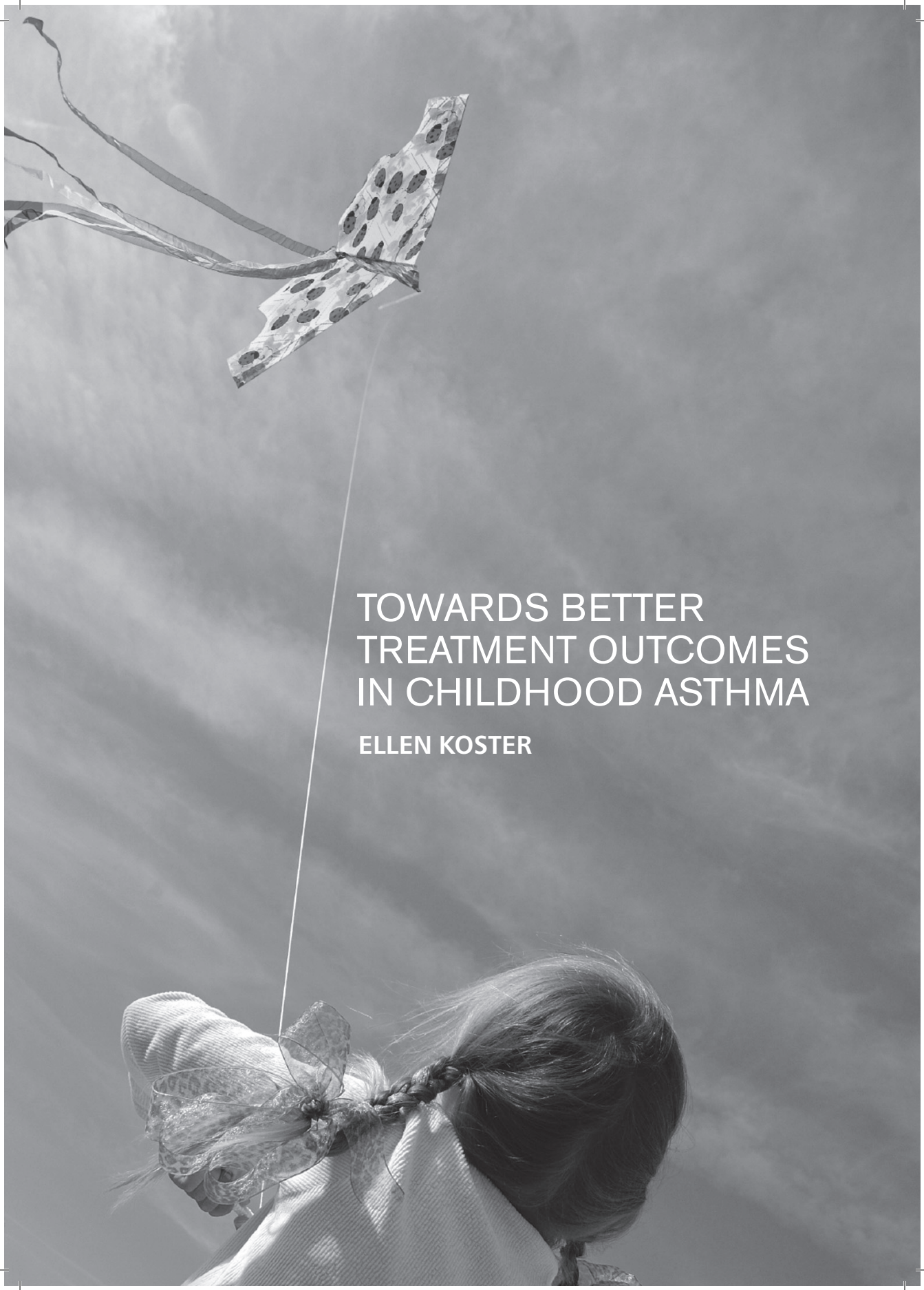

TOWARDS BETTER TREATMENT OUTCOMES IN CHILDHOOD ASTHMA

ELLEN KOSTER

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- Institute for Risk Assessment Sciences, Utrecht, the Netherlands
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TOWARDS BETTER TREATMENT OUTCOMES IN CHILDHOOD ASTHMA

OP WEG NAAR BETERE BEHANDELUITKOMSTEN VOOR KINDEREN MET ASTMA

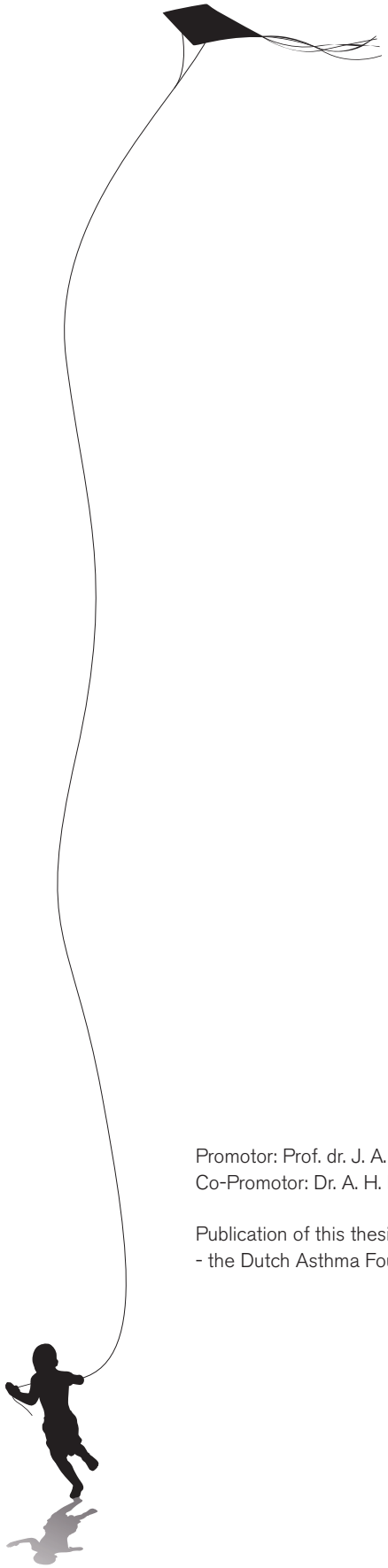
(met een samenvatting in het Nederlands)

Proefschrift
ter verkrijging van de graad van doctor aan de Universiteit Utrecht
op gezag van de rector magnificus, prof. dr. G.J. van der Zwaan, ingevolge
het besluit van het college voor promoties in het openbaar te verdedigen
op woensdag 26 oktober 2011 des middags te 4.15 uur

door

Ellen Susan Koster

geboren op 8 december 1983 te Hengelo (ov)



