequently, clinicians are very often confronted with preschool children with asthma symptoms, and only a minority of those children will actually develop asthma.[8] Early identification of children with persistent symptoms remains one of the most difficult clinical problems.[9] Therefore, in **part II** of this thesis, we evaluated which combination of perinatal factors, clinical symptoms and additional tests may be useful in clinical practice to predict which children with asthma symptoms at preschool age are most likely to develop chronic asthma.

ASTHMA AND WHEEZING DISORDERS IN CHILDREN

Asthma

Asthma is a lung disease characterized by recurrent periods of wheezing, shortness of breath, and coughing.[10] There is a strong association with chronic and mainly eosinophilic inflammation of the airways, and bronchial hyperresponsiveness. In response to specific stimuli (e.g. allergens) and nonspecific stimuli (e.g. infections, exercise) the inflamed airways can be triggered, leading to episodes with variable airflow obstruction and thereby the clinical symptoms of wheeze, shortness of breath and cough. The airflow obstruction is often reversible, either spontaneously or with specific treatment. There are three mechanisms that can cause variable airflow obstruction in asthmatics. First the airway smooth muscles contract in response to stimuli, leading to bronchoconstriction. Second, swelling of the mucosa lining the airway wall can lead to further airway narrowing. Third, an increase in mucus production can result in mucus plug formation and thereby airway obstruction. Apart from these mechanisms leading to a variable airflow limitation, irreversible changes in the airway anatomy may occur in some asthmatics, which is referred to as airway remodeling.[11] Possibly, a chronic state of inflammation is responsible for structural alterations of the airways, causing more chronic symptoms and irreversible loss of lung function.[11] The clinical symptoms of asthma are well known: recurrent attacks of wheezing, shortness of breath/ dyspnea and coughing at night, in response to a number of triggers.[12] However, the presentation may be heterogeneous and change rapidly over time, especially in children. Therefore a clear and generally accepted definition of asthma does not exist.[13] Objective tests to support an asthma diagnosis include spirometry, to assess airway obstruction and reversibility; bronchoprovocation, to assess airway responsiveness; and sputum induction to measure airway inflammation. These tests are difficult to perform in children under the age of 6 years, since active cooperation is necessary.[14] In epidemiologic studies 'asthma' is often defined as a parental report of doctors' diagnosed asthma, but also parental reports of asthma symptoms, especially wheezing, or the use of specific asthma medication are sometimes used as a proxy for asthma.[15] The use of parental reports of wheeze will introduce some uncertainty, as it is less accurate than physician-confirmed wheeze.[16]

Wheezing phenotypes in children

Wheezing is the most important symptom of asthma and highly prevalent in children, especially in the first years of life. However, only a minority of these wheezing children will develop persistent asthma. There is also a proportion of children who do not experience wheeze in the first years of life, but develops symptoms at later ages.[8] The concept that 'childhood asthma' is not a single disease entity, but comprises several heterogeneous

wheezing subtypes may be a possible explanation for contradictory findings in previous studies on asthma risk factors.[17] Therefore much effort has been put in the identification of different temporal wheezing phenotypes. Martinez *et al* introduced 4 longitudinal patterns of wheezing based on reported symptoms at the age of 3 and 6 years: 'never', 'early transient', 'persistent' and 'late onset' wheeze.[18] This classification has served as a useful model in the past decade, but may give an incomplete description of all wheezing illnesses during childhood. More recently Henderson *et al* used longitudinal latent class analysis to identify different wheezing phenotypes in the Avon Longitudinal Study of Parents And Children (ALSPAC), where wheeze was reported repeatedly from birth until 8 years of age.[19] Many different longitudinal patterns of wheeze are possible, but some patterns will occur more often than others. Latent class models can be used to identify children with similar patterns of wheeze over time. These latent classes represent different longitudinal wheezing phenotypes. The analysis was repeated in the PIAMA study, where 5 wheezing phenotypes with similar characteristics as in the ALSPAC study were found, supporting their validity.[20] Figure 1.1 depicts the probability of wheeze at every age in these 5 wheezing phenotypes identified in the PIAMA study. The prevalence of the phenotypes are shown next to the phenotypes in the legend.

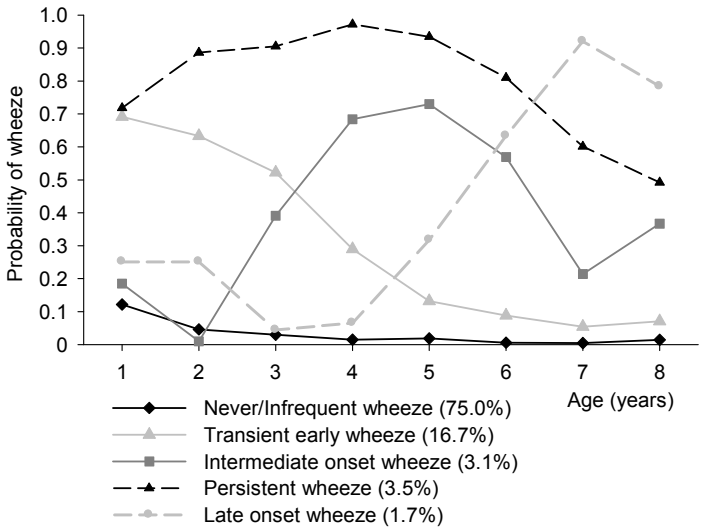

Figure 1.1. Probability of wheeze at each time point from birth to age 8 years for each wheezing phenotype in PIAMA (N = 2,810)
Adapted from Savenije & Granell *et al*.[20]

PREVENTION OF CHILDHOOD ASTHMA

Understanding the factors associated with mechanisms of asthma development is essential in order to find targets for primary prevention of specific wheezing illnesses during childhood. In spite of many cross-sectional studies, there are very few preventive measures of which a beneficial effect on asthma and allergy has been documented. The maximal possible benefit on public health of an intervention on a specific risk factor will depend on several aspects. First it is important to realize that only modifiable risk factors can be used in prevention programs. Second, the strength of the association between a risk factor and later asthma will to a large extent determine the possible benefit of an intervention. Third, also the prevalence of a risk factor in the population and the ability of an intervention to eliminate this factor from the population should be taken into account.

Perinatal factors as candidates for prevention

It has been suggested that some important risk factors for asthma development already operate during pregnancy and early in life.[4] The most consistent early risk factors for childhood asthma are probably a family history of asthma/allergies and male gender, but these factors are not modifiable and hence no candidates for interventions.[21] Previous analyses of data from the PIAMA study have identified modifiable risk factors for the development of respiratory symptoms in prenatal and early life. These risk factors included maternal nutrition during pregnancy,[22] breastfeeding,[23] air polution,[24] and maternal overweight.[25] A modifiable factor that has received much attention for its possible protective effect on later asthma development is the exposure to infections early in life. A protective effect of early infections has been suggested based on the 'hygiene hypothesis', which was first introduced by Strachan.[26] He found that the risk to develop hay fever was reduced in children with more older siblings, and proposed the association may be explained by the increased exposure to microorganisms in children with older siblings. Another factor that increases the risk of early infections and would be more easily modified is daycare attendance. However, conclusive evidence that daycare is indeed associated with a reduction in asthma/allergy on the long term is lacking.[27-31] In line with the 'hygiene hypothesis' some studies have found an increased risk of asthma development in children born by a caesarean section, possibly due the delayed microbial colonization, and this was supported by PIAMA data.[32]

Another hypothesis on the association between early life events and the development of diseases later in life was postulated by Barker *et al*, who found that children with a low birth weight were more likely to suffer from cardiovascular disease in adulthood.[33] They proposed that fetal malnutrition could lead to disproportionate fetal growth, resulting in chronic diseases in later life, especially cardiovascular diseases.[34] The association

between reduced fetal growth and childhood asthma remains much less clear.[35-37] Nevertheless a range of prenatal risk factors have been suggested to increase the risk of later asthma and allergies, such as reduced prenatal growth,[35,37] premature birth,[38] increased head circumference[39] and prenatal tobacco smoke exposure.[40]

DIAGNOSIS AND PREDICTION OF CHILDHOOD ASTHMA

Given the heterogeneous character of the disease it is difficult to diagnose asthma, especially in young children.[41] There are no standard lung function tests available for preschool children, and a diagnosis is solely based on clinical symptoms. Despite these difficulties an early asthma diagnosis is important to enable adequate treatment with bronchodilators and anti-inflammatory drugs like inhaled corticosteroids (ICS) or leukotriene receptor antagonists. On the other hand, as the majority of symptomatic preschool children will outgrow their symptoms during childhood, chronic use of ICS in all wheezing young children may lead to considerable overtreatment. Previous studies have shown that undertreatment as well as overtreatment of asthma may indeed be common in children,[42-44] but data in preschool children are scarce.[45]

Clinical prediction tools may help clinicians to better assess individual prognosis in preschool children and thereby contribute to the better management of childhood asthma. Most research on early life risk factors has focused on the etiological relation with asthma, rather than the predictive value for symptom persistence. Several birth cohorts investigated which clinical questions can be used to distinguish preschool children with persistent symptoms from those with transient symptoms.[46-48] Prediction rules developed in birth cohorts from the United States of America, the United Kingdom and Germany included clinical questions about wheezing frequency, eczema, allergic rhinitis, parental asthma, recurrent infections and gender.[46-48] Importantly, these rules were developed using information up to a fixed age (often 3 years) irrespective of the age of symptom onset. Therefore it may not be appropriate to implement the rules when they are most useful in clinical practice: at the age a child presents with symptoms for the first time. Secondly, all studies included blood tests in their algorithm.[46-50] Consequently, the derived prediction rules cannot be used to instantly calculate an individual risk profile in children presenting with symptoms, as the lab results should be awaited first. Currently no generally accepted asthma prediction tools are available for use in clinical practice.

Objective tests to diagnose asthma in preschool children

After the initial risk assessment on the basis of clinical symptoms it would be worthwhile to have additional objective tests to further improve the diagnostic and prognostic

accuracy. Standard tests to support an asthma diagnosis include spirometry and bronchoprovocation, but these tests cannot be used under the age of 5-6 years.[14] There are some objective tests available at preschool age that may help to assess the prognosis in symptomatic children.

Specific immunoglobulin E (IgE) to inhalant allergens is a well known marker for atopy and is strongly associated with both doctors' diagnosed asthma and asthma symptoms. Prospective studies have consistently shown that children with specific IgE are more likely to develop persistent asthma later in life.[48-51] Specific IgE should therefore be tested in children suspected of asthma, and the test should be taken into account when assessing the additional value of other objective tests. Two objective tests that can be easily performed in young children and may further improve the prediction of later symptoms are the measurement of eosinophilic airway inflammation using fractional exhaled nitric oxide (FeNO), and the measurement of airway resistance by the interrupter technique (Rint).

Measurement of airway inflammation using the fraction of exhaled nitric oxide

Chronic airway inflammation is a key feature of adult asthma, and reduction of the inflammation with anti-inflammatory agents is the cornerstone of asthma treatment.[10] Since the association between clinical symptoms and airway inflammation is weak at best, there may be clinical benefit in accurate monitoring of the inflammation.[52] The gold standard to assess airway inflammation is broncho-alveolar lavage or airway mucosal biopsy performed during bronchoscopy, in children usually under general anesthesia. Clearly these procedures are not suitable for routine clinical use in young children. Indirectly, airway inflammation can be measured counting inflammatory cells in induced sputum,[53] but this is difficult to perform in preschool children and the analyses are time consuming. Measurement of the fraction of nitric oxide in exhaled air (FeNO) is possible at all ages, and has been shown to strongly correlate with eosinophilic airway inflammation.[54,55] This implied that FeNO might be useful as a non-invasive test for airway inflammation, especially in young children.

In the airways, nitric oxide is produced by the oxidation of the amino acid L-arginine by NO-synthases (NOS).[56] At least three isoforms of NOS have been described; constitutive NOS (cNOS), neuronal NOS (nNOS) and inducible NOS (iNOS).[57] The inducible form iNOS has been mainly localized in the airways, alveolar epithelium, alveolar macrophages and the vascular endothelium.[56,58] Expression of iNOS can be up-regulated in inflammatory states by several cytokines (i.e. IFN, TNF, interleukin 1), which can lead to a manifold rise in NO levels.[59] (Figure 1.2)

NO is an important mediator with diverse functions, such as the regulation of airway and blood vessel diameter, through smooth muscle contraction or dilatation.[60]

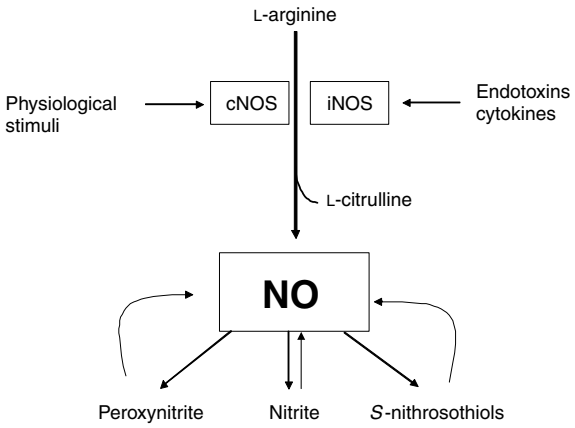

Figure 1.2. Synthesis of nitric oxide (NO) from L-arginine
cNOS, constitutive NO sythase; iNOS, inducible NO synthase. Reprinted from Pijnenburg *et al*,[55] with permission.

Nitric oxide levels in exhaled air can be measured either 'online' or 'offline'. In the online method subjects exhale directly into the NO analyzer.[61] The single breath online measurement at a constant flow of 50 ml/sec is now regarded the 'gold standard' FeNO measurement.[62] In order to compare FeNO values it is important to use standardized exhalation flows, since FeNO levels are highly flow-dependent.[63] Unfortunately this standard method is difficult to perform in young children.[62] In the offline measurement exhaled air is first collected in a NO impermeable, inert balloon, the collected gas can be analyzed afterwards.[64] Since the FeNO concentration strongly depends on the exhalation flow, a dynamic flow restrictor facilitates a constant flow and can be used in children aged 4 years or older. This method has a good correlation with the 'gold standard'.[64] In the PIAMA study FeNO was measured at the age of 4 years using the offline method with dynamic flow restrictor at a 50ml/sec exhalation flow. (Figure 1.3a) At the age of 8 years FeNO was measured using the standard online method. (Figure 1.3b) In both methods a charcoal NO scrubber was used to wash out all ambient NO from the air inhaled through the mouthpiece.

Previous studies have shown that exhaled nitric oxide levels are elevated in children with asthma, wheeze and atopy.[65-69] Most of these studies used a cross-sectional design comparing confirmed asthma cases with selected healthy controls, possibly overestimating the discriminative capacity of FeNO. Large scale epidemiological studies from the general population are scarce, especially for pre-school children.[70,71] Using data from the PIAMA study at the age of 4 years, Brussee *et al* found no significant difference in FeNO between early wheezing phenotypes, and only a small difference between asthmatics and non-asthmatics.[67] Prospective data collected until the age of 8

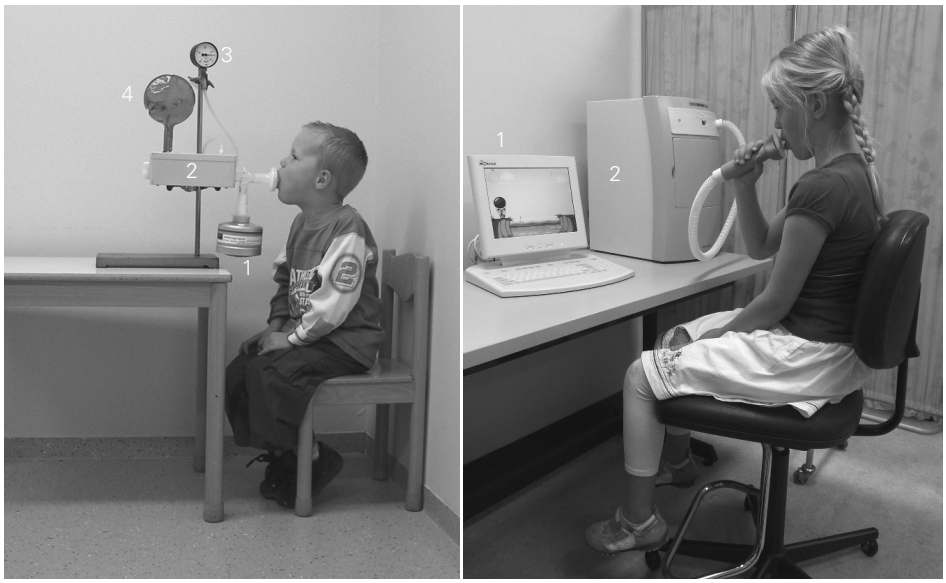

Figure 1.3. Measurement of nitric oxide fraction in exhaled air at 4 and 8 years
(a). Offline measurement in a 4-yr-old boy. 1: NO scrubber, 2: dynamic flow restrictor (50 mL/s), 3: manometer, 4: mylar balloon. (b). Online measurement of the nitric oxide fraction in exhaled air in an 8-yr-old girl. 1: computer animation gives visual feedback to maintain constant expiratory flow (50 mL/s), 2: online nitric oxide analyzer with built-in NO scrubber and dynamic flow restrictor.

years in the PIAMA study allowed us to investigate, whether a FeNO measurement in preschool children with asthma symptoms can improve prediction of the risk of asthma symptoms at older ages. A test can only be of clinical benefit when it offers additional information over the readily available clinical history or allergy tests. The added value of FeNO over other clinical parameters has not been investigated previously, and was part of our analyses.

Measurement of airway resistance by the interrupter technique

The interrupter technique (Rint) was first described by von Neergaard and Wirz in 1927.[72] It is a measure of airway resistance, calculated as the ratio of alveolar pressure and airflow at the mouth during tidal breathing. The airflow can easily be measured at the mouth, but the alveolar pressure cannot be directly assessed in a non-invasive manner. In the Rint measurement alveolar pressure is derived from the pressure measured at the mouth during a brief interruption of the airflow. The airflow is interrupted by briefly closing off the airways using a valve in the mouth piece. During the interruption alveolar pressure will rapidly equilibrate with the pressure at the mouth. Alveolar pressure just before airflow interruption can be estimated by back-extrapolating the pressure-time-curve measured in the oral cavity to the moment the valve was shut. The estimated

alveolar pressure is then divided by the airflow measured immediately prior to the airflow interruption, to calculate the Rint value.[73-75]

Figure 1.4. Measurement of interrupter resistance in a 4-year-old girl

During the Rint measurement a subject needs to sit upright and breath quietly through the measurement device, while wearing a nose clip. To reduce upper airway compliance, which is an important component of the total compliance of the respiratory system and co-determines Rint, the cheeks and chin are firmly supported by the investigator.[75] (Figure 1.4) Previous studies have shown that the measurement can be easily performed in preschool children.[76] Rint measurements correlate well with conventional methods for airway resistance, such as whole body plethysmography (Raw),[73,76,77] and have an acceptable short- and long term reproducibility within subjects,[78,79] and good interobserver agreement.[76] Cross-sectional studies have reported higher Rint in asthmatics compared to controls, although there was considerable overlap.[80-82] Previous analysis of PIAMA data at age 4 showed that Rint in persistent wheezers was higher than in children who never or transiently wheezed.[83] It is however not known whether Rint measurements can be used prospectively in preschool children, to predict the likelihood of symptom persistence at a later age.

PREVENTION AND INCIDENCE OF ASTHMA AND MITE ALLERGY STUDY

In this thesis data from the 'Prevention and Incidence of Asthma and Mite Allergy' (PIAMA) study are analyzed. The PIAMA study is a prospective birth cohort study among children recruited from the general population. The study originally consisted of two parts: 1) the Intervention Study (IS, designed to investigate the effect of house dust mite impermeable mattress covers on the incidence of asthma); 2) the Natural History Study (NHS, designed to study the natural course of asthma, allergy and respiratory symptoms).[84] (Figure 1.5) Potential participants were recruited via antenatal clinics in the northern, middle and south-western regions of the Netherlands from May 1996-July 1997. A validated screening questionnaire on maternal allergy/asthma was developed and used to categorize a total of 10,232 pregnant women into 'allergic' mothers (mothers with self-reported asthma and/or inhalant allergic disease) and 'non-allergic' mothers.[85] Based on the screening 7,862 women (2,779 allergic and 5,083 non-allergic) were invited to participate in the study, approximately 50% (n = 4,146) agreed and gave informed consent (1,327 allergic and 2,819 non-allergic). Their children were defined as high-risk and low-risk children respectively. High-risk children were allocated to either the IS (one third in the intervention group, one third in the placebo group) or to the NHS (one third). All low-risk children were allocated to the NHS. In all chapters of this thesis the complete PIAMA population (IS+NHS) was included in our analyses. As previously published, the intervention with mattress covers had only a limited effect on the actual concentration of house dust mite on the child's mattress, and it had no effect on the incidence of allergy, respiratory symptoms, or asthma.[86,87] The proportion of children with an allergic mother (31%) in the total PIAMA study (IS+NHS) was very similar to the proportion in the population of pregnant mothers from which participants were recruited (n = 10,232). Of the 4,146 participants with informed consent, 183 were lost to follow-up before any data on the child had been obtained, so that the study started with 3,963 newborn children. Attrition rate was relatively low and at age 8 years, 88% of these children were still participating in the study.

The total study population was invited to complete postal questionnaires on prenatal risk factors such as maternal smoking, diet, medication use, exposure to pets and allergen avoidance measures. Parents were also invited to complete a postal questionnaire 3 months after the child was born, and annually starting when the child was 1 year of age. These questionnaires contain data on: respiratory health of the child, siblings (adapted protocol of the International Study of Asthma and Allergy in Childhood (ISAAC))[88] and parents (ECRHS protocol),[89] perinatal characteristics of the child, general health of the child, type of feeding, housing characteristics, environmental factors, social contacts, development of height and weight, dietary habits and possible changes in many of these characteristics throughout childhood.

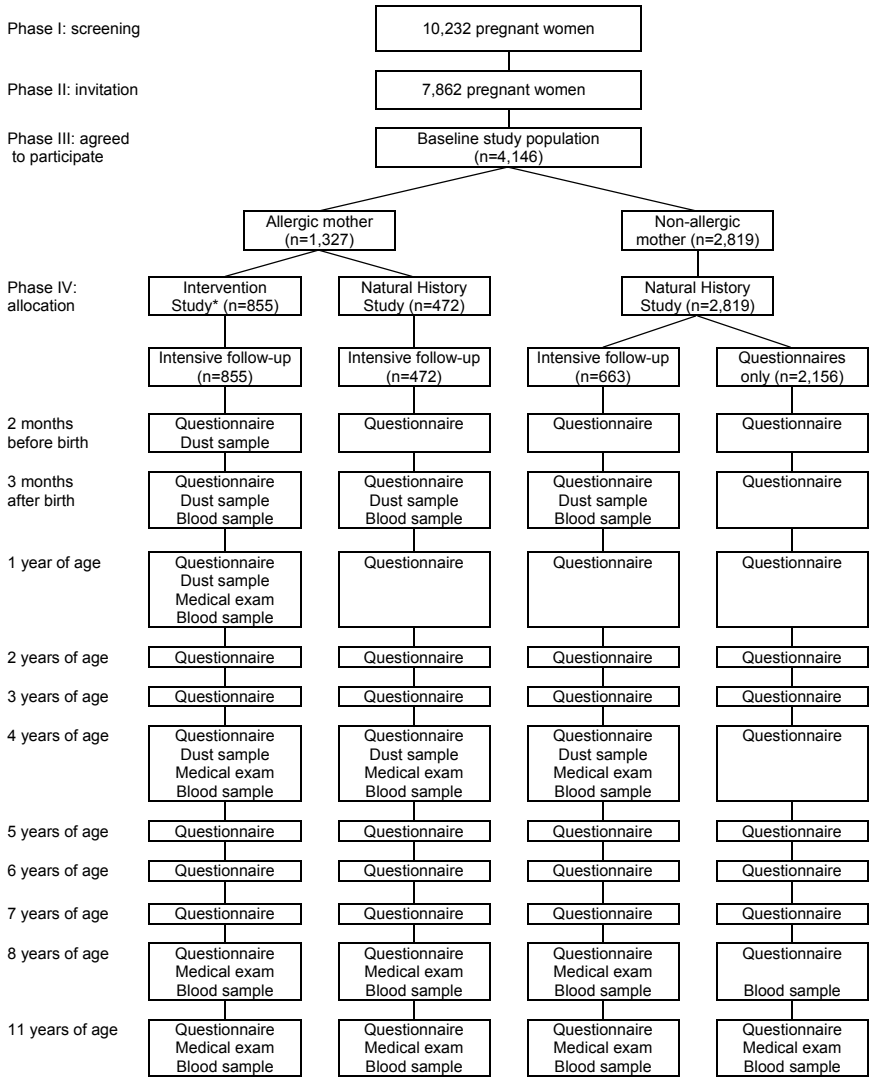

Figure 1.5. Flow chart with observation scheme at different ages in the PIAMA study
At baseline the study population consisted of n = 3,963 newborn children. *: The intervention measures were applied in 810 subjects; 416 subjects received active mattress covers and 394 subjects received placebo mattress covers.

At age 4 and 8 years a subgroup consisting of almost all high-risk children and a random subsample of the low-risk children was invited for a medical examination and collection of biological data. At 4 years 1,808 children were invited. Their medical examination included the measurement of height, weight, interrupter resistance, nitric oxide fraction in exhaled air, peak flow, and the collection of blood samples. At 8 years of age

1,554 children were invited for a hospital-based medical examination. This examination included the measurement of height, weight, interrupter resistance, nitric oxide fraction in exhaled and nasal air, lung function (Forced Expiratory Volume in 1 second; FEV1 and Forced Vital Capacity; FVC), bronchial hyperresponsiveness (methacholine provocation test) and atopy (serum immunoglobulin E and skin prick test to common inhalant allergens). Additionally at 8 years, 1,964 of the remaining low-risk children were also invited for a short community-based medical examination or house visit, in which height and weight were measured and blood samples were collected for analysis of specific immunoglobulin E to food and inhalant allergens.

AIMS OF THE STUDY

In the current thesis we aimed to investigate the following aspects of childhood asthma:

- Which (combinations of) pre- and perinatal factors are associated with the development of asthma, allergy and/or respiratory symptoms in the first 8 years of life?
- Which of the modifiable pre- and perinatal factors are the most promising targets for prevention in terms of their capacity to reduce later respiratory morbidity?
- What is the proportion of undertreatment and overtreatment of asthma symptoms in children until the age of 8 years?
- When a young child presents with wheezing, is it possible to predict the risk of persistent asthma symptoms until the age of 8 years, using only the information from a simple clinical history?
- Are FeNO, Rint and/or specific IgE in children with asthma symptoms at the age of 4 years of added value in the prediction of symptom persistence until the age of 8 years, after taking the clinical history into account?

OUTLINE OF THIS THESIS

Part I examines perinatal factors as possible etiologic risk factors for the development of childhood asthma. Chapter 2 describes the association between birth weight in children born at term and the risk of developing asthma or asthma symptoms in first seven years of life. Furthermore, it describes the effects of parental smoking on the association between birth weight and asthma symptoms. Using a similar longitudinal analysis chapter 3 presents the association of early daycare attendance and the presence of older siblings, two important determinants of respiratory infections in early life, with the development of asthma symptoms and allergy until the age of 8 years. Chapter 4 gives an overview of a wide range of perinatal factors and their relation with the development of different longitudinal wheezing phenotypes. These longitudinal wheezing phenotypes were previously defined using longitudinal latent class analysis. In chapter 5 we compare the fraction of nitric oxide in exhaled air, a marker for eosinophilic airway inflammation, between the different longitudinal wheezing phenotypes at the ages of 4 and 8 years. Part II focuses on the early diagnosis of asthma in symptomatic children and their clinical prognosis. Chapter 6 explores the agreement between prescribed asthma medication on the one hand, and asthma symptoms and doctors' diagnosis of asthma on the other hand. Chapter 7 presents a clinical prediction rule to predict the probability that preschool children with asthma symptoms will still experience asthma symptoms at the age of 8 years, using only information from a simple clinical history. In chapter 8 we investigate whether the objective tests Rint, fraction of exhaled nitric oxide (FeNO) and specific IgE could improve the prediction of later symptoms after the clinical history was taken into account. In chapter 9 we discuss the findings of this thesis in the context of other studies and evaluate their implications for clinical practice.

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Part I

Etiologic risk factors for the development of childhood asthma

Chapter 2

Respiratory symptoms in the first 7 years of life and birth weight at term: the PIAMA birth cohort

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ABSTRACT

Rationale

The relation between birth weight and respiratory symptoms and asthma in children remains unclear. Previous studies focused on a relation at separate ages. A longitudinal analysis may lead to a better understanding.

Objective

To estimate the effect of birth weight on the development and course of respiratory symptoms and asthma in the first seven years of life.

Methods

In a prospective birth cohort study, 3,628 children with a gestational age 37 weeks or more were monitored for 7 years. Parental questionnaires were used to assess respiratory health yearly. Associations of birth weight with respiratory symptoms (wheezing, cough, respiratory infections) and doctor's diagnosis of asthma were assessed in a repeated-event analysis.

Main results

Lower birth weight was associated with more respiratory symptoms (adjusted odds ratio [aOR] per kg decrease in birth weight, 1.21; 95% confidence interval [$CI_{95\%}$]: 1.09-1.34). The effect of birth weight increased from age 1 to 5, but decreased thereafter and was no longer significant at the age of 7. The effect of birth weight on respiratory symptoms was significantly greater among children exposed to tobacco smoke in their home (aOR at 5 yr: 1.52; $CI_{95\%}$: 1.23-1.87) than among non-exposed children (aOR at 5 yr: 1.21; $CI_{95\%}$: 1.02-1.44). Birth weight and doctor's diagnosis of asthma were not related (aOR: 1.06; $CI_{95\%}$: 0.82-1.37).

Conclusions

A lower birth weight in children born at term is associated with a transiently increased risk of respiratory symptoms. This effect is enhanced by environmental tobacco smoke exposure.

INTRODUCTION

Respiratory symptoms, such as wheezing, cough, and respiratory infections, are common in young children and put a serious burden on the affected children,[1] their parents, and the healthcare system. In early childhood, these symptoms are related to a predisposition to asthma only in a minority of children.[2] In the majority of children, symptoms will be transient and disappear during school age. Because the prevalence of these different phenotypes of symptoms changes rapidly during childhood, risk factors should be investigated using longitudinal data and analyses.

Size and maturity are major factors in the development of the lung. In children with diminished prenatal growth, and consequently low birth weight, a disturbed lung development is associated with a relatively small airway calibre.[3] This might cause a decreased lung function and more respiratory symptoms later in life.[2-4] Several studies have shown that prematurity is associated with lower lung function at birth and later, and a higher prevalence of respiratory symptoms in childhood.[5-9] However, pathways leading to compromised lung development in premature and term infants are almost certainly not the same. The question remains whether the development of respiratory symptoms is influenced by birth weight in infants born at term.

The association between birth weight and asthma has been extensively studied, and the results are inconsistent. This may in large part be due to different periods of follow-up and to different definitions of asthma and (low) birth weight. In addition, not all studies took pregnancy duration into account in the analysis, thereby studying the combined effect of prematurity and birth weight.[5] It has been postulated that the inverse association between birth weight and asthma as found in some studies might be explained by misclassification of transient respiratory symptoms as asthma.[10]

The aim of this study was to investigate birth weight as a predictor of respiratory symptoms and doctor's diagnosis of current asthma longitudinally in children born at term. The prospective Prevention and Incidence of Asthma and Mite Allergy (PIAMA) birth cohort data gave us the opportunity to investigate the effect of birth weight on the development and course of respiratory symptoms in children 1 to 7 years. We hypothesized that reduced intrauterine growth could lead to more respiratory symptoms as a result of smaller airways, which improve with increase in body size during childhood. We also investigated other factors that could possibly modify the effect of birth weight, such as gender, parental smoking, and allergic predisposition.

METHODS

Study population

The study population consisted of 3,628 children who participated in the PIAMA birth cohort study. Details of this study have been published earlier and are described in the data supplement.[11] Briefly, recruitment took place by means of a validated screening questionnaire,[12] distributed to 10,232 pregnant women visiting one of 52 prenatal clinics in the Netherlands. On the basis of this screening 8,033 woman were invited to participate in the study; approximately 50% (n = 4,146) agreed and gave informed consent. Their children were monitored for 8 years. Standardized questionnaires for self-completion (according to the ISAAC [International Study of Asthma and Allergies in Childhood] guidelines)[13] were sent to participating parents at the 3rd trimester of pregnancy, at the ages of 3 months and 1 year, and yearly thereafter. The present study analyzes the data up to the age of 7 years. We excluded all children with a gestational age of less than 37 weeks to avoid possible confounding effects of prematurity. In our final analyses 3,628 children were included (88%).

Definition of variables

Birth weight was obtained from the child's delivery chart, and reported in the 3-month questionnaire. Birth weight was considered as a continuous variable.

We defined the following 6 (dichotomous) outcomes, each of which were obtained at the ages of 1 to 7 years, and which pertain to the previous 12 months:

1. Wheezing: at least one episode of wheezing
2. Frequent wheezing: 4 or more episodes of wheezing (subgroup of 'wheezing')
3. Cough: at least one episode of cough at night, not associated with a cold
4. Lower respiratory tract infection (LRTI): parental report of a doctor's diagnosis of pneumonia, bronchitis or pertussis
5. Respiratory symptoms: a compound score with at least one positive score on the outcomes 'wheezing', 'cough', or 'LRTI'. If medication was prescribed for asthma this score is considered positive, because symptoms may have been suppressed
6. Doctor's diagnosis of current asthma: parental report of asthma ever diagnosed by a doctor in combination with symptoms of asthma in previous 12 months

Using questionnaire data, the following potential confounders were defined and investigated: gender, gestational age, parental education, parental history of allergies, multiparity, caesarean section, age of mother, region of birth, breastfeeding, maternal smoking during pregnancy (at least 4 weeks after onset of pregnancy), and body mass index of mother (before pregnancy). The latter two potential confounders had missing

values of 4.9% and 10.1%, respectively; in these variables, a separate category 'missing' was created. As a potential effect modifier, we also looked at exposure to tobacco smoke in the child's home 3 months after birth (not exclusively by mother). It was defined as a linear term with 3 values (never, < once a day, > once a day).

Statistical Analysis

Locally-weighted regression (LOESS) smoothing curves[14] of the crude relationship between birth weight and the different outcomes were created using S-Plus 6.0 software (Insightfull, Seattle, WA) (See Figure E2.3 (data supplement) for the outcome 'wheeze'). We compared smoothed curves with linear fits by testing for a difference between the linear fit and the smooth fit that includes both linear and smooth terms. Because no significant difference was found between linear and smooth fit (except at the age of 3 years), linearity of the association between birth weight and the logit of the outcome were assumed in all subsequent analyses.

Further analyses were performed with SAS 9.1.3 statistical software (SAS Institute, Inc., Cary, NC). Analyses of crude birth weight effect on all outcomes were first performed by logistic regression at every age separately (cross-sectional). Variables that meaningfully changed the univariate point estimate on one or more of the outcomes were included in the longitudinal models. Generalized estimating equations (GEEs) were used to investigate the longitudinal effects of birth weight on all 6 outcomes from age 1 to 7 years, taking into account the serial relations between repeated measurements in the same individual. To test for effect modification, we calculated terms for interactions between birth weight and the variables age (time trend), parental smoking, gender, and maternal allergy in the GEE model.

RESULTS

Participants

From the 4,146 included women, 187 (4.5%) dropped out before returning the first postnatal questionnaire due to various reasons (e.g., perinatal death, language barrier, not interested, moved). The children who dropped out were more likely to have an allergic mother (50% vs. 31% based on screening questionnaire). Of the 3,959 remaining children, those without a recorded birth weight (n = 45) or gestational age (n = 9), those with no data on the specified outcomes at any of the 7 years (n = 82), and those with missing data on multiparity (n = 9) were excluded from analysis. These excluded children (n = 145) were more likely than the children with complete data (n = 3,814) to have an allergic mother (51% vs. 30%), to have both parents with a low level of education (32% vs. 13%), to have a mother who smoked during pregnancy (27% vs.

17%) and to have been exposed to environmental tobacco smoke (ETS) at 3 months (43% vs. 28%). After exclusion of all children who were born prematurely (n = 186), 3,628 children remained in the final analyses.

General characteristics

Birth weights were normally distributed, even after adjustment for gestational age. Table 2.1 shows the general characteristics of the study population. Male gender, higher gestational age at birth, overweight of the mother and presence of older siblings were

Table 2.1. General Characteristics of Study Population (n = 3,628)

Characteristic	Mean ± SD or %
Birth weight (range)	3.56 ± 0.49 (1.76-5.71)
Duration of pregnancy, weeks*	40.1 ± 1.2
Age mother, years†	30.4 ± 3.9
Male gender*	51.7
Body mass index mother*	
< 25 kg/m^2	71.9
≥ 25 kg/m^2	17.9
Missing	10.1
Older sibling present*	51.5
Breastfeeding ever given†	81.7
Allergic mother	30.1
Allergic father	30.8
Born by caesarian section‡	8.0
Smoking mother during pregnancy*	
No	81.6
Yes, < 10 cigarettes/day	8.9
Yes, ≥ 10 cigarettes/day	4.5
Missing	4.9
Smoking indoors at 3 months*	
Seldom or never	56.3
Yes, but < once/week	15.4
Yes, ≥ once/week	28.3
Low education of both parents†	12.7
Region Netherlands†	
North	31.3
Central	41.0
Southwest	27.8

Study population (n = 3,628). The significance of the associations between birth weight and the other characteristics was tested using unpaired *t* test and analysis of variance (categorical variables), or Pearson's correlation coefficient (continuous variables). Body Mass Index of mother was calculated from self reported weight and height before pregnancy. Smoking during pregnancy was considered positive only if mother reported smoking at least 4 weeks after estimated date of conception. *: $p < 0.0001$, †: $p < 0.01$, ‡: $p < 0.05$.

strongly related with a higher birth weight. Maternal smoking during pregnancy was clearly associated with a reduced birth weight. A considerable proportion of children were exposed to tobacco smoke *in utero* (13.5%) and/or after birth (28.3%).

Prevalence of respiratory symptoms and asthma

Wheezing at least once was the most frequently reported symptom in the first year (21%), but showed a steep linear decline with increasing age. Frequent wheezing showed the same decline and accounted for 25 to 30% of all reported wheeze. (Figure 2.1) The symptom 'cough' was more stable over the years, with the highest reported prevalence at the age of 5 (23.2%) and the lowest at age 7 (14.9%). After the age of 2 years, cough was the most frequently reported symptom and, between the ages of 4 and 7, about 70% of the cases with 'respiratory symptoms' reported cough. The

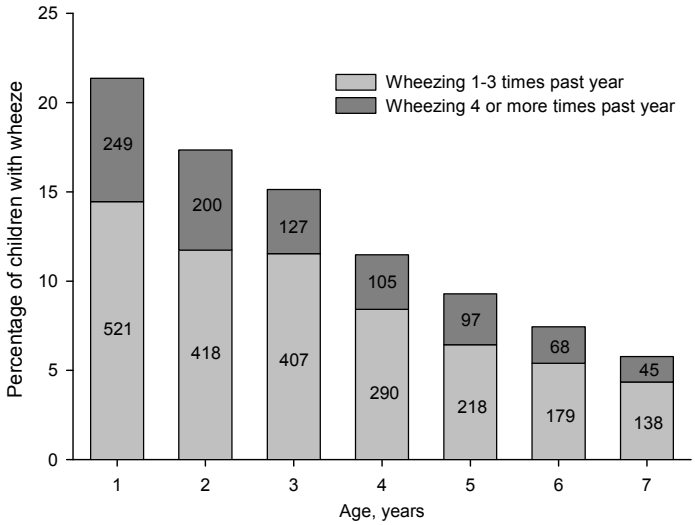

Figure 2.1. Prevalence of wheeze (of available cases) at different ages
Bars show infrequent (1-3 times, light grey) and frequent (4 or more, dark grey) wheezing episodes per year. Total height of each bar is prevalence of wheezing at least once. Number in each bar is the absolute number of children who reported infrequent wheezing or frequent wheezing.

prevalence of LRTIs declined from 16.4% in the first year to 4.4% in the seventh year. During the full 7-year follow-up, 38.9% of the study population had at least 1 wheezing episode, 51.7% reported cough at night, and 37.3% had a LRTI at any point. The compound outcome respiratory symptoms showed that the overall burden of respiratory morbidity declined from 36.8% at 1 to 20.4% at 7 years. Overall, 70% of the cohort had reported at least one of the respiratory symptoms at some point in the first 7 years of life. The prevalence of reported doctor-diagnosed current asthma showed a decline with

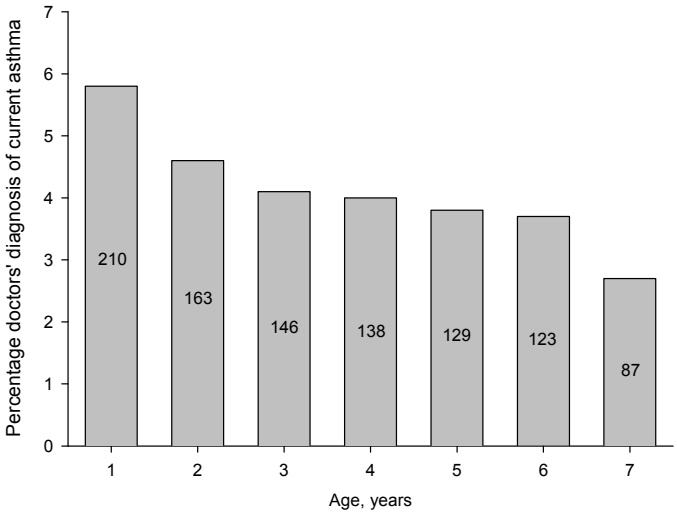

Figure 2.2. Prevalence of self-reported doctor's diagnosis of current asthma at different ages
Total height of each bar represents the prevalence; the number in each bar represents the absolute number of children with doctor's diagnosis of current asthma.

increasing age from 5.8% in the first year to 2.7% at age 7. (Figure 2.2) In total 13.6% of the children were diagnosed with asthma by a doctor at any point. Table E2.1 in the data supplement shows the annual prevalence of all outcomes.

Association of birth weight with respiratory symptoms and asthma

In crude analyses performed cross-sectionally, birth weight showed an inverse association with wheezing, frequent wheezing, cough, and LRTIs, especially between the ages of 2 to 5. Of the variables tested for confounding, the following five meaningfully changed the univariate point estimate: gender, gestational age at birth, body mass index of mother, smoking during pregnancy, and the presence of older siblings (multiparity). These variables had a comparable confounding effect on all specified outcomes, and were controlled for in the longitudinal models.