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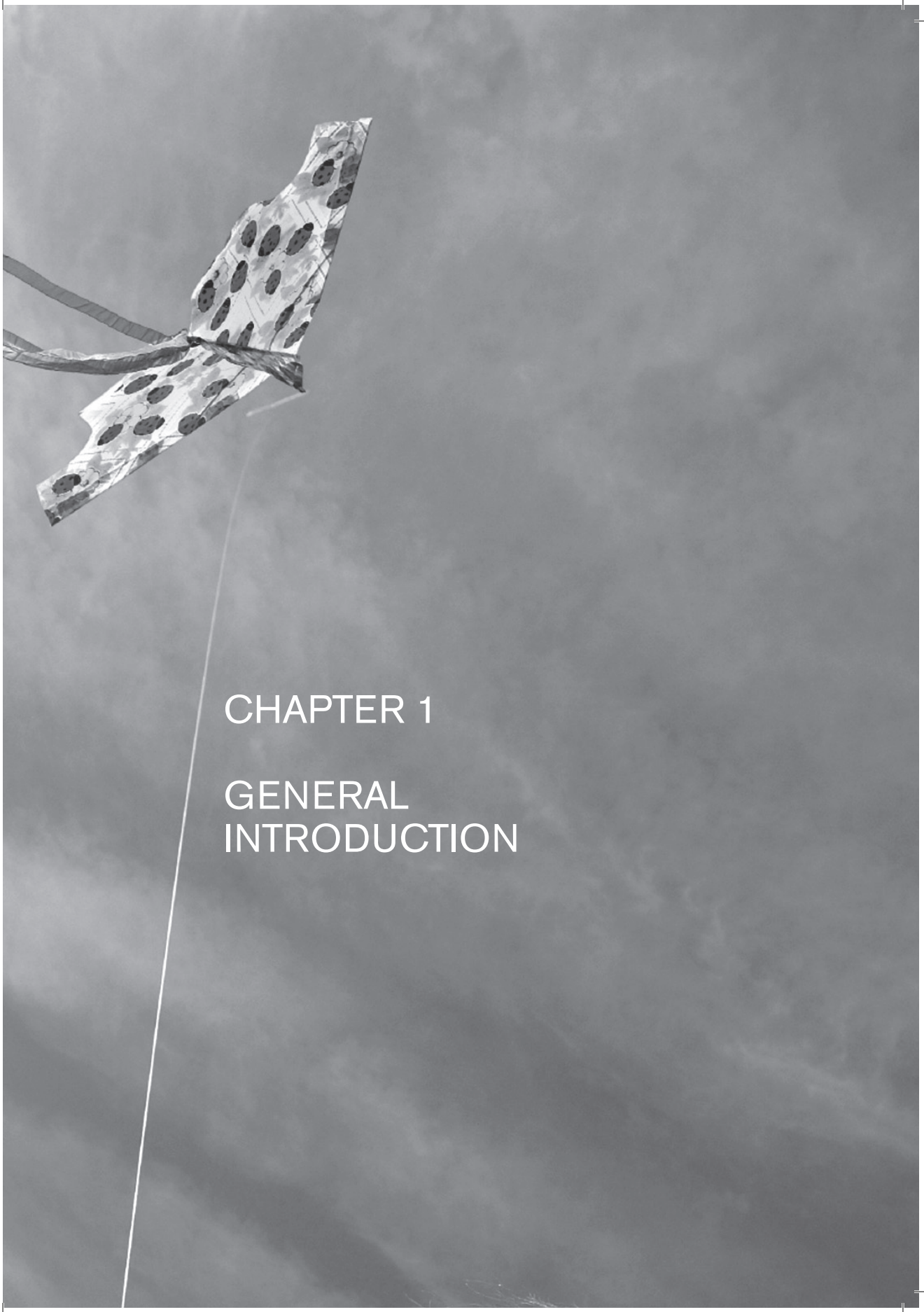
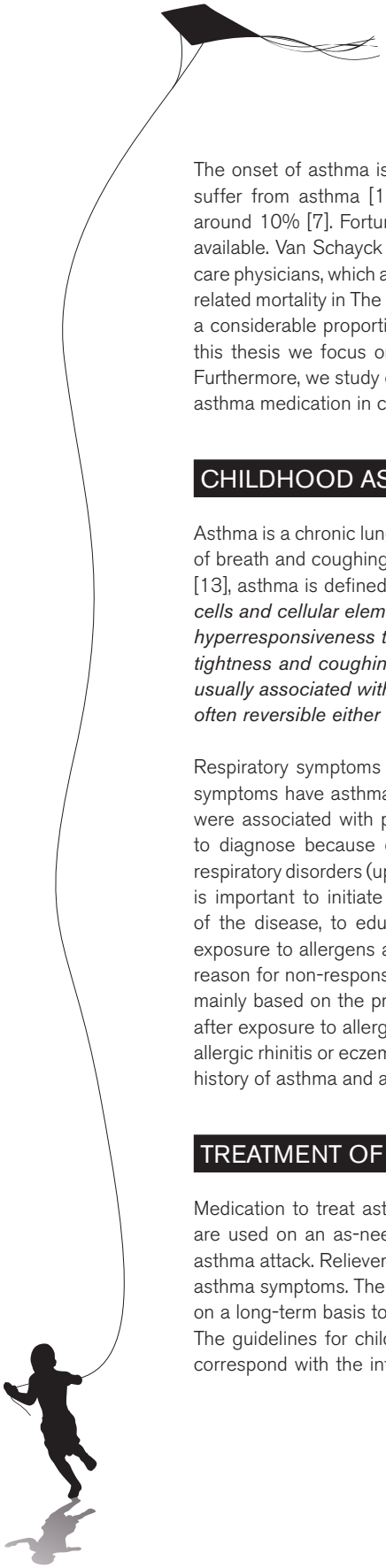
CONTENTS



CHAPTER 1

GENERAL INTRODUCTION



The onset of asthma is early in life for most patients. Up to 35% of the children worldwide suffer from asthma [1-6], whereas the prevalence of childhood asthma in Europe varies around 10% [7]. Fortunately, nowadays there are effective treatment strategies for asthma available. Van Schayck et al. [8] showed that after the introduction of guidelines for primary care physicians, which advised regular use of inhaled corticosteroids (ICS) for asthma, asthma-related mortality in The Netherlands dropped dramatically. Despite the good treatment options a considerable proportion of asthmatics still suffer from poorly controlled disease [9-12]. In this thesis we focus on factors influencing the way patients use their asthma medication. Furthermore, we study environmental and genetic factors that may influence effectiveness of asthma medication in childhood.

CHILDHOOD ASTHMA

Asthma is a chronic lung disorder characterised by recurrent episodes of wheezing, shortness of breath and coughing [13]. According to the Global Initiative for Asthma (GINA) guidelines [13], asthma is defined as: *"a chronic inflammatory disorder of the airways in which many cells and cellular elements play a role. The chronic inflammation is associated with airway hyperresponsiveness that leads to recurrent episodes of wheezing, breathlessness, chest tightness and coughing, particularly at night or in the early morning. These episodes are usually associated with widespread, but variable, airflow obstruction within the lung that is often reversible either spontaneously or with treatment."*

Respiratory symptoms are very common in childhood, but not all children with asthma-like symptoms have asthma. Zuidgeest et al. [12] showed that diagnoses different from asthma were associated with prescribing of asthma medication. Childhood asthma can be difficult to diagnose because of the disease heterogeneity and frequent co-morbidity with other respiratory disorders (upper respiratory tract infections and viral infections). A correct diagnosis is important to initiate appropriate treatment to reduce symptoms and prevent worsening of the disease, to educate children and parents how to manage symptoms and to avoid exposure to allergens and other triggers [14]. An incorrect diagnosis might be an important reason for non-response to therapy [15 16]. In young children, a diagnosis of asthma will be mainly based on the presence of symptoms, indications for allergies (increase in symptoms after exposure to allergens, IgE levels, skin-prick testing and other allergic disorders such as allergic rhinitis or eczema) and sometimes lung function measurements. Furthermore, a family history of asthma and allergic disorders makes a diagnosis of asthma more likely [13 17 18].

TREATMENT OF ASTHMA

Medication to treat asthma can be classified as reliever or controller medication. Relievers are used on an as-needed basis and act quickly to reverse bronchoconstriction during an asthma attack. Relievers do not reduce the underlying airway inflammation, which causes the asthma symptoms. Therefore, treatment with controller medication, that has to be taken daily on a long-term basis to prevent exacerbations and reduce airway inflammation, is necessary. The guidelines for childhood asthma issued by the Dutch College of General Practitioners correspond with the international GINA guidelines and describe short-acting beta-agonists

(SABA) as first-line reliever therapy [13 17 19-21]. Inhaled steroids have become first-line maintenance therapy to reduce airway inflammation and improve lung function [17 22]. Corticosteroids suppress the inflammatory response by switching off inflammatory genes that have been activated during the inflammation process. In addition, they induce transcription of genes encoding anti-inflammatory proteins [23-25]. Corticosteroids are the most effective agents available to control inflammation. When control on low ICS dose is inadequate, reasons for poor control should be examined (e.g. poor inhaler technique or poor compliance) and if indicated, ICS dose should be increased or additional therapy with long-acting beta-agonists (LABA) or leukotriene receptor antagonists (LTRA) should be considered. LABAs are primarily used as add-on therapy for ICS and LABA monotherapy should be avoided [17 22]. LTRA are an alternative anti-inflammatory treatment for persistent asthma that can be used as add-on therapy to ICS or for patients unwilling or unable to use corticosteroids [26]. An advantage of these drugs is that they can be taken orally. In the studies described in this thesis we focus on ICS treatment, as these drugs are first choice in childhood asthma management.

FACTORS INFLUENCING TREATMENT OUTCOME

The goals for successful asthma management are: (1) to achieve and maintain symptom control, (2) to maintain normal daily activities, (3) to maintain lung function as close to normal as possible, (4) to prevent exacerbations, (5) to avoid adverse treatment effects and (6) to prevent asthma-related mortality [13]. Gaining and maintaining optimal asthma control demands regular monitoring and reassessment of therapy effects. Many factors can influence effectiveness of therapy. These factors can be roughly divided into three categories: (1) environmental factors, (2) medication use related factors and (3) genetic factors.

Environmental factors

Many asthmatic patients are atopic, therefore, reduction of allergen exposure (pets, house dust mite and pollen) is of utmost importance to reach and maintain sufficient control of asthma symptoms [27]. Tobacco smoke exposure has also been associated with uncontrolled asthma which makes reduction or elimination of tobacco smoke exposure very important [16 27-30]. Home environment factors, such as exposure to air pollutants, have also been associated with asthma control [31 32]. De Boeck et al. [15] showed that successful control of asthma in children could be achieved by the use of appropriate drugs and delivery devices, good therapy adherence and avoidance of exposure to allergens and tobacco smoke. Furthermore, Farber et al. [27] discussed the importance of weight loss and treatment of co-morbidities in obese patients, because obesity is associated with asthma and may be a factor in difficult-to-control asthma.

Medication use related factors

In their review paper on causes of poor asthma control, Haugney et al. [16] described incorrect choice of inhaler and poor inhalation technique as important reasons for poorly controlled asthma. Furthermore, a patient's beliefs towards medication use (necessity of use and concerns) and therapy adherence are determinants of treatment effectiveness [16]. Low adherence rates correlate with poor asthma control [33]. Menckeberg et al. [34] found out that a patient's beliefs about medicines predicted refill adherence to ICS in adult asthmatics.

Other studies showed that parent's medication beliefs and parental perception towards their child's asthma influences asthma management [29 35]. Furthermore, a lack of knowledge about asthma and asthma management among many parents of asthmatic children has been shown [36 37]. Health care providers, such as the primary care physician or the community pharmacist, can play an important role in the process towards better treatment outcomes in childhood asthma by adequately informing patients and parents on medication use and the importance of sufficient disease management.

Pharmacogenetics of asthma medication

Besides the aforementioned factors, genetic factors may play a role in inter-individual differences in effectiveness of pharmacotherapy (pharmacogenetics). Drazen and co-workers suggested, almost a decade ago, that up to 80% of the inter-individual variance in treatment response may be due to genetic variations [38]. To date, several pharmacogenetic studies have been performed to asses the relationship between variants in genes or gene pathways and response to anti-asthma drugs [39-42]. Most of these studies have focused on the response to beta-agonists and associations have been found between polymorphisms in de beta-receptor gene (*ADRB2*) and response to beta-agonists [43-46]. Corticosteroid pharmacogenetics is hampered by the complex mechanism of action of corticosteroids. However, associations between altered ICS treatment response and polymorphisms in several genes (*CRHR1, TBX21, FCER2, STIP1, DUSP1, ORMDL3* and *NK2R*) have been described [47-55].

INITIATION OF A NEW COHORT TO STUDY ASTHMA PHARMACOGENETICS

We used different data sources for the studies presented in this thesis. However, the majority of the studies were performed within the newly initiated PACMAN-cohort study (Pharmacogenetics of Asthma medication in Children: Medication with ANti-inflammatory effects) [56]. One of the main aims of the PACMAN study is to investigate genetic factors related to treatment outcomes in childhood asthma. Several large (Dutch) cohort studies focussing on asthma or respiratory symptoms have been carried out [57-59], however, it is difficult to use the data collected within these studies for pharmacogenetic research as only a limited number of participants in these studies regularly use asthma medication. The PACMAN study aims to include 1000 children who are regular users of asthma medication. In April 2009, we started recruitment in Dutch community pharmacies. These pharmacies were selected from the Utrecht Pharmacy Panel for Education and Research (UPPER), an electronic network of community pharmacies (>1100) who are interested in cooperating in epidemiologic or pharmacy practice research. Children were selected from the pharmacy information system based on filled prescriptions for any anti-asthma drug and thereafter, selected children and their parents were invited for a visit to their own community pharmacy. During this visit the parents filled in a questionnaire, the child's lung function and fractional exhaled nitric oxide (FeNO) levels were measured and a saliva sample for DNA isolation and genotyping was collected. This method of data collection rendered a dataset including over 700 participants so far.