To account for the serial correlations among outcomes of the same individual, we used GEE models to perform longitudinal analyses on all outcomes. For crude estimates on all outcomes, see Table E2.2-7 of the data supplement. Figure 2.3 shows the adjusted odds ratios (aORs) from the longitudinal multivariate analysis for all outcomes. The overall effect of birth weight over 7 years was calculated, but the effect at every age separately from models with interaction term (age) is also presented. After multivariate adjustment, a lower birth weight remained significantly associated with more wheezing (aOR 1.17 per kg decrease in birth weight, $CI_{95\%}$: 1.01-1.35), cough (aOR 1.21; $CI_{95\%}$:

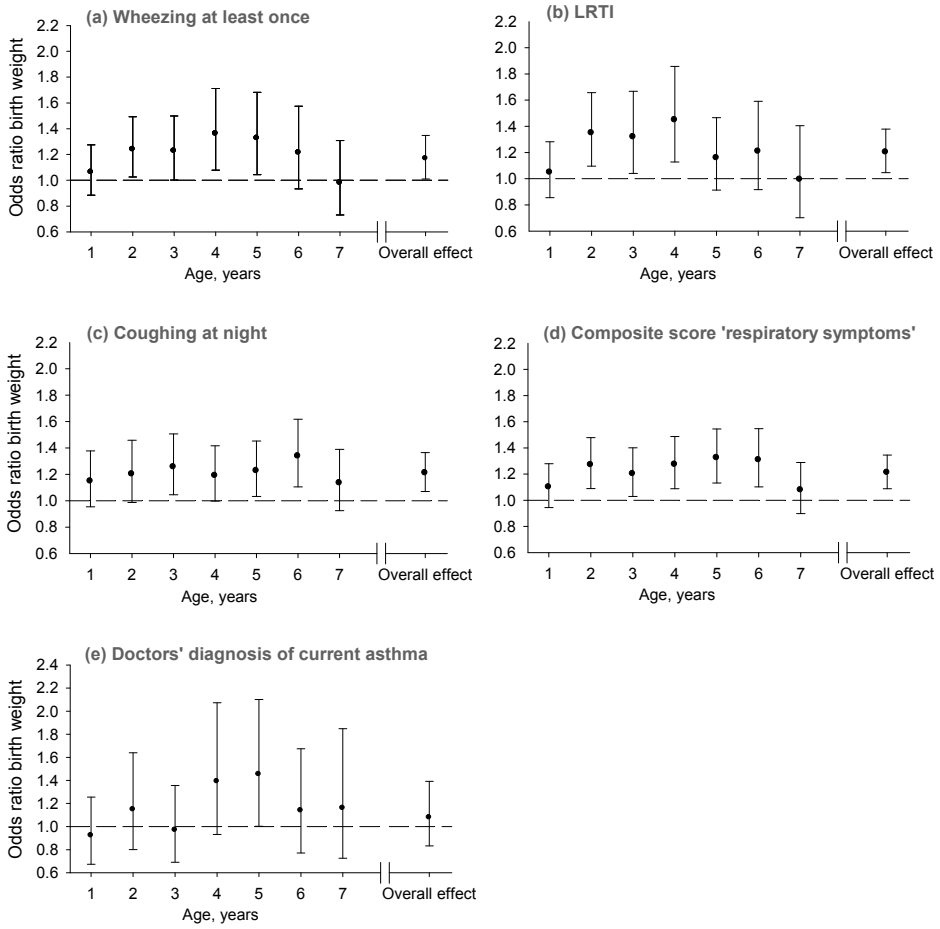

Figure 2.3. Odds ratios (ORs) and confidence intervals per kilogram decrease in birth weight for all outcomes, from longitudinal GEE model
ORs given for the overall effect and (allowing for a time trend) for each year of age separately. Estimates adjusted for gender, multiparity, body mass index of mother, smoking of mother during pregnancy, and gestational age at birth. The following outcomes are depicted: **(a)**. Wheezing at least once; **(b)**. Lower respiratory tract infections (LRTI), including pneumonia, bronchitis and pertussis; **(c)**. Cough in absence of a cold; **(d)**. Compound score 'respiratory symptoms'; **(e)**. Doctors' diagnosis of current asthma.

1.07-1.36), and respiratory infections (aOR 1.20; $CI_{95\%}$: 1.05-1.38) overall. In addition, the association with the compound score for respiratory symptoms was significant over the whole 7-year period (aOR 1.21; $CI_{95\%}$: 1.09-1.34). We investigated the aORs of birth weight at different ages, by allowing interaction of the effect with age. The inverse relation between birth weight and wheezing remained significant at the ages 2 to 5

years, with the highest aOR at age 4 (aOR 1.36; $CI_{95\%}$: 1.08-1.71). For the outcome LRTI, the aOR was also highest at the age of 4 (aOR 1.45; $CI_{95\%}$: 1.13-1.86). The aOR increased from age 1 to 4, and decreased to zero by the age of 7. Tests for this interaction between birth weight and age (time trend) in the GEE model reached significance for wheezing and LRTI (p = 0.03 and 0.05, respectively). The association between birth weight and wheezing could not be explained by the relation between birth weight and LRTI. The aOR of birth weight on cough was more stable over the years, ranging between 1.34; $CI_{95\%}$: 1.10-1.62 and 1.13; $CI_{95\%}$: 0.92-1.39. The compound score for respiratory symptoms was significantly associated with birth weight at the ages of 2 to 6 years. To assess the relative impact of children with an extreme birth weight, we performed a sensitivity analysis by excluding the 2% of the lightest and 2% of the heaviest children. ORs of the different outcomes became somewhat smaller at certain ages, but the overall pattern of the associations remained the same.

There was no significant association between birth weight and doctor's diagnosis of current asthma over all ages. (Figure 2.3e) Inspecting the effect at different ages showed a borderline significant association only at the age of 5 year (aOR 1.43 per kg decrease; $CI_{95\%}$:0.99-2.07).

Interaction with gender, maternal allergy and ETS exposure on respiratory symptoms

Modification of the effect of birth weight on respiratory symptoms by gender, atopic constitution, gestational age (wks) at birth and ETS was investigated. The effect did not differ between boys and girls, between children with and without an allergic mother, or between children with a lower and children with a higher gestational age range at birth. Separate analysis of the results of the participants who were exposed and who were not exposed to ETS in their house after birth, revealed a greater effect of birth weight in the exposed group. In the GEE model, interaction with ETS exposure at 3 months reached significance for the outcomes wheezing (p = 0.03) and respiratory symptoms (p = 0.04). In Figure 2.4, the predicted probabilities of respiratory symptoms for children with different birth weights (2.5, 3.5 and 4.5 kg) are shown in presence and absence of ETS exposure, to visualize the effect of birth weight in children of smoking and nonsmoking parents. Probabilities were calculated with given values for all confounders, as specified in the figure legend. A comparison of Figure 2.4a with 2.4b reveals the interaction effect of ETS exposure at 3 months in children who were not exposed prenatally. The difference between Figures 2.4a and 2.4c could be interpreted as the combined effect of pre- and postnatal ETS exposure. In the presence of ETS exposure (Figure 2.4b), a child with a birth weight of 2.5 kg has an approximately 45% chance each year of having respiratory symptoms between the ages 1 and 5 years, compared with 25% in a child with a birth weight of 4.5 kg. In absence of ETS exposure (Figure

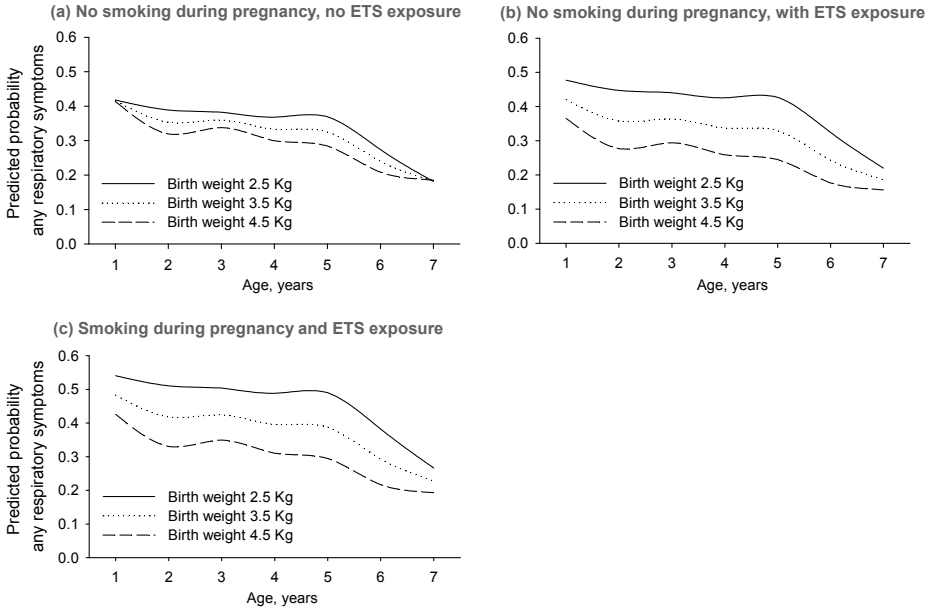

Figure 2.4. Predicted probabilities of any respiratory symptom (wheezing, cough or LRTI) for a child of male gender, 40 weeks gestational age at birth, with a non-overweight mother, and with older sibling(s)
Probabilities are predicted for a child with a birth weight of 2,500 g, 3,500 g, and 4,500 g. **(a)**. For children of whom the mother did not smoke during pregnancy, and who were not exposed to environmental tobacco smoke at 3 months. (67.3% of study population); **(b)**. For children of whom the mother did not smoke during pregnancy, but who were exposed to environmental tobacco smoke (≥ once/week) at 3 months (14.4% of study population); **(c)**. For children of whom the mother did smoke during pregnancy, and who were exposed to environmental tobacco smoke (≥ once/week) at 3 months (11.9% of study population).

2.4a), the difference in prevalence of respiratory symptoms between a 2.5- and a 4.5-kg newborn child is much smaller, about 6 percentage points. As can also be seen from Figure 2.4, a child of low birth weight (2.5 kg) has an additional 6% chance of respiratory symptoms if exposed to ETS postnatally. When exposed to ETS both pre- and postnatally the additional risk of symptoms mounts to 12%. Figure 2.5 shows the predicted probabilities of the outcome wheezing for children from both smoking and nonsmoking parents. Clearly, the effect of birth weight is larger in the children exposed to ETS, especially in the first year of life. The isolated effect of postnatal exposure appears limited after adjustment for prenatal smoking; however, 87.2% of the children exposed to tobacco smoke during pregnancy were also exposed at three months after birth. Of the children exposed to cigarette smoke in their home more than once a week, about half (48%) had a mother who smoked at least 4 weeks during pregnancy.

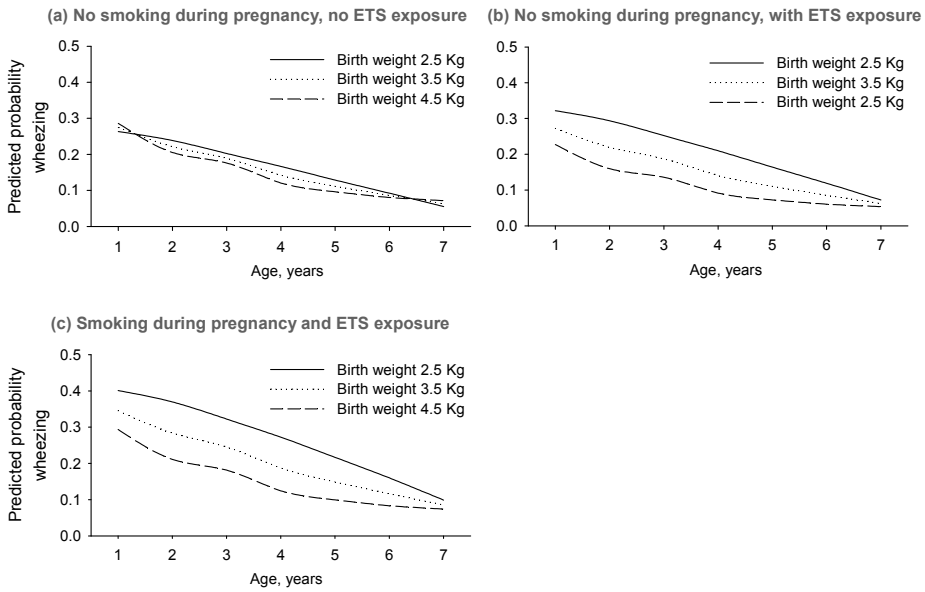

Figure 2.5. Predicted probabilities of wheeze for a male child, 40 weeks gestational age at birth, with a non-overweight mother, and with older sibling(s)
Probabilities are predicted for a child with a birth weight of 2,500 g, 3,500 g, and 4,500 g. **(a)**. For children of whom the mother did not smoke during pregnancy, and who were not exposed to environmental tobacco smoke at 3 months (67.3% of study population); **(b)**. For children of whom the mother did not smoke during pregnancy, but who were exposed to environmental tobacco smoke (≥ once/week) at 3 months (14.4% of study population); **(c)**. For children of whom the mother did smoke during pregnancy, and who were exposed to environmental tobacco smoke (≥ once/week) at 3 months (11.9% of study population).

DISCUSSION

This prospective birth cohort study shows that a low birth weight at term is significantly associated with more respiratory symptoms, including wheezing, cough, and respiratory infections in early childhood. The effect of low birth weight was amplified by exposure to ETS, and declined after the age of 5.

The effect of birth weight on respiratory symptoms has been examined in several other birth cohorts. Our findings are in concordance with results from a large birth cohort that showed an association between birth weight and wheezing at 3 years, but not at 6 months.[15] Another study in term infants did report an association in the first year of life.[16] A separate analysis on the same cohort at the age of 6 years no longer showed a significant association,[17] in line with our findings. Some other studies reported no association between birth weight and respiratory symptoms.[18,19] The dis-

crepancy might be explained by the fact that symptoms in these studies were assessed only after the age of 5. Our yearly longitudinal analysis over a period of 7 years extends our knowledge on the time trend of the association. We found an increase of the odds ratio until the age of 5 and a decrease thereafter, suggesting an association between birth weight and transient symptoms in preschool- and early elementary school-aged children. It is clear from previous publications that prematurity is associated with a decreased lung function and more asthmalike symptoms.[6-9] Unlike previous studies investigating the effect of birth weight on respiratory symptoms,[5,18-20] we excluded all premature infants from our analyses. This enabled us to separate the effect of a low birth weight in children born at term from the sequelae of prematurity, and to investigate the independent effect of birth weight. The association between birth length and respiratory symptoms was investigated, but was not significant after adjustment for birth weight. The increased risk of symptoms in term children of a lower birth weight can be due to small airways relative to lung size (dysanapsis),[3] as a result of diminished prenatal growth.[2] Although the impact of impaired fetal growth surely is related to its timing, we have no data on the timing of any possible adverse prenatal conditions. As the airways grow in absolute size with age, such children may become less apt to have symptoms, which would explain the transient nature of the increased risk. Lung function in the low-birth-weight children might remain suboptimal, even after these children become asymptomatic, as was found in children who wheezed between the ages of 1 and 6 years.[2] This is important because it has been speculated that these children may be at increased risk of respiratory morbidity when lung function decreases in later life.[21] One could also argue that disorders of fetal growth affect the immune function, thereby leading to a higher rate of respiratory symptoms.

This study does not support an effect of birth weight on the risk of a doctor's diagnosis of current asthma in children born at term. Because objective tests, including lung function, bronchial hyperresponsiveness, or allergy tests, are either difficult to perform in young children or not informative, a diagnosis of asthma in children younger than 5 is mainly based on respiratory symptoms.[22,23] As the diagnosis becomes more reliable with increasing age, one would expect less misclassification at later ages. This would lead to less dilution bias and more precise estimates of the association between birth weight and an asthma diagnosis. Our results show a decrease of the association at ages 6 and 7 years. Previous studies on the association between birth weight and asthma in children have shown contradictory results, with some reporting a positive association,[24-26] some a negative association[5,27-30] and some no association.[8,17,18] Only few of these studies excluded premature children.[17,24] The increased risk of asthma in low-birth-weight children reported by some studies might therefore be confounded by the effect of prematurity and/or bronchopulmonary dysplasia. Further inconsistencies in the results might appear because varying definitions of asthma were used in the

different studies. Our longitudinal data suggest a negative association between asthma and birth weight only at the ages 4 and 5. Possibly this association is caused by misdiagnosis of transient wheezing syndromes.[10] This is supported by the fact our study showed a strong association between birth weight and (transient) wheezing, especially at the ages 4 and 5 years.

This study reports for the first time an interaction between birth weight and ETS exposure in infancy on respiratory symptoms. To assess ETS exposure we used reported smoking in the child's home, as this is the most important single location for exposure in children. We observed a stronger effect of low birth weight in the group with ETS exposure at 3 months. The interaction with ETS could not be explained by the correlation of ETS with the level of education of the parents, which we used as a proxy of socio-economic status. Both in groups with high and low socio-economic status, the interaction between ETS and birth weight remains present. On the basis of our longitudinal models, we estimated that, in the presence of ETS exposure, a child with a birth weight of 2.5 kg suffers a nearly doubled risk of having respiratory symptoms compared with a 4.5-kg child until the age of 5 years. In a 2.5-kg child we estimated that ETS exposure pre- and postnatally is associated with a 12% increase in prevalence of wheezing and respiratory symptoms. Although several other factors, such as prematurity, other environmental pollutants, and a history of allergy, were previously reported to possibly influence the susceptibility for the effects of ETS,[31] a low birth weight was not. A possible explanation for our finding is that the lungs of a child with lower birth weight are more vulnerable to the irritating effects of ETS. Many epidemiologic studies have demonstrated that children are more sensitive to the respiratory effects of ETS exposure than adults.[32] In low-birth-weight children, a disturbed lung development could lead to immature lungs and therefore a higher susceptibility to the effects of ETS. We investigated interaction with ETS exposure established at the age of 3 months, because this variable had no missing values and it prevents bias caused by a change of parental smoking behavior due to their child's later symptoms. Tests for interaction between birth weight and ETS exposure at later ages showed similar results as ETS exposure at 3 months. This finding might suggest that ETS exposure is especially harmful for children of low birth weight, regardless of the child's age. However, another explanation is that ETS exposure at young ages has a long-lasting harmful effect in low-birth-weight children and that tracking of parental smoking behavior is responsible for the association with ETS exposure at later ages.

There are some limitations of this study that should be considered in the interpretation of the results. First, the presence of respiratory symptoms was based on parental reporting, which may lead to misclassification. We assume that this misclassification is nondifferential (independent of a child's birth weight) which may lead to dilution of the effect estimate. Especially at age 1 to 2 years this could have caused some under-

estimation, because the ISAAC questionnaires that were used are originally designed and validated for the age category of 6 to 7 years. Misclassification of parental reports of ETS exposure is less likely because previous analyses showed that ETS exposure correlated well with nicotine concentrations measured in living-room air.[33] Second, in our analyses, we adjusted for the confounding effects of smoking during pregnancy. The mechanisms by which smoking leads to more respiratory symptoms include a lower birth weight,[2] which would imply that smoking is an antecedent rather than a confounder. Hence, adding prenatal smoking to our models might have caused some overadjustment and conservative estimates of the effect. Third, a problem encountered by many investigators studying the independent effect of *in utero* and household exposure to ETS is the strong correlation between the two factors.[34] Although in our study we were able to adjust these factors for each other without the problem of colinearity, it remains difficult to prove that the interaction with birth weight found in this study is attributable to either *in utero* or postnatal exposure. Our data does suggest (especially for the compound score of respiratory symptoms) that the household ETS exposure is the actual effect modifier, but we base our conclusion largely on pathophysiologic reasoning.

Our study shows that low birth weight is an important risk factor for development and persistence of respiratory symptoms during preschool and early elementary school age. The effect of birth weight was greater in children exposed to ETS. Hence, it seems that the increased risk of symptoms in a low-birth-weight child might be considerably reduced by prevention of postnatal ETS exposure. In recent years passive smoking has been reported in numerous studies to have harmful effects on a child's health.[32] Our findings contribute to the knowledge that exposure to tobacco smoke is a public health problem with a substantial impact on respiratory health in children, especially those with a low birth weight. Despite growing public awareness the prevalence of smoking during pregnancy and frequent ETS exposure in the first years of life has remained high.[35] An active policy to reduce *in utero* and ETS exposure on infants is desirable for all new parents. Our study suggests that significant extra health benefit can be gained by focusing on parents of newborns with a low birth weight.

CONCLUSION

We conclude that a low birth weight is an important risk factor for respiratory symptoms in young children born at term, independent of other characteristics at birth. This association showed a time trend with the strongest effect at 4 years and no effect after the age of 6. We also demonstrated potentiation of the effect of birth weight by exposure to ETS. All parents should be strongly encouraged to stop smoking because it

has clear health benefits for their offspring. Our data suggest that focusing on parents of low-birth-weight children is of specific interest, because their children may be especially vulnerable to the effects of ETS.

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SUPPLEMENT DATA CHAPTER 2

METHODS

Recruitment of participants

Participating children were part of the Prevention and Incidence of Asthma and Mite Allergy (PIAMA) birth cohort study. This is a multi center study conducted in 3 different regions of The Netherlands: north (Groningen and surroundings), central (Bilthoven, Wageningen and surroundings) and southwest (Rotterdam and surroundings). Recruitment took place between March 1996 and May 1997 by means of a validated screening questionnaire,[E1] distributed by midwifes to 10,232 pregnant woman visiting one of 3 prenatal clinics. According to the results of this screening the women were divided in an allergic and a non allergic group. Women with any of the following self-reported symptoms were defined as allergic: asthma, hay fever, house dust allergy, house dust mite allergy or pet allergy. Children of allergic women were defined as 'high-risk'. All allergic women (n = 2,949) and a random sub-sample of the non-allergic women (n = 5,084) were invited to participate in the study, approximately 50% (n = 4,146) agreed and gave informed consent. The PIAMA cohort includes an 'intervention part', studying the effect of impermeable mattress covers, and a 'natural history part'. Figure E2.1 shows the flow diagram for recruitment of the intervention and the natural history arm. For the present study on the effect of birth weight, data on all 4,146 children was used. Any confounding or interaction of the birth weight effect with the intervention was investigated. None of the outcomes in this study changed meaningfully after adjust-

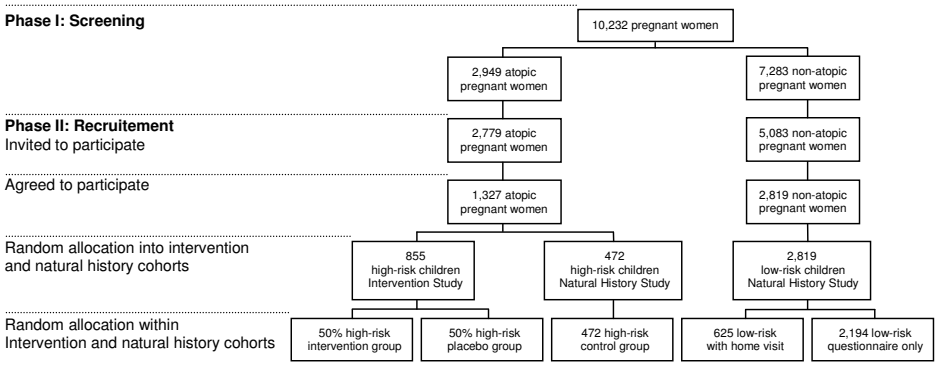

Figure E2.1. The Prevention and Incidence of Asthma and Mite Allergy (PIAMA) recruitment scheme

ment for the intervention and there was no significant interaction. The PIAMA study was approved by the Medical Ethical Committees of the participating institutions.

Data collection

For the present study data from standardized questionnaires (according to the ISAAC guidelines)[E2] were used. The questionnaires were sent to participating parents for self-completion at the 3rd trimester of pregnancy, 3 months after birth, at the age of 1 and yearly thereafter. The final questionnaire was sent at the age of 8, when also a physical examination was conducted. Information was collected on birth characteristics (e.g. birth weight, pregnancy duration, length and head circumference, season of birth), indoor environmental factors (e.g. smoking in the house, pets, housing characteristics), socioeconomic characteristics (e.g. parental education and employment status), life-style factors (e.g. feeding, child care), demographic factors (e.g. siblings, parental age/bmi) and on the child's health (e.g. allergies, eczema, respiratory symptoms, doctors' diagnosis and medication use).

Statistical Analysis

LOESS-curves of the crude relationship between birth weight and the outcome 'wheezing at least once' were created.[E3] (Figure E2.2) The logarithm of the odds of having wheeze at least once a year is plotted against birth weight for every age separately. Considering the negative monotonous relation at all ages it was decided to investigate birth weight as a continuous variable. Goodness of fit was later tested by adding the variable of birth weight-squared to the final models. This variable was not significant for any of the 6 specified outcomes.

Multiple logistic regression was used to asses a relationship between birth weight and respiratory outcomes at every age. A longitudinal analyses was then performed, combining information on the outcomes at every age in one model. In this analysis we have to take account for the fact that the repeated measurements in the same individual are correlated, in order to calculate correct standard errors and p-values of our estimates. A Generalized Estimation Equations (GEE) model was used. As correlations between repeated measurements can be expected to depend on the time lag between 2 measurements, a 6-dependent correlation matrix was modeled. This complex correlation matrix was specified, to make the model more robust against outcomes missing at random and to gain efficiency.[E4] In the models interaction with age was significant only for the effect of birth weight and multiparity. We allowed for these interactions in our final models.

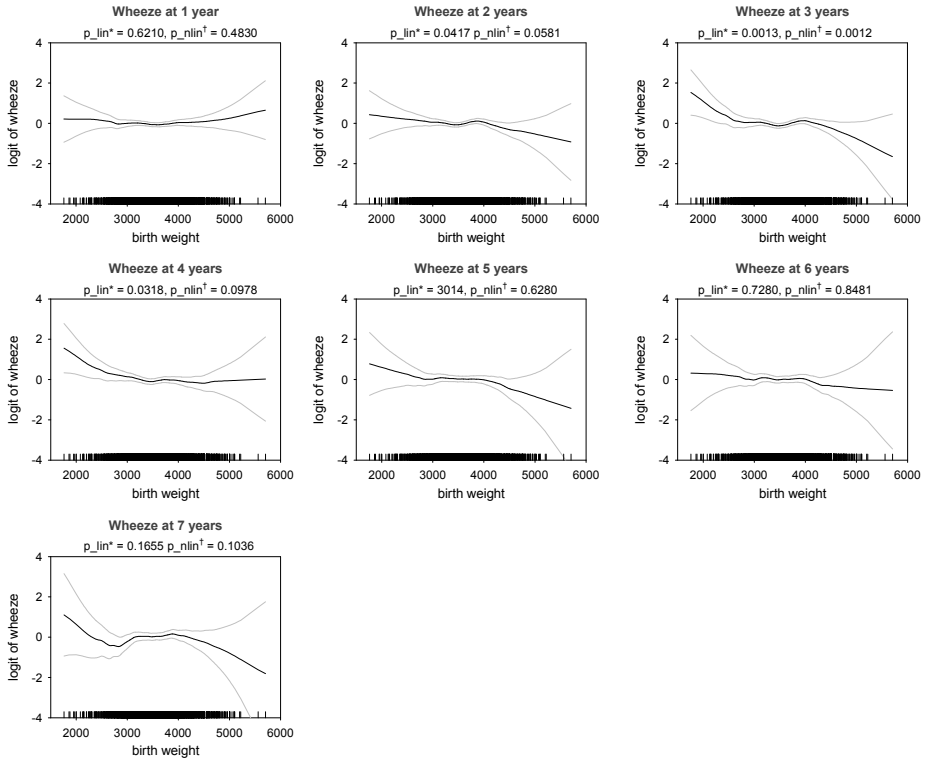

Figure E2.2. LOESS-curves of the crude effect of birth weight on wheezing (at least once) at every age
Log-transformed odds on wheezing in relation to birth weight. Considering the negative monotonous relation at all ages it was decided to investigate birth weight as a continuous variable in further analyses. * p_lin: p-value for linear association, † p_nlin: p-value for nonlinear associasion.

RESULTS

Prevalence of all 6 outcomes at the ages 1 to 7 are shown in Table E2.1. The crude and adjusted odds ratios on all outcomes per kilogram decrease in birth weight are shown in Tables E2.2-7. Multivariate models are presented both with and without adjustment for pregnancy duration. Though birth weight and pregnancy duration were highly correlated, the problem of co-linearity did not occur in the multivariate models, as can be concluded from the stable confidence intervals.

Table E2.1. Prevalence of outcomes at age 1 to 7 years

Age (yrs)	Wheezing ≥ once, % (n)	Frequent wheezing, % (n)	Coughing at night, % (n)	LRTI†, % (n)	Any respiratory symptoms, % (n)	Doctors' diagnosis current asthma, % (n)
1	21.4 (770)	6.9 (249)	17.0 (592)	16.4 (572)	36.8 (1,275)	5.8 (210)
2	17.3 (618)	5.6 (200)	15.4 (535)	13.5 (465)	32.0 (1,106)	4.6 (163)
3	15.1 (534)	3.6 (127)	19.9 (688)	11.1 (383)	33.8 (1,160)	4.1 (146)
4	11.5 (395)	3.1 (105)	21.9 (734)	8.9 (294)	32.0 (1,066)	4.0 (138)
5	9.3 (315)	2.9 (97)	23.2 (767)	10.3 (332)	32.7 (1,059)	3.8 (129)
6	7.4 (247)	2.1 (68)	18.0 (585)	7.2 (230)	25.1 (806)	3.7 (123)
7	5.8 (183)	1.4 (45)	14.9 (473)	4.4 (136)	20.4 (634)	2.7 (87)
Ever positive*	39 (1,411)	15 (549)	52 (1,875)	37 (1,352)	70 (2,547)	14 (492)

Absolute numbers of children with symptoms given in brackets. *: A positive score on this outcome at least once during 7 year follow-up. †: Lower respiratory tract infections, including doctors' diagnosed pneumonia, bronchitis, and/or pertussis.

Table E2.2. Associations between birth weight and wheezing at least once

Age	OR's ($CI_{95\%}$)*	OR's ($CI_{95\%}$)†	OR's ($CI_{95\%}$)‡
1	0.96 (0.81-1.14)	1.11 (0.93-1.33)	1.06 (0.88-1.27)
2	1.14 (0.95-1.36)	1.30 (1.09-1.56)‖	1.24 (1.03-1.49)¶
3	1.15 (0.94-1.39)	1.29 (1.06-1.57)¶	1.23 (1.00-1.50)¶
4	1.29 (1.03-1.62)¶	1.43 (1.14-1.79)‖	1.36 (1.08-1.71)‖
5	1.28 (1.02-1.62)¶	1.39 (1.10-1.76)‖	1.32 (1.04-1.68)¶
6	1.20 (0.93-1.54)	1.28 (0.98-.65)	1.21 (0.93-1.57)
7	0.98 (0.74-1.30)	1.03 (0.77-1.37)	0.98 (0.73-1.31)
Overall§	1.08 (0.95-1.23)	1.23 (1.07-1.40)‖	1.17 (1.01-1.35)¶

GEE-models, odds ratios per kilogram decrease in birth weight. *: Crude analysis. †: Adjusted for gender, body mass index of mother, smoking of mother during pregnancy and multiparity. ‡: Adjusted for previous confounders and gestational age at birth. §Overall effect at age 1-7 years (no interaction term with age). ¶:$p < 0.05$. ‖:$p < 0.01$.

Table E2.3. Associations between birth weight and frequent wheezing

Age	OR's ($CI_{95\%}$)*	OR's ($CI_{95\%}$)†	OR's ($CI_{95\%}$)‡
1	0.91 (0.70-1.19)	1.08 (0.82-1.41)	1.00 (0.75-1.33)
2	1.13 (0.84-1.52)	1.31 (0.97-1.77)	1.21 (0.88-1.66)
3	1.35 (0.92-2.00)	1.55 (1.05-2.27)¶	1.43 (0.96-2.12)
4	1.11 (0.73-1.67)	1.24 (0.82-1.87)	1.14 (0.75-1.75)
5	1.81 (1.22-2.68)‖	1.99 (1.33-2.97)**	1.83 (1.22-2.77)‖
6	1.00 (0.63-1.60)	1.09 (0.68-1.74)	1.01 (0.63-1.61)
7	0.99 (0.58-1.68)	1.06 (0.61-1.83)	0.98 (0.57-1.69)
Overall§	1.10 (0.89-1.35)	1.26 (1.02-1.56)¶	1.16 (0.92-1.47)

GEE-models, odds ratios per kilogram decrease in birth weight. *: Crude analysis. †: Adjusted for gender, body mass index of mother, smoking of mother during pregnancy and multiparity. ‡: Adjusted for previous confounders and gestational age at birth. §:Overall effect at age 1-7 years (no interaction term with age). ¶:$p < 0.05$. ‖:$p < 0.01$. **:$p < 0.001$.

Table E2.4. Associations between birth weight and coughing

Age	OR's ($CI_{95\%}$)*	OR's ($CI_{95\%}$)†	OR's ($CI_{95\%}$)‡
1	1.09 (0.92-1.30)	1.16 (0.97-.39)	1.15 (0.95-1.38)
2	1.16 (0.96-1.40)	1.22 (1.01-1.47)¶	1.20 (0.99-1.46)
3	1.23 (1.04-1.46)¶	1.27 (1.07-1.52)‖	1.25 (1.04-1.51)¶
4	1.19 (1.00-1.40)¶	1.20 (1.02-1.43)¶	1.19 (1.00-1.42)
5	1.24 (1.05-1.46)‖	1.24 (1.05-1.47)‖	1.22 (1.03-1.45)¶
6	1.38 (1.15-1.65)**	1.36 (1.13-1.63)‖	1.34 (1.10-1.62)‖
7	1.19 (0.98-1.44)	1.15 (0.94-1.40)	1.13 (0.92-1.39)
Overall§	1.20 (1.08-1.34)**	1.23 (1.10-1.37)**	1.21 (1.07-1.36)‖

GEE-models, odds ratios per kilogram decrease in birth weight. *: Crude analysis. †: Adjusted for gender, body mass index of mother, smoking of mother during pregnancy and multiparity. ‡: Adjusted for previous confounders and gestational age at birth. §Overall effect at age 1-7 years (no interaction term with age). ¶:$p < 0.05$. ‖:$p < 0.01$.
**:$p < 0.001$.

Table E2.5. Associations between birth weight and LRTI**

Age	OR's ($CI_{95\%}$)*	OR's ($CI_{95\%}$)†	OR's ($CI_{95\%}$)‡
1	0.93 (0.77-1.13)	1.07 (0.88-1.29)	1.05 (0.86-1.28)
2	1.23 (1.01-1.50)¶	1.37 (1.12-1.68)‖	1.35 (1.10-1.66)‖
3	1.24 (0.99-1.56)	1.34 (1.06-1.69)¶	1.32 (1.04-1.67)¶
4	1.40 (1.10-1.79)‖	1.47 (1.15-1.88)‖	1.45 (1.13-1.86)‖
5	1.15 (0.91-1.45)	1.18 (0.93-1.49)	1.16 (0.91-1.47)
6	1.24 (0.95-1.62)	1.23 (0.94-1.61)	1.21 (0.92-1.59)
7	1.04 (0.74-1.47)	1.01 (0.71-1.43)	0.99 (0.70-1.41)
Overall§	1.13 (1.00-1.28)	1.22 (1.07-1.39)‖	1.20 (1.05-1.38)‖

GEE-models, odds ratios per kilogram decrease in birth weight. *: Crude analysis. †: Adjusted for gender, body mass index of mother, smoking of mother during pregnancy and multiparity. ‡: Adjusted for previous confounders and gestational age at birth. §Overall effect at age 1-7 years (no interaction term with age). ¶:$p < 0.05$. ‖:$p < 0.01$. **LRTI: Lower respiratory tract infections, including doctors' diagnosed pneumonia, bronchitis, and/or pertussis.

Table E2.6. Associations between birth weight and 'Any respiratory symptoms'

Age	OR's ($CI_{95\%}$)*	OR's ($CI_{95\%}$)†	OR's ($CI_{95\%}$)‡
1	1.00 (0.86-1.15)	1.10 (0.95-1.28)	1.10 (0.94-1.28)
2	1.17 (1.01-1.36)¶	1.27 (1.10-1.48)‖	1.27 (1.09-1.48)‖
3	1.13 (0.98-1.31)	1.21 (1.04-1.40)¶	1.20 (1.03-1.40)¶
4	1.22 (1.05-1.42)‖	1.28 (1.10-1.49)‖	1.27 (1.09-1.49)‖
5	1.30 (1.12-1.50)**	1.33 (1.14-1.54)**	1.32 (1.13-1.54)**
6	1.31 (1.11-1.54)‖	1.31 (1.11-1.55)‖	1.31 (1.10-1.55)‖
7	1.10 (0.93-1.31)	1.08 (0.91-1.29)	1.08 (0.90-1.29)
Overall§	1.15 (1.05-1.27)‖	1.21 (1.10-1.34)**	1.21 (1.09-1.34)**

GEE-models, odds ratios per kilogram decrease in birth weight. *: Crude analysis. †: Adjusted for gender, body mass index of mother, smoking of mother during pregnancy and multiparity. ‡: Adjusted for previous confounders and gestational age at birth. §Overall effect at age 1-7 years (no interaction term with age). ¶:$p < 0.05$. ‖:$p < 0.01$. **:$p < 0.001$.

Table E2.7. Associations between birth weight and doctors' diagnosis of current asthma

Age	OR's ($CI_{95\%}$)*	OR's ($CI_{95\%}$)†	OR's ($CI_{95\%}$)‡
1	0.77 (0.58-1.02)	0.97 (0.72-1.31)	0.90 (0.66-1.22)
2	0.99 (0.70-1.40)	1.23 (0.87-1.75)	1.13 (0.79-1.63)
3	0.85 (0.62-1.18)	1.04 (0.75-1.44)	0.95 (0.68-1.34)
4	1.23 (0.85-1.80)	1.48 (1.00-2.18)¶	1.36 (0.91-2.02)
5	1.33 (0.95-1.88)	1.56 (1.09-2.23)¶	1.43 (0.99-2.07)
6	1.07 (0.75-1.55)	1.24 (0.85-1.81)	1.14 (0.77-1.67)
7	1.11 (0.72-1.74)	1.26 (0.79-1.99)	1.15 (0.72-1.84)
Overall§	0.95 (0.75-1.19)	1.16 (0.91-1.47)	1.06 (0.82-1.37)

GEE-models, odds ratios per kilogram decrease in birth weight. *: Crude analysis. †: Adjusted for gender, body mass index of mother, smoking of mother during pregnancy and multiparity. ‡: Adjusted for previous confounders and gestational age at birth. §:Overall effect at age 1-7 years (no interaction term with age). ¶:$p < 0.05$.

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Chapter 3

Early daycare is associated with an increase in airway symptoms in early childhood but is no protection against asthma or atopy at 8 years

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ABSTRACT

Rationale

Daycare exposes young children to more infections early in life and may thereby prevent the development of asthma and allergy.

Objective

To prospectively study the effect of daycare on the development of asthma and allergic sensitization during the first 8 years of life.

Methods

In the Prevention and Incidence of Asthma and Mite Allergy birth cohort 3,963 newborn children were followed prospectively for 8 years. Daycare use and respiratory health were assessed yearly by questionnaires. At 8 years, sensitization to airborne allergens and airway responsiveness were measured. Daycare was defined as early (aged 0-2 yr), late (aged 2-4 yr) or none (no daycare before age 4 yr). Associations of daycare and/or older siblings with asthma symptoms (wheezing, shortness of breath, and inhaled steroids taken in the last year), airway responsiveness and allergic sensitization were assessed in a longitudinal repeated-event analysis.

Results

Children with early daycare had more wheezing in the first years of life, but less wheezing and steroid use between 4 and 8 years of age. At the age of 8 years, early daycare was not protective for asthma symptoms (adjusted odds ratio [aOR], 0.99; 95% confidence interval [$CI_{95\%}$], 0.74-1.32), allergic sensitization (aOR 0.86; $CI_{95\%}$: 0.63-1.18) or airway hyperresponsiveness (aOR 0.80; $CI_{95\%}$: 0.57-1.14). The transient reduction in airway symptoms between age 4 and 8 years was only observed in children without older siblings.

Conclusion

Early daycare is associated with an increase in airway symptoms until the age of 4 years, and fewer symptoms between the ages of 4 and 8 years. We found no protection against asthma symptoms, hyperresponsiveness or allergic sensitization at the age of 8 years.

INTRODUCTION

Will parents who send their children to daycare thereby prevent the development of asthma and allergies, at the cost of more airway infections in infancy? Previous studies have not been able to give a conclusive answer to this important dilemma.

A preventive effect of daycare attendance on the development of asthma has been suggested, based on the hypothesis that infections early in life may reduce the later development of allergic diseases.[1] Daycare has been consistently associated with increased prevalence of infections in early life.[2-5] Because daycare is a modifiable factor it could be important for asthma prevention programs. However, convincing evidence that daycare is indeed associated with a reduction in asthma/allergy on the long term is lacking.[6-10] The fact that multiple studies reported an increased risk of asthma in children with infections in early childhood further adds to the controversy.[10-15]

In the Prevention and Incidence of Asthma and Mite Allergy birth cohort, daycare and the development of asthma and allergy were assessed prospectively in 3,963 children up to 8 years. At 8 years airway hyperresponsiveness and specific IgE in serum were measured. This enabled us to examine age-specific associations between daycare and the presence of older siblings on the one hand, and asthma symptoms and allergic sensitization on the other. Some of the results of this study have been previously reported in the form of an abstract.[16]

METHODS

Study population

Recruitment took place in the years 1996 to 1997. After birth the baseline study population consisted of 3,963 children. Questionnaires for parental completion, based on the ISAAC core questionnaires[17] were sent to the parents during pregnancy, at the child's ages of 3 and 12 months, and yearly thereafter up to the age of 8 years. At 8 years 3,518 children were invited for venipuncture to assess specific IgE for common allergens. Also, all children with an allergic mother (n = 988) and a random subset of children with a non-allergic mother (n = 566) were invited for a medical examination including spirometry and measurement of airway responsiveness by means of a methacholine challenge.[18] See Figure 3.1 for a population flow chart. Details of the Prevention and Incidence of Asthma and Mite Allergy study design have been published previously.[19] The study included an intervention part: 416 children (with allergic mother) were given house dust mite-impermeable mattress covers. This intervention had no effect on the incidence of allergy or respiratory symptoms at 4 or 8 years of age, nor was it associated with daycare attendance.[20] Therefore, we decided to include these children in the

present analysis. The study protocol was approved by the medical ethics committees of the participating hospitals.

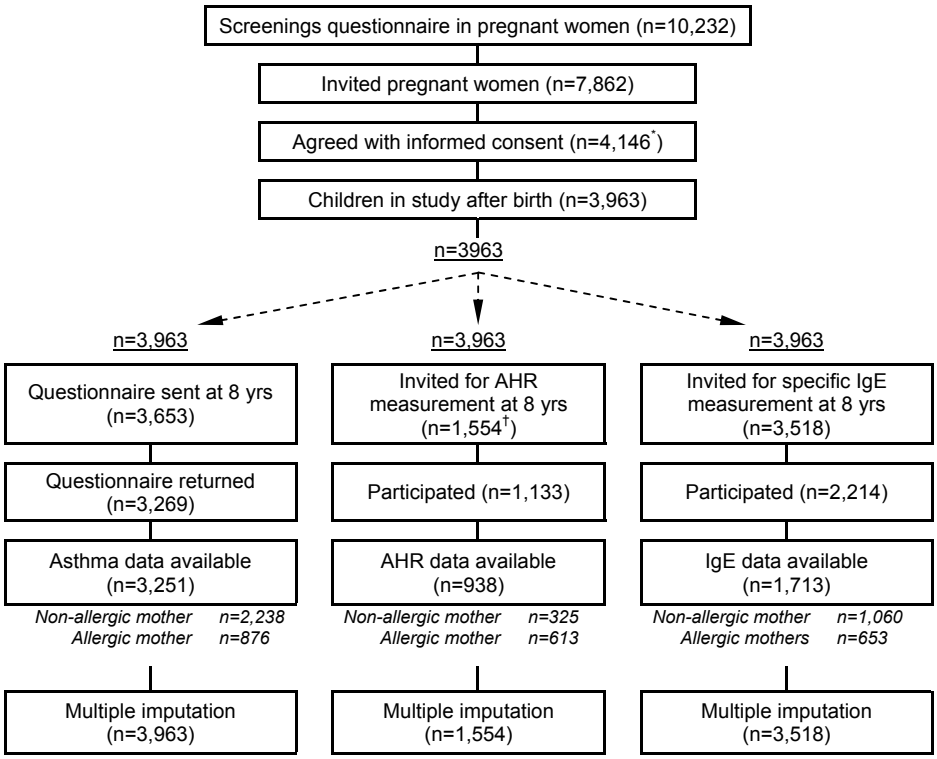

Figure 3.1. Population flow chart
All 3,963 eligible children could enter each of the 3 arms of flowchart. *: A total of 1,327 allergic mothers (32%) and 2,819 non-allergic mothers (68%) were included, which is a good reflection of the general Dutch population. †: Invited for the hospital-based medical examination, including airway hyperresponsiveness (AHR) were all children of allergic mothers (n = 988) and a random sample (22%, n = 566) of the children of nonallergic mothers who were still in the study at 8 years of age.