OBJECTIVES OF THIS THESIS

Within the context of this thesis we aimed to investigate the following aspects of asthma medication use in childhood:

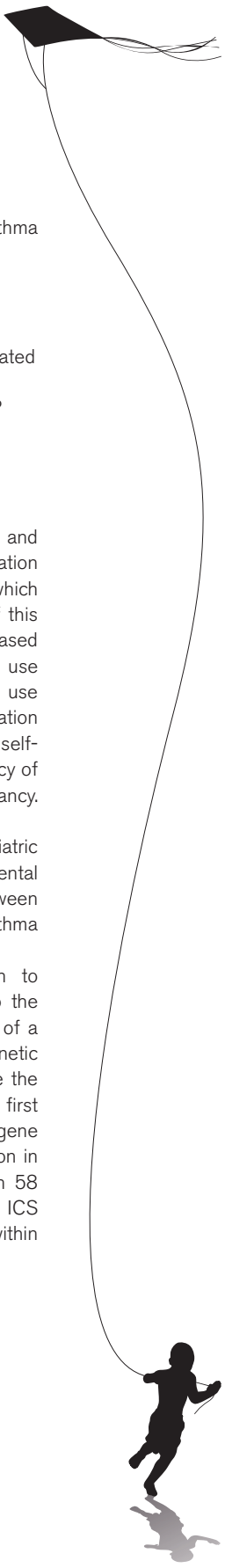
- Which medication use patterns can be described in paediatric asthmatics and which factors influence the prescription of medication?
- Which factors are associated with therapy adherence in paediatric ICS users?
- What is the proportion of uncontrolled asthma in children and which factors are associated with asthma control?
- Are there specific genetic factors (SNPs) that characterize ICS response phenotypes?

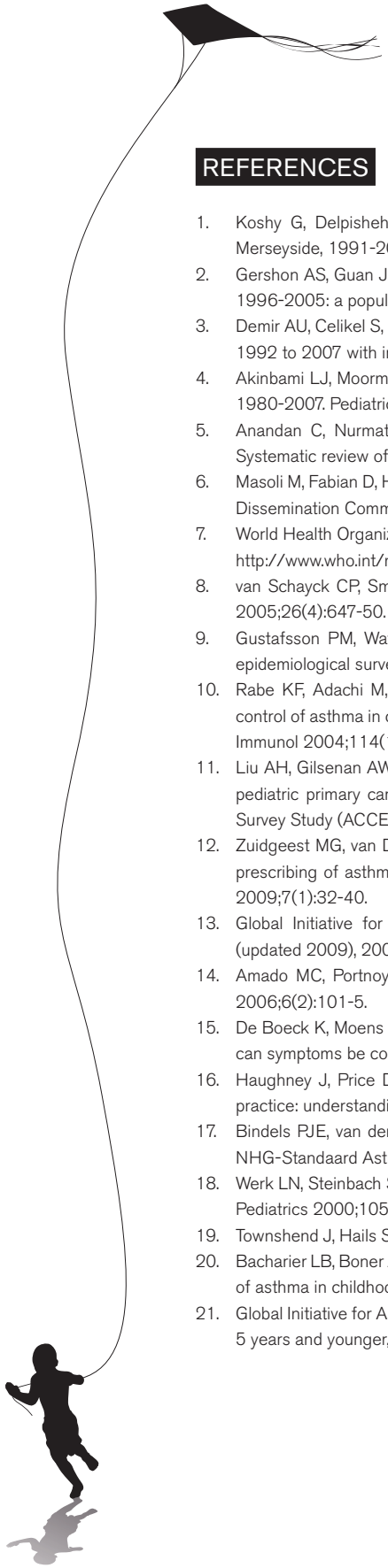
OUTLINE OF THIS THESIS

In **Part I** ("Asthma medication use") of this thesis we evaluate asthma medication use and related factors in childhood. We investigate the prevalence and patterns of asthma medication use in young children and also focus on factors that influence therapy adherence, which may be an important driver of asthma control, which is studied in the second part of this thesis. **Chapter 2** describes the formation of a new dataset (PIAMA pharmacy cohort), based on pharmacy prescription records, which can be used to study longitudinal medication use patterns. In **Chapter 3**, this dataset is used to describe patterns of asthma medication use during the first eight years of life and to study the relation between early therapy initiation and asthma outcomes at age eight. In many situations drug use is based on patient self-report or in the case of children on parental reporting. **Chapter 4** describes the accuracy of parental reported ICS use. **Chapter 5** addresses determinants of drug prescription in infancy. In **Chapter 6** we investigate aspects of therapy adherence in paediatric asthmatics.

Part II ("Control of asthma symptoms") of this thesis focuses on asthma control in paediatric patients. **Chapter 7** describes asthma control at age eight and the relation with parental perception towards the use of medication. In **Chapter 8**, we investigate agreement between measurements of current and long-term asthma control. Seasonal patterns of asthma symptoms are described in **Chapter 9**.

Part III ("Pharmacogenetics of asthma medication") gives a brief introduction to pharmacogenetics and we describe the aspects of pharmacogenetics in relation to the efficacy of ICS. **Chapter 10** discusses the future of pharmacogenetics and the use of a systems biology approach, whereas **Chapter 11** gives an overview of pharmacogenetic studies that have been performed in the field of asthma. In **Chapter 12** we describe the design and rationale of our PACMAN-cohort study. In **Chapter 13** we describe our first pharmacogenetic study, investigating the association between variation in the *FCER2* gene and ICS treatment response in asthmatics. Furthermore, we explore the role of variation in corticosteroid receptor complex genes. **Chapter 14** describes an analysis to screen 58 single nucleotide polymorphisms (SNPs) from 9 candidate genes for association with ICS treatment response. Finally, **Chapter 15** provides a general discussion of the findings within a broader perspective.