Outcome variables

Parents reported their child's airway symptoms annually at age 1 to 8 years. Data on shortness of breath and prescription of inhaled steroids were collected yearly after the age of 2 years. We defined the following (dichotomous) outcomes, pertaining to the past 12 months:

- Wheezing: at least one attack of wheezing
- Inhaled steroid prescription by a medical doctor (after age of 2 yr)
- Asthma symptoms: at least one attack of wheeze and/or at least one attack of shortness of breath and/or a prescription of inhaled steroids (after age of 2 yr)
- Allergic asthma: asthma symptoms + sensitization to at least 1 airborne allergen (at age of 8 yr)

Sensitization to airborne allergens at 8 years was defined as specific IgE of 0.70 IU/mL or greater for at least one of the following: house dust mite (Dermatophagoides pteronyssinus), cats, dogs, grass pollen (Dactylis glomerata), birch, Alternaria alternata. The cutoff was chosen because this was previously shown to be associated with clinical symptoms.[21] Frequent respiratory tract infections were defined as parental report of 3 or more serious infections in the past year. Airway responsiveness was determined according to the protocol of the European Community Respiratory Health Survey[18] and airway hyperresponsiveness (AHR) was defined as a 20% or greater decrease of FEV_1 at a cumulative dose 0.61 mg methacholine bromide or less.

Exposures and confounders

Daycare use was assessed yearly and in our final analyses children were divided in 3 groups: early daycare (first attendance between 0-2 yr), late daycare (first attendance between 2-4 yr), and no daycare. Daycare was defined as at least 4 hours a week in a professional daycare institution, where children were exposed to other children under the age of 12 years. The average group size in professional daycare centers in the Netherlands is 10 children.[22] The presence of siblings was defined as having an older sibling at time of birth (dichotomous). As potential confounders we defined: gender, gestational age, birth weight, parental education, ethnicity, mother's age, maternal allergies/asthma, breastfeeding, smoking during pregnancy, tobacco smoke exposure at home, urbanization, presence of pets and single parenthood.

Statistical analyses

Associations of daycare or older siblings with the outcomes at 8 years were analyzed by logistic regression. Generalized estimating equations (GEE) were used to study associations with respiratory outcomes longitudinally, taking into account the correlation between repeated measurements in the same individual. Interaction of exposures and confounders with age was included in the GEE model when significant, allowing associations to vary with age. The analyses were stratified for maternal allergy and gender because these variables were potential effect modifiers. Also interaction between daycare attendance and siblings was tested. Besides a complete case analysis, missing data were multiple times imputed to avoid bias, which may result from complete case analysis.[23,24]

The Multivariate Imputation by Chained Equations procedure in the statistical program R version 2.6.2 was used.[25,26] The 10 imputed datasets were analyzed and results combined using PROC MIANALYZE in SAS 9.1 (SAS Institute, Inc., Cary, NC). Data on sensitization and AHR were assessed in a subgroup. Therefore separate multiple imputation procedures were performed for these data, creating multiple imputed datasets for reported airway symptoms (n = 3,963), specific IgE (n = 3,518), and AHR (n = 1,554).

Table 3.1. General characteristics of study population

Characteristic	Baseline population with complete data (n=3,643)		Population with early daycare (n=1,112)	Population without early daycare (n=2,531)
	%	n	%	%
Female gender	48.3	1,760	47.3	48.8
Born by caesarean section†	8.5	305	9.5	8.0
Older siblings present*	50.2	1,828	60.2	45.3
Maternal smoking in pregnancy*	16.8	604	14.3	17.9
Smoking in house after birth (≥ once per week)*†	27.4	998	23.0	29.3
Breastfeeding ever given*†	82.7	3,013	87.3	80.7
Allergic mother	29.9	1,089	30.0	29.8
Allergic father	30.7	1,119	32.6	29.9
Single parenthood*†	1.1	42	1.9	0.8
Pets present at birth*	53.4	1,937	49.7	55.0
Maternal education level*†				
Low	22.7	825	11.4	27.6
Intermediate	41.9	1,528	36.6	44.3
High	35.4	1,290	52.0	28.1
Daycare attendance‡				
Early daycare (age 0-2 years)	30.5	1,112	100	-
Late daycare (age 2-4years)	46.9	1,709	-	67.5
No daycare before age 4	22.6	822	-	32.5
Urbanization (address/km^2)*†				
<1000	33.0	1,202	25.9	36.1
1000-1500	23.3	847	20.1	24.6
>1500	43.7	1,594	54.0	39.3
	mean	SD	mean	mean
Maternal age (years)*†	30.4	3.8	31.3	30.0
Duration of pregnancy (weeks)†	39.8	1.7	39.8	39.8
Birth weight child (grams)†	3,517	544	3,502	3,515

Significance of associations between characteristics and exposure variables (daycare/siblings) was tested using Chi-square test (categorical variables) or analysis of variance (continuous variables). Smoking during pregnancy was considered positive only if mother reported smoking at least 4 weeks after estimated date of conception. *: Significant association with daycare ($p < 0.05$). †: Significant association with older siblings ($p < 0.05$). ‡: Defined as at least 4 hours per week in a daycare centre with other children (under 12 yr of age) present.

RESULTS

Study population

Complete data on exposures and confounders was available in 3,643 of the 3,963 children (92%). Table 3.1 shows baseline characteristics of this group and their association with daycare and older siblings. After 8 years, 92% of the children were still in the study. In 1,445 children (36%) a questionnaire-based outcome was missing for at least one year. These children were more likely than children with complete data (n = 2,518) to have an atopic mother (39 vs. 27%) or a mother with a low level of education (31 vs. 20%), but less likely to attend daycare before the age of 5 (73 vs. 79%). Data on IgE were obtained in 1,713 children (response rate 49%) and data on airway responsiveness in 938 children (response rate 60%). The response rates were not associated with daycare. To avoid bias resulting from complete case analysis, reported results pertain to the multiple imputed datasets.

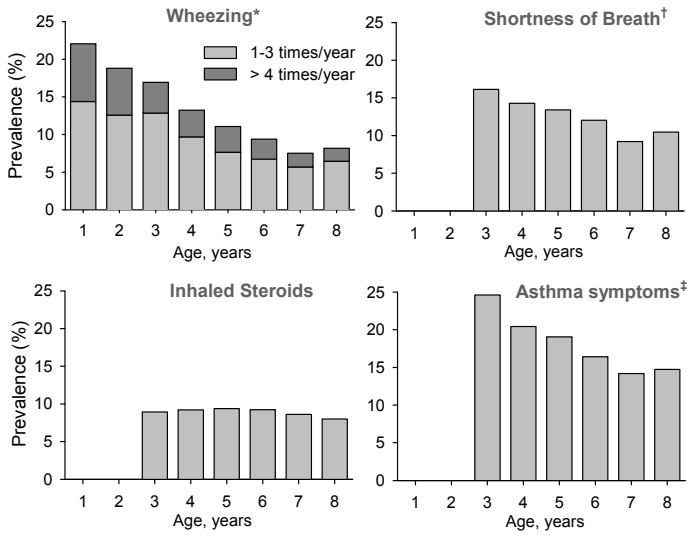

Figure 3.2. Annual prevalences of wheezing, shortness of breath, inhaled steroid prescriptions, and asthma symptoms up to the age of 8 years (n = 3,963)
*: Bars show infrequent (1-3 times, light grey) and frequent (4 or more, dark grey) wheezing episodes per year. †: At least one attack of shortness of breath in the past year ‡: Defined as at least one attack of wheeze, one attack of shortness of breath, or a prescription of inhaled steroids in the past year.

Prevalence of airway symptoms and asthma

Wheezing at least once was reported by 22% at 1 year and showed a linear decline to 8% at 7 years. (Figure 3.2) Shortness of breath showed a similar time trend, with 16% positive at 3 and 9% at 7 years. Prescription rates for inhaled steroids remained stable over time, between 8 and 9%. At 8 years, 15% of the children had at least one asthma symptom (wheezing, shortness of breath, or the use of inhaled steroids). (Table 3.2) Of the invited children, 33.6% was sensitized to 1 or more airborne allergen, and 8.9% had allergic asthma. Forty-six per cent with an allergic mother and 38% with a nonallergic mother had AHR.

Table 3.2. Prevalence of outcomes at 8 years and associations with daycare and older siblings

	Asthma symptoms§		Sensitization¶		Allergic asthma‖		AHR**	
Overall prevalence	14.7 %††	n = 429 ‡‡	33.6 %††	n = 473 ‡‡	8.9 %††	n = 147‡‡	43.0 %††	n = 402‡‡
	aOR	$CI_{95\%}$	aOR	$CI_{95\%}$	aOR	$CI_{95\%}$	aOR	$CI_{95\%}$
aOR* Older siblings†	1.14	(0.92; 1.42)	0.89	(0.73; 1.09)	0.98	(0.69; 1.40)	1.09	(0.81; 1.45)
aOR Early daycare‡	0.99	(0.74; 1.32)	0.86	(0.63; 1.18)	0.77	(0.53; 1.13)	0.80	(0.57; 1.14)
aOR Late daycare‡	0.94	(0.72; 1.23)	0.83	(0.65; 1.05)	0.82	(0.60; 1.14)	1.01	(0.82; 1.45)

*: Associations are adjusted for gender, birth weight, breastfeeding, urbanization, age and education of mother, history of allergies in mother. †: No older siblings at birth is reference group. ‡: No daycare before the age of 4 is reference group. §: Defined as at least one attack of wheeze, one attack of shortness of breath or a prescription of inhaled steroids in the past year. ¶: Defined as a specific IgE concentration ≥ 0.70 IU/ml for at least one of the tested airborne allergens. ‖: Defined as positive score on both asthma symptoms and sensitization. **Defined as a decrease ≥ 20% in FEV_1 at a cumulative dose of 0.61 mg methacholine. ††: Percentage of cases based on imputed datasets. ‡‡: Absolute number of cases based on complete dataset (not imputed).

Associations of daycare and siblings with airway symptoms, sensitization and AHR

Children with early daycare were twice as likely to experience wheezing in the first year of life, compared with children without daycare (adjusted odds ratios [aOR] at 1 year 1.89; $CI_{95\%}$: 1.50-2.39). The association changed with increasing age; at 5 years there was a trend for less wheezing in children with early daycare (aOR 0.83; $CI_{95\%}$: 0.60-1.14). At 8 years, early daycare was not associated with wheezing. Figure 3.3 shows the adjusted odds ratios of daycare for wheeze, steroid prescription, and asthma symptoms. The associations of early and late daycare are plotted with no daycare as reference. The outcome "asthma symptoms" was not available at ages 1 and 2 years, but the associations with daycare at later ages followed the same pattern as for wheeze. We found no protective effect from early daycare on asthma symptoms until 8 years (aOR at 8 years 0.96; $CI_{95\%}$: 0.73-1.29). In the early daycare group a trend for fewer prescriptions of inhaled steroids was observed between the ages of 5 and 7 years, but not at 8 years. A sensitivity analysis in which stricter outcomes were defined as "frequent wheezing

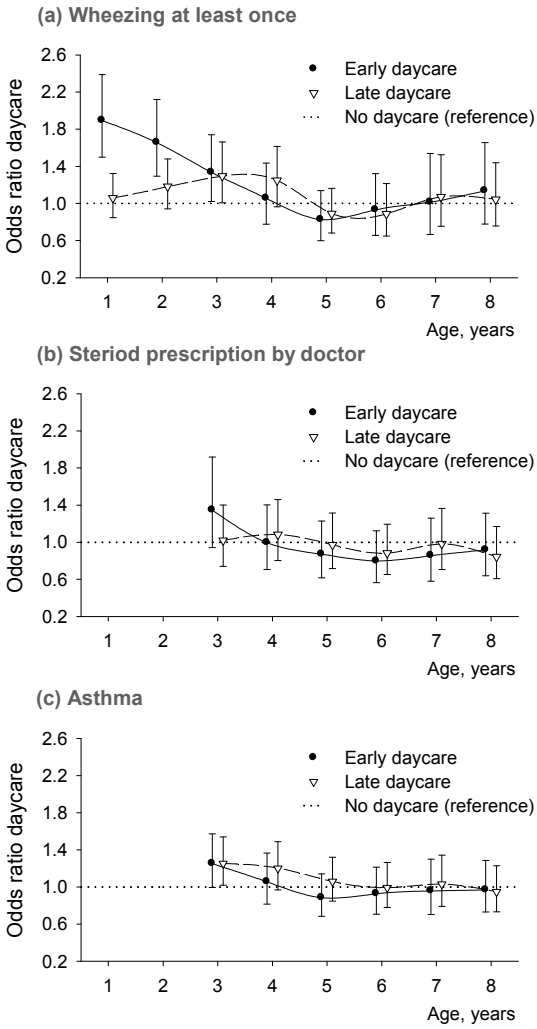

Figure 3.3. Adjusted odds ratios for longitudinal outcomes of wheezing at least once, inhaled steroid prescription, and asthma symptoms
Odds ratios and confidence intervals for early daycare and late daycare (no daycare is reference) for all outcomes, from longitudinal generalized estimating equations model. Estimates adjusted for gender, birth weight, breastfeeding, urbanization, age and education of mother, history of allergies in mother.

(≥4 episodes per year)" and "doctor's diagnosis of asthma with asthma symptoms past year" led to similar results, without any protective effect of daycare at the age of 8 years. The associations with late daycare were less pronounced and not statistically significant on any outcome. We found the effect of daycare to change gradually with the age of first attendance, without a clear window of opportunity. Also when daycare was

defined as "very early daycare" (attendance before the age of 6 months) we found no protective effect on any respiratory outcome at the age of 8 years.

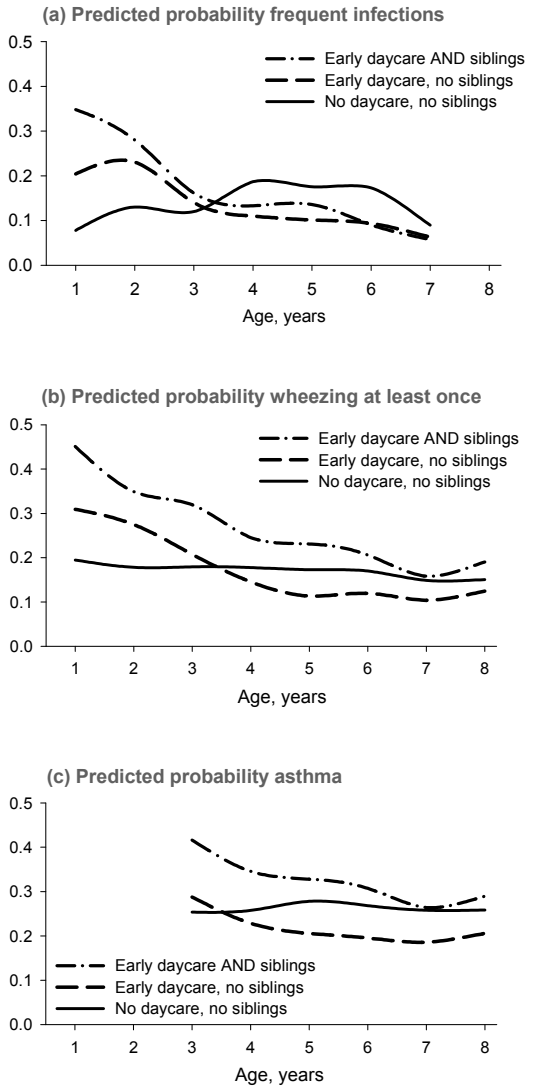

Figure 3.4. Predicted probabilities for longitudinal outcomes frequent respiratory infections, wheezing at least once, and asthma symptoms
Three lines represent children with: 1) early daycare and older siblings; 2) early daycare but no older siblings; and 3) no daycare and no older siblings. Predicted probabilities are calculated for children with: a male gender, a nonallergic mother, breastfeeding ever given, and average values for gestational age, birth weight, urbanization, age and education of mother.

In the first year of life, children with older siblings had more wheezing than first-born children (aOR 2.15; $CI_{95\%}$: 1.81-2.56). The strength of the association with wheezing, steroid prescriptions, and asthma symptoms decreased linearly with age and disappeared at 8 years. The presence of older siblings was not protective for wheeze, inhaled steroid prescriptions, or asthma symptoms at any age.

Table 3.2 shows the adjusted associations between daycare, older siblings and the clinical outcomes at 8 years. Neither daycare nor the presence of older siblings was associated with asthma symptoms, sensitization, allergic asthma, or AHR at 8 years.

Of the variables tested for confounding, the following changed the point estimate of either daycare or siblings, and were controlled for in all analyses: gender, birth weight, breastfeeding, urbanization, mother's age, mother's education and maternal allergy. We found the associations with airway symptoms in the imputed data to be weaker than in the complete case data. However, the overall pattern did not change on any outcome. Results on sensitization and AHR were not affected by the multiple imputation analysis.

Interactions between daycare and gender, atopy, or siblings

Stratified analyses were performed to investigate effect modification by gender or maternal atopy. The effects of daycare and siblings did not differ between boys and girls or between children with and without an allergic mother. We found a significant interaction between siblings and daycare for wheeze ($p < 0.001$). In Figure 3.4 the occurrence of frequent respiratory infections, wheeze, and asthma symptoms are shown for children with early daycare and older siblings (n = 486), with early daycare but no older siblings (n = 714), and without daycare and siblings (n = 330). Children with older siblings and early daycare had a more than fourfold higher risk of frequent respiratory infections and a more than twofold higher risk of wheezing in the first year compared with children without older siblings and daycare. After the age of 4 years children with early daycare and no older siblings were less likely to wheeze (significant at 5 yr), to use inhaled steroids (significant at 6 yr), and to have asthma symptoms (significant at age 5 to 7 yr) as compared with those without daycare. At the age of 8 years these associations had disappeared. Importantly, children exposed to both early daycare and older siblings experienced most infections and symptoms in early childhood, without a protective effect on wheeze, inhaled steroid prescription, or asthma symptoms until the age of 8 years. Interaction between daycare and older siblings was also significant for the outcomes sensitization and allergic asthma. In models allowing for this interaction, the associations of daycare and siblings with these outcomes remained not significant (Table 3.3).

Table 3.3. Associations of asthma symptoms and sensitization at 8 years with early daycare attendance, allowing interaction by presence of older siblings

Exposures*		Asthma symptoms‡		Sensitization§		Allergic asthma¶	
Early daycare	Siblings	aOR†	$CI_{95\%}$	aOR	$CI_{95\%}$	aOR	$CI_{95\%}$
Yes	Yes	1.32	(0.95; 1.83)	0.97	(0.68; 1.37)	1.06	(0.66; 1.68)
Yes	No	0.83	(0.61; 1.12)	0.86	(0.66; 1.13)	0.64	(0.43; 0.96)
No	Yes	0.99	(0.76; 1.29)	0.82	(0.66; 1.02)	0.81	(0.54; 1.22)
No	No	1.00	$p = 0.10$‖	1.00	$p = 0.36$‖	1.00	$p = 0.18$‖

*: No older siblings at birth in combination with no early daycare reference category. †: Odds ratios are adjusted for gender, birth weight, breastfeeding, urbanization, age and education of mother, history of allergies in mother. ‡: Defined as at least one attack of wheeze, one attack of shortness of breath or a prescription of inhaled steroids in the past year. §: Defined as a specific IgE concentration ≥ 0.70 IU/ml for at least one of the tested airborne allergens. ¶: Defined as positive score on both asthma symptoms and sensitization. ‖: Significance of F-test for combined variable (Siblings*Early daycare).

DISCUSSION

We found no evidence for a protective or harmful effect of daycare on the development of asthma symptoms, allergic sensitization, or airway hyperresponsiveness at the age of 8 years. Early daycare was associated with more airway symptoms until the age of 4 years and, only in children without older siblings, with a transient decrease in symptoms between 4 and 8 years. Children with early daycare and older siblings had the highest prevalence of respiratory symptoms in early childhood, but no decreased prevalence of asthma symptoms at any time point.

Since the postulation of the "hygiene hypothesis" by Strachan,[1] many studies have focused on the relation between daycare and the development of asthma. Studies investigating the association between reported respiratory infections in early life and development of asthma consistently found no protective effect on asthma.[5,10-15,27] We studied the effect of daycare, which is a proxy for infections.[2-5] Studying daycare has two important advantages compared to reported infections. Firstly, the relation between daycare and asthma is unlikely to be influenced by reverse causation. This is a problem with reported infections as young children with an asthmatic constitution may be more likely to experience symptoms during respiratory infections and to get a doctor's diagnosis of infection. Second, daycare is a feasible target for prevention.

Previous studies investigating the effects of daycare on the risk of asthma were mostly cross-sectional and the results differed. Discrepancies can be explained by considering the results according to age. In early childhood, daycare has been consistently associated with more respiratory infections and wheezing.[2-4,28,29] Between 3 and 5 years no association was found between daycare and asthma.[29,30] Between the ages of 4 and 14 years, a cross-sectional study reported less wheezing and asthma in children who

attended daycare.[6] A recent longitudinal study (n = 922) also reported a reduced risk of wheezing at 5 years in children with daycare.[31] Our data cover a wide age range and show a pattern of an early increase in airway symptoms followed by a later reduction. Similar results were found in the Tucson Children's Respiratory Study (n = 1,035) and in the Home Allergens and Asthma Study (n = 505).[7,32] We observed a reduction in airway symptoms associated with early daycare only in children without older siblings, in line with earlier reports of interaction between daycare and siblings.[3,4,6]

The relationship between daycare and asthma later in childhood is less clear. We found no decreased prevalence of asthma symptoms at the age of 8 years in children who attended daycare. Similarly, Nafstad and colleagues reported no association between daycare and asthma at the age of 10 years in the Oslo birth cohort (n = 2,540).[10] A retrospective study in adults aged 20-44 years reported an increased risk of asthma associated with daycare attendance.[9] In contrast, the Tucson study reported a preventive effect of daycare on the development of asthma lasting up to the age of 13 years.[7] In this study exposure was defined as daycare before the age of 6 months and information was collected retrospectively around the age of 7 years, introducing the possibility of recall bias.[7] Furthermore a significant effect on asthma was only reported for the combined effects of older siblings and daycare. To our knowledge no prospective study has reproduced these findings.

This is the first prospective study to report on the relation between daycare and AHR. We found no significant decrease in AHR in children exposed to daycare or older siblings in early childhood. The high prevalence of AHR could imply that the cutoff at a cumulative dose 0.61 mg or less of methacholine bromide was not strict enough for our population. However, in a separate sensitivity analysis where responsiveness was defined as the dose-response slope (% change in FEV_1 in response to total methacholine bromide dose), a continuous variable, we found no significant association with early daycare. AHR is a feature of asthma,[33] and has strong predictive value for the long-term prognosis.[34,35] The findings that daycare was not associated with asthma symptoms at 8 years and did not affect AHR strongly support our conclusion that early daycare does not prevent asthma.

The relationship between daycare attendance and atopy remains unclear despite earlier studies. Several studies reported a lower prevalence of sensitization in children with daycare, as measured by skin prick test reactivity[7,14,30] or specific IgE.[14,28] Other studies found no significant association,[9,10,36,37] or even increased sensitization, after daycare.[31,38] These studies vary in methodology: some investigated skin tests, others specific IgE, and different allergies were assessed. Furthermore, the definition of daycare and the age at which outcomes were assessed varied. We found no significant effect of daycare or siblings on sensitization measured by the presence of specific IgE. We found a trend of less asthma with sensitization in children with early daycare, and

without siblings. Likewise, Krämer and colleagues reported lower rates of sensitization and asthma associated with early daycare in a cross-sectional study in Eastern Germany, in a subgroup of children without siblings.[6] Possibly children with older siblings are already exposed to more infections early in life, reducing any additional effects of daycare. However, if infections would protect, one would expect a dose-response relationship with the largest protection in children with the highest microbial burden at an early age. Our results show that children with older siblings and early daycare indeed experienced most infections in infancy, but did not develop less asthma or sensitization, thus arguing against a causal relationship.

Strong points of our study are the large cohort with a prospective design, long follow-up with very good compliance, and collection of objective data including AHR and specific IgE. Because the study population is a fairly random representation of the Dutch population, our results can be generalized. However, in the population invited for AHR measurement there was an overrepresentation of children with an allergic mother. Because we found no interaction by allergy of the mother on the association between daycare and AHR, this does not compromise generalizability of our findings. There is evidence that the age of entry into daycare may influence the natural course of asthma.[6,31] It has been suggested that a window of opportunity exists earlier in life, (e.g., in the first 6 months of life).[7] We found no support for this in our data. Early daycare was defined as attendance before the age of 2 years, but the majority of these children (59%) visited daycare before the age of 6 months. Also, when the definition was limited to attendance before the age of 6 months there was no significant association with any of the studied outcomes at 8 years.

Some limitations should be considered in the interpretation of our results. First, in the absence of a gold standard, the definition for asthma remains arbitrary. Apart from the sensitive definition "asthma symptoms" we performed sensitivity analyses with outcomes "frequent wheezing (≥4 times/year)" and "doctor's diagnosis of asthma with asthma symptoms past year". Early daycare was associated with a higher prevalence of all these outcomes in the first years of life, but there was no association at the age of 8 years. Second, airway symptoms were based on parental reports. Especially at early ages this may have led to some misclassification. Most likely any misclassification would be independent of the exposure to daycare (i.e., nondifferential), causing underestimation of the effect. Because we found strong correlations especially in the first years of life, we consider it unlikely that our results have been biased by misclassification of early symptoms. Parents with an atopic history may report wheeze more accurately and this could influence our results. However, we found that atopy of the mother had no confounding effect nor did it modify the effect of daycare on any of the outcomes. Furthermore it should be realized that apart from early infections, multiple other pathways might explain a relation between daycare and later asthma symptoms.

Finally, children were followed up to the age of 8 years. Strictly, we can therefore not exclude a protective effect of early daycare on symptoms at later ages. Our data are not suggestive of such an effect.

What are the practical implications of our findings? We found that early daycare is associated with a transient decrease in wheezing after the age of 4 years, but not with the development of allergic sensitization, AHR, or asthma symptoms at 8 years. Apparently, the incidence of asthma cannot be reduced by promoting early daycare attendance. Early daycare merely seems to shift the burden of respiratory morbidity to an earlier age, where it is more troublesome than at a later age. Hence, early daycare should not be promoted for reasons of preventing asthma and allergy.

CONCLUSION

Early daycare is associated with an increase in airway symptoms until the age of 4 years, and fewer symptoms between 4 and 8 years. We found no protection against asthma symptoms, hyperresponsiveness, or allergic sensitization at the age of 8 years.

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Chapter 4

Perinatal risk factors for wheezing phenotypes in the first 8 years of life

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ABSTRACT

Rationale

A novel data-driven approach was used to identify wheezing phenotypes in preschool children aged 0-8 years, in the PIAMA birth cohort. Five phenotypes were identified: never/infrequent wheeze, transient early wheeze, intermediate onset wheeze, persistent wheeze, and late onset wheeze. It is unclear whether these phenotypes have different perinatal risk factors.

Objectives

To assess the associations of wheezing phenotypes with perinatal factors, and to identify possible targets for prevention.

Methods

In the PIAMA study (n = 3,963) perinatal factors were collected at 3 months, and wheezing was assessed annually until the age of 8 years. Associations between the 5 wheezing phenotypes and perinatal risk factors were assessed using weighted multinomial logistic regression models. Odds ratios were adjusted for confounding variables and calculated with 'never/infrequent wheeze' as the reference category.

Results

Complete data on outcome and all confounding variables were available for 2,728 children. Risk factors for transient early wheeze (n = 455) were male gender, maternal and paternal allergy, low maternal age, high maternal body mass index, short pregnancy duration, smoking during pregnancy, presence of older siblings, and daycare attendance. Risk factors for persistent wheeze (n = 83) were male gender, maternal and paternal allergy, and not receiving breastfeeding for at least 12 weeks. Intermediate onset wheeze (n = 98) was associated with a lower birth weight and late onset wheeze (n = 45) with maternal allergy.

Conclusion

The association with perinatal factors varied between different wheezing phenotypes, which suggests different underlying pathophysiology. Some of the identified risk factors are modifiable and may be important targets for prevention programs.

INTRODUCTION

The rapid rise in asthma prevalence at the end of the last century is most likely caused by a change in environmental factors, affecting individuals with a genetic susceptibility for asthma. A crucial window for the effect of such environmental factors appears to exist in prenatal and early postnatal life.[1] Understanding the mechanisms of asthma development is essential in order to find targets for primary prevention. Unfortunately, conclusive evidence on the relevant major risk factors is still lacking. A likely explanation for contradictory findings in previous studies is that 'childhood asthma' does not refer to a single disease entity, but comprises several distinct wheezing phenotypes, each of which may have different risk factors.[2]

Much effort has been put in identifying different wheezing phenotypes. Martinez *et al* described longitudinal patterns of 'transient early', 'persistent' and 'late onset' wheeze, based on reported symptoms between the ages of 0-3 and 3-6 years.[3] Henderson *et al* more recently used longitudinal latent class analysis to identify different wheezing phenotypes in the Avon Longitudinal Study of Parents And Children (ALSPAC), where wheeze was reported repeatedly from birth until 8 yrs.[4] The same analysis was applied in the Prevention and Incidence of Asthma and Mite Allergy (PIAMA) study, and 5 wheezing phenotypes with similar characteristics as in the ALSPAC study were found, supporting their validity.[5]

The aim of the present study was to investigate pre- and perinatal risk factors for wheezing phenotypes in the PIAMA study. If specific wheezing illnesses are associated with different risk factors, this would support the hypothesis that they have a differential etiology and pathophysiology. Understanding of these complex associations may lead to the discovery of targets for primary prevention of specific wheezing illnesses during childhood.

METHODS

Study population

The PIAMA study is a prospective birth cohort study in Western, middle and Northern areas of the Netherlands. Recruitment took place in the years 1996 and 1997. The baseline study population consisted of 3,963 children. Questionnaires for parental completion, based on the ISAAC core questionnaires were sent during pregnancy, at 3 months, and yearly after birth up to the age of 8 years. More details of the PIAMA study design are given in the online supplement and have been published previously.[6] The study protocol was approved by the medical ethics committees of the participating hospitals.

Phenotypes of wheeze

Wheeze was assessed annually with the question: "Has your child had wheezing or whistling in the chest in the last 12 months?" at the age of 1 to 8 years, and answered with yes or no. Therefore $2^8 = 256$ different longitudinal patterns of wheeze were possible. In order to identify children with similar patterns of wheeze over time, longitudinal latent class analysis (LLCA) was used. With LLCA wheezing phenotypes were derived in the PIAMA and the ALSPAC birth cohort, and results were previously reported.[4,5] The derived phenotypes in these independent cohorts were comparable, supporting the validity of the phenotypes.[5] Including only children with complete data on wheezing at every age in the PIAMA cohort, the following five phenotypes of wheeze were identified: never/infrequent wheeze, early wheeze, intermediate onset wheeze, persistent wheeze and late onset wheeze.

Perinatal factors

Candidate perinatal risk factors were selected based on previous literature and availability within our dataset, and included parental characteristics (n = 5), child characteristics (n = 5), prenatal exposures (n = 3), and postnatal exposures (n = 6). Apart from exposure to fine particulate matter ($PM_{2.5}$) and study region, all investigated risk factors were defined using questionnaire data. Maternal age, maternal body mass index (BMI), pregnancy duration, birth weight, and exposure to $PM_{2.5}$ were included as continuous variables. Maternal BMI was calculated on the basis of maternal height and weight before pregnancy, as reported by the mother. Study region was considered a possible confounder and included as a variable with 4 categories. All other items were analyzed as dichotomous variables. High educational level was defined as a completed bachelor and/or master study. Breastfeeding was defined positive if the child was breastfed for at least 12 weeks. Individual exposure to $PM_{2.5}$ was assessed as the annual average concentrations at the birth address, and estimated by land-use regression models. These models were based on four two-week measurements of $PM_{2.5}$ at 40 sites throughout the study area, and details were previously described.[7] A detailed definition of all perinatal factors is given in the online supplement.

Statistical analysis

All analyses were carried out using SAS 9.1 (SAS Institute, Inc., Cary, NC). The association between phenotypes of wheeze and all perinatal factors was investigated using a weighted multinomial logistic regression model (SAS PROC CATMOD). The analyses were weighted for the probability that a child belonged to a certain phenotype (individual posterior membership probability), to minimize bias due to misclassification of the wheezing phenotypes. All factors were inspected adjusting for gender. Secondly, multivariable models were built, adjusted for the following confounding variables (based

on literature and correlations within our dataset): gender, parental allergy, pre- and postnatal smoke exposure, breastfeeding, daycare, pregnancy duration, older siblings, birth weight, maternal age, study region, parental level of education. Finally, modification of the associations between all perinatal factors and wheezing phenotypes was investigated for gender of the child, and maternal allergy. These factors were selected, because interaction of gender and maternal allergy with several other perinatal factors has been previously reported.[8-10]

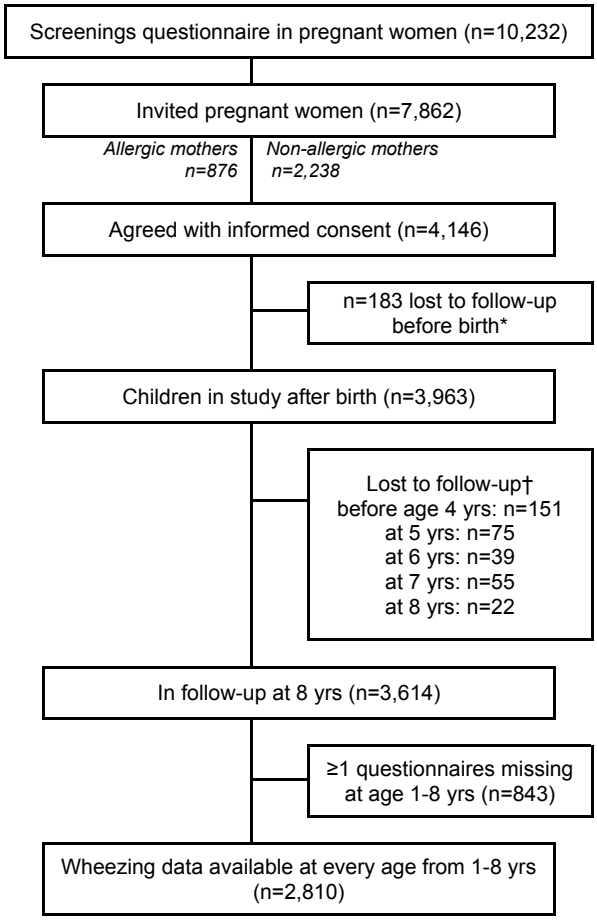

Figure 4.1. Population Flow Chart
*: Lost to follow-up due to various reasons; perinatal death, language barrier, not interested, moved, etc. †: Various reasons (e.g. moved, personal reasons, repeated non-response without known reason).

RESULTS

Study population

Of the 3963 children included at baseline 3,614 were still in follow-up at 8 years and 2,810 (71%) had complete data on wheezing at every single year. This was taken as the study population for the present analyses. A detailed flowchart is presented in Figure 4.1. Table 4.1 shows general characteristics of the study population (n = 2,810). This population was comparable to the baseline population (n = 3,963) with respect

Tables 4.1. Description of the study population

Variable	n / N	%
General characteristic		
Female gender	1,360 / 2,810	48.4
Allergic mother	750 / 2,810	26.7
Allergic father	850 / 2,810	30.3
Maternal education (% high)	1,046 / 2,807	37.3
Paternal education (% high)	1,166 / 2,792	41.8
Study region		
North	891 / 2,810	31.7
Middle	765 / 2,810	27.2
East	426 / 2,810	16.2
West	728 / 2,810	25.9
Maternal smoking during pregnancy	421 / 2,780	15.1
Premature birth (<37 wks)	131 / 2,805	4.7
Low birth weight (<2500 g)	89 / 2,805	3.1
Breastfeeding ever given	2,334 / 2,789	83.7
Older siblings present (≥1)	1419 / 2,810	50.5
	mean / N	IQR
Maternal age (yrs)	30.7 / 2,804	5.0
Maternal body mass index (kg/m^2)	22.8 / 2,626	3.7
Particulate matter ($PM_{2.5}$) (ug/m^3)	16.9 / 2,806	2.0
Health outcomes at 8 yrs	n / N	%
Wheezing phenotype*		
Never/infrequent	2,109 / 2,810	75.0
Transient early	470 / 2,810	16.7
Intermediate onset	85/ 2,810	3.1
Persistent	99 / 2,810	3.5
Late onset	47 / 2,810	1.7
Allergic sensitization inhaled allergens†	389 / 1,434	27.1

*: Frequency (n) of each wheezing phenotype is calculated as the sum of the membership probability of all children for that phenotype. †: Defined as specific IgE concentration ≥ 0.7 IU/ml for at least one of the following inhaled allergens: house dust mite (Dermatophagoides pteronyssinus), cats, dogs, grass pollen (Dactylis glomerata), birch, Alternaria alternata.

to gender, prematurity, birth weight, older siblings and allergy of the father. However, children in the study population had parents with a higher level of education, more often received breastfeeding and were less likely to have a mother who smoked during pregnancy, compared to the children who were excluded. The percentage of missing data on perinatal factors in the study population was less than 1% for all variables, apart from maternal BMI, which had 6.5% missings. In the multivariable analysis 13 potentially confounding variables were included. Eighty-two children (2.4%) had a missing value for at least one of these variables.

Wheezing phenotypes

The percentages of children for each phenotype are presented in Table 4.1. Seventy-five percent of all children belonged to the group that never/infrequently wheezed. Of the children who wheezed, the largest group had transient early wheeze (16.7%), late onset wheeze being the least prevalent phenotype (1.7%). Figure 4.2 depicts the age-specific probability of wheeze for all phenotypes.

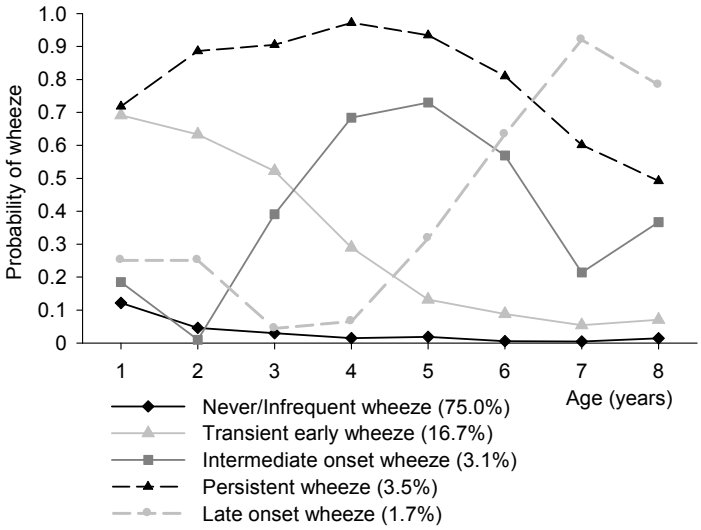

Figure 4.2. Probability of wheeze at each time point from birth to age 8 years for each wheezing phenotype in PIAMA (N = 2,810)
Prevalence of the phenotypes is shown next to the phenotypes in the legend. Figure from Savenije & Granell *et al*,[5] with adjustments

Association of perinatal risk factors with wheezing phenotypes

Parental risk factors

A family history of allergy was significantly associated with a higher risk of transient early wheeze, persistent wheeze and late onset wheeze, but not intermediate onset wheeze (Table 4.2). A higher maternal body mass index (BMI) was significantly associated with more transient and persistent wheezing.

Child characteristics

Gender, birth weight, pregnancy duration and admittance to a hospital after birth were associated with at least one wheezing phenotype, but caesarean section was not. Boys more often had transient early and persistent wheeze than girls. Children with a lower birth weight were more likely to develop transient early and intermediate onset wheeze. A short pregnancy duration and admittance to a hospital after birth were both associated with more transient early wheeze.

Prenatal exposures

Smoking during pregnancy was the only prenatal exposure variable linked with a wheezing phenotype. It significantly increased the risk for transient early wheeze, and had a non-significant trend for more persistent wheeze with a similar odds ratio. (Table 4.3)

Postnatal exposures

Breast feeding for at least 12 weeks significantly decreased the risk of persistent wheeze, while older siblings and daycare attendance in the first year increased the risk of transient early wheeze. Environmental tobacco smoke did not significantly increase the risk of any wheezing phenotype. Air pollution as measured by $PM_{2.5}$ appeared to be associated with phenotypes with a higher probability of wheezing, especially intermediate onset and persistent, but this was not significant.

Multivariable analyses

In order to exclude confounding by correlated risk factors for wheezing we performed a multivariable analysis. All factors that remained significantly associated with one or more wheezing phenotypes are presented in figure 4.3. In the adjusted analysis a parental history of allergy and male gender remained the strongest risk factors for wheezing during childhood, and were significantly associated with more transient early and persistent wheeze. Two modifiable maternal factors were also associated with wheezing phenotypes. First, children from an older mother were less likely to develop transient early wheeze, but tended to develop more late onset wheeze. Second, a high maternal BMI was significantly associated with more transient early wheeze and

Table 4.2. Crude associations of parental and child characteristics at birth with wheezing phenotypes

	Never/ infrequent wheeze (n=2109)	Transient Early wheeze (n=470)	Intermediate Onset wheeze (n=85)	Persistent wheeze (n=99)	Late Onset wheeze (n=47)
Parental characteristics					
Maternal allergy*					
Frequency, %	24.8	29.5	32.8	40.4	45.0
OR ($CI_{95\%}$)		1.27 (1.02-1.59)†	1.49 (0.93-2.36)	2.06 (1.36-3.12)†	2.49 (1.39-4.47)†
Paternal allergy*					
Frequency, %	28.1	36.2	32.7	40.2	42.3
OR ($CI_{95\%}$)		1.46 (1.18-1.80)†	1.25 (0.79-1.99)	1.74 (1.15-2.63)†	1.88 (1.05-3.34)†
Parental educational level (≥1 parent high level)					
Frequency, %	53.5	51.2	51.1	50.3	21.8
OR ($CI_{95\%}$)		0.91 (0.74-1.11)	0.91 (0.59-1.40)	0.87 (0.58-1.31)	0.76 (0.42-1.35)
Age mother‡					
Average	30.7	30.4	30.3	30.7	31.1
OR ($CI_{95\%}$)		0.90 (0.79-1.03)	0.83 (0.62-1.11)	1.00 (0.76-1.30)	1.14 (0.78-1.68)
Body Mass Index mother (kg/m^2) ‡§					
Average	22.7	23.1	23.0	23.4	23.0
OR ($CI_{95\%}$)		1.16 (1.03-1.30)†	1.10 (0.86-1.42)	1.26 (1.01-1.57)†	1.10 (0.79-1.53)
Child characteristics					
Gender (male)					
Frequency, %	50.7	57.8	57.1	66.7	51.7
OR ($CI_{95\%}$)		1.41 (1.15-1.73)†	1.37 (0.89-2.12)	2.06 (1.34-3.16)†	1.10 (0.62-1.96)
Birth weight (per kg)					
Average	3.55	3.48	3.42	3.55	3.48
OR ($CI_{95\%}$)		0.75 (0.63-0.91)†	0.63 (0.43-0.93)†	0.91 (0.62-1.33)	0.77 (0.45-1.33)
Pregnancy duration (per week)					
Average	40.0	39.6	39.8	39.7	39.8
OR ($CI_{95\%}$)		0.87 (0.82-0.92)†	0.97 (0.85-1.11)	0.91 (0.81-1.02)	0.96 (0.80-1.15)
Caesarian section					
Frequency, %	8.6	8.1	8.9	8.6	9.3
OR ($CI_{95\%}$)		0.92 (0.64-1.33)	1.03 (0.48-2.20)	0.96 (0.47-1.99)	1.08 (0.39-3.00)
Admitted to hospital after birth					
Frequency, %	11.2	15.4	13.0	12.6	13.4
OR ($CI_{95\%}$)		1.41 (1.06-1.70)†	1.16 (0.61-2.22)	1.09 (0.59-2.02)	1.22 (0.51-2.90)

Never/ infrequent wheeze is reference category, all analyses were weighted for the probability that a child belongs to a certain phenotype (individual posterior membership probabilities). All odds ratios adjusted for gender, not for other possible confounders. OR ($CI_{95\%}$); Odds ratio with 95% confidence interval. *: Defined positive if any of the following items were reported: asthma, hay fever, house dust allergy, house dust mite allergy, or pet allergy. †: Associations with a significance level of $p < 0.05$. ‡: Odds ratio expressed per inter quartile range increase. §: Based on reported maternal weight and height before pregnancy.

borderline significantly associated with persistent wheeze. Low birth weight remained strongly associated only with intermediate onset wheeze, and a short pregnancy with more transient early wheeze. Smoking during pregnancy, older siblings and daycare

attendance remained significantly associated with a higher risk of transient early wheeze. Breastfeeding for at least 12 weeks was the only significant protective factor for persistent wheeze.

Effect modification was tested for gender as well as maternal allergy on all perinatal factors listed in Table 4.2 and 4.3, but no significant interaction was found for any of these factors.