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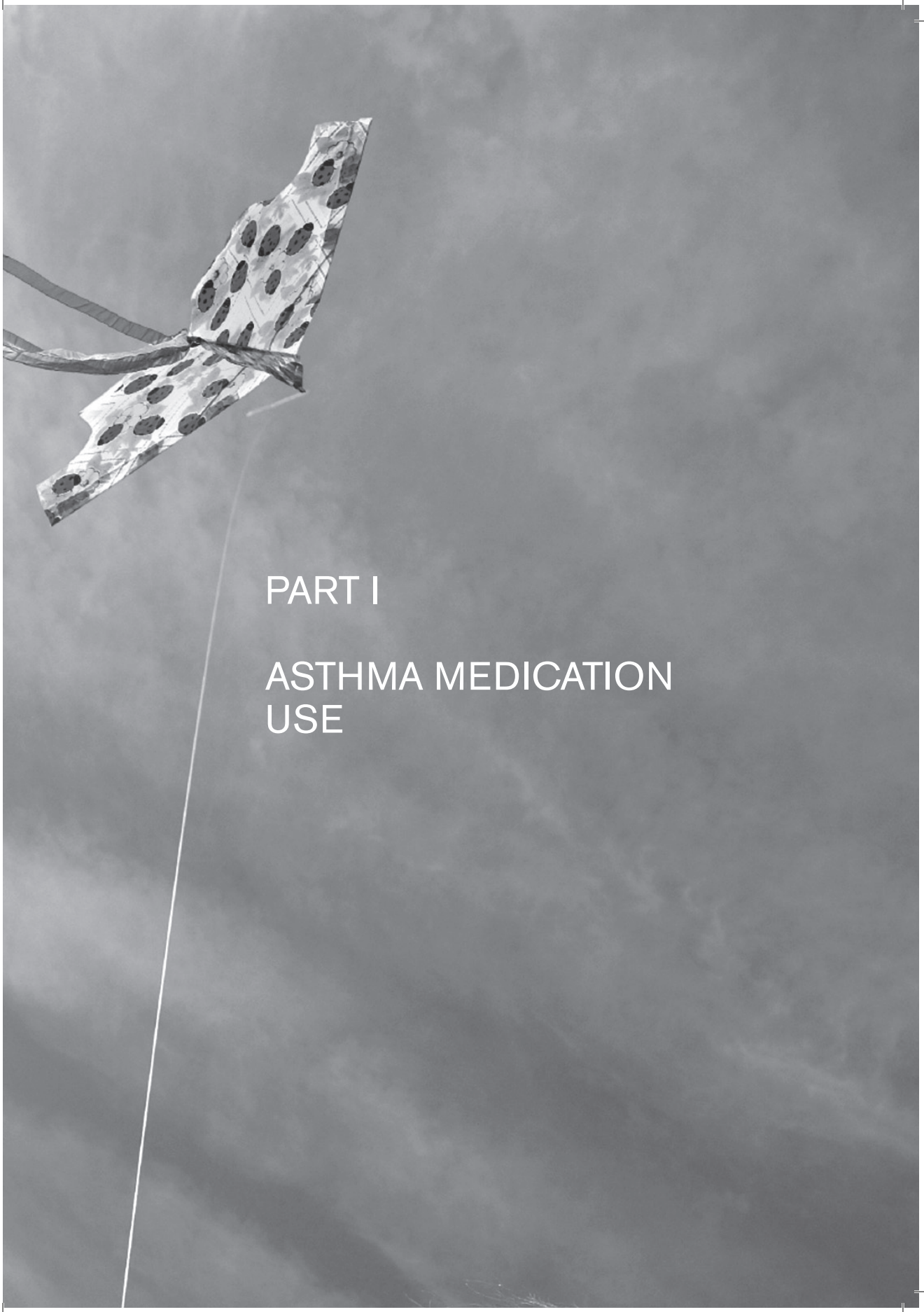
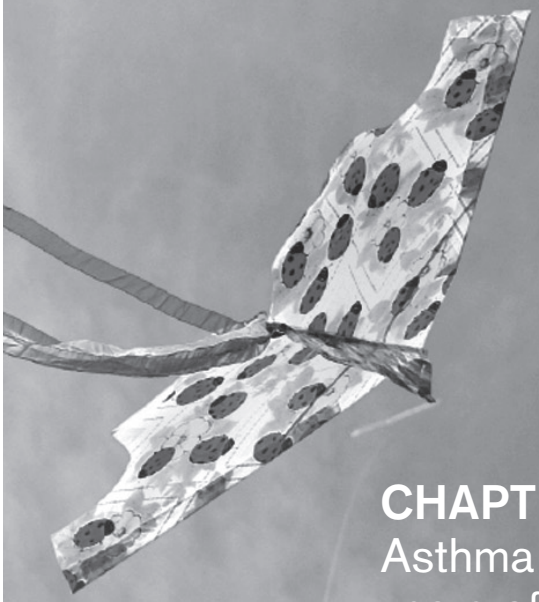


PART I

ASTHMA MEDICATION USE



CHAPTER 2
Asthma therapy during the first 8 years of life: a PIAMA cohort study

Mira G.P. Zuidgeest [1,2], Ellen S. Koster [1], Anke-Hilse Maitland- van der Zee [1], Henriëtte A. Smit [2], Bert Brunekreef [3,4], Hubert G. M. Leufkens [1], Gerard H. Koppelman [5], Dirkje S. Postma [6], Johan C. de Jongste [7], Maarten O. Hoekstra [8], Madelon Bracke [1] and the PIAMA-study group

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ABSTRACT

Objective: Many studies evaluated asthma medication use in children in a cross-sectional manner, yet little is known about longitudinal use patterns. This study describes the formation of a longitudinal dataset on asthma medication use and shows first results regarding the prevalence and incidence of medication use.
Methods: The PIAMA (Prevention and Incidence of Asthma and Mite Allergy) study is a prospective birth cohort study among 3963 Dutch children. Recruitment took place in 1996-1997. The data of the PIAMA birth cohort study were complemented with pharmacy data. Prescription information of family members was used to determine whether medication histories were complete from birth until age 8. The prevalence and incidence of asthma medication use was studied in children for whom complete medication histories were available.
Results: A first prescription for asthma medication was filled before age 8 by 280 (36%) children, with 88% starting therapy before age 5. Of all children who started therapy, 91.1% received short-acting beta-agonists and 61.1% inhaled corticosteroids.
Conclusion: The applied method of data collection rendered a dataset including 777 children with complete medication histories for their first 8 years of life. This dataset provides the opportunity to study longitudinal medication use patterns. First analyses show that asthma medication is initiated in a rather high percentage of children in this cohort and mainly at an age at which an asthma diagnosis cannot yet be firmly established.

INTRODUCTION

Several cohort studies have investigated the natural history of wheeze, which led to the identification of a number of wheezing phenotypes, such as transient, nonatopic and atopic wheezing [1, 2]. Though these phenotypes require a different therapeutic approach, they can only be discriminated retrospectively and are therefore of no direct use in treatment decision. Therefore, treatment decision is often based on risk factors for persistence of symptoms, including family history, the presence of eczema, passive smoking and sensitization to aeroallergens. These risk factors have been incorporated in recent guidelines on management of childhood asthma [3-6]. Additionally, treatment of respiratory symptoms in young children is often a therapeutic trial, where initial assessment and regular reassessment of the child's treatment response and need for therapy is essential [3-6]. This is expected to lead to distinct treatment patterns in young children with respiratory symptoms. However, studies evaluating the appropriateness of treatment with asthma medication in children are mainly cross-sectional in nature [7-11] or focus on the group of children with persistent asthma or physician diagnosed asthma [12-14].
To answer research questions such as (1) how many young children actually receive asthma medication and at which age therapy is initiated, (2) whether the guideline recommendations lead to differences in treatment between wheezing phenotypes and (3) what the actual treatment patterns are in young children with respiratory symptoms, a database with longitudinal information on asthma medication use has been created. This database is nested within the PIAMA (Prevention and Incidence of Asthma and Mite Allergy) study. In this study, we describe the formation of this new dataset and answer the first research question regarding prevalence and incidence of asthma medication use.

METHODS

Study design

We aimed to create a database with longitudinal information on asthma medication use within the ongoing PIAMA study. The PIAMA study is a prospective birth cohort study among 3963 Dutch children. Details of the study design have been published previously [15]. Recruitment took place between 1996 and 1997. A screening questionnaire was distributed among 10232 pregnant women visiting one of 52 prenatal clinics (Figure 1). Based on this screening 7862 women were invited to participate in the study; 4146 agreed and gave written informed consent. One hundred eighty-three participants were lost to follow-up before any data on the child had been obtained, therefore the PIAMA study started with 3963 newborn children. Data on respiratory and allergic symptoms, potential risk factors for asthma and allergy and demographic factors were collected by postal questionnaires. Questionnaires were sent to the participating parents during the last trimester of pregnancy, at the child's age of 3 months, at the age of 1 and annually thereafter. An extensive medical examination was carried out at age 8 in a subset of the population. Children of atopic mothers were overrepresented in this subset. The medical examination included measurement of bronchial hyperresponsiveness (BHR) and sensitization against common allergens.