Table 4.3. Crude associations of perinatal exposures with wheezing phenotypes

	Never/ infrequent wheeze (n=2109)	Transient Early wheeze (n=470)	Intermediate Onset wheeze (n=85)	Persistent wheeze (n=99)	Late Onset wheeze (n=47)
Prenatal exposures					
Smoking mother during pregnancy*					
Frequency, %	13.9	19.5	18.1	19.1	13.7
OR ($CI_{95\%}$)		1.51 (1.16-1.96)†	1.37 (0.78-2.42)	1.48 (0.88-2.48)	0.99 (0.42-2.30)
Antibiotics by mother during pregnancy*					
Frequency, %	8.8	9.7	7.0	10.7	6.7
OR ($CI_{95\%}$)		1.12 (0.80-1.58)	0.79 (0.34-1.84)	1.26 (0.65-2.43)	0.75 (0.23-2.37)
Meconium in amniotic fluid					
Frequency, %	19.7	17.4	17.8	20.5	21.9
OR ($CI_{95\%}$)		0.86 (0.65-1.15)	0.88 (0.47-1.66)	1.07 (0.61-1.85)	1.14 (0.52-2.50)
Early postnatal exposures					
Breastfeeding at least 3 months					
Frequency, %	48.8	43.6	43.3	36.3	43.3
OR ($CI_{95\%}$)		0.82 (0.67-1.01)	0.81 (0.52-1.25)	0.61 (0.40-0.93)†	0.80 (0.45-1.44)
Older siblings present (≥1)					
Frequency, %	49.3	56.2	45.5	55.7	45.2
OR ($CI_{95\%}$)		1.31 (1.07-1.60)†	0.85 (0.55-1.32)	1.28 (0.85-1.92)	0.85 (0.47-1.52)
Daycare attendance in first year					
Frequency, %	24.3	31.5	20.5	19.2	18.3
OR ($CI_{95\%}$)		1.42 (1.14-1.77)†	0.80 (0.47-1.37)	0.73 (0.44-1.22)	0.70 (0.33-1.47)
Environmental tobacco smoke exposure‡					
Frequency, %	24.5	26.4	27.2	29.4	20.7
OR ($CI_{95\%}$)		1.11 (0.88-1.39)	1.15 (0.70-1.87)	1.29 (0.83-2.01)	0.80 (0.39-1.64)
Pets at 3 months					
Frequency, %	50.0	48.4	50.6	56.2	39.2
OR ($CI_{95\%}$)		0.94 (0.77-1.15)	1.02 (0.66-1.58)	1.28 (0.85-1.92)	0.65 (0.36-1.17)
Particulate matter ($PM_{2.5}$) at birth address (ug/m^3)§					
Average	16.8	17.0	17.1	17.1	17.0
OR ($CI_{95\%}$)		1.15 (0.97-1.35)	1.26 (0.88-1.80)	1.28 (0.92-1.79)	1.17 (0.73-1.88)

Never/ infrequent wheeze is reference category, all analyses were weighted for the probability that a child belongs to a certain phenotype (individual posterior membership probabilities). All odds ratios adjusted for gender, not for other possible confounders. OR ($CI_{95\%}$); Odds ratio with 95% confidence interval. *: Based on parental reports during second trimester of pregnancy. †: Associations with a significance level of $p < 0.05$. ‡: In the child's house; more than 1 cigarette per week. §: Odds ratio expressed per inter quartile range increase in $PM_{2.5}$ concentration.

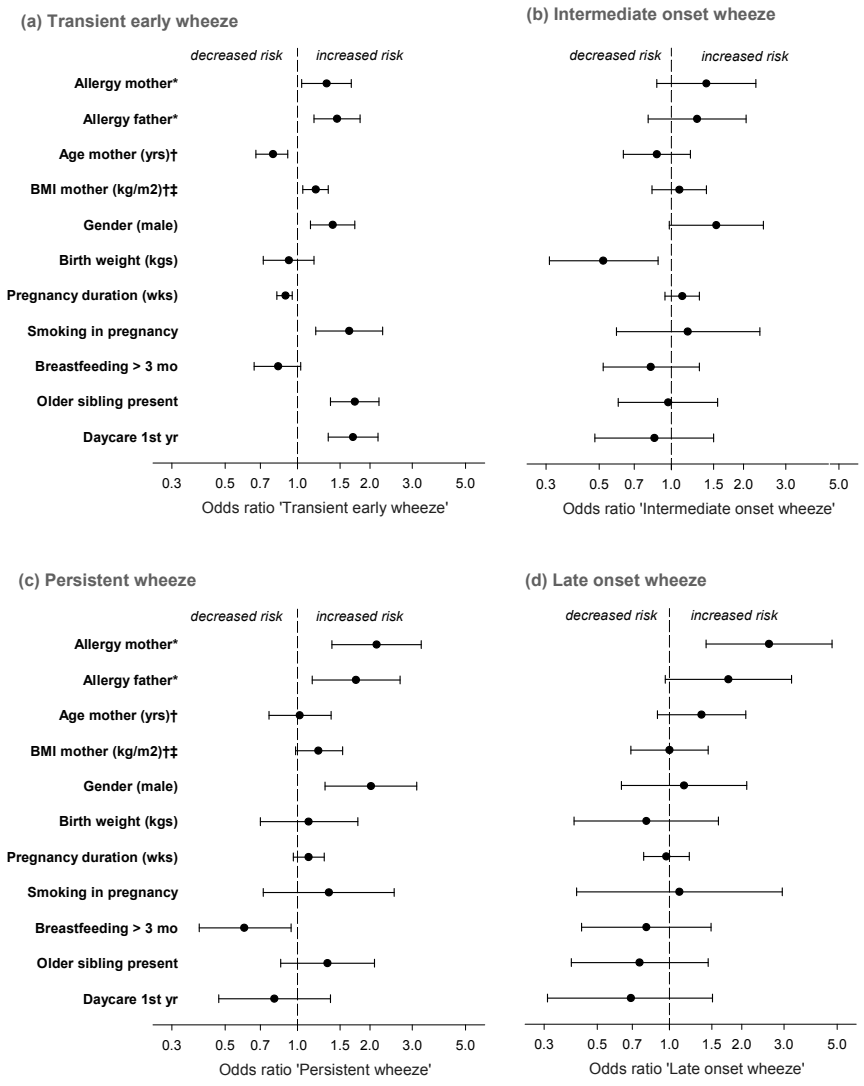

Figure 4.3. Odds ratios of perinatal risk factors after multivariable adjustment
Never/ infrequent wheeze is reference category, all analyses were weighted for the probability that a child belongs to a certain phenotype (individual posterior membership probabilities). Total N = 2,728. *: Defined positive if any of the following items were reported: asthma, hay fever, house dust allergy, house dust mite allergy, or pet allergy. †: Odds ratios given per interquartile range increase. ‡: Body Mass Index.

DISCUSSION

Wheezing phenotypes were differentially and sometimes uniquely associated with perinatal risk factors. Compared to children with never/infrequent wheeze, intermediate onset wheeze occurred significantly more often in children with a lower birth weight and persistent wheeze in children who were not breastfed for at least 12 weeks. Transient early wheeze was associated with a wider range of modifiable pre- and postnatal risk factors, namely maternal age, maternal BMI, pregnancy duration, smoking during pregnancy, and daycare attendance in the first year. These findings support the hypothesis that different longitudinal wheezing patterns may have different underlying pathophysiologies. Furthermore these risk factors are modifiable and may therefore be important targets for future prevention programs.

Risk factors for transient early wheeze

The phenotype transient early wheeze was associated with several perinatal factors, including a family history of allergy, in line with two previous studies on wheezing phenotypes.[11,12] A possible explanation for the association between parental asthma and transient wheeze in their offspring, could be that these transient wheezers are at increased risk of developing asthma symptoms again at adult age. A long term follow-up of transient early wheezers confirmed this hypothesis, and suggested it may be explained by tracking of a lower lung function into adulthood.[13,14]

Multiple modifiable factors were associated with transient wheezing, among which low maternal age and high maternal BMI. Both factors are strongly correlated with socioeconomic status, which may explain part of the association. However, the fact that the relations were significant after adjustment for a wide range of possible potential confounders, indicates that a more direct effect may be present as well. A recent study reported maternal obesity as an independent risk factor for wheezing in early childhood: the authors acknowledged the possibility of residual confounding, but hypothesized that in utero exposure to systemic inflammation in obesity may influence the developing immune system of the child.[15] Another explanation for the association could be that maternal obesity is linked to obesity in their offspring, which may be an independent risk factor for the wheezing symptoms.[16] Previous studies consistently linked low maternal age with an increased risk of wheezing in childhood, speculating this could be explained by both social and biological factors.[17,18]

Daycare attendance and the presence of older siblings were both associated with transient early wheeze, probably because they increase the risk of respiratory infections.[19,20] Daycare attendance is a modifiable factor and given its strong association with transient early wheeze it is a possible candidate for prevention strategies. Importantly, daycare attendance seems to cause a shift in the burden of wheezing illness to an

earlier age. The significant increase in transient early wheezing is accompanied by a non-significant trend for less late onset wheeze.[11,12] Unfortunately, this does not necessarily imply that daycare causes a long-lasting preventive effect on wheezing, as we have previously shown using the PIAMA data.[19]

Smoking during pregnancy has been consistently reported as a prenatal risk factor for childhood wheezing.[21] Most studies found a positive association with transient early and/or persistent wheeze.[12,22-24] Our results are consistent with this in part, and show that in utero smoke exposure was most strongly associated with transient early wheeze. An association with persistent wheeze was not demonstrated in our data. Previous animal studies have demonstrated that tobacco smoke can have a direct negative influence on lung growth and maturation.[25] Apart from this direct effect, prenatal smoking may indirectly cause morbidity mediated by a lower birth weight. We here reported the independent effect of smoking, adjusted for birth weight. Avoidance of prenatal smoking may be a promising preventive interventions as it could lead to a reduction in the prevalence of transient early wheeze and at the same time reduce the prevalence of low birth weight, which appears to be associated with intermediate onset wheeze.

Risk factors for intermediate onset wheeze

A lower birth weight was the only significant risk factors for intermediate onset wheeze. Birth weight cannot be directly influenced, but several modifiable factors have been reported to influence birth weight, most notably smoking during pregnancy.[26] We cannot easily compare our findings with other studies, because the outcome of intermediate onset wheeze was not used previously. Conflicting results on the effect of birth weight on wheezing and asthma in previous studies may well have been caused by differences in outcome definitions, particularly the age of outcome assessment.[27-29] Intermediate onset wheeze was first described in the ALSPAC study and an independent analysis of the PIAMA data revealed a group of children with a similar pattern of wheeze.[4,5] The clear differences between risk factors for intermediate onset and other wheezing phenotypes may imply a different underlying pathophysiology. Possibly children with a lower birth weight are prone to wheezing due to disturbed airway growth and, hence, smaller airways. The fact that the prevalence of wheezing decreases in this group after the age of 5 years fits well with this hypothesis, because children may become less susceptible for wheezing illnesses when their airways increase in size.[3] Why this specific phenotype becomes manifest after a symptom-free interval of several years remains to be elucidated.

Risk factors for persistent wheeze

For persistent wheeze the most striking risk factor was parental allergy, in line with previous literature.[3,11,12,22,23] Another finding consistent with previous literature is that in the studied age range boys were twice more likely to have persistent wheeze than girls.[3,11,23] Controversy exists about the effects of breastfeeding.[30,31] We found that breastfeeding for at least 3 months significantly reduced the risk of persistent wheezing for at least 8 years, with an odds ratio of 0.6 (p = 0.03). Odds ratios for other wheezing phenotypes were non-significant, but all showed a trend towards protection. This implies that breastfeeding reduces the overall prevalence of wheezing, rather than causing a shift in age of onset. Therefore breastfeeding remains an important target in the prevention of asthma symptoms in the first 8 years of life. Previous studies suggested that there is interaction between breastfeeding, maternal allergy, and the risk of asthma. We did not find evidence for this, in line with a previous paper on breastfeeding and childhood asthma using PIAMA data.[30] Multiple pathways could explain the lower risk of wheezing in breastfed children. Breast milk may influence the infant's immune system directly by immunomodulatory factors, or indirectly by affecting the infant's mucosal maturation and thereby the intestinal microflora.[32,33]

Risk factors for late onset wheeze

In the multivariate analyses maternal allergy was the only significant risk factors for late onset wheeze, paternal allergy was borderline significant. No modifiable risk factors for late onset wheeze were detected. This stresses the importance of genetic constitution in the development of late onset wheeze, but also suggests a lack of perinatal targets for the prevention of it. However, it should be noted that the small sample size may have reduced the power to detect any significant differences. To estimate long-term benefits of any preventive strategies, follow-up is needed to know which of the wheezing phenotypes is most strongly related with asthma symptoms in adolescence and/or adulthood. Late-onset wheezers are often considered as true asthmatics,[34] but follow-up of the Tucson cohort has shown that persistent wheezers may actually be more likely to become asthmatic adults than those with late onset wheeze.[14]

The possible impact of an intervention on a risk factor depends not only on its odds ratio, but also on the prevalence of a risk factor in the population. The attributable fraction (AF) takes this into account, and is given in the online supplement. (Table E4.1) The AF of a risk factor is 'the proportion of children with a specific wheezing phenotype that could be prevented by eliminating the risk factor from the population', but it is likely an overestimation of the actual effect of an intervention on this risk factor. Therefore interventions on the risk factors for transient early wheeze given in Table E4.1 will have only a limited impact, with <10% reduction in transient early wheeze. Breastfeeding had the highest AF of all modifiable risk factors (25.6%), suggesting that it may indeed be the single most important target for interventions to reduce the prevalence of persistent wheeze.

Strengths of the current study are the large sample size with high follow-up rate, and the longitudinal design. Regular follow-up with annual questionnaires that start before birth, eliminated the risk of recall bias. Such bias may have affected the results of the few previous studies investigating risk factors for wheezing phenotypes.[12,22,24,31] We used an unbiased and data driven approach to prospectively identify several distinct wheezing phenotypes within our cohort. The use of annual recordings of wheeze allowed us to detect new wheezing phenotypes, in a period that wheezing prevalence changes rapidly. Correct classification of wheezing phenotypes remains crucial to understand the complex associations with a wide range of perinatal risk factors.

Some considerations should be made when interpreting our results. We found differential associations with the wheezing phenotypes, suggesting different pathophysiologies. Causality however cannot be proven using observational data. We adjusted our results for many possible confounders, but we cannot exclude that some unknown or residual confounding influenced the results. Furthermore, despite the large size of the PIAMA birth cohort, relatively few children were classified as late onset wheezers, with limited power to detect significant associations with perinatal risk factors. To a lesser extent this may have also affected the results for intermediate onset and persistent wheeze.

We found different patterns of risk factors for the wheezing phenotypes, and this could imply that these phenotypes are indeed different disease entities, with different pathophysiologies. This should be taken into account in the search for prevention strategies. Our data show that most modifiable risk factors may have an influence on transient early wheeze. Prevention of transient early wheeze will have the biggest impact on symptoms in the first years of life. It is the most prevalent wheezing phenotype and there is evidence that these children are at increased risk for adult asthma as well.[14] Prenatal smoking is a promising target for prevention, as it was associated with transient early wheeze and simultaneously causes a lower birth weight, which may lead to more intermediate onset wheeze. The promotion of breastfeeding for at least 3 months could have a large impact on persistent wheeze, which has a high risk of chronic asthma lasting into adulthood.[14]

In conclusion, we found that the association of perinatal factors with wheezing phenotypes was not the same for different phenotypes. This suggests that the underlying pathophysiology of these wheezing phenotypes may be different. We identified modifiable risk factors that may be important targets for prevention of specific wheezing phenotypes.

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Supplement data Chapter 4

METHODS

Recruitment of participants

All children participated in the Prevention and Incidence of Asthma and Mite Allergy (PIAMA) birth cohort study. This multicentre study was conducted in 3 different regions of The Netherlands: north (Groningen and surroundings), central (Bilthoven, Wageningen and surroundings) and southwest (Rotterdam and surroundings). Recruitment took place between March 1996 and May 1997 by means of a validated screening questionnaire,[E1] distributed by midwifes to 10,232 pregnant woman visiting one of 52 prenatal clinics. According to the results of this screening the women were divided in an allergic and a non-allergic group. Women with any of the following self-reported symptoms were defined as allergic: asthma, hay fever, house dust allergy, house dust mite allergy or pet allergy. Children of allergic women were defined as 'high-risk'. Based on the screening 7,862 women (2,779 allergic and 5,083 non-allergic) were invited to participate in the study, approximately 50% (n = 4,146) agreed and gave informed consent (1,327 allergic and 2,819 non-allergic). The PIAMA cohort includes an intervention part, studying the effect of impermeable mattress covers, and a natural history part. (See Figure E2.1, data supplement chapter 2) In the present study we considered children from both the intervention and the natural history part. As previously published, the intervention had no effect on the incidence of allergy or respiratory symptoms, including the symptom of wheezing.[E2] After birth, the study population consisted of 3,963 children. Eligible for the present study were children with complete data on wheezing at every age from 1-8 years (n = 2,810). In these children 5 wheezing phenotypes were previously indentified using Longitudinal Latent Class Analysis.[E3]

Perinatal factors

In total 19 possible perinatal risk factors were investigated. The following parental characteristics were included: maternal allergy, paternal allergy, parental educational level, maternal age, and maternal body mass index (BMI). Parents were considered 'allergic' if they reported any of the following items: asthma, hay fever, house dust allergy, house dust mite allergy, pet allergy. Information on education of both parents was combined in one dichotomous variable (low vs. high), and considered 'high' if at least one of the parents completed a bachelor or masters study. Maternal BMI was calculated on the basis of maternal height and weight before pregnancy, and was reported by the mother in the questionnaire send when the child was 1 year old. The odds ratios of both maternal age

and BMI were calculated per interquartile range increase. The child characteristics that were investigated were: gender, birth weight, pregnancy duration, caesarean section, and whether a child was admitted to a hospital directly after birth. The odds ratios for gender were calculated with girls as the reference group. Estimates for birth weight were calculated per kilogram increase, and estimates for pregnancy duration per week increase. Three prenatal exposures were investigated: prenatal smoking, prenatal use of antibiotics (both assessed during the second trimester of the pregnancy), and exposure to meconium in the amniotic fluid which was asked when the child was three months old. The investigated postnatal exposures included breastfeeding, the presence of older siblings, daycare attendance in first year, environmental tobacco smoke exposure, the presence of pets in the household, and exposure to particulate matter ($PM_{2.5}$) at the child's birth address. Breastfeeding (BF) was assessed as the total number of weeks that breastfeeding was given. In a previous PIAMA publication this variable was divided in three categories (no BF, 0-16 wks BF, more than 16 wks BF), but due to the small sample size in some wheezing phenotypes it was dichotomized in this paper: no breastfeeding or less than 12 weeks vs. at least 12 weeks.[E4] Daycare was defined as at least 4 hours per week in a professional daycare institution, where children were exposed to other children under the age of 12 years. The average group size in professional daycare centers in the Netherlands is 10 children. Environmental tobacco smoke was assessed when the child was three months old, and defined as exposure to tobacco smoke at least once a week in the living room of the child's house.

Statistical analysis

All analyses were carried out using SAS 9.1 (SAS Institute, Inc., Cary, NC). As outcome in this study we used wheezing phenotypes, that were previously derived in the PIAMA study using longitudinal latent class analysis (LLCA). This statistical approach attempts to identify groups of children within the population (latent classes) that have a similar pattern of wheeze over time. This is done by selecting subgroups within which the occurrence of wheeze at a certain time point, is statistically independent of the occurrence of wheeze at another point in time. Rather than appointing every individual child to a single latent class (i.e. phenotype), the model calculates for every child the probability of belonging to each of the identified latent classes. This probability is called the 'individual posterior membership probability'. Since 5 latent classes (wheezing phenotypes) were identified in the PIAMA study, 5 posterior membership probabilities were calculated for every child. The association between phenotype of wheeze and all perinatal factors was investigated using a weighted multinomial logistic regression model (SAS PROC CATMOD). These multinomial models were weighted for the individual posterior membership probabilities, in order to minimize the risk of bias due to phenotype misclassification.

Attributable Fraction

The aim of our study was to find possible interventions to prevent wheezing illnesses in childhood. In order to assess the maximal possible effect of a prevention program on any modifiable risk factor, it is important to consider the strength of the association with the outcome, but also the prevalence of the risk factor in the population. Both aspects are taken into account in the attributable fraction (AF). The AF indicates the excess number of children with a wheezing phenotype associated with a specific risk factor, and is expressed as a proportion of the total number of children with the wheezing phenotype. We calculated the AF for all modifiable factors that were significantly associated with a wheezing phenotype in the multivariate analysis. The AF of a risk factor was estimated by calculating the expected number of cases using the regression coefficients of the multivariable model, after elimination of that (dichotomous) risk factor from the population.[E5] The AF of continuous variables was estimated by changing the population distribution to a degree that would be clinically plausible. Therefore the following changes were modeled in the continuous variables: 1) maternal age was increased with 5 yrs, but only in mothers < 30 yrs of age; 2) maternal BMI was decreased to a maximum of 25 kg/m^2 for all mothers; 3) pregnancy duration was increased to a minimum of 39 wks for all children; and 4) birth weight was increased with 200 grams in all children.

RESULTS

Attributable Fraction

The results for the attributable fraction of all risk factors that were significant in the multivariable analysis are given in Table E4.1. Transient early wheeze had the most significant risk factors after multivariable adjustment. Parental allergy and gender were significant risk factors, but as these factors are not modifiable, these are no targets for interventions. The presence of older siblings had the highest AF, but also this factor would in practice not be usable for an intervention. The AF of the other significant risk factors for transient early wheeze was small (≤10%). The AF could be interpreted as 'the proportion of children with a specific wheezing phenotype that could be prevented by completely eliminating the risk factor from the population'. However, there are two important assumptions in this interpretation. First, the association of the risk factor with the wheezing phenotype is assumed to be completely causal. Causality cannot be proven using observational data. Results were adjusted for known confounders, but it cannot be excluded that some unknown or residual confounding influenced our results. Secondly, it is unlikely that an intervention in the general population could achieve a complete elimination of any of the investigated risk factors. Therefore the AF is an

Table E4.1. Attributable fraction of risk factors after multivariable adjustment*

Risk factor	Transient Early wheeze (n=455)	Intermediate Onset wheeze (n=83)	Persistent wheeze (n=98)	Late Onset wheeze (n=45)
Maternal allergy[†]	5.9 %	-	20.2 %	28.0%
Paternal allergy[†]	9.5 %	-	16.0 %	-
Age mother[‡]	9.6 %	-	-	-
Maternal BMI (kg/m^2)[‡§]	2.2 %	-	-	-
Gender (male)	13.1 %	-	33.0 %	-
Birth weight (per kg)	-	12.3%	-	-
Pregnancy duration (per wk)	3.8 %	-	-	-
Smoking mother pregnancy	5.8 %	-	-	-
No breastfeeding for ≥3 mths	-	-	25.6 %	-
Older sibling present (≥1)	19.5 %	-	-	-
Daycare in first year	10.8 %	-	-	-

Total N = 2,728. Never/ infrequent wheeze (n = 2,047) is reference category. Frequencies (n) of wheezing phenotypes calculated as the sum of the individual posterior membership probability of all children for that phenotype, all analyses weighted for individual posterior membership probability. *Analyses adjusted for: gender, parental allergy, pre- and postnatal smoke exposure, breastfeeding, daycare, pregnancy duration, older siblings, birth weight, maternal age, study region, parental level of education. [†]: 'Allergy' defined positive if any of the following items were reported: asthma, hay fever, house dust allergy, house dust mite allergy, or pet allergy. [‡]: Odds ratios given per interquartile range increase. [§]: BMI is Body Mass Index.

overestimation of the actual effects of a prevention program on public health. Taking these considerations into account, the proportion of wheezing that may be avoided by interventions on perinatal risk factors is probably limited.

The only significant risk factor for intermediate onset wheeze was birth weight. The most important modifiable factor that can influence birth weight is smoking during pregnancy. In our data smoking during pregnancy led on average to a 200 gram reduction in birth weight, which is why we calculated the AF by modeling a 200 gram increase in birth weight. The AF of 12.3% is considerable, but should be interpreted with caution, as it may be very difficult to actually influence birth weight on a population level.

Parental allergy and male gender were associated with persistent wheeze, but are not modifiable. Breastfeeding was the only modifiable significant risk factor for persistent wheeze. It had the highest AF (23.8%) of all modifiable factors, which can be explained by the strong odds ratio, in combination with the high proportion of mothers that did not give breastfeeding for at least three months (over 50%). Therefore breastfeeding remains an important target in the prevention of asthma symptoms in the first 8 years of life.

The outcome of late onset wheeze was significantly associated only with maternal allergy. As this is not a modifiable factor, we could not estimate the possible impact of any intervention to reduce late onset wheeze.

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Chapter 5

Childhood wheezing phenotypes are associated with FeNO in atopic children at age 8

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ABSTRACT

Rationale

Fractional exhaled Nitric Oxide (FeNO) is a biomarker of eosinophilic airway inflammation. Using longitudinal latent class analysis, 5 wheezing phenotypes have been identified, characterized by different age of onset and prognosis. We hypothesize that these different wheezing phenotypes have distinct patterns of airway inflammation.

Objectives

To assess FeNO at 4 and 8 years in children with different phenotypes of wheeze and atopy.

Methods

Children participated in the PIAMA study, a prospective birth cohort in The Netherlands. Respiratory health was assessed yearly by questionnaires until the age of 8 years, these data were used to identify 5 wheezing phenotypes. Associations between FeNO measured at 4 and 8 years and wheezing phenotypes were investigated using weighted linear regression.

Results

Complete data on wheezing phenotypes and FeNO at 4 years was available in 462 children. Compared to the phenotype of never wheeze, FeNO at 4 years was higher only in intermediate onset wheeze. Data on wheezing phenotype and FeNO at 8 years was available for 803 children. Compared to never wheeze, FeNO at 8 years was significantly higher in persistent, intermediate onset, and late onset wheeze. Stratified analyses showed that the increase in FeNO in persistent, intermediate and late onset wheeze was only present in children with allergic sensitization at 8 years.

Conclusion

FeNO measured at 8 years was associated with specific wheezing phenotypes, but only among atopic children. We speculate that the role of eosinophilic inflammation in the pathophysiology of wheezing phenotypes differs between atopic and non-atopic children.

INTRODUCTION

The fraction of nitric oxide in exhaled air (FeNO) is a non-invasive biomarker of eosinophilic airway inflammation with excellent reproducibility.[1-5] Recent studies have shown that FeNO is a useful test both in large population-based studies, as in clinical asthma management.[6,7] Elevated FeNO was found in children and adults with asthma and atopy, [4,6,8,9] overlapping with the distribution in normals.[10,11] We previously reported FeNO in 4-year-old children from the Prevention and Incidence of Asthma and Mite Allergy (PIAMA) cohort, and found no association with classic wheezing phenotypes as described by Martinez in preschool children.[11,12] However, FeNO may be influenced by atopy, which can develop later in life.[4,13-16] The PIAMA birth cohort study provided the opportunity to study FeNO in relation to phenotypes of wheeze in a large group of children recruited from the general population. One of the special features of PIAMA is the yearly respiratory health assessment, which can be used to define temporal phenotypes of wheeze. Recently, temporal phenotypes of wheeze were identified by longitudinal latent class analysis (LLCA) in the ALSPAC study, and these phenotypes were differently associated with atopy and lung function.[17] This analysis was repeated in the PIAMA study with comparable results.[18] FeNO has not been studied in relation to phenotypes identified using this novel approach. We hypothesized that the different wheezing phenotypes are characterized by differences in eosinophilic inflammation, which would be reflected by differences in FeNO measured at the age of 4 and 8 years. Because atopy is a determinant of FeNO, we stratified our analysis for atopy.[4]

MATERIALS AND METHODS

Study design

The Prevention and Incidence of Asthma and Mite Allergy study is a prospective birth cohort study in The Netherlands. Recruitment took place in 1996-1997 through prenatal clinics; 7,862 pregnant women were invited to participate, 4,146 (53%) agreed and gave informed consent. Children were labeled as high-risk (n = 1,327) and low-risk (n = 2,819), based on the atopic status of the mother.[19] Respiratory health and asthma symptoms of the children were assessed yearly by questionnaires, partly based on the ISAAC core questionnaires, along with data on demographics and a wide range of asthma risk factors.[20] All high-risk children and a subgroup of low-risk children were invited for FeNO measurement at the age of 4 years (n = 1,808) and 8 years (n = 1,554). A detailed description of the PIAMA study design was previously published.[11,21] The study protocol was approved by the medical ethics committees of the participating medical centers.

Study population

At the age of 4 years all high-risk (n = 1,173) and a random sample of low-risk children (n = 635) were invited for a medical examination, including offline FeNO measurement. Of those 1,808 children 1,269 attended the examination, and an exhaled air sample was obtained in 939 children. Off-line FeNO measurements of sufficient quality were obtained in 595 children (63%) at age 4.[11] At 8 years also all high-risk children still in follow-up (n = 988) and a similar, random sample of low-risk children (n = 566) were invited for a hospital-based medical examination including online FeNO measurement. Of these 1,554 children 1129 (73%) gave informed consent and attended the examination. In 39 children a FeNO measurement could not be performed due to device failure. Of the remaining 1,090 children at least 1 successful FeNO measurement was obtained in 976 children (90%), all of whom were included in our analyses. The remaining 114 children were unable to exhale at a constant flow during FeNO measurement. Finally, wheezing phenotypes were defined only if children had complete data on wheezing for every year. A detailed flowchart of the study population with complete data on wheezing phenotype and FeNO at 4 years (n = 462) and 8 years (n = 803) is presented in Figure 5.1.

Measurements

FeNO in 4-year-old children was measured offline by the balloon method,[22] according to European Respiratory Society (ERS) / American Thoracic Society (ATS) guidelines.[2,11] FeNO in 8-year-old children was measured online using the NIOX chemiluminescence analyzer (Aerocrine AB, Solna, Sweden) according to ERS and ATS guidelines.[1] We previously found good agreement between these on- and offline FeNO measurements.[23] At 8 years blood was drawn to assess sensitization to airborne allergens, defined as specific IgE of $\geq$ 0.70 IU/mL for at least one of the following allergens: house dust mite (Dermatophagoides pteronyssinus), cats, dogs, grass pollen (Dactylis glomerata), birch, Alternaria alternata.

Phenotypes of wheeze

Longitudinal latent class analysis was used by Savenije *et al* to define wheezing phenotypes in PIAMA in early childhood, as originally published by Henderson *et al.*[17] Five wheezing phenotypes were identified in the first 8 years of life: never/infrequent wheeze (75%), transient early wheeze (17%), intermediate onset wheeze (3.1%), persistent wheeze (3.5%) and late onset wheeze (1.7%). These phenotypes were comparable with those identified in the ALSPAC cohort.[18] The five phenotypes are graphically depicted in Figure 4.2, chapter 4.

Statistical analysis

All analyses were carried out in SAS 9.1 (SAS Institute, Inc., Cary, NC). The associations between FeNO at 4 and 8 years and phenotypes of wheeze were investigated with weighted linear regression models (SAS PROC GENMOD). FeNO data were log-

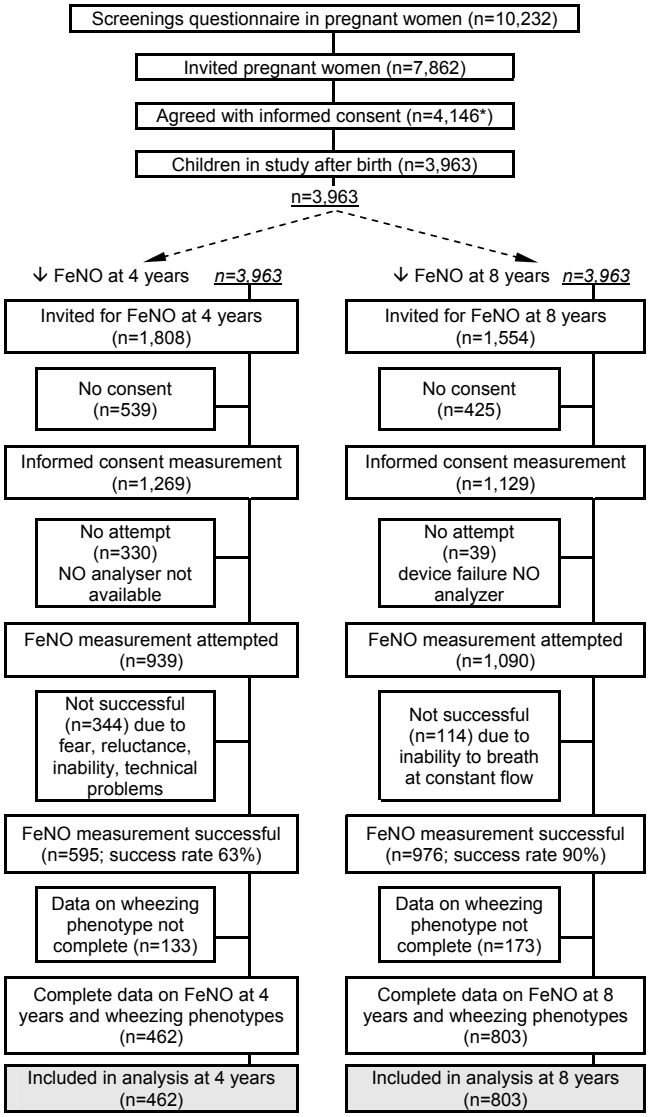

Figure 5.1. Flow chart of study population at 4 and 8 years
Flow chart of the number of children participating in the study. *: These 4146 consisted of 1327 atopic (32%) and 2819 non-atopic mothers (68%), which is a good reflection of the general Dutch population.

transformed, to achieve a normal distribution for linear regression analyses and back-transformations were used to calculate geometric mean FeNO for the latent phenotypes of wheeze. Due to the stratified study design, all analyses were performed for the total study population as well as for the high-risk and low-risk children separately. The analyses were also stratified for allergic sensitization at 8 years, because specific IgE is an important determinant of FeNO.[4] Individual membership probabilities (each child gets a probability to belong to each phenotype) derived from LLCA were used as weight factors in the linear regression models to minimize the risk of misclassification of the wheezing phenotypes. Gender, recent symptoms of cold, steroid use, study region, education of the mother and exposure to environmental tobacco smoke were considered as potential confounders. Confounders were included in the models based on their association with wheezing phenotypes, or if they changed the effect estimate by more than 10%.

RESULTS

General characteristics of the study population

Baseline characteristics at 4 and 8 years are given in Table 5.1. Due to the study design, this population was overrepresented with high-risk children compared to the total PIAMA population. Compared to those invited for medical examination at 8 years (n = 1,554), children with complete data at 8 years had a higher level of maternal education and lower prevalence of prenatal smoking. However, differences were small, and with respect to other general characteristics the groups were similar. (Table 5.1) Finally, among children with complete data, the proportion of never/infrequent wheeze and late onset wheeze was somewhat lower than in the total population with complete data.

Associations of FeNO values at 4 years and phenotypes of wheeze

Phenotypes of wheeze were derived from yearly respiratory health assessments from birth up to 8 years. Geometric mean FeNO in never/infrequent wheeze was lower than in the remaining 4 phenotypes, but with considerable overlap. (Table 5.2) The adjusted geometric mean FeNO value (95% confidence interval [$CI_{95\%}$]) was highest in intermediate onset wheeze 12.1 ($CI_{95\%}$: 9.8-15.0) ppb compared to never/infrequent wheeze 8.8 ($CI_{95\%}$: 8.3-9.4) pbb ($p < 0.001$). The predictive value of FeNO at age 4 for wheezing phenotypes was limited, as a result of the large overlap of FeNO between groups.

Table 5.1. General characteristics of study population

	Invited for exam at 8 yrs (n=1,554)	Complete data at 4 yrs (n=462)	Complete data at 8 yrs (n=803)
Gender (% females)	49	49	51
Study region			
West	31	49	31
Middle	37	37	41
North	32	15	28
Maternal education level			
Low	22	19	19
Middle	42	42	41
High	36	39	40
Caesarean section	9	9	10
Atopic* mother	64	63	64
Atopic* father	32	32	32
Exposure to pets in 1st yr	48	45	46
Older siblings (% present)	48	49	47
Daycare attendance in 1st yr	24	27	26
Smoking during pregnancy	16	13	14
Exposure to environmental tobacco smoke†	16	15	15
Inhaled steroid use†	9	7	8
Doctors' diagnosis asthma†	12	12	11
Phenotypes of wheeze‡			
Never/infrequent wheeze	72.5	70.0	71.0
Early wheeze	17.1	20.3	18.3
Intermediate onset wheeze	3.4	3.9	3.8
Persistent wheeze	4.4	4.2	4.9
Late onset wheeze	2.5	1.5	2.0
Specific IgE inhalant allergen			
Positive for at least 1 of the 6 tested allergens§	30	29	28

*: Defined as a positive report of hayfever, allergy and/or asthma. †: Reported at the age of 8 years. ‡: Defined using longitudinal latent class analysis as previously described,[18] known in 1,165/1,554 children invited at 8 years. §: The following 6 inhalant allergens were tested for: house dust mite (Dermatophagoides pteronyssinus), cats, dogs, grass pollen (Dactylis glomerata), birch, Alternaria alternata.

Associations of FeNO values at 8 years among different phenotypes of wheeze

Also at 8, there was considerable overlap in FeNO between all phenotypes. FeNO was highest when wheeze started later in life and persisted longer. The adjusted geometric mean FeNO in transient early wheeze and never/infrequent wheeze was similar (9.0 vs. 9.6 ppb, NS). Compared to never/infrequent wheeze (9.6 ($CI_{95\%}$: 8.9-10.3) ppb), FeNO was significantly higher in intermediate onset wheeze: 17.4 ($CI_{95\%}$: 14.0-21.6) ppb (*p*

Table 5.2. Crude and adjusted geometric mean FeNO per phenotype of wheeze at 4 and 8 years

	FeNO at 4 years (n=462)			FeNO at 8 years (n=803)		
Phenotype of wheeze	n*	Crude	Adjusted ($CI_{95\%}$)†	n*	Crude	Adjusted ($CI_{95\%}$)†
Never/infrequent	324	9.0 (8.7; 9.3)	8.8 (8.3; 9.4)	570	10.2 (9.8; 10.5)	9.6 (8.9; 10.3)
Early	94	9.6 (8.6; 10.7)	9.2 (8.0; 10.6)	147	9.5 (8.5; 10.5)	9.0 (7.8; 10.4)
Intermediate onset	18	12.3 (10.2; 14.9)‡	12.1 (9.8; 15.0)‡	31	19.2 (16.0; 22.9)‡	17.4 (14.0; 21.6)‡
Persistent	20	10.1 (8.4; 12.1)	9.4 (7.7; 11.6)	40	13.2 (11.2; 15.6)‡	12.5 (10.2; 15.2)‡
Late onset	7	10.0 (7.6; 13.3)	9.3 (6.9; 12.6)	16	22.7 (17.9; 28.7)‡	20.7 (15.8; 27.1)‡

Analyses were weighted for the probability that a child belongs to a certain phenotype (individual posterior membership probabilities). *: Frequency (n) of each wheezing phenotype is calculated as the sum of the membership probability of all children for that phenotype. †: Geometric mean FeNO value (95% confidence interval) in ppb per phenotype of wheeze, adjusted for gender, recent symptoms of cold, study region, education of the mother and exposure to environmental tobacco smoke. ‡: $p < 0.05$ for difference in comparison with never/infrequent wheeze.

< 0.001), persistent wheeze: 12.5 ($CI_{95\%}$: 10.2-15.2) ppb ($p < 0.001$) and late onset wheeze: 20.7 ($CI_{95\%}$: 15.8-27.1) ppb ($p < 0.001$). The crude and adjusted geometric mean FeNO for each wheezing phenotype is given in Table 5.2 and the distributions are shown in Figure 5.2. None of the potential confounders changed the association between FeNO and phenotypes of wheeze to a meaningful degree. Steroids were

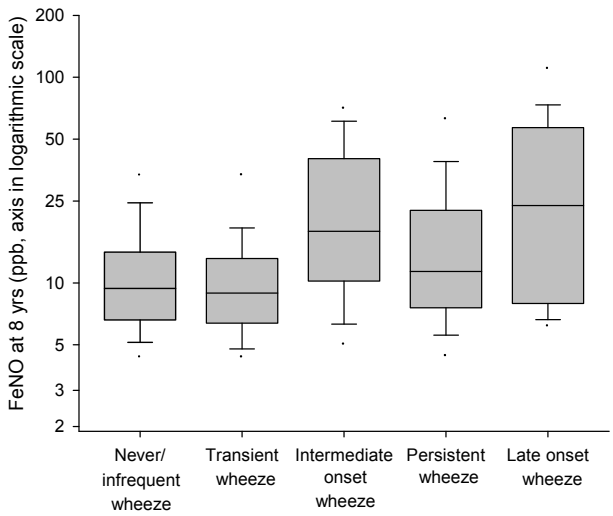

Figure 5.2. Box plots of FeNO at 8 years per phenotype of wheeze
Horizontal lines indicate the geometric mean FeNO. Upper/lower limits of the box, outer lines, and dots represent the 25th/75th, the 10th/90th, and the 5th/95th percentiles, respectively. Data are weighted for the probability that a child belongs to a certain phenotype (individual posterior membership probabilities).

mainly used in intermediate-, late onset- and persistent wheeze, and this might lead to underestimation of the differences between these phenotypes and the reference group. We think that this is not the case because exclusion of children using steroids at 8 years led to similar results. In order to investigate whether the association between FeNO and the wheezing phenotypes may be caused solely by the association between FeNO and wheeze at the age that FeNO was measured, we performed a sensitivity analyses adjusting for current wheeze at the age of 8 years. This adjustment did not alter the associations between

FeNO and phenotypes of wheeze

FeNO values and phenotypes of wheeze in atopic and non-atopic children We performed stratified analyses based on atopy of the mother. The associations between FeNO and phenotypes were similar. However, we found significant interaction with allergic sensitization of the children themselves at the age of 8 years. Among children with elevated specific IgE, FeNO at 8 years was low in never/infrequent wheeze and transient early wheeze, and significantly elevated in the remaining persistent phenotypes. In children without elevated specific IgE for inhalant allergens, FeNO at 8 years was not associated with phenotypes of wheeze. Because the numbers of children with low specific IgE were small for all wheezing phenotypes except never/infrequent, phenotypes with persistent symptoms (intermediate onset wheeze (n = 6), persistent wheeze (n = 16) and late onset wheeze (n = 4)) were combined for this analysis. Table 5.3 shows that, among atopic children, this combined phenotype had a significantly higher FeNO (21.8 ($CI_{95\%}$: 16.4-29.1) ppb) than never/infrequent wheeze (12.9 ($CI_{95\%}$: 11.1-14.9) ppb, $p <$ 0.001), while no such association was present in non-atopic children. This interaction is illustrated in Figure 5.3. The differences in FeNO between atopics and non-atopics

Table 5.3. Adjusted geometric mean FeNO at 8 years per phenotype of wheeze stratified for atopy

	Atopy - (n=474)		Atopy + (n=188)	
Phenotype of wheeze	n*	Adjusted ($CI_{95\%}$)†	n*	Adjusted ($CI_{95\%}$)†
Never/infrequent	359	8.1 (7.5; 8.7)	107	12.9 (11.1; 14.9)
Early	90	7.5 (6.5; 8.8)	34	11.0 (8.1; 14.9)
Combined persistent phenotypes‡	26	7.7 (6.3; 9.5)	47	21.8 (16.4; 29.1)§

Atopy of the child defined as specific IgE of ≥ 0.70 IU/ml for at least one inhalant allergen at the age of 8 years. Analyses were weighted for the probability that a child belongs to a certain phenotype (individual posterior membership probabilities). *: Frequency (n) of each wheezing phenotype is calculated as the sum of the membership probability of all children for that phenotype. †: Geometric mean FeNO value (95% confidence interval) (ppb) per phenotype of wheeze stratified for atopy at 8 years, adjusted for gender, recent symptoms of cold, study region, education of the mother, and exposure to environmental tobacco smoke. ‡: Three phenotypes with persistent symptoms (intermediate onset wheeze, persistent wheeze and late onset wheeze) were combined in this analysis. §: $p < 0.05$ for difference in comparison with never/infrequent wheeze.

were greatest for never/infrequent wheeze ($p < 0.001$) and the combined phenotype ($p < 0.001$), but also significant for transient early wheeze ($p < 0.001$). (Figure 5.3)

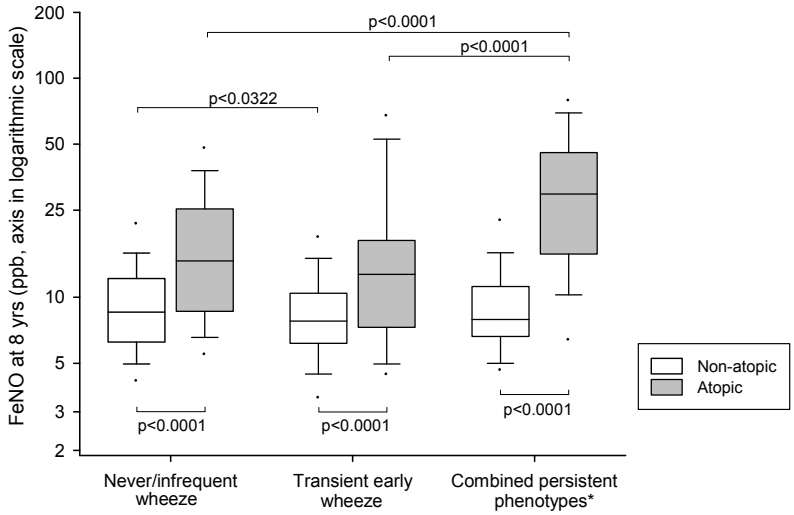

Figure 5.3. Box plots of FeNO at 8 years per phenotype of wheeze, stratified for atopy of the child defined as a specific IgE of ≥ 0.70 IU/ml for at least one of the tested inhalant allergens at the age of 8 years. Horizontal lines indicate the geometric mean FeNO. Upper/lower limits of the box, outer lines, and dots represent the 25th/75th, the 10th/90th, and the 5th/95th percentiles, respectively. Data are weighted for the probability that a child belongs to a certain phenotype (individual posterior membership probabilities). Only significant ($p < 0.05$) differences are depicted. *: Three phenotypes with persistent symptoms ('intermediate onset wheeze', 'persistent wheeze' and 'late onset wheeze') were combined in this analysis.