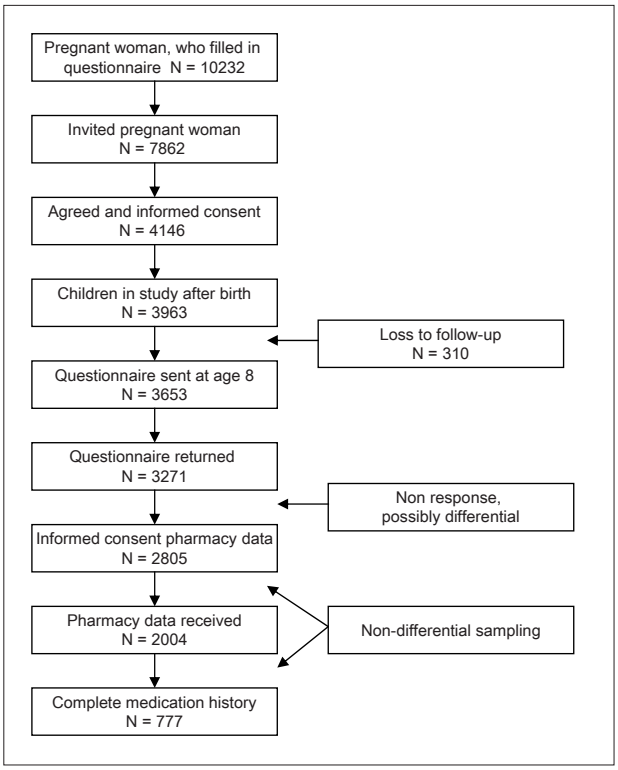
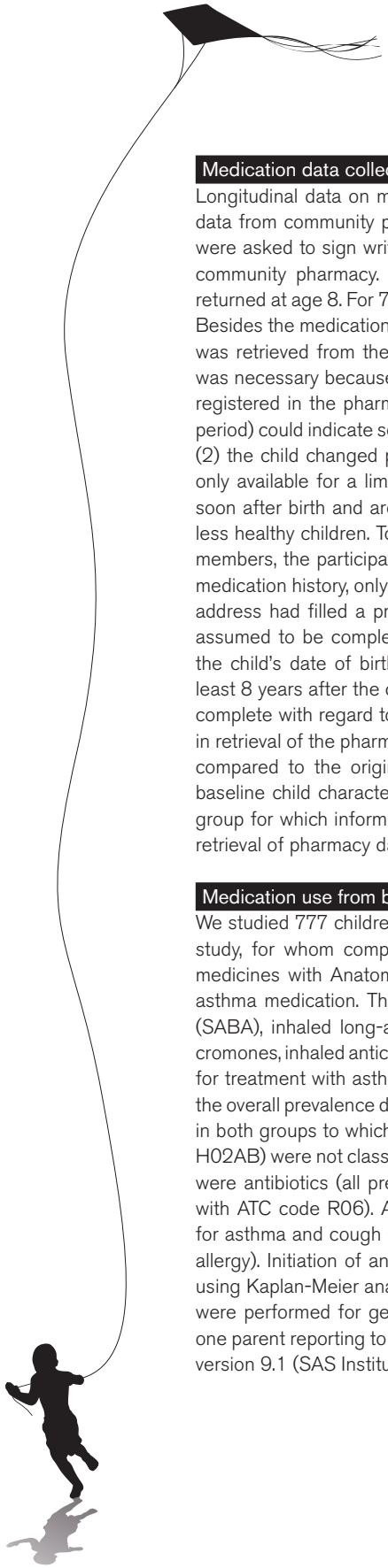


Figure 1. Flowchart of study population



Medication data collection

Longitudinal data on medication retrieval has been collected at age 8 through prescription data from community pharmacy records. In the annual questionnaire sent at age 8, parents were asked to sign written consent for retrieval of their child's medication history from their community pharmacy. Informed consent was given in 2805 of the 3271 questionnaires returned at age 8. For 777 children these pharmacy data were complete from birth until age 8. Besides the medication history of the child, limited prescription information on the family level was retrieved from the pharmacy to check if the medication histories were complete. This was necessary because a time period in which a child does not have any prescriptions while registered in the pharmacy (especially at the beginning and the end of the data collection period) could indicate several things: (1) the child did not receive any medication in that period, (2) the child changed pharmacy, or (3) the medication data in that specific pharmacy were only available for a limited time period. Including only children who received a prescription soon after birth and around age 8 would bias the study population towards a population of less healthy children. To tackle this problem without invading the privacy of the child's family members, the participating pharmacists were asked to extract, besides the child's complete medication history, only the first and the last date at which a family member living at the same address had filled a prescription at the pharmacy. The medication history of the child was assumed to be complete if the first prescription of a family member was recorded before the child's date of birth, and the last prescription of the child or a family member was at least 8 years after the child's date of birth. In the Netherlands, pharmacy records are virtually complete with regard to outpatient medication use [16]. To define whether the nonresponse in retrieval of the pharmacy data led to differences in the composition of the study population compared to the original PIAMA population we compared three groups with respect to baseline child characteristics: (1) children with complete pharmacy data (n = 777), (2) the group for which informed consent was given (n = 2805), and (3) the population eligible for retrieval of pharmacy data, which was the total PIAMA population at age 8 (n = 3271).

Medication use from birth up to age 8

We studied 777 children born in 1996 or in 1997 who participate in the PIAMA birth cohort study, for whom complete medication histories from birth until age 8 were available. All medicines with Anatomical Therapeutic Chemical (ATC) code R03 were considered to be asthma medication. This R03 group includes inhaled and oral short-acting beta2-agonists (SABA), inhaled long-acting beta2-agonists (LABA), inhaled corticosteroids (ICS), inhaled cromones, inhaled anticholinergics and montelukast [17]. The yearly prevalence was calculated for treatment with asthma medication in general and for specific medication groups, as was the overall prevalence during the first 8 years of life. Combination preparations were calculated in both groups to which the individual components belonged. Oral corticosteroids (ATC code H02AB) were not classified as primary asthma treatment. Other medication groups of interest were antibiotics (all prescriptions with ATC code J01) and antihistamines (all prescriptions with ATC code R06). Antihistamines were further subdivided into medications mainly used for asthma and cough (deptropine and promethazine) and all other antihistamines (used for allergy). Initiation of any asthma medication or ICS between birth and age 8 was analysed using Kaplan-Meier analyses. For the initiation of any asthma medication, subgroup analyses were performed for gender and parental asthma. Parental asthma was defined as at least one parent reporting to have (had) asthma. All statistical analyses were performed using SAS, version 9.1 (SAS Institute, Cary, NC).

RESULTS

Formation of longitudinal medication use dataset

Informed consent was given in 2805 of the 3271 questionnaires returned at age eight. Primary reason for not giving informed consent was that the child had never used any prescription drugs (83%). Furthermore, parents reported other reasons for not given informed consent (14%), they did not fill in or did not know the name of their community pharmacy (1%) and in some cases the form was not signed (2%). Pharmacy information was retrieved from community pharmacies for 2004 children. From 801 pharmacies we did not receive any information; most of these pharmacies (97%) did not give any response. Other reasons for not receiving the extraction were that the pharmacy no longer existed, the pharmacist was not able to perform the data extraction or the selected children were not registered to the pharmacy. For 777 children these pharmacy data were complete from birth until age eight. For the remaining 1227 children we did not have eight years of complete follow-up due to changing pharmacies (after migration) or there was no information on family members in the pharmacy or the pharmacy information system did not contain data for the complete eight year follow-up period.

Characteristics of the study population

A total of 777 children were included in this study. Our study population corresponded very well with the original PIAMA population with respect to gender, ethnicity, parental educational level, degree of urbanisation, parental asthma, and birth weight. No statistical significant differences in these characteristics were found between the three groups (Table 1). Children in our study population were primarily of Dutch origin (97.2%), 51.7% was male and 12.7% had at least one parent with reported asthma.

Table 1. Study population characteristics in relation to the original PIAMA cohort

	Study population (n = 777)	Informed consent for pharmacy data retrieval (n = 2805)	Original PIAMA study population at age 8 a (n = 3271)
Gender, % male	51.7	50.9	51.5
Ethnicity, % Dutch [b]	97.2	95.1	94.9
Maternal educational level, % low [c]	21.2	21.2	21.5
Paternal educational level, % low [c]	25.7	24.4	24.6
Degree of urbanisation, mean (SD) [d]	3.2 (1.2)	2.9 (1.3)	2.9 (1.3)
Parental asthma, % [e]	12.7	13.7	13.5
Birth weight in g, mean (SD)	3532 (533)	3517 (533)	3524 (534)
Maternal smoking during pregnancy, %	13.2	16.0	15.7

[a] This is the eligible population for retrieval of medication history, [b] Based on both the country of birth of the mother and the self-reported ethnicity of the mother, if both Dutch, [c] Educational level: low = primary, lower vocational and lower general; intermediate/high = senior high school, intermediate and high vocational and university, [d] Degree of urbanisation: groups are defined based on the address density per km²: >=2500, 1500-<2500, 1000-<1500, 500-<1000, <500, [e] Father or mother or both reported to have (had) asthma

Prevalence of asthma medication use

The prevalence of asthma medication use in the study population is shown in Table 2. Thirty-six percent of all children received at least one prescription for asthma medication in the years from birth until age 8. The prevalence was somewhat higher in the first years of life (varying around 12%) than at ages 5 to 7 (just above 10%). The high overall prevalence compared to the yearly prevalence indicates that in different years different children received asthma medication.

When investigating specific medication groups, we found that SABAs and ICSs were the most frequent prescribed drugs. The prevalence of SABA treatment steadily declined with age from 11.6% at age 0 to 6.8% at age 7, whereas ICS therapy was lower in the first year of life (4.8%) and fluctuated from 6.4% to 8.8% from age 1 to 7. Anticholinergics were mainly used at a young age, with a decline in prevalence of 5.5% in the first year to almost 0% (0.1%) at age 7. Only few children received montelukast, cromones or theophylline. The LABA group was the sole asthma medication group to show a modest rise in prevalence with increasing age (from 0.3% at age 0 to 1.2% at age 7).

Table 2. Prevalence of asthma medication use within the study population (n = 777)

	Age								
	0	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**0-7**
Asthma medication	12.7	12.9	11.5	12.7	12.9	10.7	10.2	10.2	36.0
Medication groups									
SABA	11.6	10.6	9.5	9.3	9.9	7.3	7.0	6.8	32.8
ICS	4.8	6.4	7.2	8.8	7.9	8.0	7.9	7.0	22.0
LABA	0.3	0.3	0.3	0.5	0.6	0.5	1.0	1.2	2.8
PSL	5.5	4.0	2.8	2.2	2.1	1.2	0.5	0.1	11.2
Other [a]	0.3	0.1	0	0.1	0	0	0.3	0.3	1.0
Other medicines									
Oral corticosteroids	0.6	0.6	1.0	0.9	1.0	0.8	0.4	0	3.9
Antihistamines	20.9	18.0	10.0	8.6	11.7	7.5	5.9	7.6	48.3
Deptropine and promethazine	18.0	13.1	5.8	5.0	6.8	2.6	1.7	2.3	35.8
Antibiotics	30.6	39.1	34.4	28.6	33.2	23.6	17.0	17.9	81.7

[a] Other = montelukast, cromones and/or theophylline, SABA = short-acting beta2-agonist; ICS = inhaled corticosteroids; LABA = long-acting beta2-agonist, PSL = parasympathicolytics

Other medicines

A majority of the children (81.7%) received at least one prescription for antibiotics in the first 8 years of life. Furthermore, a high use of antihistamines was found at a young age, which can be explained by a high prevalence of deptropine and promethazine (18.0%, traditionally used for cough and asthma instead of allergy), which rapidly declined with age. The prevalence of use of all other antihistamines prescribed was fairly constant over the years. Oral corticosteroid use was low at all ages.

Time until first prescription

Figure 2 shows the results from the Kaplan-Meier analysis. Of the 280 children receiving a first prescription for asthma medication before age 8, 77.9% initiated this therapy within the first 4 years of life. With respect to ICS treatment, 68.9% of children ever receiving ICS started this treatment before the age of 5 years. Initiation of asthma therapy was significantly associated with male gender, with 41.3% of the boys receiving asthma medication before age 8 versus 26.6% of the girls ($p < 0.005$). A similar trend, though not reaching significance was found for children with an asthmatic parent, i.e. 43.9% of children with an asthmatic parent started asthma medication versus 34.6% of children without parental asthma ($p = 0.08$).

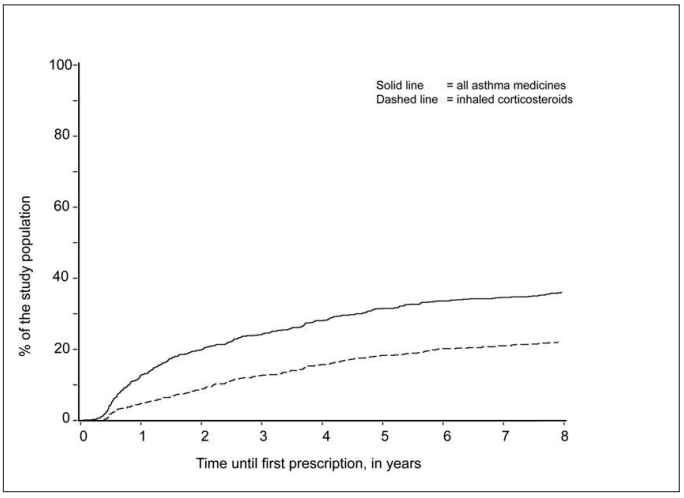


Figure 2. Cumulative prevalence of all children receiving at least one asthma medication prescription during 8 years of follow-up