DISCUSSION

We examined FeNO at 4 and 8 years in relation to phenotypes of wheeze and atopy. We found that FeNO at 4 years was higher only in intermediate onset wheeze compared to never wheeze. The association between phenotypes of wheeze and FeNO measured at 8 years was much stronger. FeNO at 8 years was significantly higher in persistent phenotypes of wheeze, including intermediate onset, persistent and late onset wheeze, only among children with allergic sensitization at 8 years.

FeNO and wheezing phenotypes

Previous studies have reported increased FeNO in asthmatic children,[8,24-27] while others did not confirm this.[28-30] A possible explanation for these discrepancies is that 'asthma' comprises several phenotypes that may or may not share the same inflammatory

mechanisms.[12,17,31,32] Lumping all these phenotypes together as a single disease entity might hamper our efforts to understand the etiology and pathophysiology of specific phenotypes.[32,33] That there are such differences has been suggested in studies that assessed airway inflammation using either broncho-alveolar lavage (BAL)[34,35] or FeNO.[36] Only few studies investigated the association between FeNO and wheezing phenotypes in early childhood. Moeller *et al* defined phenotypes of wheeze on the basis of a clinical symptom score and found higher FeNO levels in preschool children with persistent wheeze.[37] This is in line with our present findings. Brussee *et al* found in the PIAMA study only a weak association of FeNO at 4 years with phenotypes of wheeze up to that age, with slightly higher FeNO levels in children who wheezed at the age of 4 years, compared to those who never wheezed.[11] In the present study there was only a weak association between phenotype and FeNO at 4 years. This could be explained by an increase in eosinophilic airway inflammation with age,[38] becoming detectable only after the age of 4 years. Alternatively, differences in the methods of FeNO measurement at 4 and 8 years might be involved, but we earlier found that these on- and offline methods give similar results,[39] so this seems unlikely. At 8 years, eosinophilic airway inflammation was present only in the phenotypes with persistent symptoms. Elevated FeNO levels were especially pronounced in the phenotypes with onset of wheezing after the age of 2 years.

FeNO and atopy

We found a strong association between atopy and FeNO. This is a consistent finding in earlier studies. [8,25,28,29] Some authors suggested that the association between asthma and FeNO may be entirely explained by atopy, suggesting that measuring FeNO is of limited use in order to assess whether a child has asthma.[40] The present study showed that the presence of atopy modified the association between wheezing phenotypes and FeNO, and this is in line with previous studies.[9,28,41,42] FeNO is a biomarker of atopy and the 'allergic' asthma phenotype rather than of any type of asthma. This may partly explain why FeNO-guided asthma treatment has proven to be of limited success in studies that did not take atopic status into account.[9] In the present study FeNO levels differed substantially between the wheezing phenotypes in atopic children at 8 years, which suggests that FeNO can be helpful to differentiate between wheezing phenotypes in atopic children. Furthermore it shows that all wheezing phenotypes occur in atopic and non-atopic children, but that the pathophysiology of wheeze in these two groups is probably different, with a predominance of eosinophilic inflammation selectively in atopic children with persistent phenotypes of wheeze. Other mechanisms may play a role in the pathophysiology of transient or persistent wheeze in non-atopic children. These include smaller airway caliber and/or neutrophilic airway inflammation.[34,35]

Strengths and limitations

A strong point of our study is that we assessed wheezing prospectively, without the possibility of recall bias, and that the wheezing phenotypes were discovered without pre-specified constraints in two large birth cohorts, using longitudinal latent class analysis.[17,18,43] Well-standardized FeNO measurements,[44] objective assessment of atopy at 8 years, and the large size of the PIAMA cohort with good follow-up allowed us to detect significant differences in FeNO in less common phenotypes, even after stratification for atopy.

A point of consideration in the interpretation of the data is that some children were using inhaled steroids while FeNO was measured, which potentially decreased FeNO.[4] However, any such effect seems limited because a sensitivity analysis after exclusion of steroid users did not change the results. In addition, one should take into account the possibility that the reported association between FeNO and phenotypes of wheeze solely depends on the relation between FeNO and current wheeze at the age of 8 years. This seems unlikely, because adjustment for current wheeze at 8 years did not alter any of the associations between FeNO and phenotypes of wheeze. Furthermore, the small sample size of FeNO measurements at the age of 4 years should be noted, especially in the late onset wheeze phenotype. In the stratified analyses of FeNO at 8 years we presented the 3 phenotypes with persistent symptoms as a combined phenotype, due to the low sample size of non-atopic wheezers. Importantly, results remained similar both in size and significance when these phenotypes were analyzed separately. Because FeNO was measured at 4 and 8 years, we cannot exclude that FeNO might have been increased in transient early wheeze before the age of 2, at the time when wheeze was still present.[41,45]

Implications

FeNO measured at 8 years differed between specific wheezing phenotypes in the first 8 years of life, but only among atopic children. Our findings at 8 years may help to understand the pathophysiology of different wheezing phenotypes in early childhood.

CONCLUSION

FeNO measured at 8 years differed between wheezing phenotypes, only in atopic children. Hence, we speculate that the pathophysiology of wheezing phenotypes differs between atopic and non-atopic children, with eosinophilic airway inflammation in atopic, but not in non-atopic children with persistent wheeze. Whether or not eosinophilic inflammation is indeed causally involved in the pathogenesis of wheeze remains to be shown.

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Part II

Early diagnosis and prognosis of childhood asthma

Chapter 6

Asthma symptoms and medication in the PIAMA birth cohort: evidence for under- and overtreatment

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ABSTRACT

Objective

Under- and overtreatment of asthma may be a serious problem especially in young children, but the evidence is scarce and no longitudinal data are available. Our aim was to longitudinally investigate whether inhaled medication use in young children was in agreement with asthma symptoms.

Methods

Data were used from the 'Prevention and Incidence of Asthma and Mite Allergy' birth cohort, consisting of 3,963 children born in the Netherlands. Between age 2 and 8 years children were followed-up using annual postal questionnaires. Age-specific prevalences of asthma symptoms were assessed and compared with reported use of inhaled bronchodilators and/or corticosteroids.

Results

The proportion of current wheeze decreased with age. About a third of 'current wheezers' did not use any inhaled medication during the years in which symptoms were reported. At 8 years 30% of children with reported 'severe current asthma symptoms' were not using inhaled corticosteroids. On the other hand, up to 50% of children with inhaled corticosteroids for at least 2 years did not report any wheezing during those 2 years.

Conclusions

The proportion of symptomatic children without appropriate treatment was substantial throughout childhood, even when parents reported prolonged or severe symptoms. Treatment of asymptomatic children with inhaled corticosteroids increased with age and accounted for up to a third of all inhaled steroid use at 8 years. These findings suggest that under- and overtreatment of asthma in children was common.

INTRODUCTION

Asthma symptoms are highly prevalent in children but an asthma diagnosis is difficult at young age when symptoms are nonspecific. Nonetheless it is important that asthmatic children are adequately treated with bronchodilators (BD) and inhaled corticosteroids (ICS) or leukotriene receptor antagonists, as these reduce symptoms and improve quality of life.[1-3] On the other hand, the majority of symptomatic preschool children will outgrow their symptoms during childhood.[4] Long-term prescription of ICS in children, without regular evaluation, may lead to overtreatment.

Indeed some previous studies have shown evidence that considerable undertreatment as well as overtreatment of asthma exists in children.[5-9] Only few studies have addressed under- and overtreatment in preschool children, in whom it is particularly difficult to make an accurate asthma diagnosis.[7-9] Importantly, previous studies were all cross-sectional in design and could not follow symptoms and medication use over multiple years.[5-13]

Using the longitudinal data from the 'Prevention and Incidence of Asthma and Mite Allergy' (PIAMA) birth cohort we investigated whether medication use corresponded with parental report of asthma symptoms in children aged 2 to 8 years. By inspecting medication use among children with asthma symptoms of different severity, type and duration we estimated the rate of undertreatment. Reports of asthma symptoms among long-term ICS users were assessed to evaluate possible overtreatment.

PATIENTS AND METHODS

Study population

Recruitment of the PIAMA cohort took place in 1996-1997 by means of a screening questionnaire distributed to 10,232 pregnant women visiting one of 52 prenatal clinics in The Netherlands.[14] Based on this screening 7,862 women were invited and 4,146 (53%) agreed and gave informed consent. Their children were followed-up annually for 8 years, using postal questionnaires for parental completion, based on the International Study of Asthma and Allergies in Childhood (ISAAC) core questionnaires.[15] A detailed description of the study has been published elsewhere.[16]

Definitions

Parents were asked about their child's airway symptoms every year. Data on shortness of breath and prescription of specific types of inhaled medication was collected only after the age of 2 years. Several symptom outcomes were defined at the ages of 3-8 years, each pertaining to the past 12 months. 'Current wheeze' was assessed with

the question: "Has your child had wheezing or whistling in the chest in the last 12 months?", and 'Current shortness of breath' with: "Has your child had tightness of the chest or shortness of breath in the last 12 months?" These symptoms were considered 'frequent' if parents reported 4 or more episodes in the past year. A range of asthma symptoms was inspected in relation to medication use. A child was considered to have 'Severe current asthma symptoms' when the parents reported wheezing in the past year AND an episode of wheeze or shortness of breath severe enough to limit speech to 2 words at the time and/or 4 or more episodes of wheeze/shortness of breath severe enough to keep the child awake at night. All national and international guidelines used during the study period advise maintenance therapy with ICS in children with severe asthma symptoms.[1,17] 'Doctors diagnosed current asthma' was assessed using the question: "Did a doctor ever diagnose asthma in your child AND has your child had asthma in the last 12 months?".

Information on asthma medication was collected by the question: "Has your child had medication for lung problems prescribed by a doctor in the last 12 months?" Parents were asked to specify the medication from a list that included generic and brand names of all medication registered for asthma treatment in the Netherlands. Inhaled asthma medication use was categorized annually in 4 groups: 1) no inhaled medication; 2) only BD; 3) only ICS; 4) BD and ICS. Cromones were not advised by national asthma guidelines and rarely prescribed during the study (<0.2%). Leukotriene receptor antagonists were introduced in Dutch guidelines in 2003 and only 7 children used montelukast at 8 years.[17] Therefore, only BD and ICS were considered in our analyses.

Statistical analyses

Proportions of children with inhaled medication and different types of asthma symptoms were compared at every year of age using SAS software version 9.1 (SAS Institute, Inc., Cary, NC). Longitudinal data allowed us to differentiate children with incidental wheezing, from those with wheezing in 2 consecutive years. Inhaled medication in asymptomatic children may imply either overtreatment, or a good treatment response. During the study period, guidelines recommended to reduce and eventually withdraw ICS in children who have become completely free of wheeze using 3-monthly intervals.[18,19] Therefore we considered ICS use in at least 2 consecutive years without a single reported episode of symptoms as suggestive for overtreatment. We performed complete case analyses for every time point, excluding children with missing data at a certain age from the analysis only at that specific age.

RESULTS

Study population

From the 4,146 included mothers, 183 (4.5%) dropped out before returning any postnatal questionnaires due to various reasons (e.g. stillbirth, language barrier, not interested, moved). General characteristics of the study population at baseline (n = 3,963) are given in Table 6.1. Of the baseline population 199 children (5.0%) were lost to follow-up before the age of 3 years. The remaining 3,764 returned at least 1 questionnaire between the age of 3-8 years and were included in the analyses. The age-specific response rates are given in Table 6.1: 3,033 children (77% of baseline) completed a questionnaire every single year from age 3-8. Children who were lost to follow-up before age 3 (n = 199) or missed at least 1 questionnaire thereafter (n = 731) were similar to children with complete data (n = 3,033) with respect to gender, pregnancy duration, delivery by caesarean section, proportion with older siblings, and allergy of the father. However, children with complete data had a higher level of parental education, were more likely to be ever breastfed, and less likely to be exposed to cigarette smoke during pregnancy (data not shown). The average response rate between 3-8 years was 88% from baseline and at 8 years 3,314 children (84%) returned a completed questionnaire.

Table 6.1. Population characteristics at baseline and annual response rate

Characteristic	n (%)
Baseline population	3,963 (100)
Gender (boy)	2,056 (52)
Preterm birth	190 (5)
Caesarean section	332 (9)
Older siblings (present)	1,994 (51)
Allergic mother	1,237 (31)
Allergic father	1,217 (31)
Breastfeeding ever given	3,200 (82)
Education mother (low)	894 (23)
Smoking in pregnancy	556 (14)
Questionnaire returned at age: (% from baseline)	
3 yrs	3,693 (92)
4 yrs	3,561 (90)
5 yrs	3,518 (89)
6 yrs	3,472 (88)
7 yrs	3,373 (85)
8 yrs	3,314 (84)
every age (3-8 yrs)	3,033 (77)

Prevalence of asthma symptoms and inhaled medication

Current wheezing at least once was reported by 15.8% at 3 years and showed a gradual decline to 5.9% at age 7. (Table 6.2) The prevalence of shortness of breath and markers for symptom severity such as speech limiting, nightly or frequent symptoms all decreased with age. The prevalence of 'doctors diagnosed current asthma' did not decrease and had the highest prevalence at 8 years. BD use decreased with age and ICS use varied between 7-8% between ages 2 and 8 years. When ICS were prescribed after the age of 2, they were often continued for multiple years. Figure 6.1 shows that at the age of 5, only 20% of children using ICS started ICS in that year. At 8 years over 70% of ICS users had been using ICS for at least 3 years.

Table 6.2. Age-specific prevalence of symptoms and medication use

	Asthma symptoms (%)					
	age 2-3 yrs	age 3-4 yrs	age 4-5 yrs	age 5-6 yrs	age 6-7 yrs	age 7-8 yrs
Wheezing						
Current wheezing	15.8	11.9	9.6	7.8	5.9	6.7
Frequency						
1-3 times per year	12.0	8.7	6.6	5.6	4.5	5.3
≥4 times per year	3.8	3.2	3.0	2.2	1.4	1.4
Shortness of breath						
Current shortness of breath	15.0	12.9	12.1	10.4	7.5	8.9
Frequency						
1-3 times per year	11.2	9.1	8.1	7.5	5.5	6.6
≥4 times per year	3.8	3.8	4.0	2.9	2.0	2.3
Speech limiting symptoms	3.3	2.5	1.9	1.5	1.1	0.9
Awake at night due to symptoms	13.0	11.4	10.1	7.6	5.5	5.1
Doctors diagnosed current asthma	4.2	4.2	4.0	3.9	2.8	4.6
	Inhaled medication (%)					
	age 2-3 yrs	age 3-4 yrs	age 4-5 yrs	age 5-6 yrs	age 6-7 yrs	age 7-8 yrs
Bronchodilators	12.9	10.5	10.7	9.3	7.8	7.5
Inhaled corticosteroids	8.0	7.8	8.1	7.7	6.8	6.5
Bronchodilators and inhaled corticosteroids	6.3	6.3	6.7	6.1	5.4	4.7

Undertreatment

Which proportion of symptomatic children indeed received asthma medication? Figure 6.2 depicts the age-specific proportions of inhaled medication among children with different definitions of asthma symptoms. Among current wheezers at 3 years 29% used both ICS and BD, and 43% did not use any inhaled medication that year. (Figure 6.2a) Medication use increased until 5 years. At 7 and 8 years over 30% of current wheezers did not use inhaled medication. Medication use among children who reported current

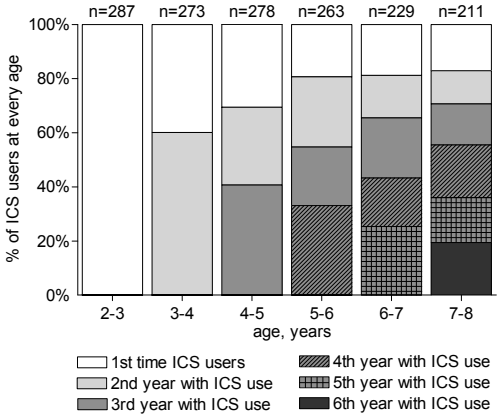

Figure 6.1. Number of years that inhaled corticosteroids were used after the age of 2 years
ICS: Inhaled corticosteroids. Numbers above bars represent the absolute number of children who used inhaled corticosteroids that year. Example: At age 3-4 yrs n=273 children were using ICS; 60% of those children were using ICS for the 2nd year in a row, 40% started using ICS at age 3-4 yrs for the 1st time. Note: only inhaled corticosteroid use after the age of 2 yrs was taken into account.

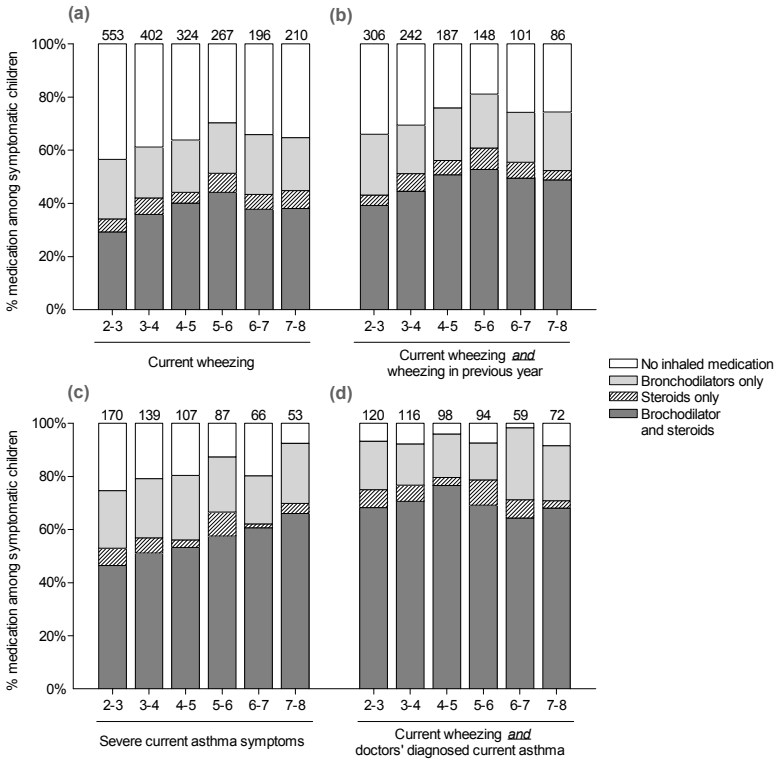

Figure 6.2. Proportion of inhaled medication among children with symptoms of different duration and severity
(a) Current wheezing; **(b)** Current wheezing and wheezing in previous year; **(c)** Severe current asthma symptoms; **(d)** Current wheezing and doctors' diagnosed current asthma. Numbers above bars represent the absolute number of children who reported symptoms that year.

wheezing in 2 consecutive years is given in Figure 6.2b. The proportion receiving medication was only slightly higher in this subgroup and at 8 years 30% was still not using any inhaled medication. The number of children with severe current asthma symptoms was smaller, n = 53 at 8 years. The proportion that used ICS in this group increased with age, but at 8 years 30% did not use ICS. (Figure 6.2c) Our data further indicate that over 90% of the untreated children with severe asthma symptoms consulted a medical doctor in the year of symptoms. Consequently, it is likely that parents indeed took their symptomatic child to the doctor, and that the majority of the undertreatment might have occurred because doctors did not adequately recognize or treat asthma symptoms. In children with a current doctors' diagnosis of asthma there appeared to be less undertreatment. Among children with doctors' diagnosed current asthma, more than 90% of the current wheezers was using inhaled medication, and 70-80% used ICS, irrespective of age (Figure 6.2d).

Overtreatment

Of all children who received ICS at the age of 3 years 67% reported current wheezing, the majority less than 4 episodes per year. (Figure 6.3a) Among ICS users the proportion of children who did not wheeze increased over time to well over 50% at age 7 and 8 years. Less than half of children with current ICS use reported 'doctors diagnosed current asthma'. This was still the case at 8 years, at which point the majority of ICS users had been using ICS for several years. (Figure 6.3b) Considering that ICS may not have been used for the entire year, e.g. as part of a medication trial to confirm an

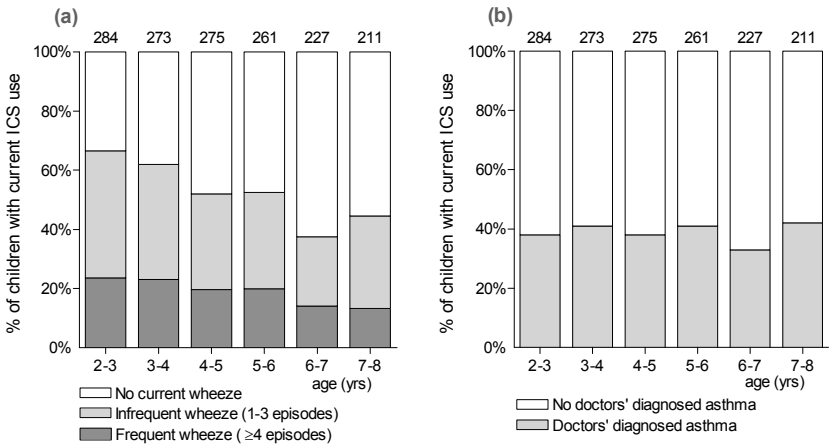

Figure 6.3. Proportion of asthma symptoms among children currently using inhaled corticosteroids **(a)** Current wheezing, with frequency; **(b)** Doctors' diagnosed asthma with symptoms. ICS: Inhaled corticosteroids. Numbers above bars represent the absolute number of children who reported current inhaled corticosteroid use that year.

asthma diagnosis,[18,19] we inspected symptoms in children with ICS over multiple years. Figure 6.4a depicts wheezing symptoms in the past 2 years among children who were using ICS in 2 consecutive years. At the age of 3-4 years, 22% of these children did not report a single episode of wheezing for the entire 2 year period. At the age of 8 years this percentage had increased to 50% (n = 72). This group represented 36% of all children using ICS and 2.3% of the entire study population at 8 years. Wheezing is not the only symptom for which ICS are prescribed, and therefore we also considered 'current shortness of breath'. Among children who reported ICS use at the age of 7 and 8, 26% did not report a single episode of wheezing or shortness of breath during that 2 year period. (Figure 6.4b) At least 80% of children with ICS but no symptoms had reported wheezing prior to ICS initiation (data not shown).

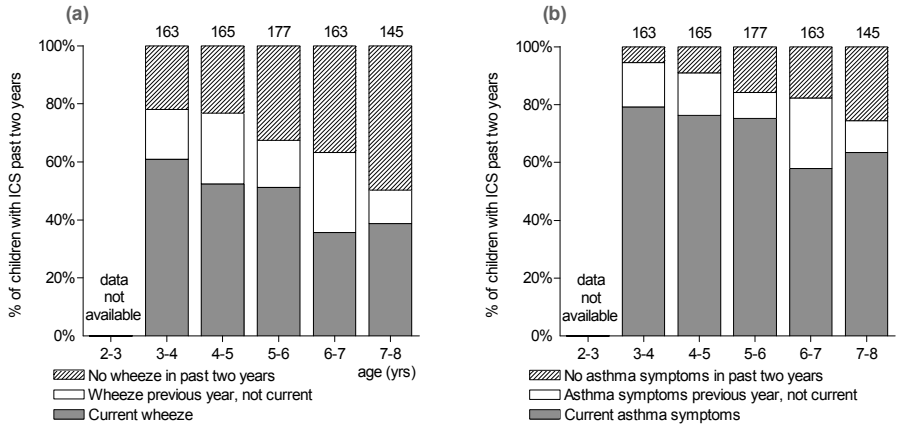

Figure 6.4. Reported symptoms among children using inhaled corticosteroids for at least 2 consecutive years
(a) Wheezing symptoms; **(b)** Asthma symptoms (wheezing and/or shortness of breath). ICS: Inhaled corticosteroids. Numbers above bars represent the absolute number of children who were using inhaled corticosteroids for at least 2 consecutive years.

DISCUSSION

Our data on 6 years longitudinal follow-up showed a substantial proportion of asthma undertreatment, even in children with prolonged or severe symptoms, which decreased only little with age. At the same time a considerable proportion of all children with long-term ICS use had not reported any asthma symptoms for the past 2 years, which is suggestive for overtreatment. This possible overtreatment increased with age and accounted for one third of all prescriptions at the age of 8 years.

Undertreatment

Evidence that inadequate treatment of asthma symptoms may be a serious problem has been shown in population surveys in Europe as well as the United States.[7,8] Most previous studies on the adequacy of asthma treatment in children included school children of 8 years and older.[5,6,10-13] Given the occurrence of different wheezing phenotypes at preschool age, findings from those studies cannot be generalized to younger children. In preschoolers, undertreatment may be more common than in older children.[7,9] A limitation in all previous studies was the cross-sectional design. Our longitudinal data allowed us to investigate the course of symptoms and medication use over multiple years. Considering that an asthma diagnosis becomes more reliable in older children, we expected that the rate of undertreatment would decrease with age. Indeed the proportion of current wheezers without any medication decreased slightly until the age of 5, but remained stable thereafter around 35%. We found that the dissociation between symptoms and treatment was not limited to short time periods. Among children who wheezed in 2 consecutive years, a third did not use any inhaled medication. In children with severe asthma symptoms the use of ICS has been strongly recommended by national and international asthma guidelines throughout the study period.[1,18-20] We found that ICS use increased gradually with age in children with severe symptoms, but that still more than one third of children with severe asthma symptoms did not use ICS at 8 yrs.

Undertreatment may be due to problems in diagnosing asthma in children, and this is supported by our finding that 40% of all current wheezers did not use any inhaled medication, compared to only less than 10% in those with a doctor's diagnosis of asthma. Therefore it seems important that guidelines not only focus on medical treatment, but also on the ability of doctors to recognize and correctly diagnose asthma in children.

Overtreatment

Little is known about the rates of overtreatment, as most surveys included only patients with documented asthma.[5,8,10,12,13] Treatment without apparent indication was documented in up to 23% of preschool children.[6,9] We found rates increasing to 50% at 8 years. Evidently, the absence of symptoms may be a desired effect of the medication indicating good treatment rather than overtreatment, and this can only be assessed using longitudinal data. According to guidelines at the time of the study period treatment with ICS should be evaluated with 3-month intervals, and stepped down and eventually stopped in asymptomatic patients, especially young children.[18,19] Hence, we reasoned that children with ICS during 2 consecutive years without a single episode of wheeze were probably overtreated. The proportion of children with ICS but no wheezing in the past 2 years gradually increased, and most of these children had reported asthma

symptoms prior to ICS initiation. This suggests that overtreatment resulted from failure to withdraw ICS while symptoms disappear.

A novel finding was the high proportion of children using ICS without 'doctors diagnosed current asthma', often for 2 years or more. Recent guidelines indeed promote ICS before an asthma diagnosis is made, but only as a short trial to support the diagnosis.[20,21] The prolonged mismatch between diagnosis and treatment confirms that doctors regularly prescribe ICS without the parents being aware of an asthma diagnosis.[22] This is unfortunate, as good asthma education improves management of childhood asthma,[23] and effective communication about the diagnosis and treatment helps to prevent both under- and overtreatment.

Strengths and limitations

This is the first longitudinal study to report on possible under- or overtreatment of asthma in children. Previous cross-sectional studies may have classified any mismatch between symptoms and medication use as incorrect treatment. Our longitudinal data allowed us to quantify the extent of avoidable under- or overtreatment during multiple years, taking into account reported symptoms and medication use in previous years. Secondly, considering the large sample size, high follow-up and annual response rates, our results may be extrapolated to the general Dutch population and, perhaps, other Western European countries.[8] If any bias resulted from the relatively few missing questionnaires, it most likely led to an underestimation of the mismatch between symptoms and treatment. Missing data occurred more frequently in children whose mother had a low level of education, which is a risk factor for incorrect treatment.[24]

An important limitation of our study is that symptoms were based on parental report. This limitation is shared with all previous surveys, and resembles clinical practice, where asthma management in children is also based on parental report. Likewise, medication use was recorded using parental report. To estimate the reliability of these data, we compared our findings with those in a subgroup of 777 children, for whom medication data were retrieved directly from the child's pharmacy. This comparison showed very high agreement (> 97%) between parental reported medication use and pharmacy prescription data, suggesting that parental self-report of medication use is an appropriate source of data to asses ICS use in children.[25] Finally, we are not informed about medication dosage and frequency. Therefore we cannot exclude that some symptomatic children received insufficient ICS doses.[6,8]

Implications

To avoid undertreatment, it may be worthwhile to actively screen children for asthma symptoms in primary and secondary care. Easy-to-use clinical scores to predict the likelihood of persistent asthma symptoms in preschool children may be helpful for this

purpose.[26,27] An improvement in symptoms could be caused by a treatment effect, but also by the natural course of asthma, especially if the improvement is prolonged.[28] If symptoms disappear completely, ICS should be gradually withdrawn in order to avoid overtreatment.[19] More recent guidelines emphasized the importance of this advice for preschool children.[29] The high percentage of ICS users without symptoms during multiple years implies that a critical reappraisal of the continuation of ICS may be worthwhile, especially in school aged children.

CONCLUSION

We found evidence for under- and overtreatment in children with asthma. Undertreatment decreased with age, and at 8 years one third of children with asthma symptoms did not receive appropriate medication, even if symptoms were prolonged or severe. At the same time up to one third of children chronically using ICS did not report any asthma symptoms for the past 2 years. These findings suggest that over- and undertreatment of asthma in children is common.

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Chapter 7

Predicting the long term prognosis of children with symptoms suggestive of asthma at preschool age

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ABSTRACT

Rationale

Clinicians have difficulty in diagnosing asthma in preschool children with suggestive symptoms.

Objective

We sought to develop a clinical asthma prediction score for preschool children who have asthma-like symptoms for the first time.

Methods

The Prevention and Incidence of Asthma and Mite Allergy birth cohort followed 3,963 children for 8 years. Between 0 to 4 years, 2,171 children (55%) reported 'wheezing' and/or 'coughing at night without a cold'. In these children possible predictor variables for asthma were assessed at the age respiratory symptoms were first reported. Asthma was defined as wheezing, inhaled steroid prescription, or a doctor's diagnosis of asthma at both age 7 and 8 years of age.

Results

Eleven percent of children with symptoms at 0 to 4 years had asthma at 7 to 8 years. Eight clinical parameters independently predicted asthma at 7 to 8 years of age: male gender, postterm delivery, parental education and inhaled medication, wheezing frequency, wheeze/dyspnea apart from colds, respiratory infections, and eczema. In 72% of the cases, the model accurately discriminated between asthmatic and nonasthmatic children. A clinical risk score was developed (range, 0-55 points). Symptomatic children with a score of less than 10 points had a 3% risk, whereas children with a score of 30 points or greater had a 42% risk of asthma.

Conclusion

A risk score based on 8 readily available clinical parameters at the time preschool children first reported asthma-like symptoms predicted the risk of asthma at 7 to 8 years of age.

INTRODUCTION

Most preschool children with symptoms suggestive of asthma do not have asthma and are unlikely to respond to asthma treatment.[1,2] If simple clinical parameters could identify children with early symptoms who have a high risk of asthma, this would allow for better targeting of secondary prevention measures and treatment for those children who are most likely to benefit. Also, doctors could be more restrictive when prescribing treatment to those who probably have transient conditions other than asthma.

A number of factors that may help to identify early-onset asthma have been reported, including a family history of atopy, eczema, and wheezing or wheezing frequency.[3-8] Only few studies predicted asthma in children at the age when symptoms occurred.[4,5] Recently, Frank *et al* followed 201 wheezing children and reported 'wheeze after exercise' and 'family history of atopy' as the only significant predictors of later wheeze.[5] However, since these data were not prospectively collected from birth, these authors were unable to exclusively select children with a first episode of symptoms. This would be important because prediction of asthma becomes clinically relevant as soon as suggestive symptoms first appear.

The aim of the present study was to develop a prediction rule for the development of asthma in children who have their first symptoms between the ages of 0 and 4 years, that could be useful in primary care settings, and that is based on data collected prospectively over a long period of time. For this purpose, we used data from the Prevention and Incidence of Asthma and Mite Allergy (PIAMA) birth cohort, in which 3,963 children were followed from birth up to the age of 8 years and in which adherence was very good. In children who had reported symptoms suggestive of asthma between the ages of 0 and 4 years, the combination of factors that best predicted asthma at the age of 7 to 8 years was assessed by using variables that can be easily obtained in practice. The resulting model was translated into a simple score that is feasible in a primary or secondary care setting to quantify the likelihood of development of asthma.

METHODS

Study population

Recruitment took place in 1996-1997. A screening questionnaire was distributed to 10,232 pregnant women who attended one of 52 prenatal clinics in the Netherlands. Based on this screening, 7,862 women (2,779 allergic and 5,083 nonallergic) were invited to participate in the study; 4,146 agreed and gave written informed consent. Questionnaires for parental completion, partly based on the International Study of Asthma and Allergies in Childhood core questionnaires,[9] were sent to the parents during pregnancy,

when the children were aged 3 and 12 months, and yearly thereafter up to the age of 8 years. The eligibility criterion for the present study was at least one positive response to the following questions in the annual questionnaires at age 1 to 4 years: "Has your child had wheezing or whistling in the chest in the last 12 months?", "Has your child had cough during the night, when he/she did not have a cold or a chest infection, in the last 12 months?", or both. Children who did not have these early symptoms were not eligible and were excluded from our analyses. Details of the study design have been published previously.[10] The PIAMA birth cohort study included an intervention part investigating the effect of mite-impermeable mattress covers. Of the population eligible for the current study, 242 (11%) children had used these covers. Because this had no effect on any of the investigated associations, these children were included in the final analyses. A screening questionnaire was used to select only atopic mothers for the intervention part. Importantly, the final study population (including the intervention and the natural history part) was not enriched for atopic mothers. See the Methods section and Figure E7.1 in the data supplement for further details on study design. The study protocol was approved by the medical ethics committees of the participating university hospitals.

Predictor variables

Based on previous literature and availability within our dataset we, selected 26 candidate predictor variables. All variables had to be easy to assess in general practice and not involve invasive tests or time-consuming measurements. Parental questionnaire data were used to asses all variables at the age of first report of symptoms. To mimic clinical practice, when composing the predictor variables, we only used information gathered up to the year of first presentation. Candidate predictor variables were family history of atopic diseases (6 variables tested), perinatal factors (8 variables tested), environmental factors (4 variables tested), and the child's pattern of symptoms (8 variables tested). (Table 7.1) Age at first symptoms was defined as a categorical variable. Parental report of number of serious infections per year was recorded in 3 classes (0, 1-2, and $\geq$3) and included in the model as a linear variable, with the assumption that the effect of frequent infections was 1.5 times as strong as the effect of infrequent infections. All remaining predictors were included as dichotomous variables. For both categorical variables "wheezing" and "pregnancy duration" 2 dichotomous dummy variables were included in the model. The symptom "dyspnea" was not asked for at 1 or 2 years because it was considered unreliable at this age. Therefore the variables "wheezing and/or dyspnea apart from colds" and "exercise-induced wheezing and/or dyspnea" were scored negative by default in children aged 1 or 2 years.

Outcome measures

The following 3 items of the questionnaires were used for the case definition of asthma: 1) at least one episode of wheezing; 2) inhaled steroids prescribed by a medical doctor; 3) doctor's diagnosis of asthma (a parental report of a doctor's diagnosis of asthma at any time and a parental report of asthma during the past 12 months). In the analyses children were only considered positive for asthma if they had one or more positive items at age 7 and one or more positive items at age 8 years. Thereby we aimed to select only children with clinically relevant chronic asthma symptoms.

Data analyses

Univariate associations between candidate predictor variables and the outcome of asthma were investigated using SAS software version 9.1 (SAS Institute Inc., Cary, NC). In the eligible study population the overall proportion of missing data on candidate predictor variables was very low (<1%) and follow-up to the age of 8 years was 95%. In a standard complete case analysis, subjects with one or more missing values (on any variable) are excluded from analysis. Missing data were imputed using the Multivariate Imputation by Chained Equations package in the statistical program R (version 2.5.1) to avoid any bias that may result from such a complete case analysis.[11,12] Multivariable analysis was performed by backward stepwise logistic regression, to select combinations of predictor variables. Likelihood ratio statistics were used as a criterion for selection. Candidate predictors were removed from the model if the corresponding *p*-value was greater than .01. Interaction terms between different candidate predictors were tested if effect modification was considered likely based on previous literature. Interaction with age and "atopic mother" was tested for all predictor variables. Calibration of the model was assessed both graphically and with the Hosmer-Lemeshow Chi-Square statistic. The final model's ability to discriminate between asthmatic and nonasthmatic children was assessed by using the C-index (equivalent of area under the curve in receiver operator characteristic curve). We considered a C-index of greater than 0.7 to be acceptable for a prognostic model.[13] Internal validation of the final model was performed by using the bootstrap resampling technique.[14,15] For 200 bootstrap samples, a new model was selected according to the above procedure and tested on the original sample. This enabled us to estimate the bias (i.e., optimism) caused by overfitting. Regression coefficients and the C-statistic of the final model were corrected for the calculated optimism. These regression coefficients were multiplied by a factor of 10 to create a simple risk score for use in clinical practice. Multivariate regression analyses and model validation were performed using R version 2.5.1 (Free Software Foundation, Inc., Boston, Mass).[16]

Table 7.1. General characteristics of study population of children who reported symptoms between age 0 to 4 years and univariate relationship with asthma at 7 to 8 yrs

	All children* (n=2,171) n (%)	Asthma at 7-8 yrs (n=240) n (%)	OR ($CI_{95\%}$) for asthma	*p*-value†
Age at onset of symptoms‡				
0-1 year	1,157 (53)	143 (60)	1.7 (1.0-2.9)	0.042
1-2 years	423 (19)	47 (20)	1.5 (0.9-2.7)	ns
2-3 years	366 (17)	33 (14)	1.2 (0.7-2.2)	ns
3-4 years (=reference)	225 (10)	17 (7)	ref.	-
Reported symptom(s)				
Cough at night§	1,314 (62)	137 (57)	0.8 (0.6-1.1)	ns
Wheezing				
No wheezing (=reference)	941 (43)	62 (26)	ref.	-
1-3 times/year	860 (40)	98 (41)	1.8 (1.3-2.5)	<0.001
>3 times/year	370 (17)	80 (33)	3.9 (2.7-5.6)	<0.001
Family history				
Parental asthma	389 (18)	67 (28)	1.9 (1.4-2.6)	<0.001
Parental inhaled medication	372 (17)	77 (32)	2.6 (1.9-3.5)	<0.001
Parental hay fever	885 (41)	129 (54)	1.8 (1.4-2.4)	<0.001
Sibling asthma	129 (6)	22 (9)	1.7 (1.1-2.8)	0.025
Sibling hay fever	57 (3)	10 (4)	1.7 (0.9-3.5)	ns
Sibling eczema	402 (19)	51 (21)	1.2 (0.9-1.7)	ns
Perinatal factors				
Male gender	1,196 (55)	162 (68)	1.8 (1.4-2.4)	<0.001
Caesarean section	191 (9)	31 (13)	1.6 (1.1-2.5)	0.018
Low birth weight (<2500 g)	94 (4)	15 (6)	1.6 (0.9-2.8)	ns
Breastfeeding ever	1,793 (83)	190 (79)	0.8 (0.6-1.1)	ns
Older sibling(s)	1,109 (51)	134 (56)	1.2 (0.9-1.6)	ns
Medium/low education parent(s)¶	1,659 (76)	199 (83)	1.6 (1.1-2.2)	0.013
Smoking during pregnancy‖	325 (15)	41 (17)	0.8 (0.6-1.2)	ns
Delivery				
Term (=reference)	1,948 (90)	204 (85)	ref.	-
Preterm (<37 wks)	118 (5)	15 (6)	1.2 (0.7-2.2)	ns
Post-term (>42 wks)	105 (5)	21 (9)	2.1 (1.3-3.5)	0.003
Environmental factors at age of report of symptoms				
Daycare	819 (38)	71 (30)	0.7 (0.5-0.9)	0.006
Smoking in parental house**	632 (29)	67 (28)	0.9 (0.7-1.3)	ns
Pet ownership	1,221 (56)	128 (53)	0.9 (0.7-1.1)	ns
Avoidance pets (due to allergy)	606 (28)	98 (41)	1.9 (1.5-2.5)	<0.001
Child's other symptoms at age of report of symptoms				
Wheezing/dyspnea apart from colds§††	79 (4)	20 (8)	2.9 (1.7-4.9)	<0.001
Wheezing/dyspnea exercise induced††	68 (3)	14 (6)	2.2 (1.2-3.9)	0.013
Nose symptoms§	908 (42)	126 (53)	1.6 (1.2-2.1)	<0.001
Resp. tract infections‡‡				
No infections (=reference)	655 (30)	43 (18)	ref.	-
1-2 times/year	993 (46)	111 (46)	1.8 (1.2-2.6)	<0.001

	All children* (n=2,171) n (%)	Asthma at 7-8 yrs (n=240) n (%)	OR ($CI_{95\%}$) for asthma	*p*-value†
≥3 times/year	523 (24)	86 (36)	2.8 (1.9-4.1)	<0.001
Eczema				
Doctor's diagnosis ever	809 (37)	127 (53)	2.1 (1.6-2.7)	<0.001
Eczematous rash present§§	465 (21)	89 (37)	2.4 (1.8-3.2)	<0.001
Ever admitted for respiratory problem	65 (3)	17 (7)	3.0 (1.7-5.3)	<0.001

All numbers and odds ratios refer to imputed dataset (n = 2,171). *: All children in eligible study population reported wheezing and/or coughing at night at least once between the age of 0 and 4 years. †: Significance calculated using Wald Chi-square test. ‡: *p* for trend (Cochran-Armitage test) = 0.012. §: In period without a cold, flu or chest infection. ¶: Defined as an education below the level bachelor/masters (HBO/University in Dutch system) for at least one of the parents. ‖: Defined as any smoking, at last 4 weeks after estimated date of conception. **: Defined as smoking in the child's house more than once a week. ††: Data only available for children aged 3 or 4 years at first report of symptoms; at age 1 and 2 considered negative by default. ‡‡: Parental report of number of serious respiratory, throat, nose, and/or ear infections such as flu, infection of the throat, infection of the middle ear, sinusitis, bronchitis or pneumonia in the last 12 months. §§: Defined as parental report of itchy rash on at least one of following locations: folds of elbows, behind knees, around ears or eyes, in front of ankles.

RESULTS

Study population

Of the 4,146 included mothers, 183 (4.5%) dropped out before returning the first post-natal questionnaire for various reasons (e.g., stillbirth, language barrier, not interested, moved). Of the 3,963 remaining children, 2,171 (55%) reported an episode of wheezing, coughing at night, or both between the ages of 0 to 4 years. Because only those children with early symptoms were eligible, the population for the present analysis consisted of 2,171 children. In more than half of the children, first symptoms were reported before the age of 1 year. (Table 7.1) The symptom of coughing at night (62%) was slightly more prevalent than wheezing (57%). At the age of 8 years, complete data on the outcome measure was available from 1,921 children (88% of the eligible study population). Complete data on all predictor variables was available from 1,854 (85%) children. The percentage of missing data per individual predictor variable did not exceed 2%. Children with at least 1 missing value on predictor variables, outcome variables, or both (n = 486) were less likely than children with complete data (n = 1,685) to have parents who both had a high level of education (19 vs. 25%), to have been breastfed (79 vs. 84%) and to attend daycare (33 vs. 40%), and more likely to have been exposed to cigarette smoke during pregnancy (19 vs. 13%) and have had a low birth weight (8 vs. 4%). This shows that complete case analyses would refer to a study population that is not representative of the original study population. To match the distribution

of characteristics of the original dataset, these variables were taken into account in constructing the imputed dataset. Thus our results pertain to the imputed dataset, including all 2,171 children.

Outcome asthma

Two hundred and forty (11%) children had symptoms, medication, or both at the ages of both 7 and 8 years and were thus defined as cases. (Table 7.2) The prevalence of asthma symptoms and medication use at the age of 7 years was similar to the prevalence at the age of 8 years. Only 10% of the children with asthma (n = 25) exclusively reported wheezing; the majority (90%) also used medication, had a doctors' diagnosis of asthma, or both. Asthma prevalence in the imputed dataset was 1.5% higher than in the complete dataset. Considering all children initially included in the birth cohort (n = 3,963), 85% of the children with asthma at 7 to 8 years reported cough, wheeze, or both at some point in the first 4 years of life. Apparently, children without these early symptoms accounted for only 15% of asthma diagnoses at 7 to 8 years of age.

Table 7.2. Prevalence of features of asthma at age 7 and 8 years

Age	Wheezing at least once		Inhaled steroid prescriptions		Doctor's diagnosis of asthma*		Asthma† (positive on ≥ 1 item)	
	%	*(n)*	*%*	*(n)*	*%*	*(n)*	*%*	*(n)*
7 yrs	10.0	(216)	11.9	(259)	5.0	(108)	16.7	(362)
8 yrs	11.0	(239)	11.0	(238)	6.0	(130)	17.0	(370)
Both at 7 and 8 yrs	4.8	(105)	7.9	(172)	3.4	(73)	11.1	(240)

All numbers refer to imputed dataset (n = 2,171). *: Defined as a parental report of a doctor's diagnosis of asthma ever, in combination with parental report of asthma in the past 12 months. †: Only children with ≥ 1 item positive at age 7 and 8 years were considered to have asthma in subsequent analyses.

Univariate analysis

The risk of asthma decreased with increasing age of report of first symptoms. A family history of asthma, hay fever, or both was clearly associated with an increased risk of asthma, especially parental use of inhaled medication. Male gender, caesarean section, low level of parental education, relatively long pregnancy duration, and avoidance of pets because of allergies in the family were all significantly associated with a higher prevalence of asthma in this population of children with early symptoms. Children attending daycare at the age that symptoms were first reported had a lower prevalence of asthma at 7 to 8 years of age. Apart from the symptom "cough at night" (which was an inclusion criterion) all symptoms investigated were significantly positively associated with asthma. Results of all univariate analyses are given in Table 7.1.

Multivariate analysis

In the multivariate analysis male gender, postterm delivery, parental education, parental inhaled medication, wheezing frequency, wheezing/dyspnea apart from colds, respiratory tract infections, and eczema remained as independent predictors of asthma. (Table 7.3) Age did not significantly contribute nor did interaction terms of predictors with age. Odds ratios for individual predictors are reported before and after internal validation. The discriminative power of the model, represented by the C-index, was 0.72 after validation. A clinical prediction score was developed by assigning points for each predictor variable based on its regression coefficient. (Table 7.3) A score for each individual child was calculated using the equation shown in the legend of Table 7.3. The score ranged from 0 to 55, with a median of 15.5. Figure 7.1 depicts the predicted risk of asthma at 7 to 8 years for every prediction score value. The risk of asthma increases with an increasing prediction score. In Figure 7.2 the predicted risk is compared with the observed risk in our population per score category with 5-point intervals. Because only a minority (2%)

Table 7.3. Multivariate prediction model for asthma

Predictor variable	Before validation* OR (95% CI)	After validation* OR (95% CI)	Points in prediction score¶
1 Male gender	1.7 (1.3-2.3)	1.6 (1.2-2.1)	4.6
2 Post-term delivery	2.3 (1.3-4.0)	2.1 (1.2-3.6)	7.3
3 Medium/low education parent(s)	1.6 (1.1-2.3)	1.5 (1.1-2.2)	4.2
4 Inhalation medication parent(s)	2.4 (1.8-3.3)	2.2 (1.6-3.0)	7.7
5 Wheezing frequency†			
1-3 times/yr	1.6 (1.1-2.3)	1.5 (1.1-2.1)	4.2
≥ 4 times/yr	2.8 (1.9-4.2)	2.5 (1.7-3.6)	9.1
6 Wheezing/dyspnea apart from colds‡	2.3 (1.3-4.1)	2.0 (1.1-3.7)	7.1
7 Serious infections†			
1-2 times/yr	1.7 (1.3-2.2)	1.6 (1.2-2.1)	4.6
≥ 3 times/yr	2.2 (1.4-3.3)	2.0 (1.3-3.0)	6.9
8 Doctor's diagnosis of eczema and eczematous rash present	2.6 (1.9-3.5)	2.3 (1.7-3.1)	8.2 +
C-index§	0.743	0.717	55.1
Hosmer-Lemeshow (p-value)	0.822	0.538	

*: Internal validation performed using bootstrapping method. †: 'None' is reference category: 0 points. ‡: Only available for children aged 3 or 4 years, in younger children negative by default. §: Equivalent of 'Area Under Curve' in 'Receiver Operator Characteristic' (ROC) curve. ¶: Points calculated based on regression coefficients after validation (log(OR) multiplied by a factor 10). Individual risk score can be calculated using following equation: Individual score = 4.6 x Gender (boy=1, girl=0) + 7.3 x Postterm delivery (yes=1, no=0) + 4.2 x Medium/low education at least 1 parent (yes=1, no=0) + 7.7 x Inhalation medication by at least 1 parent (yes=1, no=0) + 4.2 x Infrequent wheezing (yes=1, no=0) + 9.1 x Frequent wheezing (yes=1, no=0) + 7.1 x Wheezing/dyspnea apart from colds (yes=1, no=0) + 4.6 x Infrequent serious infections (yes=1, no=0) + 6.9 x Frequent serious infections (yes=1, no=0) + 8.2 x Diagnosis eczema and rash present (yes=1, no=0)

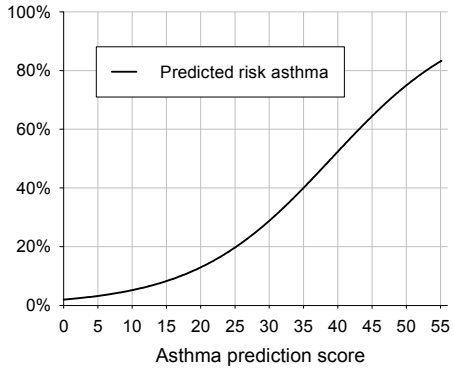

Figure 7.1. Predicted risk of asthma development at 7 to 8 years by prediction score

of children had a score of greater than 35 points, they were combined into a single category. Figure 7.2 shows that the model is well calibrated, which is confirmed by the Hosmer-Lemeshow test. (Table 7.3) Of the 561 children with a predictor score of less than 10, only 18 (3%) had asthma at the age of 7 to 8 years. In contrast, 50% (17/34) of the children with a score of at least 35 at first presentation of symptoms had developed asthma by the age of 7 to 8 years. Dichotomizing the prediction score at a certain cutoff

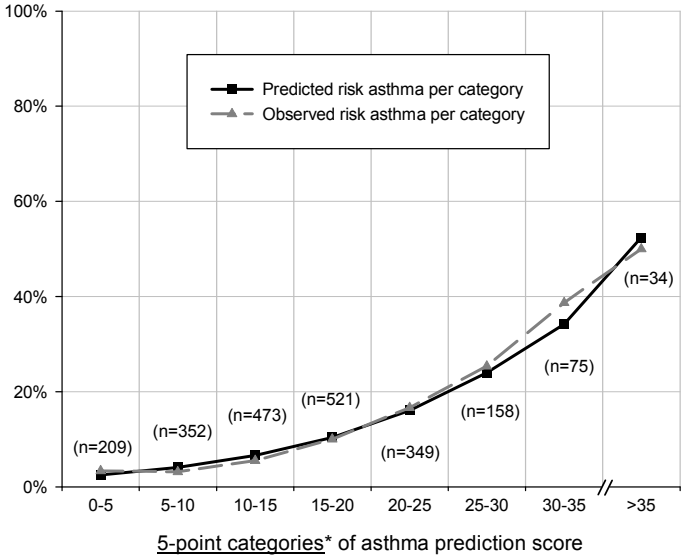

Figure 7.2. Predicted and observed risk of asthma development at 7 to 8 years per prediction score category
Number of children observed per category given between brackets. Expected risk estimated using the average score per 5 point category. *: Estimate for the merged category (≥35 points) is weighed by the number of children actually observed at each 5 point subcategory between 35-55.

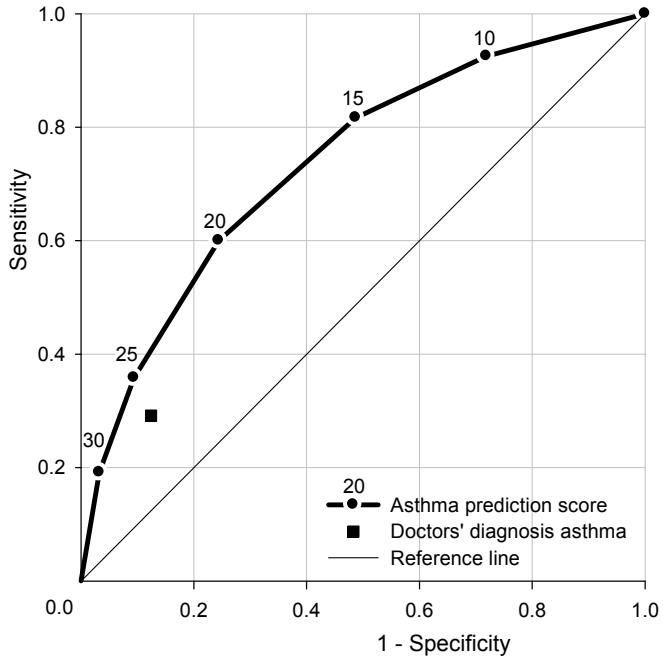

Figure 7.3. ROC-curve of categorised prediction score on outcome asthma at 7 to 8 years
Cutoff values of prediction scores are reported in curve (dots). Area under the ROC-curve (C-index) for the categorised score is 0.736 (before validation). Also sensitivity and specificity for doctor's diagnosis of asthma at the age when symptoms were first reported (between 0 to 4 yrs) are displayed (square).

value leads to a test with a dichotomous (positive/negative) result, which could be used to assist in clinical decisions in general practice. Sensitivity and specificity corresponding to ascending cutoff values are graphically displayed in a receiver operator characteristic curve. (Figure 7.3) Table 7.4 further reports the predictive values and percentages of children with positive scores at the different cutoff values. For comparison with current practice, the predictive value of a doctor's diagnosis of asthma in the year when symptoms were first reported is given in Figure 7.3 and Table 7.4. Thereby it should be noted that for a "doctor's diagnosis of asthma", all information that is available in clinical practice can be considered (e.g., allergy tests, lung function tests, medication trial, referral to secondary/tertiary medical centers). Our prediction rule merely uses simple items from the medical history and, with an appropriate cutoff, performs better. The proportion of children with asthma at 7 to 8 years of age accounted for by children at highest risk according to the prediction rule is plotted in Figure 7.4. It can be seen from this figure that half of the population with the highest prediction score account for 80% of all asthma cases at 7 to 8 years of age. By selecting a cutoff of 20 points, 28% of the population will have a positive score (high risk) and this group accounted for 60% of all asthma cases.

Table 7.4. Test characteristics at various cutoff points of prediction score for asthma at 7 to 8 yrs

Cutoff value	N positive test (%)*	Sensitivity	Specificity	Positive predictive value	Negative predictive value
≥ 10	1,610 (74)	93	28	14	97
≥ 15	1,137 (52)	82	51	17	96
≥ 20	616 (28)	60	76	23	94
≥ 25	268 (12)	36	91	32	92
≥ 30	109 (5)	19	97	42	91
≥ 35	34 (2)	7	99	50	90
Doctors' diagnosis asthma†	310 (14)	29	88	23	91

*: Number (%) of children within our dataset with a positive test result at different cutoff values. A 'positive test' is defined as an individual score equal to or above the chosen cutoff value. †: Defined as a parental report of a doctor's diagnosis of asthma at the age symptoms were first reported (between 0 to 4 yrs).

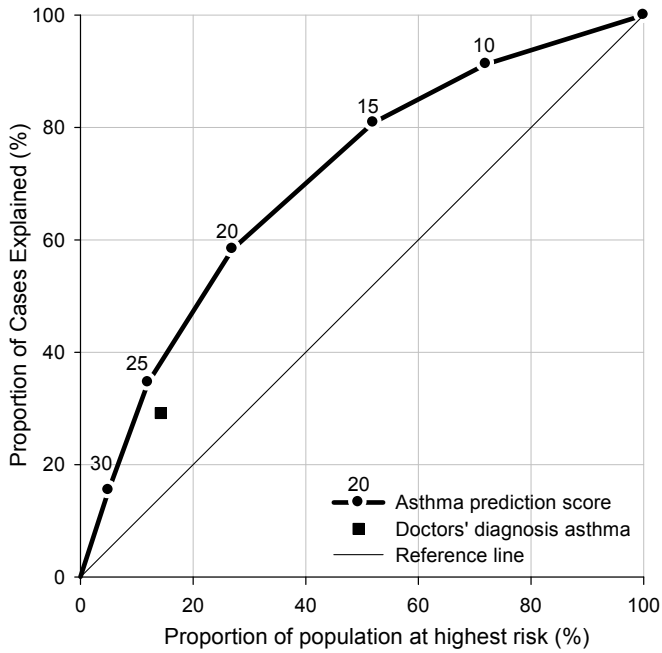

Figure 7.4. Proportion of children with asthma at 7 to 8 years explained by the proportion of children at highest risk, according to the prediction score
Cutoff values of prediction scores are reported in the curve (dots). Also data for doctor's diagnosis of asthma at the age when symptoms were first reported (between 0 to 4 yrs) are displayed (square).

DISCUSSION

In this study we developed a prediction rule for the risk of asthma at 8 years of age, to be used when preschool children present with symptoms suggestive of asthma for the first time. From a large prospective database, we identified 8 easily obtainable clinical parameters that best characterized the risk of asthma at 7 to 8 years of age.

Algorithms to predict development of asthma in children have been reported previously. However, differences in design and analysis should be considered when comparing the results. Although some authors developed a model for use in the general population,[3,17] others restricted their analysis to a selection of children with early symptom, such as wheeze or cough.[4-7] We restricted our analysis to children who reported "wheezing", "coughing at night without a cold or chest infection", or both. We based our selection on these symptoms because they are prevalent in young children and suggestive of childhood asthma.[18,19] Another important consideration is that most studies developed a prediction rule using all available information at a fixed age.[3,6,7,17] Consequently, these prediction rules are valid only for this fixed age rather than at the age of symptom onset. In a recent study by Frank *et al*, the investigators followed children of different ages with a parental report of wheeze.[5] In 201 children aged 0 to 4 years, they found only "wheeze after exercise" and "history of eczema or hayfever" to be predictive for wheezing after at least 6 years of follow-up. Interestingly, wheezing frequency or severity was not associated with symptom persistence. In their study data were not collected longitudinally from birth. Therefore the analysis could not be focused on the age of first symptoms, when prediction of asthma becomes relevant. Also, the fact that 51% of their original population was lost to follow-up might have been a source of bias.[5]

How do our findings compare with those from previous studies? A risk index developed in the Tucson Children's Respiratory Study also included wheezing frequency, eczema, and parental asthma as major criteria.[3] This study reported wheezing apart from colds as a minor risk factor. The German Multicentre Allergy Study birth cohort reported male gender and parental atopy as independent predictors for symptom persistence in children with wheeze before the age of 3 years,[7] and an algorithm to predict asthma from the Isle of Wight birth cohort included parental history of asthma and frequency of respiratory infections.[6] All these earlier studies also included invasive allergy tests in their algorithm. This was also done in a Dutch case-control study by Eysink *et al*.[4] For reasons of practical applicability, we included only clinical information that is easily obtainable in a primary care setting. In contrast to earlier studies, we found postterm delivery as a predictor of asthma development. The fact that we found this association does not necessarily imply a causal relationship. It may well be explained by correlated risk factors, such as meconium aspiration, caesarean section, birth order

and/or high birth weight.[20-23] High socioeconomic status was consistently linked with a lower prevalence of childhood asthma,[24,25] but this is the first study to report it as an independent predictor in a prognostic model.

The predictive power of our rule is not easily comparable to other studies because of differences in study design and objective. The test characteristics of the stringent risk index by Castro-Rodriguez *et al* to predict asthma at 8 years are similar to our prediction rule at a cutoff of 30 points.[3] (Table E7.1 data supplement) Considering that our rule predicted asthma in children at a much younger age (mean, 1.8 vs. 3 yrs), did not require any laboratory tests, and was corrected for over-optimism by bootstrapping, we propose that our prediction rule can have an added value over the currently available risk scores. Second, we compared the index by Castro-Rodriguez *et al* with our prediction score by applying both algorithms to our own dataset using only variables from clinical history (eosinophils not included) at the age symptoms were first reported. (Data supplement and Table E7.2 for further details.) We found that our prediction rule had better predictive power in symptomatic preschool children at the age of first symptoms.

A strong point of our study is its size. The PIAMA birth cohort is the largest birth cohort used to predict asthma in children. Further strengths are good adherence and little missing data. In combination with the multivariate imputation, this limits the risk of selection bias.[12,26,27] Unlike most previous studies, we performed internal validation by bootstrapping, which leads to more realistic estimates of regression coefficients and discriminative power of the model.[15] By closely following clinical practice in the selection of our eligible population and predictor variables, we have developed a clinical tool that can be used at the moment it is of most clinical relevance. Finally, since the original study population of the PIAMA birth cohort is a reflection of the general population, our results may be valid for the Netherlands and, perhaps, other Western countries.

There are some limitations that should be considered when interpreting our results. First, information on dyspnea in children younger than 3 years is lacking in our database and difficult to retrieve reliably from parental reports. We cannot exclude that such information would contribute to the prediction. Consequently, we may have underestimated the predictive value of this variable. Second, in the absence of a universal gold standard, the definition for asthma remains arbitrary. We decided to consider asthma as reported asthma symptoms, medication, or both at both ages 7 and 8 years because we aimed to predict chronic disease with clinical relevance. In a sensitivity analysis in which asthma was defined as doctor's diagnosis plus symptoms at 7 to 8 years of age, our prediction rule did not lose any of its predictive power (C-index 0.752).

We developed our prediction rule in a population selected on the basis of symptoms reported by questionnaire. Will these children indeed see a general practitioner? We propose that most parents will visit a general practitioner when their children have wheezing, nightly cough, or both, especially when their children are young (1-2 years of

age). This is supported by our data: 1,577 (73%) children received a doctor's diagnosis of a respiratory illness in the year of first symptoms. Clearly, these children consulted a medical doctor with respiratory symptoms, and the prediction rule could have been used. A validation of our prediction rule in this subgroup showed that it had a similar predictive value (C-index 0.726) and that all included questions remained significant predictors of later asthma at 7 to 8 years (p = 0.05, see data supplement and Table E7.3 for details). This strongly supports that our prediction rule is relevant and valid for children presenting in general practice. Before our prediction rule can be implemented, an external validation on a separate dataset should be performed.

Wheezing and coughing are very common in children.[19] More than half of the children in this birth cohort reported one of these symptoms before the age of 5 year, the majority already in the first year of life. Treatment with inhaled medication could be effective for some but is costly and can be troublesome to administer to young children, and there is little evidence base for the use of inhaled corticosteroids in viral induced intermittent wheeze.[2] It remains a challenge for the clinician in general practice to differentiate children with transient symptoms from children who will have chronic asthma, especially if only a few risk factors are present. This is illustrated by the fact that in our birth cohort only 10% of children who received asthma medication in the first year of life were still using this medication 3 years later.[28] A trial with asthma medication could be helpful to confirm the diagnosis,[29] but spontaneous improvement while taking steroids can easily be misinterpreted as a favourable treatment effect. Despite these difficulties, clinicians will need to make treatment decisions. Our prediction score may help to improve the accuracy of the prognosis and reduce undertreatment or overtreatment in individual children, simply by combining readily available clinical information. The positive predictive value of our prediction score was twice as high as that of reported doctor's diagnoses at the same age, with similar negative predictive values. Apparently, the combination of 8 simple clinical questions had a better predictive value than the complete workup that is current practice for preschool children. We therefore propose that our prediction score could serve as a practical and useful tool for clinicians who deal with preschool children presenting with symptoms suggestive of asthma, especially in a primary care setting. At a cutoff of 15 points, half of the children will have a negative test result. Because 96% of these children will not develop asthma, this might well justify a conservative approach with reassurance of the parents. Increasing cutoff points could be used to imply different clinical actions, such as a specific IgE test, start of a trial with inhaled medication, or even referral to secondary care. Optimal cutoff points may vary between different settings, and an extensive analysis falls outside the scope of this article. Nonetheless, a reliable risk assessment in individual children is an essential step towards individualized asthma therapy.

In conclusion, we have developed an asthma prediction score that uses 8 easily obtainable clinical parameters and can be used in preschool children who present with asthma symptoms to predict asthma at the age of 7 to 8 years. Children with a low score (<10) at onset of symptoms had a risk of only a few per cent of having asthma at 8 years, whereas children scoring 30 or higher had a risk of greater than 40%. The score could potentially be helpful for prognosis and treatment decisions in these young children.

ACKNOWLEDGMENTS

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Supplement data Chapter 7

METHODS

All children participated in the Prevention and Incidence of Asthma and Mite Allergy (PIAMA) birth cohort study. This multicentre study was conducted in 3 different regions of The Netherlands: north (Groningen and surroundings), central (Bilthoven, Wageningen and surroundings) and southwest (Rotterdam and surroundings). Recruitment took place between March 1996 and May 1997 by means of a validated screening questionnaire,[E1] distributed by midwifes to 10,232 pregnant woman visiting one of 52 prenatal clinics. According to the results of this screening the women were divided in an allergic and a non-allergic group. Women with any of the following self-reported symptoms were defined as allergic: asthma, hay fever, house dust allergy, house dust mite allergy or pet allergy. Children of allergic women were defined as 'high-risk'. Based on the screening 7,862 women (2,779 allergic and 5,083 non-allergic) were invited to participate in the study, approximately 50% (n = 4,146) agreed and gave informed consent (1,327 allergic and 2,819 non-allergic). The PIAMA cohort includes an intervention part, studying the effect of impermeable mattress covers, and a natural history part. Only high-risk children could participate in the intervention study. As previously published, the intervention had no effect on the incidence of allergy or respiratory symptoms.[E2] Furthermore the intervention was no significant predictor of asthma in our study, and had no significant interaction with any of the candidate predictor variables tested. Therefore, we considered the complete PIAMA study population (both the intervention and the natural history part) in the present study. After birth, this study population consisted of 3963 children. Importantly, the proportion of children with an allergic mother (31%) in the PIAMA is very similar to the proportion in the general Dutch population. For the current study only children with a positive response to the questions in the annual questionnaires at age 1-4 years "Has your child had wheezing or whistling in the chest in the last 12 months" and/or "Has your child had cough during the night, when he/she did not have a cold or a chest infection, in the last 12 months" were eligible. Figure E7.1 presents how the final study population was arrived at in the form of a flowchart.

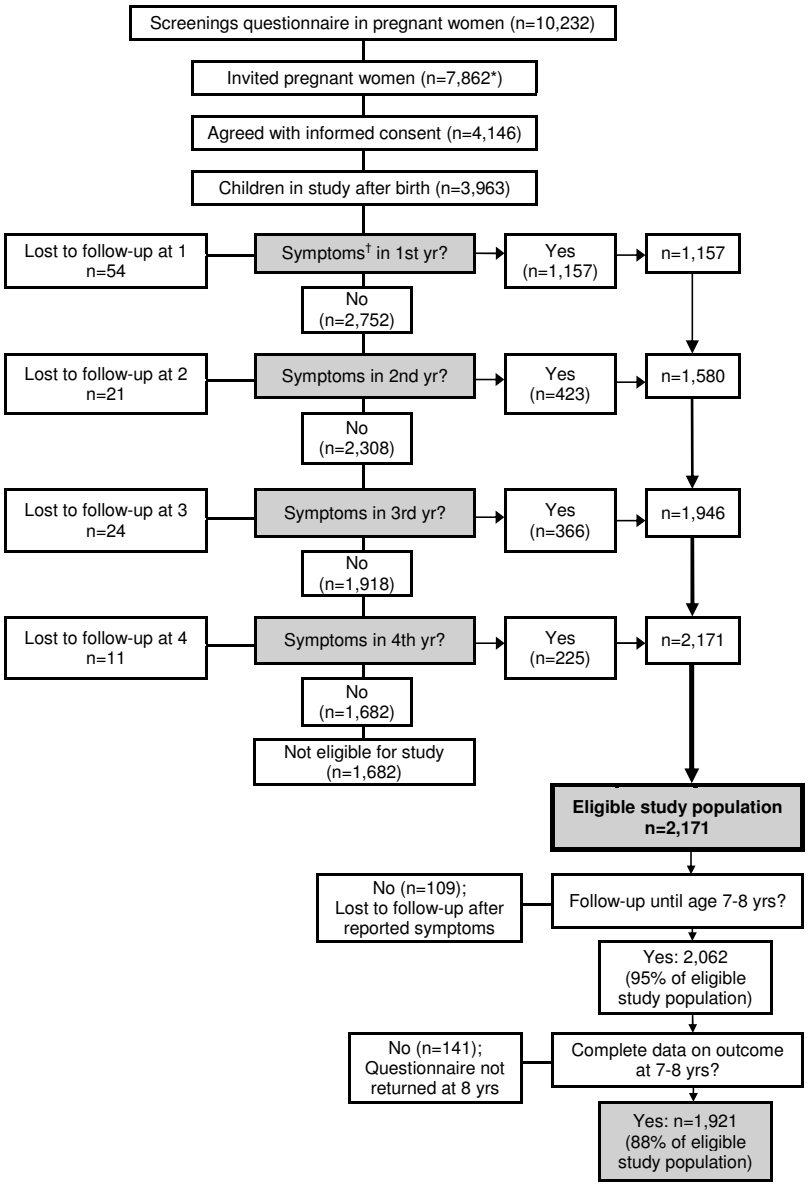

Figure E7.1. Flowchart of study population

*: 2,779 allergic and 5,083 non-allergic mothers were invited, as determined before initiation of the study based on a power calculation. Of the 4,146 women that were included in the study, the proportion of allergic women (31%) was very similar to that in the general Dutch population. †: Symptoms were defined as a positive response to the questions "Has your child had wheezing or whistling in the chest in the last 12 months" and/or "Has your child had cough during the night, when he/she did not have a cold or a chest infection, in the last 12 months". Reasons for loss to follow-up included: not motivated, illness of child, repeated non-response, moved, personal reasons.

RESULTS

Comparison with previous literature

Due to differences in study design and objective the predictive power of our rule is not easily comparable to other studies. Castro *et al* developed a risk index for the prediction of asthma in preschool children.[E3] An important difference between their study and ours is that they predicted asthma in children at the age of three years, regardless of the age of onset of symptoms. Secondly, their algorithm included blood eosinophils. We choose not to include any invasive and/or time consuming tests because our aim was to develop a prediction rule with which a doctor can instantly calculate the risk of later asthma. Despite these differences it is worthwhile to compare the yield of their algorithm with our risk score. Table E7.1 gives the test characteristics of the loose and stringent risk index as reported by Castro *et al* to predict asthma at 8 years.[E3] The same information is given for our prediction rule at a cut-off of 21 and 30 points (which leads to comparable groups of children with a positive test). It can be seen that sensitivity and specificity as well as predictive values are similar at both cut-off values. In summary we found similar test characteristics of the two algorithms despite the following differences: (1) the majority of children in our study was 1 year old at the time of prediction (compared to 3 years in paper by Castro *et al*); (2) our prediction rule did not include any invasive tests (such as blood eosinophils); (3) unlike the study by Castro *et al*, our study adjusted for over-optimism by using an internal bootstrap validation; (4) our study used a more strict definition of asthma. Therefore we propose that our prediction rule can have an added value over this currently available risk score.

Table E7.1. Comparison of predictive yield of PIAMA risk with predictive yield as reported by Castro *et al.*

Risk score	N positive test (%)	Sensitivity	Specificity	Positive predictive value	Negative predictive value
PIAMA score at cut-off ≥ 21	506 (23)	54	80	25	93
Loose index Castro et al.*	180 (23)	51	82	29	91
PIAMA score at cut-off ≥ 30	109 (5)	19	97	42	91
Stringent index Castro et al.*	39 (5)	16	97	44	88

*: Taken from table 2, 5 and 6 of paper by Castro *et al*.[E3] Note that prediction was performed at the fixed age of 3 years, that a lab test (eosinophilia) was included, and that sensitivity, specificity and negative predictive values were calculated using the complete study population (including children who never wheezed).

Secondly we aimed to assess whether the new clinical predictor variables that were found are of added value over those previously reported. We did this by comparing the variables included in the paper by Castro *et al* with those in our prediction rule, using our own dataset. Apart from eosinophil counts we annually collected all the information

included in the risk index by Castro: infrequent wheezing, frequent wheezing, doctors' diagnosis of parental asthma, doctors' diagnosis of eczema, allergic rhinitis, and wheezing in absence of a respiratory infection. Using information up to the year of first symptoms and following their algorithm (without considering the factor eosinophilia) we calculated the risk of later asthma for every child within our own dataset. This resulted in three categories: (1) low risk of asthma: 'negative loose index'; (2) intermediate risk of asthma: 'positive loose index but negative strict index'; and (3) high risk of asthma: 'positive strict index'. We then compared the risk score according to Castro with our own internally validated PIAMA risk score. For this purpose, we also categorized our continuous PIAMA risk score in three identical categories (at similar cut-off points as the Castro risk score). The C-index of the Castro risk score was 0.628 vs. the PIAMA risk score 0.696. This implies that while using easily obtained variables from clinical history, the PIAMA score could better discriminate between asthmatic and non-asthmatic children. Importantly, by restricting the PIAMA score to only 3 categories predictive information was lost. In clinical practice a continuous score could provide a more accurate individual risk assessment. Indeed, with the PIAMA score as a continuous variable the C-index rises to 0.740. Detailed results of this analysis including sensitivity, specificity and predictive values of the two risk scores are given in Table E7.2. This table illustrates that all indices are higher in our prediction score than in the risk index by Castro *et al*. Apparently the use of the additional variables (gender, pregnancy duration, infection frequency, parental education, and parental inhaled medication rather than parental asthma) is of added value in the prediction of later asthma.

Table E7.2. Comparison of risk index by Castro *et al* with the PIAMA risk score categorised to three equal categories within the PIAMA dataset (using only variables from clinical history)

	Castro risk score*					PIAMA risk score				
	n (%)	Sens	Spec	PPV	NPV	n (%)	Sens	Spec	PPV	NPV
Low risk†	1,573 (72)					1,555 (72)				
Intermediate risk‡	402 (19)	49	75	20	92	416 (19)	60	76	23	94
High risk§	196 (9)	20	92	25	90	196 (9)	31	93	37	92
C-index¶			0.628					0.696		
Hosmer-Lemeshow			0.190					0.856		

*The algorithm as described by Castro et al. [E3] was followed, without considering the variable of eosinophilia. Only information up to the age of first presentation was used. †: Low risk of asthma: 'negative loose index'. ‡: Intermediate risk of asthma: 'positive loose index but negative strict index'. §: High risk of asthma: 'positive strict index'. ¶: Equivalent of 'Area Under Curve' in 'Receiver Operator Characteristic' (ROC) curve.

External validity of prediction rule

We developed our prediction rule in a population selected on the basis of symptoms reported by questionnaire. Will these children indeed see a general practitioner? We

assume that most parent will present at a general practitioner when their child has respiratory symptoms such as wheezing or coughing at night without a cold, especially if the child is very young. (The majority of children were under the age of 1 year when they had first symptoms.) This is an important assumption, because a general practitioner will only be able to use a prediction rule if children seek medical attention. To check the assumption and thereby the external validity of our results we performed a validation of our prediction rule in a subgroup of children who reported a doctors' diagnosis of a respiratory illness in the same year as they had symptoms. Of these children we can be certain that they presented at a medical doctor, with respiratory symptoms. The following doctors' diagnosed respiratory illnesses were included: bronchitis, pneumonia, pertussis, flu, throat infection, croup, pseudo-croup, otitis media and asthma. Of the 2,171 children 1,577 (73%) reported at least one of these diagnoses in the year of first symptoms. The percentage of children with asthma at 7-8 years in this subgroup (11.7%) did not differ significantly from the percentage in the total population (11.0%).

Table E7.3 gives the results of the developed model in the total population and in the subgroup with a doctors' diagnosis of a respiratory illness. It can be seen from the table that the regression coefficients of all variables remained more or less stable. The level of significance of some factors decreased. This can be partly explained by the weaker associations with asthma at 7-8, but also partly by the decrease in sample size (n =

Table E7.3. Validation of multivariate model in subgroup of children with a doctors' diagnosis of respiratory illness in the year of first symptoms (n = 1,577)

Predictor variable	Total population (n=2,171) OR (95% CI)	*p* - value	Validation in subgroup (n=1,577) OR (95% CI)	*p* - value
1 Male gender	1.7 (1.3-2.3)	<0.001	1.9 (1.3-2.6)	<0.001
2 Post-term delivery	2.3 (1.3-4.0)	0.003	2.0 (1.1-3.7)	0.039
3 Medium/low education parent(s)	1.6 (1.1-2.3)	0.010	1.5 (1.0-2.2)	0.057
4 Inhalation medication parent(s)	2.4 (1.8-3.3)	<0.001	2.3 (1.6-3.2)	<0.001
5 Wheezing frequency*				
1-3 times/yr	1.6 (1.1-2.3)	0.007	1.4 (1.0-2.1)	0.078
≥ 4 times/yr	2.8 (1.9-4.2)	<0.001	2.7 (1.8-4.1)	<0.001
6 Wheezing/dyspnoea apart from colds†	2.3 (1.3-4.1)	0.007	2.7 (1.4-5.0)	0.002
7 Serious infections*				
1-2 times/yr	1.7 (1.3-2.2)	<0.001	1.5 (1.1-2.0)	0.028
≥ 3 times/yr	2.2 (1.4-3.3)	<0.001	1.8 (1.2-2.7)	0.028
8 Doctor's diagnosis of eczema and eczematous rash present	2.6 (1.9-3.5)	<0.001	2.2 (1.6-3.2)	<0.001
C-index‡	0.743	-	0.726	-
Hosmer-Lemeshow (p-value)	0.822	-	0.805	-

*: 'None' is reference category: 0 points. †: Only available for children aged 3 or 4 years, in younger children negative by default. ‡: Equivalent of 'Area Under Curve' in 'Receiver Operator Characteristic' (ROC) curve.

1,577 vs. n = 2,171). Most factors remained strong and very significant independent predictors of asthma at 7-8. The overall predictive power of the model decreases slightly from 0.743 to 0.726.

In conclusion, we find that all variables in our original prediction rule were also significant predictors in this subpopulation of children with a doctors diagnosis of a respiratory illness. This strongly supports our assumption that the prediction rule that was developed in this study has good validity in children who present at a medical doctor in the year of first symptoms.

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Chapter 8

Prediction of asthma in symptomatic preschool children using exhaled nitric oxide, Rint and specific IgE

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ABSTRACT

Rationale

For clinicians it remains very difficult to predict whether preschool children with symptoms suggestive of asthma will develop asthma in later childhood.

Objective

To investigate whether measurement of fraction of exhaled nitric oxide (FeNO), interrupter resistance (Rint), or specific immunoglobulin E (IgE) in 4-year-old children with suggestive symptoms can predict asthma symptoms up to age 8 years.

Methods

Children were recruited from the PIAMA birth cohort. All children with symptoms suggestive of asthma at age 3 or 4 years, who were invited for medical examination at age 4 (n = 848) were eligible. Associations of FeNO (n = 308), Rint (n = 482) and specific IgE (n = 380) at 4 years with wheezing and asthma at the ages of 5-8 years were assessed using repeated measurement analyses. The added predictive value of these objective tests was then investigated by including parameters for clinical history in the model.

Results

FeNO and specific IgE measured at 4 years were associated with wheezing and asthma at 8 years. Both tests remained significant predictors also after mutual adjustment and adjustment for clinical history: odds ratio on wheezing at 8 years for FeNO (^{10}log-scale, per interquartile range) 1.6 ($CI_{95\%}$:1.1-2.2) and for specific IgE 2.8 ($CI_{95\%}$: 1.9-4.1). Rint was significantly associated with wheezing at age 6, but not at 7 and 8 years.

Conclusions

In preschool children with symptoms suggestive of asthma, both FeNO and specific IgE measured at age 4, but not Rint, improved the prediction of asthma symptoms until the age of 8 years, independent of clinical history.

INTRODUCTION

In preschool children, asthma-like symptoms such as wheezing and coughing are highly prevalent.[1] Unfortunately, children with transient symptoms are not easily distinguished from those with persistent asthma on the basis of reported symptoms. Objective tests to support an asthma diagnosis include spirometry, to assess airway obstruction and reversibility; bronchoprovocation, to assess airway responsiveness; and sputum induction to measure airway inflammation, a hallmark of asthma.[2] These tests are difficult to perform in children under the age of 6 years since active cooperation is necessary.[3] Non-invasive measurement of the fraction of exhaled nitric oxide (FeNO) has received much interest due to its ability to reflect eosinophilic airway inflammation.[4,5] Several studies have shown elevated FeNO values in children with asthma or atopy.[6-12] Moeller *et al* showed that FeNO could distinguish between the different phenotypes of preschool wheezers.[13] However, prospective data on the prognostic value of FeNO in preschool children on later symptoms are scarce. In clinical practice, measurement of FeNO would only be worthwhile if it offers an added predictive value over information that is already available, such as a standard clinical history. This has not been investigated so far. Interrupter resistance (Rint) measurement can be easily performed in young children and correlates with asthma symptoms.[14-16] Specific immunoglobulin E (IgE) to inhalant allergens in young children is associated with the risk of persistence of asthma symptoms.[17-20]

The aim of our study was to prospectively investigate whether FeNO, Rint and/or specific IgE at the age of 4 years in children with respiratory symptoms could be used to predict asthma symptoms up to the age of 8 years. We used data from the 'Prevention and Incidence of Asthma and Mite Allergy' (PIAMA) birth cohort.[21] Children were eligible if they reported 'wheeze', 'shortness of breath' or 'nightly cough without a cold' at the age of 3 or 4 years. FeNO, Rint and specific IgE were measured at the age of 4 years and children were followed until the age of 8 years. The added value of the tests to predict asthma symptoms until the age of 8 was calculated.

METHODS

Study population

Recruitment of the PIAMA cohort took place in 1996-1997; 7,862 pregnant women were invited to participate; 4146 women (53%) agreed (1,327 allergic and 2,819 non-allergic, based on a validated screening questionnaire).[22] Their children were followed up for 8 years, using questionnaires for parental completion, partly based on the International Study of Asthma and Allergies in Childhood (ISAAC) core questionnaires.[23] At 4 years,

all children with an allergic mother and a random sample of children with a non-allergic mother were invited for a medical examination, including measurement of FeNO, Rint and specific IgE levels (n = 1,808). A detailed description of the study design has been published previously.[21] The study protocol was approved by the medical ethics committees of the participating university hospitals. For the current study, we selected all children who were invited for the medical examination at 4 years and who reported at least one respiratory symptom suggestive of asthma (wheeze, shortness of breath, or cough at night without a cold) in the questionnaire at age 3 or 4 years (n = 848) (Figure 8.1).

FeNO, Rint and specific IgE measurement

FeNO at age 4 was measured offline according to European Respiratory Society (ERS)/ American Thoracic Society (ATS) guidelines.[24] For every child, duplicate exhaled air samples and an ambient air sample were collected in Mylar balloons, and analyzed using a chemiluminescence analyzer (Sievers NOA 280B, Boulder, Colorado, USA). FeNO was expressed in parts per billion (ppb). Rint was measured in kPa/l with MicroRint (MicroMedical, Rochester, Kent, UK) during expiration, with occlusion of the airway at peak expiratory flow. Median values for at least five acceptable measurements were calculated. Detailed descriptions of both measurement techniques were previously published.[8,16,25] Sensitization was defined as specific IgE of $\geq$ 0.70 IU/ml for at least one of 6 tested airborne allergens. To assess the added predictive value of these measurements over reported clinical history, three variables previously shown to have the highest predictive value on later symptoms were selected:[26] 1) Allergic mother (yes/no); 2) Doctors diagnosis of eczema ever until age 4 (yes/no); 3) Wheezing frequency during 4th year of life (no/ 1-3 times per year/ $\geq$ 4 times per year). Mothers were considered 'allergic' if they reported any of the following items: asthma, hay fever, house dust allergy, house dust mite allergy, pet allergy.

Outcomes

Based on data from the annual questionnaires at age 5 to 8 years the following dichotomous outcomes were defined, each pertaining to the past 12 months:

- Wheezing: at least one episode
- Inhaled steroid prescription by a medical doctor
- Doctors diagnosis of asthma: a parental report of a doctors' diagnosis of asthma at any time AND a parental report of asthma symptoms during the past 12 months

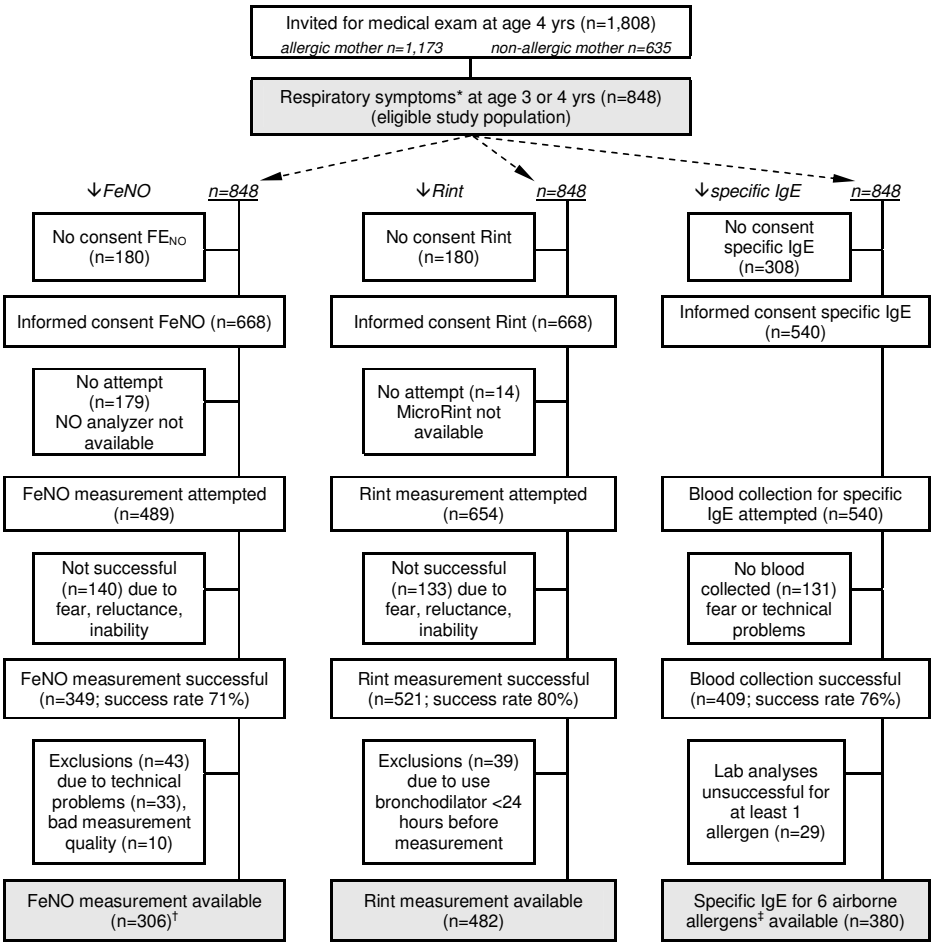

Figure 8.1. Flow chart of study population
All 848 eligible children enter each of the 3 arms of the flowchart. *: Respiratory symptoms were defined as a positive response to at least one of the following 3 questions: "Has your child had wheezing or whistling in the chest in the last 12 months?" and/or "Has your child had tightness of the chest or shortness of breath in the last 12 months?" and/or "Has your child had cough during the night, when he/she did not have a cold or a chest infection, in the last 12 months?" †: Out of 306 children with available FeNO 51 used steroids at the age of 4 years; these children were not excluded from analysis. ‡: The following 6 airborne allergens were tested for: House dust mite (Dermatophagoides pteronyssinus), cat, dog, grass pollen (Dactylis glomerata), birch, and Alternaria alternata.

Statistical analysis

Univariate associations between FeNO, Rint and specific IgE measurements and respiratory outcomes were investigated with logistic regression. FeNO values had a right-skewed distribution; a normal distribution was achieved after ^{10}log-transformation.

Generalized estimating equations (GEE) were used to take into account the correlation between repeatedly measured outcomes in the same individual. Variables for clinical history were included in the model to examine additional predictive value the tests over clinical history. The study design was stratified for allergy of the mother and any interaction with this variable was investigated. After a complete case analysis, missing data were multiple times imputed to check for any bias that could result from complete case analysis.[27,28] The 'Multivariate Imputation by Chained Equations' (MICE) procedure in the statistical program R version 2.6.2 was used to create 10 imputed datasets (n = 848).[29,30] Results were combined using PROC MIANALYZE in SAS 9.1 (SAS Institute, Inc., Cary, NC). Finally, a model was built including clinical history, specific IgE and FeNO, which allowed us to estimate the added predictive value of FeNO over the combination of clinical history and specific IgE. The data supplement provides a detailed description of study design, analysis and the multiple imputation procedure (Table E8.1).

RESULTS

Study population

At the age of 4 years 1,808 children were invited for medical examination, of whom 848 (46%) reported at least one of the following symptoms at 3 or 4 years: wheeze (n = 379), shortness of breath (n = 385), or nightly cough (n = 592). The eligible study population therefore consisted of 848 children. An acceptable FeNO measurement was obtained from 306, Rint from 482, and specific IgE from 380 children. (Figure 8.1) We compared children with and without available values for these measurements separately, with respect to baseline characteristics and symptoms at 8 years. (Table 8.1) Children with available FeNO values (n = 306) were similar to children without (n = 542), the only difference being educational level of the mother. Children with Rint values (n = 482) were also more likely to have a mother with higher education than children without Rint measured (n = 366), and had significantly less symptoms at the age of 8. Children with IgE data were more likely to be boys, to have an allergic mother, older siblings and to be exposed to cigarette smoke in utero, compared with children with missing IgE data. With respect to symptoms at baseline and at 8 years there were no major differences. The distribution of clinical symptoms and objective tests that were used to predict later asthma symptoms is given in Table 8.2.

Predictive value of FeNO

A higher FeNO at 4 years was associated with significantly more wheezing and steroid use between the ages of 5 and 8 years. The association remained more or less stable over the 4 year follow-up period, with an odds ratio per interquartile range (IQR) in-

Table 8.1. General characteristics of eligible study population

Baseline characteristics	Eligible study population (N=848)		FeNO (N=306)	Rint (N=482)	Specific IgE (N=380)
	n/N	%	%	%	%
Gender (female)	391/848	46	47	47	41†
Allergic mother	567/848	67	66	66	72†
Allergic father	289/846	34	35	35	35
Maternal education level					
Low	195/832	23	21	20	23
Middle	366/832	44	41	43	43
High	271/832	33	38*	36*	34
Caesarean section	86/834	10	10	11	7†
Pets in the house (at birth)	473/844	56	54	55	54
Older sibling present	422/846	50	49	52	59†
Smoking during pregnancy‡	147/831	18	18	17	21*
Smoking in house (at age 4)§	191/798	24	26	23	24
Symptoms at baseline (age 3-4)					
Wheeze	379/848	45	47	45	48
Shortness of breath	385/848	45	48	46	47
Cough at night¶	592/848	70	73	70	69
Symptoms at age 8 yrs					
Wheeze	104/730	14	13	13	14
Inhaled steroid use	109/722	15	15	12†	16
Doctors' diagnosis asthma‖	55/700	8	8	6†	9

For each variable children with and without available data were compared. Significance of differences was tested using a Chi-square test. *:p <0.05; †:p < 0.01. ‡: Positive if mother reported smoking at least 4 weeks after estimated date of conception. §: Smoking in the child's house more than once a week. ¶: In period without a cold, flu or chest infection. ‖: Defined as a parental report of a doctor's diagnosis of asthma ever, in combination with parental report of symptoms in the past 12 months.

Table 8.2. Variables used to predict asthma symptoms

Clinical symptoms and objective tests		
Clinical symptoms	*n/N*	*%*
Allergic mother	567/848	67
Wheezing in 4th year of life		
Infrequent (1-3 times/yr)	179/848	21
Frequent (>3 times/yr)	64/848	8
Doctors' diagnosis eczema	388/848	46
Objective tests	*Median (range)*	*IQR†*
FeNO in ppb* (n=306)	9.05 2.40 - 45.9	6.30
^{10}log-transformed FeNO	0.96 0.38 - 1.66	0.29
Rint in kPa.L^{1} (n=482)	0.93 0.14 - 1.99	0.32
	n/N	%
Positive specific IgE	102/380	27

*: Particles per billion; †: Inter quartile range.

crease in ^{10}log(FeNO) on 'wheezing' at 8 years of 2.1 (95% confidence interval [$CI_{95\%}$]: 1.3-3.3). (Figure 8.2) A significant association with doctors' diagnosis of asthma was present only at age 7. Inclusion of three variables for clinical history in the model had very little effect on the odds ratios: FeNO remained significantly associated with later symptoms, independent of clinical history. Multiple imputed data analyses produced similar associations. (Data supplement, Figure E8.1) Fifty-one children used steroids at the age of 4, which may have influenced FeNO measurement. In a sensitivity analysis, exclusion of these children from the analysis led to slightly stronger associations with the outcomes 'doctors diagnosis of asthma' and 'steroid use'. A charcoal NO scrubber was used while collecting exhaled air to eliminate an influence of ambient NO on FeNO measurements, but some association between FeNO and ambient air NO remained. Adjustment of our models for ambient NO did not change our results.

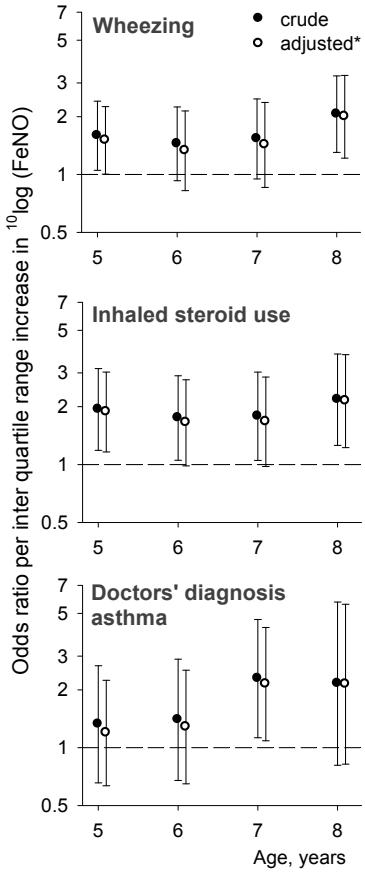

Figure 8.2. Predictive value of FeNO measured at 4 yrs on all outcomes at 5-8 yrs
Black dots are crude odds ratios for FeNO (per IQR increase in ^{10}log(FeNO)). *: Open dots are adjusted for clinical history (allergic mother/ doctors' diagnosis of eczema/ wheezing at age 4).

Predictive value of Rint

Rint values at age 4 were associated with symptoms at age 5 and 6. The association decreased after the age of 6 and was no longer significant at 8 years. (Figure 8.3) Adjustment for clinical history further decreased the association between Rint and all outcomes. The adjusted odds ratio per IQR increase Rint for wheezing at 8 years was 1.1 ($CI_{95\%}$: 0.7-1.6). Multiple imputed analyses did not change this. (Data supplement, Figure E8.2)

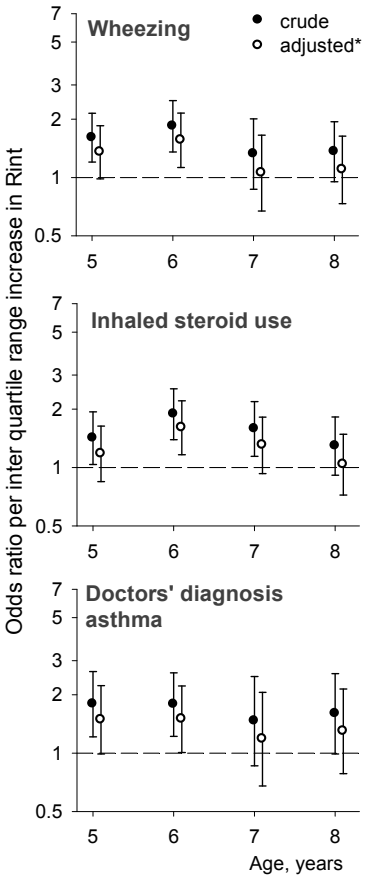

Figure 8.3. Predictive value of Rint measured at 4 yrs on all outcomes at 5-8 yrs
Black dots are crude odds ratios for Rint (per IQR increase). *: Open dots are adjusted for clinical history (allergic mother/ doctors' diagnosis of eczema/ wheezing at age 4).

Predictive value of specific IgE

A positive specific IgE to any airborne allergen had a strong association with later symptoms. (Figure 8.4) The association remained stable over the 4 year follow-up. At age 8 the odds ratio for 'wheezing' was 6.6; $CI_{95\%}$: 3.5-12.7. When clinical history was taken into account the odds ratios decreased considerably, but the associations remained strongly significant for all outcomes (OR 4.3; $CI_{95\%}$: 2.1-9.1 for wheezing at 8 years).

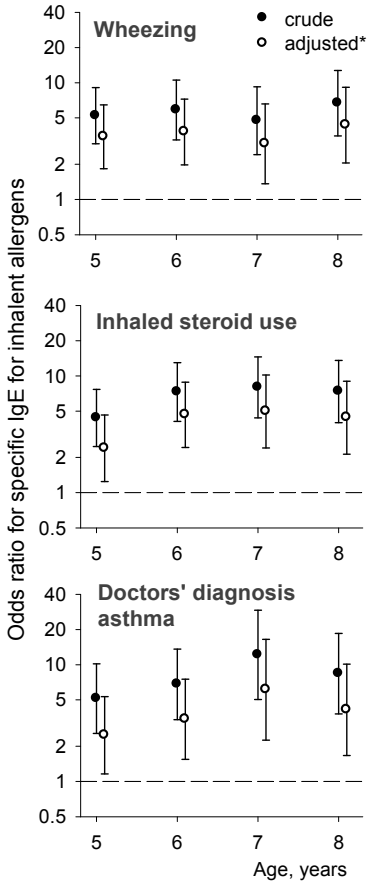

Figure 8.4. Predictive value of specific IgE measured 4 yrs on all outcomes at 5-8 yrs
Black dots are crude odds ratios for specific IgE. *: Open dots are adjusted for clinical history (allergic mother/ doctors' diagnosis of eczema/ wheezing at age 4).

Odds ratios for specific IgE decreased considerably on all outcomes in the multiple imputed analysis. (Data supplement, Figure E8.3) Since such analyses are less likely to be influenced by selection bias, these estimates are probably more reliable. In the imputed analyses specific IgE remained the strongest predictor of later symptoms, even when clinical history was taken into account.

Combination of clinical history, FeNO and specific IgE

These results imply that both FeNO and specific IgE, independent of clinical history at 4, significantly improve the prediction of asthma symptoms up to the age of 8 years. The question remains whether FeNO still has an added value, when clinical history and specific IgE are already known. We investigated this in a combined model which included FeNO, specific IgE and clinical history. Complete data on all variables was available from 185 children; the eligible population comprised 848 children. Analyses on the outcome 'wheezing' at age 8 were performed in both populations. (Table 8.3) Estimates

Table 8.3. Predictive value FeNO and specific IgE on 'wheezing' (with mutual adjustment and adjustment for clinical history)*

	FeNO†	Specific IgE
n = 185 FeNO and IgE both available	1.65 ($CI_{95\%}$: 0.94 - 2.89) p=0.078	2.86 ($CI_{95\%}$: 1.38 - 5.91) p=0.005
n = 848 Total eligible population	1.57 ($CI_{95\%}$: 1.10 - 2.23) p=0.016	2.78 ($CI_{95\%}$: 1.90 - 4.07) p<0.001

Odds ratios (95% confidence interval) of FeNO and specific IgE on the outcome of 'wheezing', using complete case data (n=185) and multiple imputed data (n=848). *: Odds ratios for FeNO and specific IgE were mutually adjusted for each other, and for three variables on clinical history (allergic mother/ doctors diagnosis of eczema/ wheezing at age 4). †: Odds ratios for FeNO are calculated per interquartile range increase in ^{10}log-transformed FeNO.

for FeNO and specific IgE were constant in both analyses. The level of significance increased in the multiple imputed analysis, but even in the complete case analysis (n = 185) FeNO remained borderline significant. (Table 8.3) Importantly, we found no interaction between FeNO and either 'specific IgE' or 'allergic mother'. To visualize the clinical implications of our findings, the predicted probability of wheezing is plotted

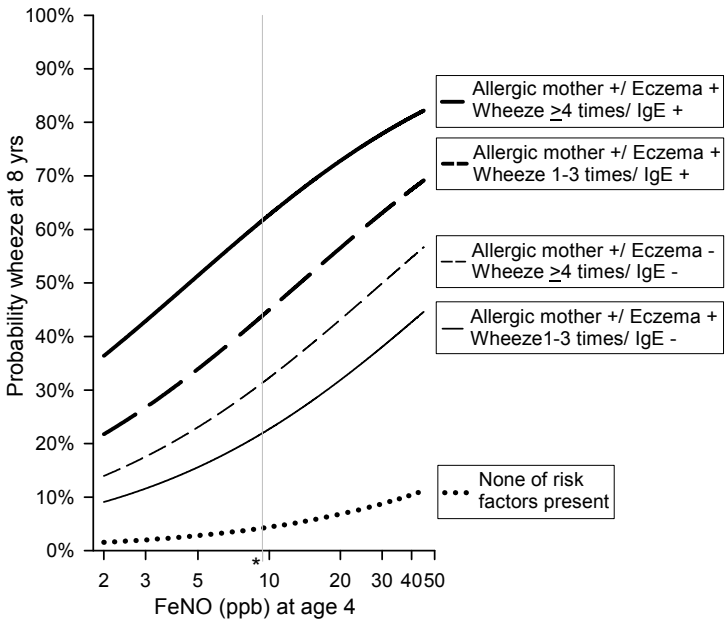

Figure 8.5. Predicted probability of 'wheezing' depending on FeNO at 4 yrs
Predicted probability for 'wheezing' at age 8 is modelled for FeNO values (unit: parts per billion) measured at age 4, adjusted for specific IgE, allergic mother, doctors' diagnosis of eczema and wheezing frequency at age 4. Different lines represent children with different subsets of risk factors. Horizontal axis in log-scale. *: Vertical gray line represents geometrical mean FeNO in study population and can be used to determine the pre-test probability of wheeze at 8 for individual children.

against FeNO values measured at 4 yrs. (Figure 8.5) Different lines represent children with different subsets of risk factors. When all risk factors are absent, FeNO appears to be of limited clinical value, because the post-test probability remains low even at higher FeNO values. However, in children with an intermediate or high pre-test probability of later wheezing, a FeNO-test at the age of 4 could change the post-test probability to a degree that would be relevant in clinical practice. For example, in children with an allergic mother, eczema and specific IgE, but with infrequent wheezing at 4 (thick dashed line, Figure 8.5) risk of asthma symptoms at 8 ranged from 25-70% depending on FeNO measured at 4 years.

DISCUSSION

We show that in preschool children with symptoms suggestive of asthma, measurement of FeNO and specific IgE could predict later asthma symptoms, up to the age of 8. Both tests had an added predictive value, independent of each other and of clinical history.

Several studies investigating the association between FeNO and airway symptoms in children reported a higher FeNO in wheezing and asthmatic children.[6,7,9,11] Malmberg and colleagues even reported an 86% sensitivity and 92% specificity of FeNO to diagnose asthma, clearly superior to conventional bronchoprovocation testing and spirometry.[9] Importantly, these studies selected children already known to have asthma, and healthy controls. Such a case-control design is likely to cause selection bias with overestimation of a test's diagnostic performance.[31] In unselected groups of schoolchildren much lower diagnostic performance of FeNO was reported.[12,32] Age is important when comparing different studies, due to heterogeneous respiratory disorders and changing FeNO during childhood.[33,34] In children aged 4 years higher FeNO values were measured in recurrent wheezers and in children with doctors' diagnosed asthma, with considerable overlap.[8,10] Moeller *et al* showed that FeNO could be used to distinguish between different phenotypes of preschool wheezers, based on their clinical history up to the age of 4. However, FeNO will only have a clinical benefit if it offers additional information compared to clinical history.[13] Previous studies were cross-sectional, and very little is known about the predictive value of FeNO on later asthma in preschool children. We performed a 4 year follow-up and took the child's clinical history into account to assess the true additional predictive value of FeNO on later asthma. This reflects the clinical setting in which FeNO would be used. Our prospective analysis shows that FeNO measured at 4 years indeed improved prediction of asthma until the age of 8 years.

Cross-sectional studies have reported higher Rint in asthmatics compared to controls, with considerable overlap.[14,15,35] Previous analysis of our own data at age 4 showed

a higher Rint in persistent wheezers, compared to children who never or transiently wheezed.[25] The only prospective study on the predictive value of Rint measurements was performed in a cohort of 110 children diagnosed with asthma (aged 2-5 yrs) and found no association between Rint and asthma medication after 3 years follow-up.[36] Our study recruited children from a population-based cohort, based on reported respiratory symptoms, and results were similar: Rint at 4 did not discriminate between children with or without symptoms at 8 years. Although Rint was associated with symptoms up to 2 years after measurement, it was not useful in the long-term management of individual children. Thirty-nine children (7%) were excluded because bronchodilators were used within 24 hours prior to Rint measurement. When these children were included, the predictive power of Rint was even smaller. Nonetheless, selective exclusion of this group of high risk children may have decreased the power to detect an association of Rint with later symptoms.

Prospective studies showed that children with positive specific IgE for inhalant allergens were more likely to develop wheeze[19] and asthma[18] in childhood. Even when information from patient history is considered, specific IgE improved prediction of later asthma.[17,20] Our results show that the predictive value of specific IgE measured at 4 years remains high until the age of 8. Importantly, our data allowed us to investigate the independent added value of specific IgE and FeNO measurements at 4. Several authors reported raised FeNO levels in atopic rather than asthmatic children.[32,37] Consequently, it has been proposed that any predictive value of FeNO on asthma might be explained by its correlation with specific IgE.[38] Our combined analysis shows that even though FeNO and specific IgE are correlated, they both independently contributed to the prediction of later asthma symptoms.

Major strengths of this study are its longitudinal design and large sample size. In the selection of the eligible population and the statistical analysis we aimed to mimic the clinical setting. This enabled us to estimate the true added value of the tests and hence their clinical relevance.

Selection bias could have resulted from incomplete data. However, we found that children with complete data did not differ from those with incomplete data. Furthermore, a multiple imputed analysis including all eligible children led to similar results and did not change any of our conclusions (data supplement). Secondly, the PIAMA study is an unselected birth cohort, but due to overrepresentation of children with an allergic mother in the medical examination at 4, our eligible population included more children with an allergic mother. If the predictive value of FeNO, Rint or specific IgE differs between children with and without an allergic mother, this would limit generalizability of our findings. We consider this unlikely since there was no interaction between any of the tests and the variable 'allergic mother'. Hence, our findings may be generalizable to all children in the general population with asthma symptoms at 3-4 years. Although

steroid use is well known to decrease FeNO, we decided to include 51 (17%) children who used inhaled corticosteroids at 4. This may have led to some underestimation of the predictive power of FeNO, but we preferred this over selective exclusion of 51 high risk children. Finally, in order not to overestimate the added prognostic value of FeNO, Rint and specific IgE over a clinical history we tested which variables for clinical history were the best prognostic indicators for later symptoms, and added the 3 variables with the strongest association in our multivariable analysis.[39] Including a variable for wheezing phenotype (multi-trigger wheeze/ viral induced wheeze/ no wheeze) in our models did not decrease the predictive ability of any of the three investigated tests.

Do our findings have clinical relevance? In the management of wheezing preschool children a first step will always be to take a full medical history. Secondly, a specific IgE test has the highest predictive value for symptom persistence. FeNO measurement as a next step can significantly improve prediction of later asthma symptoms, especially in children with an intermediate pre-test risk.

In conclusion, we demonstrated that in preschool children with symptoms suggestive of asthma, both FeNO and specific IgE at age 4 predicted asthma symptoms until the age of 8 years independently, and independent of clinical history. Specific IgE had the highest predictive value, the added value of FeNO was limited. Nevertheless, in children with an intermediate or high risk, FeNO substantially changed the risk of later wheezing and asthma to a degree that may be relevant in clinical practice.

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Supplement data Chapter 8

METHODS

Recruitment of participants

All children participated in the Prevention and Incidence of Asthma and Mite Allergy (PIAMA) birth cohort study. This multicentre study was conducted in 3 different regions of The Netherlands: north (Groningen and surroundings), central (Bilthoven, Wageningen and surroundings) and southwest (Rotterdam and surroundings). Recruitment took place between March 1996 and May 1997 by means of a validated screening questionnaire,[E1] distributed by midwifes to 10,232 pregnant woman visiting one of 52 prenatal clinics. According to the results of this screening the women were divided in an allergic and a non-allergic group. Women with any of the following self-reported symptoms were defined as allergic: asthma, hay fever, house dust allergy, house dust mite allergy or pet allergy. Children of allergic women were defined as 'high-risk'. Based on the screening 7,862 women (2,779 allergic and 5,083 non-allergic) were invited to participate in the study, approximately 50% (n = 4,146) agreed and gave informed consent (1,327 allergic and 2,819 non-allergic). The PIAMA cohort includes an intervention part, studying the effect of impermeable mattress covers, and a natural history part. In the present study we considered children from both the intervention and the natural history part. As previously published, the intervention had no effect on the incidence of allergy or respiratory symptoms.[E2] Furthermore there was no association between the intervention and the measurements of FeNO, Rint or specific IgE. At 3 months and 4 years all high-risk children (n = 1,327) and a subsample of the low-risk children (n = 625) would be invited for a medical examination. (See Figure E2.1, data supplement chapter 2, for flow chart) Eligible for the present study were only children who were invited for the medical examination at 4 years (n = 1,808) and who, in addition, had a positive response to at least one of the following 3 questions in the questionnaire at age 3 or 4 years:

- Has your child had wheezing or whistling in the chest in the last 12 months?
- Has your child had tightness of the chest or shortness of breath in the last 12 months?
- Has your child had cough during the night, when he/she did not have a cold or a chest infection, in the last 12 months?

In total n = 848 children fulfilled these criteria and were included in the study population.

Table E8.1. Associations between predictor variables, outcomes, and covariates in children with missing and children with available data

	Predictor variables					
	FeNO at 4		Rint at 4		Specific IgE at 4	
Variables used in imputation	$N_{missing}$ = 542	$N_{available}$ = 308	$N_{missing}$ = 366	$N_{available}$ = 482	$N_{missing}$ = 447	$N_{available}$ = 380
	% usable cases	*r*(FeNO)	% usable cases	*r*(Rint)	% usable cases	*r*(Specific IgE)
Predictor variables						
FeNO at age 4	-	n.a.	16	0.12	39	0.22
Rint at age 4	44	0.12	-	n.a.	40	0.05
Specific IgE at age 4	26	0.22	27	0.05	-	n.a.
Model covariates (clinical history)						
Wheezing frequency at age 4	91	0.05	89	0.21	91	0.21
Atopy of mother	100	0.03	100	0.05	100	0.03
Eczema at age 4	95	0.08	94	0.02	95	0.16
Outcome variables (wheezing/ steroid use/ shortness of breath/ asthma diagnosis)						
At age 5	90	0.15	89	0.16	89	0.21
At age 6	88	0.14	86	0.21	86	0.22
At age 7	85	0.13	80	0.18	82	0.30
At age 8	84	0.20	81	0.11	82	0.25
Variables related to missingness of predictor variables*						
Maternal age	100	0.08	100	0.02	100	0.06
Maternal education	98	0.05	98	0.02	98	0.05
Paternal education	95	0.07	95	0.04	95	0.07
Degree of urbanization	100	0.03	100	0.08	100	0.01
Study region	100	0.11	100	0.09	100	0.01
Presence older siblings	100	0.01	100	0.02	100	0.17
Caesarian section	99	0.05	99	0.09	99	<0.01
Pregnancy duration	99	0.13	100	0.06	99	0.05
Eczema siblings	99	<0.01	99	0.04	99	0.10
Variables related to value of predictor variables*						
Avoidance of allergens indoors	99	0.02	99	0.03	99	0.15
Wheezing age 1	94	0.02	95	0.13	94	<0.01
Wheezing age 2	97	0.02	96	0.12	96	0.08
Wheezing age 3	97	0.02	96	0.13	98	0.08
Shortness of breath age 4	92	0.10	90	0.10	91	0.13
Steroid use age 4	89	0.08	87	0.14	88	0.09
Asthma diagnosis age 4	81	0.04	79	0.06	78	0.10
Blood eosinophils at age 4	37	0.23	25	0.09	0	n.a.
FeNO measured at age 8	45	0.16	39	0.01	42	0.28
Rint measured at age 8	57	<0.01	49	0.44	52	0.10
Specific IgE measured at age 8	56	0.12	47	0.08	46	0.39

*: Predictor variables are FeNO, Rint and Specific IgE measured at age 4.

Data collection

Questionnaires were sent to participating parents for self-completion at the 3rd trimester of pregnancy, 3 months after birth, at the age of 1 and yearly thereafter. The final questionnaire was sent at the age of 8. Information was collected on birth and socioeconomic characteristics; indoor environmental, lifestyle, and demographic factors; and on the child's health in at every age (e.g. allergies, eczema, respiratory symptoms, doctors' diagnoses and medication use).

FeNO Measurement at age 4

Exhaled FeNO was measured offline by the balloon method, according to European Respiratory Society/American Thoracic Society guidelines.[E3] Children were asked to take a deep breath through a charcoal NO-scrubber, and to exhale immediately into a collection device employing dynamic flow restriction, using a two-way valve. Exhalation flow was kept constant at 50 $mL \cdot s^{-1}$ over a pressure range of 5–20 cm H_2O. Mouth pressure was monitored during the measurement using a manometer. After discarding dead space air for 3–4 s, exhaled air was collected in a NO impermeable 150 ml Mylar balloon (Jurjen de Vries BV, Leeuwarden, the Netherlands).[E4] For every child, duplicate exhaled air samples and a sample of ambient air were collected. Balloons were sealed, stored and analysed within 8 h with a chemiluminescence analyser. Exhaled FeNO is expressed in particles nitric oxide per billion (ppb). In the western and middle areas of the Netherlands, a Sievers NOA 280 analyser (Boulder, CO, USA; sensitivity 0.5 ppb, detection range 0.5–500,000 ppb, sample flow 200 $ml \cdot min^{-1}$, sampling rate $4 \cdot s^{-1}$ (middle), $20 \cdot s^{-1}$ (western)), was used. In the northern area, an Ecophysics CLD 700 AL analyser (Ecophysics, Basel, Switzerland; sensitivity 1 ppb, detection range 0–50,000 ppb, sample flow rate 700 $ml \cdot min^{-1}$, data sampling rate $0.5 \cdot s^{-1}$) was used. A check for systematic differences between the different NO analysers was performed by measuring balloons filled with calibration gas on all analysers on the same day. This analysis revealed a good agreement, with no significant systematic differences between the analysers.[E5]

Rint measurement at age 4

Measurements were carried out using the MicroRint (Micro Medical Ltd, Rochester, UK), a portable device including a shutter and pneumotachograph, connected to a palmtop computer with an online display showing mouth pressure, time of shutter closure, Rint values, and the median value of all Rint data recorded during one session. Its software calculates Rint (expressed in $kPa.L^{1}$) using the back extrapolation technique to t = 15 ms after shutter closure during 100 ms. A maximum of 10 tracings can be gathered for each measurement. Daily calibrations of pressure and flow (volume) were carried out using a manometer and a 2-L precision pump. All measurements were carried out with a filter

(Micro Medical Ltd) in place for reasons of hygiene, and to prevent dysfunction of the pneumotachograph due to any saliva.[E6]

Specific IgE measurement at age 4

Specific immunoglobulin E (IgE) to inhalant allergens (house dust mite (*Dermatophagoides pteronyssinus*), cat, dog, grass (*Dactylis glomerata*), birch (*Betula verrucosa*) and mould (*Alternaria alternata*)) were determined by a Radio Allergo Sorbent Test. Atopy was defined as specific IgE concentration >0.70 $IU \cdot mL^{-1}$ for at least one inhalant allergen.

Statistical Analysis

Logistic regression was used to investigate the relationship between the three measurements (FeNO, Rint and specific IgE) and respiratory outcomes assessed at the ages of 5, 6, 7 and 8 years. A longitudinal analyses was then performed, combining information on the outcomes at every age in one model. In this analysis we have to take into account that the repeated measurements in the same individual are correlated, in order to calculate correct standard errors and p-values of our estimates. A Generalized Estimation Equations (GEE) model was used. As correlations between repeated measurements can be expected to depend on the time lag between 2 measurements, a 3-dependent correlation matrix was modelled. Interaction of the measurements with age (as a categorical variable) was included in the GEE model, in order to allow the associations to vary with age.

Besides a complete case analysis, a multiple imputed analysis was performed to investigate and correct for any bias that may result from complete case analysis.[E7] The imputed datasets were created following the guidelines as described by van Buuren *et al*.[E8] Apart from all variables in the final models, the following variables (due to their strong association with the missing data) were used in the imputation matrix: region, urbanisation, parental education, age mother, pregnancy duration, mode of delivery, number of siblings, pet avoidance due to allergy, symptoms of wheeze/dyspnea/eczema, eosinophilic count at age 4, Rint measurements at age 8, total and specific IgE measured at age 8, FeNO measured at age 8. The benefit of an imputed analysis depends on two factors: 1) the strength of the association between the imputed variable and variables used for the imputation; and 2) the availability of complete data on these variables used for imputation. In Table E8.1 these data are summarized in two columns for each predictor variable (FeNO/ Rint/ Specific IgE). The first column represents the % of usable cases, which is the proportion of children with missing data on the predictor variable that have data available on the variable used for imputation.[E8] The second column represents the correlation (Spearman' s coefficient) between the predictor variable and the variable used for imputation, among the children with available data. Using the 'Multivariate Imputation by Chained Equations' (MICE) procedure

in the statistical program R version 2.6.2, ten imputed datasets with each n = 848 subjects were created.[E9,10] All 10 datasets were analysed using above specified GEE models and results were then combined using PROC MIANALYZE in SAS 9.1 (SAS Institute, Inc., Cary, NC).

RESULTS

Results from the multiple imputation analyses are reported here. Figure E8.1 displays the odds ratios of ^{10}log-transformed FeNO (per IQR increase) on all outcomes. Odds ratios at different ages were calculated by including an interaction term with age (as a categorical variable) in the model. Compared with complete case analysis none of the associations changed direction, but most associations decreased slightly in strength.

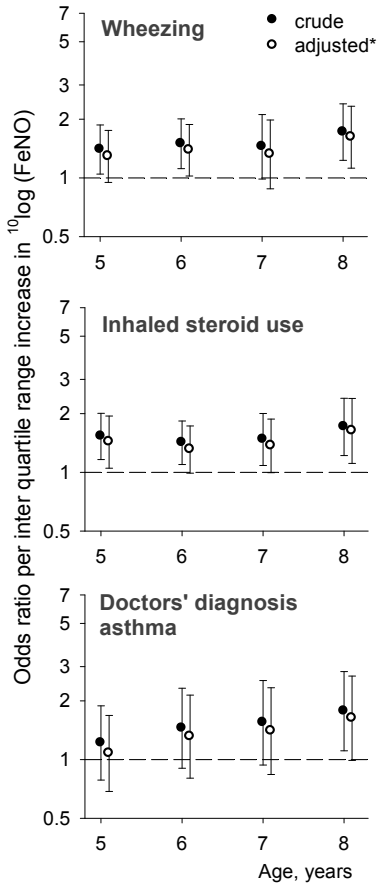

Figure E8.1. Predictive value of FeNO measured at 4 yrs on all outcomes at 5-8 yrs, using multiple imputation analysis
Black dots are crude odds ratios for FeNO (per IQR increase in ^{10}log(FeNO)). *: Open dots are adjusted for clinical history (allergic mother/ doctors' diagnosis of eczema/ wheezing at age 4).

Confidence intervals narrowed, which can be explained by the fact that multiple imputation makes it possible to use all available data. All odds ratios that were significant in the complete case analysis remained significant in multiple imputed analysis.

Figure E8.2 gives the odds ratio of Rint (per IQR increase) on all outcomes. There were very little differences between results from complete case and multiple imputation analysis.

Figure E8.3 and gives the odds ratios of a positive specific IgE on all outcomes. Compared with complete case analysis all odds ratios of IgE decrease in strength in the multiple imputed analyses, especially for the outcomes 'steroid use' and 'doctors diagnosis of asthma. This decrease is much more pronounced than for the other 2 tests: FeNO and Rint. Again we see that confidence intervals are decreased in the multiple imputed analysis. None of the associations change direction and all odds ratios remain strongly significant.

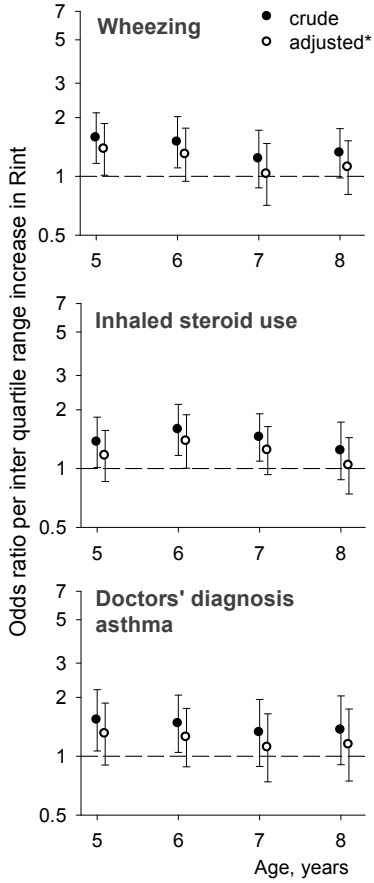

Figure E8.2. Predictive value of Rint measured at 4 yrs on all outcomes at 5-8 yrs, using multiple imputation analysis
Black dots are crude odds ratios for Rint (per IQR increase). *: Open dots are adjusted for clinical history (allergic mother/ doctors' diagnosis of eczema/ wheezing at age 4).

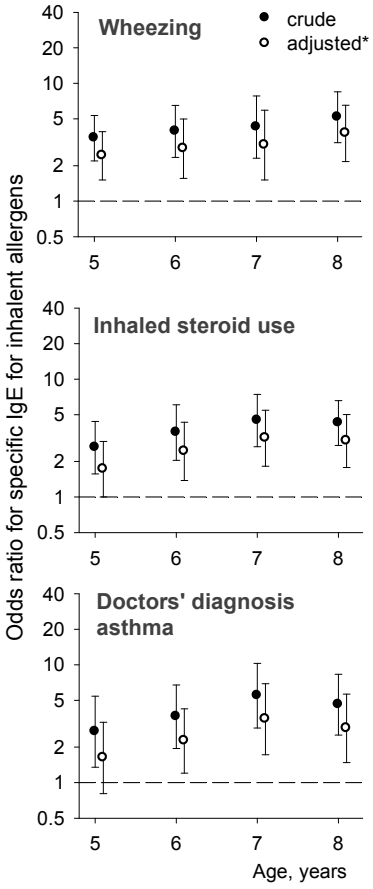

Figure E8.3. Predictive value of specific IgE measured 4 yrs on all outcomes at 5-8 yrs, using multiple imputation analysis
Black dots are crude odds ratios for specific IgE. *: Open dots are adjusted for clinical history (allergic mother/ doctors' diagnosis of eczema/ wheezing at age 4).

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Chapter 9

General discussion

Chronic, recurrent respiratory symptoms constitute one of the most common problems presented to general practitioners and pediatricians.[1] Symptoms such as wheezing and coughing are extremely common in young children, and the prevalence only increased in the last decades.[2] Large birth cohort studies in different parts of the world have aimed to gain understanding of the causes and the natural history of asthma symptoms, particularly wheeze, in children.[3-5] The PIAMA study is one of these, and previous analyses of this cohort have led to significant advances in our knowledge on different aspects of childhood asthma.[6] Two major themes with respect to wheezing in preschool children remain largely unresolved, and we aimed to address both these themes in this thesis. First, there is still no conclusive evidence on which environmental factors may have contributed to the recent rise in the prevalence of asthma and allergies. This knowledge is essential in the search for new ways to prevent asthma development in the future. Second, how can infants and preschool children with transient symptoms be distinguished from those who will actually develop asthma. In the present chapter we will discuss the main findings of the reported studies, in the context of current literature. We will emphasize the scientific and clinical implications of our findings, and make suggestions on direction of future research.

STUDY DESIGN

Strengths and limitations

The PIAMA study is a prospective birth cohort study in the Netherlands. Mothers of the participating children were selected in the second trimester of pregnancy, and a wide range of data on pre- and post natal exposures was collected before the children experienced respiratory symptoms for the first time. That is important, because it excludes the risk of recall bias, which could be an issue in cross-sectional or case-control studies. The availability of data on many different perinatal factors allowed us to investigate these as possible risk factors for asthma, but also to include these in the analysis as possible confounders. This reduces the risk of confounding bias, but residual confounding cannot be excluded entirely, because there may have been unknown or incompletely assessed confounders.

The PIAMA study participants were recruited from the general Dutch population. Maternal allergy was used as a selection variable for allocation of participants to the different study arms, but the total PIAMA study population was not enriched with allergic mothers. It should be realized that some selection bias may have occurred due to selective non-response. Compared to the general Dutch population, the PIAMA study population contained more parents with a high education, more parents of Dutch ethnic origin, and fewer parents who smoked. Furthermore, information bias may have

occurred due to selective loss to follow-up. The attrition rate was very low and about 90% of the children were still in follow-up at the age of 8 years. However, children who were lost to follow-up or had missing data in at least one questionnaire, more often had parents with a lower level of education. Therefore results from the PIAMA study may not be completely generalizable to the general population, especially with respect to prevalence estimates of asthma symptoms. However, many of the reported associations are probably valid, because it is not likely that all associations are substantially different among children form a high or low socio-economic status. Furthermore, if there was evidence for effect modification, the analyses could be stratified and corrected for parental education, as well as a range of correlated variables. In a study with a follow-up of multiple years, the risk increases that some children have missing data on at least one questionnaire or specific question. Traditionally, statistical models would remove all subjects with a single missing value on the analyzed variables from the analysis. This method of 'complete case analysis' can lead to considerable bias, by selection of the study population.[7,8] The risk of bias is reduced by using statistical modules that make it possible to also include subjects with some missing data in the analyses, such as single or multiple imputations.[9] These techniques were used in chapter 3, 7 and 8 of this thesis.

A limitation of observational research in general, is that it is not possible to prove causality between any of the reported risk factors and the development of asthma.[10] Only in a randomized controlled trial the risk of reverse causation and residual confounding can be fully eliminated. It should be noted that some factors in practice cannot be studied using a randomized design, for example the number of older siblings. In that case an observational study is the best achievable study design, and causality simply cannot be proven.

Longitudinal research

A prospective design with repeated measurements may be especially important in pediatric asthma research. Recent longitudinal studies have made clear that childhood asthma can be highly variable with respect to symptoms as well as time course.[4,11-13] The prevalence of wheeze decreases rapidly in the first years of life, and only a minority of preschool children with wheeze will develop asthma lasting into adulthood.[11,12] On the other hand, children who are free of symptoms during infancy and preschool years, may develop asthma after the age of 4 years.[11,12] The varying course of asthma symptoms in early life complicates research in this field in two important ways. First, it affects the search for early risk factors for childhood asthma. If the associations between risk factors and asthma symptoms change over time, then the result of a study may depend on the age at which the outcome 'asthma' is assessed. Previous studies on risk factors for asthma have repeatedly shown contradictory results, which may partly be explained

by differences in the age of outcome assessment. In chapter 2 and 3 of this thesis we investigated the effects of birth weight, daycare attendance, and older siblings, on the prevalence of asthma symptoms.[14,15] Indeed, both studies confirmed that the associations between risk factors and asthma symptoms change over time. Second, the fact that asthma symptoms are so nonspecific and variable in young children complicates an early asthma diagnosis.[16] Both issues underline the importance of longitudinal data collection and analysis. Therefore, in the PIAMA study respiratory health was annually assessed between the age of 0-8 years, and longitudinal statistical methods were used to analyze the data.

ASTHMA AND WHEEZING PHENOTYPES IN CHILDHOOD

We found the associations between risk factors and asthma symptoms to change with age, as described in both chapter 2 and 3. This could be explained by different mechanisms.[14,15] First, it is possible that all wheezing disorders in childhood are in principle a single disease, but with a variable presentation. The age at which wheezing starts might be explained by the time that a child is exposed to certain risk factors, such as respiratory infection. For example, a child attending daycare in the first year of life may wheeze at that age because of exposure to many respiratory pathogens. The same child not attending daycare may start to wheeze at 4 years, when confronted with a high infection load in elementary school.

A second possibility however, may be that wheezing disorders in childhood are not a single disease, but comprise a heterogeneous group of several discrete disease entities.[17] In current literature, this is certainly the most widely accepted view on childhood wheezing and asthma.[3,18-20] The Tucson group first introduced the concept of different wheezing phenotypes, based on the age of symptom onset.[11] Their longitudinal classification in early transient, persistent and late onset wheeze has become widely accepted.[21-24] Indeed, if perinatal risk factors are differentially associated with different wheezing phenotypes, this would explain their changing association with age. The last decade several phenotype classifications have been suggested, based on clinical symptoms (e.g. type, age, and severity of symptoms) and/or objective measures (e.g. bronchial hyperresponsiveness and atopy).[18,19,25-27] In the PIAMA study wheezing phenotypes were recently defined using a data-driven statistical method called longitudinal latent class analysis (LLCA). Because there is no gold standard for the definition of wheezing phenotypes, and the underlying disease processes are insufficiently understood, it is difficult to validate these phenotypes.[20] An important finding supporting the validity of the phenotype classification by LLCA is the fact that the wheezing phe-

notypes in PIAMA were similar to those previously found in the independent ALSPAC birth cohort.[28]

Question remains however, whether the wheezing phenotypes in PIAMA represent disease entities with a different underlying pathophysiology. In chapter 4 we found differential associations of the wheezing phenotypes with perinatal risk factors. The newly identified phenotype of intermediate onset wheeze was the only phenotype significantly associated with a lower birth weight. Daycare attendance and older siblings only increased the risk of transient early wheeze. Breastfeeding on the other hand appeared to decrease the risk of wheezing throughout childhood. These differential associations could imply that different causal mechanisms play a role in the development of the wheezing phenotypes, but causality cannot be proven on the basis of these observational data.[10] Furthermore, the smaller sample size in the less frequent phenotypes may be responsible for some of the non-significant results. Replication of our findings in a large independent birth cohort would be worthwhile, to increase statistical power and reduce the risk of chance findings.

Asthma is associated with various patterns of chronic airway inflammation, and if differences in types of inflammation could be demonstrated between children with different wheezing phenotypes, this would support the evidence that childhood asthma consists of several disease entities with a different pathophysiology. Indeed results from our study on exhaled nitric oxide (FeNO), a marker of eosinophilic airway inflammation, showed different levels of FeNO among the different wheezing phenotypes (chapter 5). FeNO levels at 8 years were higher in the phenotypes with a high probability of wheeze after the age of 4 years. These findings could imply that eosinophilic inflammation is present only in wheezers with persistent symptoms, and that different inflammatory mechanisms may be responsible for other transient phenotypes. However, we did not measure FeNO in the first years of life and therefore we cannot exclude that FeNO may have been elevated also in transient wheezers at the age they had wheeze. Indeed two previous studies have shown elevated levels of FeNO in infants, preceding respiratory symptoms in the first years of life.[29,30] Furthermore, recent findings suggest that even within the same children the type of airway inflammation may change over time (A. Bush, London, personal communication).

Our study (chapter 5) further showed that FeNO levels differed between atopic and non-atopic children. FeNO was elevated in the phenotypes with a high probability of wheeze after the age of 4 years, but only in the subgroup of atopic children. Therefore, our findings suggest that the pathophysiology of wheezing also differs between atopic and non-atopic children.[27] This implies that an asthma phenotype definition based only on the time course of symptoms may not be accurate, and that a definition which includes markers for atopy may better reflect the underlying disease entities. In this context it should be realized that although wheezing is considered the most important

clinical symptom of asthma, 'wheezing' and 'asthma' are not synonymous. Several other characteristics may be considered as determinants of asthma phenotypes, such as allergy, cough, need for medication, or symptom severity.[19,26,27,31] Including these items may lead to the discovery of different asthma phenotypes (fewer or more), useful for basic researchers investigating the etiology of asthma, and perhaps eventually for physicians treating patients with asthma – and for the patients themselves.[20]

EARLY RISK FACTORS AND THE PREVENTION OF CHILDHOOD ASTHMA

The most consistent risk factor for asthma in children is a family history for atopy and/or asthma. It has been known for a long time that genetic predisposition plays a crucial role in the development of asthma.[32] The patterns of inheritance suggest that multiple genes are involved, that interact with each other and with environmental factors.[33] A second consistent finding in epidemiologic data confirmed in the PIAMA study is that boys more often have asthma symptoms than girls, at least before puberty.[34] Although genetic and gender differences are two major risk factors for asthma development, these factors are rather stable over time and cannot be easily influenced. This implies that it is unlikely that these factors can explain the recent rise in childhood asthma, nor could they be used as possible targets for asthma prevention. Therefore we will not focus on these risk factors in this discussion.

Following the rise in asthma prevalence, research groups all around the world have tried to find lifestyle and environmental factors that could explain the pandemic, in order to find possible targets for asthma prevention. So far there are only few modifiable perinatal factors for which there is convincing evidence that they increase the risk of asthma. In the PIAMA study several perinatal risk factors for asthma development (e.g. exposure to house dust mite,[35] presence of pets,[36] breastfeeding,[37] caesarean section,[38] nutrition during pregnancy,[39] maternal[40] and childhood[41] overweight) have been previously studied and extensively discussed. This discussion focuses on the perinatal factors that were investigated in this thesis, and that may have implications for the prevention of childhood asthma. This thesis presents detailed analyses on the age-specific associations between birth weight, exposure to tobacco smoke, daycare attendance and older siblings on the one hand and the development of respiratory infections, asthma symptoms, airway hyperresponsiveness and allergy on the other hand. Furthermore it present a combined analysis of these and several other perinatal risk factors (e.g. caesarean section, pets, breast feeding, pregnancy duration, maternal age and body mass index), investigating their association with longitudinally defined wheezing phenotypes.

Smoking

Probably the most unequivocal risk factor for asthma symptoms in childhood is parental smoking. In line with numerous previous reports,[42] we found that smoking during pregnancy significantly increased the risk of childhood wheeze, especially transient early wheeze (chapter 4). Furthermore, in chapter 2 we showed that in children with a low birth weight the effects of smoking (during pregnancy and after birth) significantly increased the risk of respiratory symptoms in the first 7 years of life.[14] The adverse effects of smoking are evident, but it should also be noted that these effects are not large enough to explain the overall increase in asthma prevalence. Results from chapter 4 suggest that cessation of smoking would lead to 5.8% reduction in transient early wheeze.

Breastfeeding

The evidence on the effect of breastfeeding is less clear. Although some studies have reported a higher prevalence of asthma in breastfed children, most studies support a protective effect on asthma and allergies.[43,44] We found breastfeeding to be the only factor with a significant protection of persistent wheeze, with a population attributable risk of about 25%. Assuming a directly causal association, this implies that if all mothers would breast feed their children for at least 3 months, the prevalence of persistent wheeze in the general population could decrease from 3.5% to 2.6%. Based on these findings, in combination with results from previous studies, we propose that breastfeeding is one of the most important targets for asthma prevention.

Birth weight

We found that a low birth weight, which is a marker of reduced intrauterine growth, was associated with more respiratory symptoms after the age of 2 years. Possibly reduced intrauterine growth is associated with a disturbed development of the immune system, leading to an increased risk of atopy and wheeze after the age of 2 years. On the other hand, children with a lower birth weight may simply have smaller airways that are more easily obstructed during infections, and are therefore more prone to airway symptoms. However, this hypothesis cannot explain why symptoms become manifest after a symptom-free interval of several years. Unfortunately, birth weight is not a factor that is easily modifiable. The most important modifiable factor that can positively influence birth weight is smoking during pregnancy, which further supports the possible positive effects of smoking cessation.

Other factors

For many perinatal risk factors (e.g. early exposure to pet allergens, house dust mite, caesarean section, maternal age or body mass index) there is still no conclusive evi-

dence on the association with childhood asthma and allergy. This is mainly due to the fact that several epidemiological studies have led to conflicting results. Partly these differences may be explained by the previously mentioned difficulties in the definition of 'childhood asthma'. Many studies have used proxy outcomes for 'asthma', such as allergy, atopy, wheeze, shortness of breath, medication use, emergency room visits, or a combination of several of these items. Importantly, many studies have used a cross-sectional design, and the age of outcome assessment varied greatly. Another possible explanation for the conflicting findings, are the genetic differences between the investigated populations. In the presence of certain genes an environmental factor may increase the risk for asthma development, while in the absence of this gene the environmental factor could be protective. This interaction with genetics has been previously shown for several possible risk factors, including exposure to environmental tobacco smoke, air pollution and bacterial endotoxins.[45,46]

Hygiene hypothesis

Several hypotheses have been proposed to explain the recent rise in the prevalence of asthma and allergies.[47] Probably the best-known explanation in both the scientific and lay community, is the concept that increased exposure to microbes in early life may have a preventive effect on the development of asthma and allergies.[48] This association was first reported by Strachan in 1989, who found that children with more siblings developed fewer allergies than children from smaller families.[49] Later immunological research found a plausible mechanism for this association. Infections early in life may cause a shift in the T_H1/T_H2 balance, from Th2 cells (that are involved in the pathogenesis of allergy) towards Th1 cells.[50,51] Hence, the absence of infections could lead to insufficient development of T_H1 immunity. Recent immunological analyses have challenged this concept, and it is likely that other immune mechanisms are also involved.[52] Nevertheless, the combination of the epidemiological and immunological findings led to the postulation of the *hygiene hypothesis*, which states that the reduced exposure to infectious diseases in recent times may be responsible for the rising prevalence of asthma and allergies.[49] The hygiene hypothesis has been subject to much debate in scientific literature.[52] Some epidemiological studies clearly contradict the hypothesis. First there are several studies that have consistently shown that infections in early life are associated with an increased risk of later asthma, rather than a decreased risk.[53-58] It should be noted that these results could be explained by reverse causation; children with a predisposition for asthma may be more susceptible to symptomatic respiratory infections, and this would explain their positive association.[52] In chapter 3 we reported the associations between daycare, siblings and the development of asthma and allergy.[15] Both daycare and older siblings were strong risk factors for respiratory infections. Our results show that children with older siblings and early daycare experienced

most infections in the first year of life, but had no protection against asthma or allergy at the age of 8 years. Because there is no association between asthmatic constitution and daycare attendance or older siblings, these results cannot be explained by reverse causation. On the other hand there have also been several studies that show a protective effect of exposure to infections. For example, children exposed to high levels of bacterial endotoxins have a decreased risk of allergic asthma.[59-61] Furthermore, analysis of PIAMA data has shown that children born by caesarean section may have an increased risk for asthma, especially if they have allergic parents.[38] This association may be explained by delayed colonization with microbes after caesarean section, which could predispose the child to later asthma and allergy development. These conflicting results do not necessarily mean that the hygiene hypothesis should be discarded. They do however suggest, that the reality is more complex than the original hypothesis suggested.[49] The effect of early infections with common respiratory viruses on the development of asthma and allergy is not necessarily the same as the effect of exposure to specific bacteria and their endotoxins. Future research will need to focus on exposure to specific microorganisms, rather than infections in general, in order to find novel preventive or therapeutic strategies. For example, recent studies suggest that infections with human rhinovirus could be one of the most important risk factors for childhood wheezing and possibly the development of chronic asthma.[62] Unfortunately, to date there are still no practical implications that can be deduced from the hygiene hypothesis, for the prevention of asthma and allergies.[52]

Practical implications

Based on literature and our own results in this thesis there is enough evidence to make some recommendations for current clinical practice.

- There is sufficient evidence to strongly advise against smoking during pregnancy. The effects on the incidence of asthma symptoms in childhood are limited, but highly significant. Also smoking in the presence of children should be discouraged, especially for children with a low birth weight, who are more vulnerable for the effects.
- Early daycare attendance exposes children to more infections in early life, and is not associated with the prevalence of asthma at 8 years. Parents of children with a high risk of morbidity form respiratory infections may be advised to consider individual child care, irrespective of the child's risk of developing asthma later.[63]
- Mothers should be advised to breast feed their child. Breastfeeding for 3-4 months reduces the risk of asthma symptoms in the first 8 years of life. The protective effect appears to be stable over 8 years and is present in children with and without a family history of allergy.

EARLY DIAGNOSIS AND PREDICTION

The variable nature of respiratory symptoms in early childhood complicates the diagnosis and treatment of asthma in young children.[16] Previous studies have aimed to predict which children are at high risk of developing asthma, primarily for prevention purposes.[64,65] The hypothesis was that if children with asthma symptoms were treated aggressively with anti-inflammatory medication at a young age, this could change the course of asthma development and reduce symptoms in later life. Unfortunately, three independent trials have conclusively shown that aggressive treatment in symptomatic young children reduces current symptoms, but does not lead to a reduction in symptoms after medication is withdrawn.[66-68] Early aggressive treatment is therefore not effective as a secondary prevention strategy.

It remains important for clinicians to be able to diagnose asthma at an early age, because it allows for adequate treatment. Analysis of data from the PIAMA study (chapter 6) shows that a delayed diagnosis of asthma is associated with higher rates of undertreatment, in line with previous cross-sectional reports.[69,70] Furthermore, we found a considerable risk of overtreatment with inhaled corticosteroids in preschool children. Most preschool children will only suffer from transient airway symptoms. If symptoms subside in children using inhaled corticosteroids, it remains unclear whether this resulted from a positive treatment effect, or simply from the natural course of the wheezing symptoms. Our findings emphasize the importance of making an accurate risk assessment of symptom persistence in young children before inhaled steroids are initiated. Furthermore, it stresses the need to perform regular follow-up in young children who use inhaled steroids. If children remain free of symptoms, inhaled steroids should be decreased and eventually stopped, in order to avoid overtreatment (this thesis).[71] Regular follow-up is actually advised by the GINA as well as national asthma guidelines, but apparently this advise is often not followed in clinical practice.[72,73]

Diagnosis of asthma using clinical symptoms

Several research groups have created algorithms from reported clinical symptoms to predict which symptomatic young children will develop asthma.[12,64,74-79] Many differences between these studies make it difficult to compare the derived prediction rules. Study design, study population, definition of predictor and outcome variables, outcome prevalence, and prediction rule cut-off points all differ between the reported studies, and this may affect their results. A common finding of previous studies is that it remains difficult to make an accurate asthma prognosis in preschool wheezing children on the basis of simple clinical findings. The reported positive predictive values are fairly low, around 35-55%.[64,74,77,80]

The reported independent predictors for the development of chronic asthma vary between the different studies, but some predictor variables are reported in multiple studies. Wheezing history appears to be the most important risk indicator for symptoms persistence.[12,64,76,77] Eczema of the child,[12,64,77] a family history of atopy,[12,64,76-78] frequency of respiratory infections[74,78] and wheezing in the absence of a cold[64,77] were also previously reported significant predictors. Our study (chapter 7) confirms these variables as the most important predictors for persistent asthma, and presents a practical new algorithm that provides a prediction at the time of first wheeze, based on a weighted appreciation of the most important clinical factors.[80] Before this can be implemented in clinical practice external validation in an independent cohort should be performed. If externally validated, the rule may be used in primary care to make an individual assessment of a child's risk of persistent asthma. Given the limited predictive power, a prediction rule with clinical symptoms should not be used as the only means to diagnose chronic asthma, but may be used to guide further clinical decisions. Several options are possible, such as a conservative approach with reassurance of the parents, further investigations, start of a trial with inhaled medication or referral to specialist care.

The use of FeNO in the early diagnosis of asthma

An important clinical research question was whether FeNO measurement in preschool children with wheeze is helpful in the diagnosis and prediction of childhood asthma. Previous studies on this subject led to varying conclusions. Several studies have shown elevated FeNO in school-age children with asthma or asthma symptoms. Sivan *et al* even reported an 80% sensitivity and 92% specificity for an asthma diagnosis.[81] In preschool children fewer studies were performed, but Malmberg *et al* also found an 86% sensitivity and 92% specificity to diagnose asthma in a selected population of asthmatics and healthy controls.[82] These high predictive indices are in contrast with reports from two population based studies, that found much smaller differences in FeNO between children with and without asthma symptoms.[83,84]

In order to estimate the clinical benefit of a test, some aspects in the study design need to be considered. First, comparison of a selected group of asthmatics with healthy controls poorly reflects clinical practice, where many children present with intermediate symptoms, without a clear distinction between cases and controls.[85] Second, a test that differentiates between asthmatics and non-asthmatics is not useful in a setting where it is already known which children are asthmatic. In practice, an important potential of an asthma test is whether it can predict if a child will still suffer from asthma in the coming years. Third, FeNO will only be clinically useful if it adds new information to the available information, such as clinical symptoms, lung function and allergy tests.

In chapter 8 we aimed to estimate the added predictive value of FeNO at 4 years on asthma symptoms until 8 years, taking into account these considerations. Although we found that FeNO was higher in children with persistent symptoms, the added predictive value was smaller than some previous reports suggested.[81,82,86] This suggests that in clinical practice the value of FeNO as a diagnostic test for asthma may be more limited than originally proposed.[87] Nevertheless it is a simple, non-invasive test that does contain useful supplementary information. Especially in symptomatic preschool children with intermediate risk of persistent asthma, FeNO helps to predict the risk of symptom persistence (this thesis).

The use of Rint in the early diagnosis of asthma

Several studies have reported that Rint measurements are associated with asthma symptoms in children, and that Rint could be used to measure the effects of beta-2-agonists.[88-90] The value of Rint in the early diagnosis of asthma remains less clear. No studies assessed the additional value of Rint to other clinical information. One previous study investigated the predictive value of Rint on the need for asthma medication in the following three years, but this was in a selected population of 'asthmatic' children aged 2-7 years.[91] The authors concluded that a high Rint measurement was not a risk factor for symptom persistence. In chapter 8 we found similar results in a population based sample of symptomatic preschool children. Rint was significantly associated with symptoms in the 2 years following the measurement, but no longer thereafter. After clinical symptoms were taken into account, the additional value of a Rint measurement appeared to decrease even further. We therefore conclude that there is currently no evidence that Rint is a useful test to diagnose asthma in preschool children.

There may be several explanations for the fact that Rint does not appear to be useful in the diagnosis and prognosis of wheezing preschool children. First, resistance of the upper airways including the oral cavity is probably not associated with asthma symptoms, but it can have a considerable effect on the Rint measurement, making the results less reliable.[92] Secondly, it should also be noted that in the PIAMA study most children performed lung function tests during a clinically stable period. Possibly, Rint measured during a period with severe symptoms could have a stronger predictive value on later symptoms. On the other hand, it could be reasoned that the airway resistance as measured by Rint, is also reflected by reported clinical symptoms. In that case Rint would not add any additional information over a readily available clinical history.

The use of specific IgE in the early diagnosis of asthma

There is little doubt that the presence of specific IgE is predictive for asthma, as several longitudinal studies have confirmed the association between atopy and asthma development in children.[12,76,79,93] It is also clear that specific IgE is a useful test in the

diagnosis and prognosis of asthma in young children, especially IgE against inhalant allergens,[76,79] but at a young age IgE against food allergens such as hens egg is also valuable.[94] Results from the PIAMA study confirmed this: the presence of specific IgE at 4 years was strongly associated with asthma symptoms until the age of 8 years, even after clinical history was taken into account (this thesis). Therefore specific IgE for inhalant allergens should have a place in clinical practice when preschool children are suspected of asthma.

CONCLUSIONS

Asthma symptoms are very common in childhood, and affect more than half of all preschool children in the Netherlands. We found that early daycare attendance and older siblings are associated with more infections and asthma symptoms in the first years of life, but do not protect against asthma or allergy at the age of 8 years. Children with a lower birth weight had more respiratory symptoms between the age of 2-7 years, especially if they were exposed to environmental tobacco smoke. The promotion of breastfeeding seems the most promising target for the prevention of asthma, as it appeared to reduce the prevalence of wheeze throughout childhood.

Even if preventive measures are successfully applied, asthma symptoms will remain a common problem in young children, and an early asthma diagnosis is important in order to start adequate treatment. The use of a simple clinical prediction rule at the onset of symptoms can help to assess the risk of symptoms persistence in preschool children. Specific IgE and FeNO can be assessed, as they independently improve the prediction of symptom persistence. Notwithstanding the positive predictive values of these tests, it remains difficult to accurately identify those children who will develop persistent asthma, and therefore regular follow-up is important, also to avoid under- or overtreatment.

DIRECTIONS FOR FUTURE RESEARCH

In the PIAMA study the association between a wide range of early risk factors and the development of respiratory symptoms in childhood has been investigated. Little is known on the early risk factors for adolescent or adult onset asthma. In order to gain more insight in the changes that take place during adolescence, further follow-up of the PIAMA cohort would be valuable. Follow-up is also needed to find out what the long term prognosis is of the longitudinal wheezing phenotypes that were derived in from PIAMA study.

Future research should try to take into account the problems of defining 'childhood asthma'. Previous literature and studies in this thesis have made clear that asthma is a highly variable syndrome that may comprise several distinct disease entities. An accurate definition of the outcome 'asthma' is essential in the search for etiologic risk factors for its development. Therefore efforts should be made to find meaningful and universally accepted criteria for asthma phenotypes, that can be used in epidemiological studies and, perhaps in clinical care. Epidemiological research in a prospective observational study is a powerful tool to detect risk factors for asthma symptoms, and to generate hypotheses on causal mechanisms. However, to understand the underlying pathophysiology, we need to combine this knowledge with the discoveries in the field of basic immunology and human genetics. Better understanding of basic immunology can help to define meaningful asthma phenotypes, and this can improve precision of basic and genetic research into causes and mechanisms. Any implications of such insights for clinical practise will need to be addressed in appropriate clinical trials.

We presented an asthma prediction rule using 8 clinical characteristics, to improve the early diagnosis of asthma in preschool children. Before this rule is implemented in primary care, the rule should be prospectively validated in an independent cohort.

We have shown that FeNO can be a used to improve the diagnosis of asthma in symptomatic preschool children. Future research may focus on other tests that can further improve the identification of preschool children with a high risk of persistent asthma symptoms. In order to be suitable for young children, such a test should not require active cooperation and preferably be non-invasive. Possibly, the analysis of volatile substances in exhaled air, or inflammatory mediators in exhaled breath condensate may reveal new biomarkers for inflammatory airways disease, not limited to the eosinophilic phenotype, and this may be important for predicting the persistence of asthma symptoms and the effect of treatment.

Development of Genome Wide Association (GWAS) analysis has created the opportunity to detect association between hundred-thousands of genes and the development of asthma in birth cohorts, such as PIAMA. This field of genetic epidemiology aims to find new "asthma genes". Understanding of how these genes and their products interact with environmental factors, may lead to the identification of causal pathways in the pathophysiology of asthma. This could pave the way to entirely new therapeutic interventions, as well as preventive strategies. Furthermore, the possibility that newly identified genes or gene-products could be used to improve an early asthma diagnosis should be further explored.

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Chapter 10

Summary
Samenvatting
Affiliations Co-authors

SUMMARY

In this thesis we investigated which pre- and perinatal factors are associated with the development of childhood asthma and allergy, and which of those may be used to prevent asthma. Secondly, we investigated if it is possible to diagnose asthma in very young children, who reported symptoms for the first time. Therefore we assessed the predictive value of information from clinical history, and of three additional tests that can be performed in preschool children: 1) fraction of nitric oxide in exhaled air (FeNO); 2) interrupter resistance (Rint); and 3) specific immunoglobulin E (IgE).

Chapter 1 is a general introduction to the different aspects of childhood asthma that were investigated in this thesis. It also provides the aims of the studies that were performed and describes the outline of the thesis.

Chapter 2 describes a study on the association between birth weight and the development and course of respiratory symptoms and asthma in the first 7 years of life. Children born at term in the PIAMA birth cohort (n = 3,628) were followed for 7 years and respiratory health was assessed every year. We demonstrated that a lower birth weight was associated with more respiratory symptoms between the age of 1 to 7 years. However, the association decreased with age and was no longer significant at 7 years. The effect of birth weight on respiratory symptoms was significantly greater among the children who were exposed to tobacco smoke, than among those who were not exposed.

We concluded that a lower birth weight in children born at term is associated with a transiently increased risk of respiratory symptoms, and that this effect is enhanced by environmental tobacco smoke exposure.

Chapter 3 reports a study on the associations between daycare early in life, the presence of older siblings, and the development of asthma and allergic sensitization in the first 8 years of life. Previous studies have shown that both daycare and older siblings expose young children to more infections early in life, and that this might have a preventive effect on the later development of asthma and allergy. In the PIAMA birth cohort (n = 3,963) respiratory health was assessed yearly, and at the age of 8 years allergic sensitization (specific IgE) and airway responsiveness were measured. We found that children who attended daycare before the age of 2 years had more respiratory infections and symptoms in the first years of life, and fewer infections and symptoms between 4 and 8 years. At 8 years however, early daycare was not protective for asthma symptoms, allergic sensitization or airway hyper-responsiveness.

We concluded that early daycare is associated with an increase in airway symptoms until the age of 4, but does not offer a lasting protection against asthma or allergy.

Chapter 4 describes the results of an analysis of the association between a wide range of perinatal factors and the development of different longitudinal patterns of wheezing (wheezing phenotypes). Five longitudinal wheezing phenotypes were previously defined using longitudinal latent class analysis; never/infrequent wheeze (n = 2,047), transient early wheeze (n = 455), intermediate onset wheeze (n = 98), persistent wheeze (n = 83), and late onset wheeze (n = 45). We found the following independent perinatal risk factors for transient early wheeze: male gender, maternal and paternal allergy, low maternal age, high maternal body mass index, short pregnancy duration, smoking during pregnancy, presence of older siblings, and daycare attendance. Risk factors for persistent wheeze were male gender, maternal and paternal allergy, and breastfeeding for less than 12 weeks. Intermediate onset wheeze was only associated with a lower birth weight and late onset wheeze only with maternal allergy.

Therefore we conclude that wheezing phenotypes are differentially and sometimes uniquely associated with perinatal risk factors, which could imply that these phenotypes have different underlying pathophysiologies. Some of the identified risk factors are modifiable, and may be important targets for asthma prevention.

In chapter 5 we compared FeNO, a biomarker for eosinophilic airway inflammation, measured at the ages of 4 and 8 years, between the different longitudinal wheezing phenotypes. Compared to the phenotype of never/infrequent wheeze, FeNO at 4 years was significantly higher only in intermediate onset wheeze, but the difference was relatively small. At 8 years, FeNO was significantly higher in the phenotypes of persistent, intermediate onset, and late onset wheeze, in comparison to never/infrequent wheeze. Stratified analyses further showed that the increased FeNO at 8 years in persistent, intermediate and late onset wheeze was only present in children with allergic sensitization.

We concluded therefore that FeNO at 8 years is associated with specific wheezing phenotypes, but only among atopic children. We speculated that the pathophysiology of wheezing phenotypes may differ between atopic and non-atopic children, and that eosinophilic airway inflammation may be present only in atopic children.

Chapter 6 presents a study on the agreement between prescribed asthma medication on the one hand, and asthma symptoms and doctors' diagnosis of asthma on the other hand in children aged 2 to 8 years. We found that about a third of the children with current wheeze did not use any inhaled medication during a given year with symptoms. Even among children with severe asthma symptoms at 8 years, 30% reported that they

did not use inhaled corticosteroids, the first choice treatment for persistent asthma. On the other hand, about half of the children who were using inhaled corticosteroids for at least 2 years did not report any wheeze during those 2 years.

We concluded that under- and overtreatment of asthma symptoms seemed common in children aged 2 to 8 years. The prevalence of overtreatment increased with age, implying that regular follow-up in children using asthma medication remains important.

Chapter 7 presents a study in which a clinical asthma prediction score was developed, to diagnose asthma in preschool children who have suggestive symptoms for the first time. In the PIAMA study children who had asthma symptoms (wheeze and/or cough at night) for the first time before the age of 5 years were selected (n = 2,171). In these children, possible predictor variables for asthma at 8 years were assessed at the age that the respiratory symptoms were first reported. We found 8 clinical parameters that independently predicted asthma at 8 years. A clinical risk score was developed which ranged from 0-55 points. Children with a score <10 points had 3% risk, whereas children with a score ≥30 points had 42% risk of asthma at 8 years.

We concluded that the asthma prediction score could serve as a useful tool for clinicians when preschool children present with asthma symptoms, especially in primary care.

In chapter 8 we investigated whether the objective tests FeNO, Rint, and specific IgE could be used in children with respiratory symptoms at the age of 4 years to predict asthma symptoms until the age of 8 years. Thereby we assessed whether these tests had any added predictive value over the information from a standard clinical history, which is readily available. We found that FeNO and specific IgE measured at 4 years were significantly associated with wheezing and the use of inhaled steroids at 8 years. Moreover, specific IgE was also associated with a doctors' diagnosis of asthma at 8 years. Both tests remained significantly associated with these outcomes at 8 years, even after mutual adjustment, and after adjustment for a clinical history at 4 years. Rint measured at 4 years was significantly associated with wheezing at age 6, but no longer at 7 and 8 years.

From these findings we concluded that it may be useful to measure both FeNO and specific IgE at 4 years in children with symptoms suggestive for asthma. The information from these tests can help to identify those children who will develop persistent asthma symptoms, even after a clinical history is taken into account.

SAMENVATTING

In dit proefschrift hebben we onderzocht welke pre- en perinatale factoren geassocieerd zijn met de ontwikkeling van astma en allergie op de kinderleeftijd, en welke factoren gebruikt zouden kunnen worden voor de preventie van astma. Ten tweede hebben we onderzocht of het mogelijk is om astma te diagnosticeren in jonge kinderen, op het moment dat zij voor het eerst klachten rapporteren. Daarvoor hebben we de voorspellende waarde bepaald van informatie uit de anamnese, en van drie additionele testen die uitgevoerd kunnen worden bij kinderen onder de 5 jaar, namelijk: 1) meting van de concentratie van stikstof monoxide in uitademingslucht (afgekort als FeNO); 2) meting van de luchtwegweerstand met behulp van de 'interrupter resistance' techniek (afgekort als Rint); en 3) meting van specifiek immunoglobuline E in het bloed (afgekort als specifiek IgE).

Hoofdstuk 1 is een algemene introductie in de verschillende aspecten van astma op de kinderleeftijd, die onderzocht werden in dit proefschrift. Hier worden bovendien de doelen van de uitgevoerde studies en de verdere opzet van het proefschrift beschreven.

Hoofdstuk 2 beschrijft een studie naar de associatie tussen geboortegewicht en de ontwikkeling van luchtweg symptomen en astma in de eerste 7 levensjaren. A term geboren kinderen in de PIAMA studie (n = 3628) werden gevolgd gedurende 7 jaar en klachten van de luchtwegen werden jaarlijks geregistreerd. We hebben aangetoond, dat een lager geboortegewicht geassocieerd was met meer luchtwegklachten tussen de leeftijd van 1 tot 7 jaar. De associatie nam echter af met de leeftijd en was niet meer significant op de leeftijd van 7 jaar. Het effect van geboortegewicht op luchtwegklachten was significant groter onder de kinderen die waren blootgesteld aan sigarettenrook, in vergelijking met de kinderen die daar niet aan waren blootgesteld.

We concludeerden dat een lager geboortegewicht in a term geboren kinderen geassocieerd is met een tijdelijk toegenomen risico op luchtwegklachten. Dit effect wordt versterkt door blootstelling aan sigarettenrook.

Hoofdstuk 3 beschrijft een studie naar de associatie tussen kinderdagopvang op jonge leeftijd, de aanwezigheid van een oudere broer of zus, en de ontwikkeling van astma en allergie in de eerste 8 levensjaar. Eerdere studies hebben laten zien dat zowel kinderdagverblijf als de aanwezigheid van een oudere broer of zus ertoe leiden dat kinderen op jonge leeftijd meer luchtweginfecties doormaken. Volgens de 'hygiëne hypothese' kunnen deze vroege infecties de kans op de ontwikkeling van astma en allergie verkleinen. In het PIAMA geboorte cohort (n = 3963) werden luchtwegklachten jaarlijks geregistreerd, op de leeftijd van 8 jaar werden bovendien allergische sensiti-

satie (specifiek IgE) en bronchiale hyperreactiviteit bepaald. We zagen dat de kinderen die vóór de leeftijd van twee jaar naar kinderdagverblijf gingen, meer luchtweginfecties en symptomen hadden in de eerste levensjaren. Ze hadden daarentegen minder luchtweginfecties en symptomen tussen de leeftijd van 4 en 8 jaar. Op de leeftijd van 8 jaar bleek kinderdagverblijf niet beschermend te zijn voor astma symptomen, allergische sensitisatie of bronchiale hyperreactiviteit.

Wij concludeerden dat kinderdagverblijf op jonge leeftijd geassocieerd is met meer luchtwegsymptomen vóór de leeftijd van 4 jaar, maar geen blijvende bescherming biedt tegen astma of allergie.

Hoofdstuk 4 beschrijft de resultaten van een analyse van de associatie tussen een breed scala aan perinatale factoren en de ontwikkeling van verschillende longitudinale patronen van piepende ademhaling (piepende fenotypen). Vijf longitudinale piepende fenotypen werden in een eerder onderzoek gedefinieerd, met behulp van de statistische methode 'longitudinal latent class analysis'; 1) kinderen die nooit/zelden piepende ademhaling hadden ('never/infrequent wheeze', n = 2047); 2) kinderen met piepende ademhaling uitsluitend in de eerste 3 levensjaar ('transient early wheeze', n = 455); 3) kinderen waarbij piepende ademhaling begon na de leeftijd van 2 jaar ('intermediate onset wheeze', n = 98); 4) kinderen die op alle leeftijden van 0-8 piepende ademhaling hadden ('persistent wheeze', n = 83); en 5) kinderen waarbij het piepen begon na de leeftijd van 4 jaar ('late onset wheeze', n = 45). We vonden de volgende onafhankelijke risicofactoren voor 'transient early wheeze': mannelijk geslacht, allergie van vader of moeder, lage leeftijd van moeder, hoge body mass index van moeder, korte zwangerschapsduur, roken tijdens de zwangerschap, aanwezigheid van oudere broer/zus, en kinderdagverblijf in het 1e jaar. Risicofactoren voor 'persistent wheeze' waren mannelijk geslacht, allergie van vader of moeder, en het geven van borstvoeding gedurende minder dan 12 weken. 'Intermediate onset wheeze' was geassocieerd met een laag geboortegewicht en allergie van moeder.

We concludeerden dat de 5 piepende fenotypen verschillende associaties hadden met de onderzochte perinatale risico factoren. Sommige risicofactoren waren met slechts één van deze fenotypen geassocieerd. Dit zou kunnen betekenen dat de piepende fenotypen in feite verschillende ziektes zijn, met ieder een andere onderliggende pathofysiologie. Aangezien sommige risicofactoren beïnvloedbaar zijn, zouden ze mogelijk gebruikt kunnen worden als aangrijppunt in astma preventieprogramma's.

In hoofdstuk 5 hebben we de waarden van FeNO, een biomarker voor eosinofiele luchtwegontsteking, vergeleken tussen de verschillende longitudinale piepende fenotypen. In vergelijking met het fenotype van 'never/infrequent wheeze', was FeNO op 4 jaar significant hoger in het fenotype 'intermediate onset wheeze', al was het verschil

relatief klein. Op de leeftijd van 8 jaar was FeNO significant hoger in de fenotypen 'persistent', 'intermediate onset' en 'late onset wheeze', in vergelijking met 'never/ infrequent wheeze'. Gestratificeerde analyses toonden aan, dat de hogere FeNO waarden op 8 jaar in de fenotypen 'persistent', 'intermediate onset' en 'late onset wheeze' uitsluitend aanwezig waren bij kinderen met allergische sensitisatie.

We concludeerden daarom dat FeNO op 8 jaar geassocieerd is met specifieke piepende fenotypen, maar alleen in atopische kinderen. We speculeerden dat de pathofysiologie van de piepende fenotypen verschilt tussen atopische en non-atopische kinderen; eosinofiele ontsteking speelt mogelijk uitsluitend een rol in de ontwikkeling van piepende ademhaling bij atopische kinderen.

Hoofdstuk 6 presenteert een studie naar de overeenstemming tussen voorgeschreven astma medicatie aan de ene kant en astma diagnose en symptomen aan de andere kant, in kinderen van 2-8 jaar oud. We zagen dat ongeveer een derde van de kinderen met piepende ademhaling geen inhalatiemedicatie gebruikten, in het jaar dat ze de symptomen hadden. Zelfs onder de kinderen die op de leeftijd van 8 jaar ernstige astmatische klachten hadden rapporteerde 30% dat ze geen inhalatiecorticosteroïden gebruikten, terwijl dat de eerste keus behandeling is voor persisterend astma. Tegelijkertijd rapporteerde ongeveer de helft van alle kinderen die ten minste 2 jaar inhalatiecorticosteroïden gebruikten, dat ze gedurende die 2 jaar geen enkele keer last hadden gehad van een piepende ademhaling.

We concludeerden hieruit dat onder- en overbehandeling van astmasymptomen een veel voorkomend probleem lijkt te zijn in kinderen van 2-8 jaar. De prevalentie van overbehandeling nam met de leeftijd toe, hetgeen impliceert dat regelmatige follow-up in kinderen met astmamedicatie belangrijk blijft.

In **hoofdstuk 7** werd een klinische voorspelregel ontworpen om astma te diagnosticeren. De regel kan gebruikt worden bij kinderen onder de 5 jaar, die voor de eerste keer symptomen hebben die bij astma zouden kunnen passen. In de PIAMA studie werden alle kinderen met astmatische symptomen (piepende ademhaling en/of nachtelijk hoesten) onder de 5 jaar geselecteerd (n = 2171). In de kinderen werden mogelijke prognostische variabelen voor de ontwikkeling van astma bepaald, op de leeftijd dat ze de symptomen voor het eerst hadden. Wij vonden dat astma op de leeftijd van 8 jaar voorspeld kon worden met 8 onafhankelijke klinische parameters. Een klinische risico score werd ontwikkeld, met een range van 0-55 punten. Kinderen met een score van <10 punten hadden 3% risico op het ontwikkelen van astma op 8 jaar, terwijl bij kinderen met een score van ≥30 punten dit risico 42% was.

We concludeerden dat deze klinische astma voorspelregel een handig hulpmiddel kan zijn voor artsen, wanneer jonge kinderen zich presenteren met astma symptomen, met name in de eerstelijnszorg.

In **hoofdstuk 8** hebben we onderzocht of de objectieve testen FeNO, Rint en specifiek IgE nuttig zijn, om in kinderen met luchtweg symptomen op de leeftijd van 4 jaar te voorspellen of deze symptomen op 8 jaar nog steeds aanwezig zullen zijn. Daarbij hebben we bovendien bepaald, of deze testen een additionele voorspellende waarde hadden, boven de informatie die standaard in een klinische anamnese gevraagd wordt. We hebben gevonden dat de testen FeNO en specifiek IgE gemeten op de leeftijd van 4 jaar, significant geassocieerd waren met piepende ademhaling en het gebruik van inhalatiecorticosteroïden op 8 jaar. Specifiek IgE was bovendien geassocieerd met dokters diagnose van astma op de leeftijd van 8 jaar. Ook na correctie voor elkaar en voor de klinische anamnese op 4 jaar, bleven beide testen significant geassocieerd met de uitkomstmaten op 8 jaar. Rint op 4 jaar was significant geassocieerd met piepende ademhaling op 6 jaar, maar op 7 en 8 jaar niet meer.

We concludeerden hieruit dat het nuttig kan zijn om FeNO en specifiek IgE te meten op de leeftijd van 4 jaar, in kinderen met symptomen suggestief voor astma. Ook nadat de klinische anamnese bekend is, kan de informatie van beide testen helpen bij het identificeren van de kinderen die persisterende astma symptomen ontwikkelen.

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Dankwoord

DANKWOORD

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Curriculum vitae

CURRICULUM VITAE

Daan Caudri was born in Eindhoven on January 22nd, 1981. In 1999 he passed his secondary school exam (VWO) at the "Collegium Marianum" in Venlo. In the same year he started his medical training at the Medical Faculty of the Erasmus University of Rotterdam. In the years 2001-2002 he was treasurer of the STOLA foundation (Stichting Stages in Ontwikkelingslanden). In 2003 he performed a research project together with S.F van den Heuvel on 'tuberculosis and HIV' in a rural hospital in Manyemen, Cameroon (supervisors: Dr. J.L. Nouwen, Dr. C. Timah). In 2005 he started a masters in Clinical Epidemiology at the Netherlands Institute for Health Sciences (Nihes) in Rotterdam, for which he attended two courses at the Harvard school of Public Health in Boston, USA. In 2006 he obtained his Master of Science degree in Clinical Epidemiology and received the Nihes Award 2006 for first author of the best research paper (supervisor: Prof.dr. J.C. de Jongste). After obtaining his medical degree (cum laude) in 2007, he worked for 8 months as a resident (ANIOS) pediatrics at the Erasmus MC – Sophia Children's Hospital in Rotterdam. In November 2007 he enrolled in the residency program in pediatrics at the Erasmus MC – Sophia Children's Hospital in Rotterdam (head pediatrics Prof.dr. A.J. van der Heijden, Dr. M. de Hoog). He combined his clinical training with a research fellowship (AGIKO) at the department of Pediatric Pulmonology, under supervision of Prof.dr. J.C. de Jongste. His research project was funded by the Netherlands Organisation for Scientific Research (NWO) 'Toptalent scholarship', which he received in 2007. The research performed during this period is presented in this thesis.

In may 2009 he married Stephanie Fijten, who is a medical doctor. Together they live in Rotterdam.

List of publications

LIST OF PUBLICATIONS

Caudri D, Wijga A, Gehring U, Smit HA, Brunekreef B, Kerkhof M, Hoekstra MO, Gerritsen J, de Jongste JC. Respiratory symptoms in the first 7 years of life and birth weight at term: the PIAMA Birth Cohort. Am J Respir Crit Care Med 2007;175(10):1078-85

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Caudri D, Wijga A, Schipper CMA, Hoekstra MO, Postma DS, Koppelman GH, Brunekreef B, Smit HA, de Jongste JC. Predicting the long-term prognosis of children with symptoms suggestive of asthma at preschool age. J Allergy Clin Immunol 2009;124(5):903-10

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Savenije* OE, Granell* R, Caudri D, Koppelman GH, Smit HA, Wijga A, de Jongste JC, Brunekreef B, Sterne JA, Postma DS, Henderson J, Kerkhof M. Comparison of childhood wheezing phenotypes in two birth cohorts: PIAMA and ALSPAC. *both authors contributed equally. J Allergy Clin Immunol 2010; revision.

Caudri D, Wijga AH, Smit HA, Koppelman GH, Kerkhof M, Hoekstra MO, Brunekreef B, de Jongste JC. Asthma symptoms and medication in the PIAMA birth cohort: evidence for under- and overtreatment. Submitted

Caudri D*, van der Valk RJP*, Savenije OEM, Koppelman GH, Smit HA, Wijga AH, Postma DS, Kerkhof M, Brunekreef B, de Jongste JC. Childhood wheezing phenotypes are associated with FeNO in atopic children at age 8. *both authors contributed equally. Submitted.

Caudri D, de Jongste JC. Exhaled nitric oxide and childhood asthma
J Pediatr 2010 Mar;156(3):514 (letter)

PhD Portfolio

PhD PORTFOLIO:
SUMMARY OF PhD TRAINING AND TEACHING

Erasmus MC Department: General Paediatrics – Paediatric Pulmonology
Research School: Nihes
PhD period: 1 December 2007 – 3 December 2010
Master of Science Clinical Epidemiology 2005 – 2006
Promotors: Prof.dr. J.C. De Jongste, Prof.dr. H.A. Smit
Co-promotor: Dr. A.H. Wijga

General academic courses	Year	Workload (ECTS)
Biomedical English Writing and Communication	2009	4.0
Research skills		
Master of Science Clinical Epidemiology, Nihes including elective/in depth courses: . Repeated measurements in clinical studies . Missing values in clinical research . Paediatric clinical epidemiology . Prognostic research . Ethical basis in health care delivery . Decision analysis in clinical research	2005-2006	40
Weekly research meeting, department Paediatric Pulmonology Erasmus MC - Sophia	2007-2010	2.0
Weekly radiology meeting, department Paediatric Pulmonology Erasmus MC - Sophia	2007-2010	1.0
Lectures on longitudinal data analysis, department Statistics Erasmus MC	2009-2010	0.5
Seminars and workshops		
TULIPS Workshop 'Grant writing & Successful Team Building'	2010	1.4
Dag voor de jonge onderzoekers, NVK, Veldhoven	2008, 2009	0.6
Generation R symposium (oral presentation)	2009	1.0
Netherlands Respiratory Society (NRS) symposium	2008, 2009	0.6
Jaarsymposium Astma Fonds (poster presentation)	2008, 2009	1.0
Symposium 60-jarig bestaan Nederlandse Vereniging voor Allergologie (oral presentation)	2008	1.0
(Inter)national conferences		
'Werkgroep Epidemiologisch Onderzoek Nederland' congress (WEON), Groningen, Netherlands (oral presentation)	2008	1.4
18th European Respiratory Society, Berlin, Germany (oral presentation: awarded 'Paediatric Respiratory Epidemiology Abstract Award')	2008	1.4
'Werkgroep Epidemiologisch Onderzoek Nederland' congress, Amsterdam, Netherlands (2 poster presentations)	2009	1.0
15th European Union for School and University Health and Medicine (EUSUHM) congress 2009, Leiden, Netherlands (oral presentation)	2009	1.4
19th European Respiratory Society, Vienna, Austria (oral presentation)	2009	1.4
American Thoracic Society, San Diego, USA (poster presentation)	2010	0.5
American Thoracic Society, New Orleans, USA (2 poster presentation & oral presentation: awarded 'ATS Travel Award')	2010	2.0
20th European Respiratory Society, Barcelona, Spain (invited speaker symposium 'Early Origins of Adult Lung Disease – Early Origin of Asthma')	2010	2.0

Teaching activities		
Supervising medical students practical	2007-2010	0.5
Supervising Master's theses (1 student)	2009-2010	3.0
Statistical advice/consultations	2010	3.0
Other		
Peer review of articles for scientific journals	2009-2010	1.4
Clinical work Paediatric Pulmonology, outpatient clinic	2007-2010	2.0