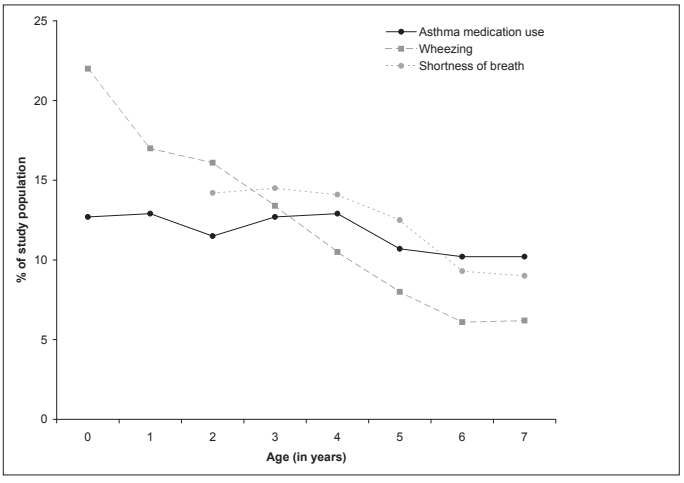
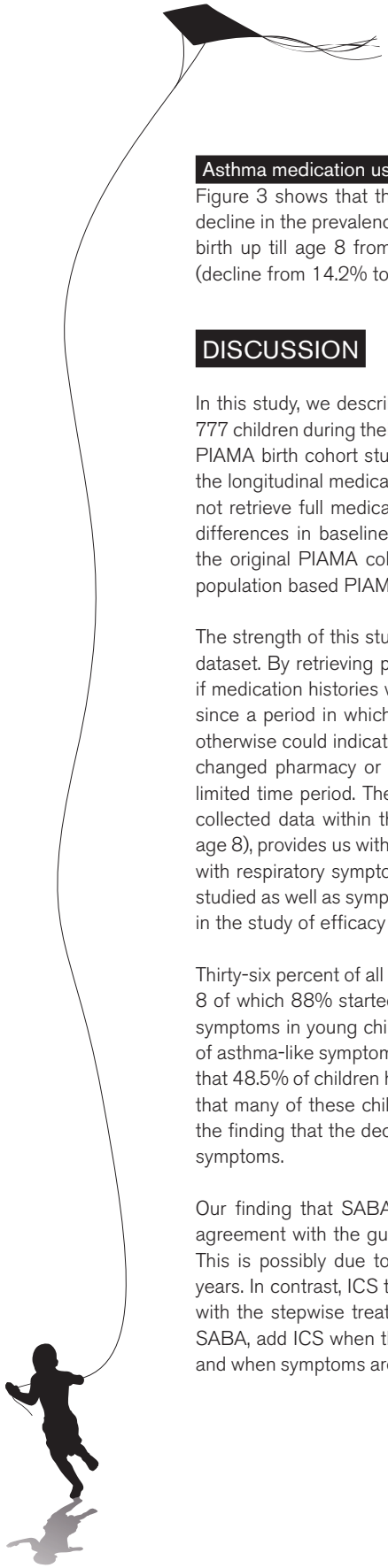


Figure 3. Asthma medication use and respiratory symptoms during the first 8 years of life



Asthma medication use and respiratory symptoms

Figure 3 shows that the decline in medication use (as shown in Table 2) correlates with a decline in the prevalence of respiratory symptoms. The prevalence of wheezing declines from birth up till age 8 from 22.0% to 6.2%. The same trend is shown for shortness of breath (decline from 14.2% to 9.0%).

DISCUSSION

In this study, we described the formation of a dataset with complete medication histories of 777 children during their first 8 years of life. We also showed the first results from the enriched PIAMA birth cohort study with pharmacy data, collected with the aim to render insights into the longitudinal medication use patterns in children from birth until age 8. Although we could not retrieve full medication histories for all children within the PIAMA cohort, no significant differences in baseline child characteristics were found between our study population and the original PIAMA cohort, indicating that our study population is a good reflection of the population based PIAMA cohort.

The strength of this study is the longitudinal design and the completeness of the medication dataset. By retrieving prescription information from family members, we were able to check if medication histories were completely extracted from birth until age eight. This is important, since a period in which a child does not have any prescriptions registered in the pharmacy otherwise could indicate that the child did not receive any medication in that period, the child changed pharmacy or medication data in that specific pharmacy were only available for a limited time period. The complete follow-up from birth until age 8, combined with the other collected data within the PIAMA study (annual questionnaires and medical examination at age 8), provides us with the opportunity to study patterns of asthma medication use combined with respiratory symptoms in a longitudinal design. Onset and duration of treatment can be studied as well as symptom characteristics. Together this information can for example be used in the study of efficacy of asthma medication in children.

Thirty-six percent of all children received a first prescription for asthma medication before age 8 of which 88% started therapy before age 5, which reflects the high burden of respiratory symptoms in young children published in the literature. Bisgaard et al. reported a prevalence of asthma-like symptoms of 32% in children aged one to five [18] and Martinez et al. reported that 48.5% of children have wheezed before the age of six [2]. This study shows that it is likely that many of these children receive treatment for their symptoms. This is also confirmed by the finding that the decline in asthma medication use correlated with a decline in respiratory symptoms.

Our finding that SABA and ICS were the main asthma medication used in children is in agreement with the guidelines [3-6]. The prevalence of SABA treatment declined with age. This is possibly due to the group of transient wheezers becoming asymptomatic over the years. In contrast, ICS therapy and LABA use are lower in very young children. This is in line with the stepwise treatment plan in the guidelines, which recommend to start therapy with SABA, add ICS when the child has certain risk factors and start LABA only in older children and when symptoms are more severe [3-6]. Also, LABA is not registered for use in very young

children. The decline in prevalence of anticholinergics from 5.5% to almost 0% could be explained by the fact that SABA has become the first choice bronchodilator in all guidelines, minimizing the place in therapy for the anticholinergics. The significant association between male gender and initiation of asthma therapy is conform previous findings in a population based cross-sectional study that showed that boys were more often treated with asthma medication and diagnosed as asthmatics [19]. Previous studies have consistently reported that the prevalence of wheeze and asthma is higher in boys than girls, while this pattern changes in adolescence [20]. Therefore, more frequent initiation of asthma treatment in boys is in agreement with these observations.
Both Goodman et al. [10] and Clavenna et al. [9] evaluated asthma medicine use cross-sectionally and showed that most children received only occasional prescriptions, presumably for mild illnesses and diseases other than asthma. A longitudinal approach makes it possible to evaluate whether the infrequent fills are within the group of transient wheezers. The difference in yearly and overall prevalence found in this study already shows that many children do not continue using asthma medication after a first prescription.

CONCLUSION

In conclusion, the described method of data collection resulted in a dataset containing longitudinal data on 777 children of the PIAMA study, which is a good reflection of the original PIAMA cohort. This data collection shows the high prevalence of asthma medication treatment in children before age 8 (36%) and provides opportunities to evaluate the appropriateness of this treatment in a longitudinal way.

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CHAPTER 3
Patterns of asthma medication use: Early asthma therapy initiation and association with asthma outcomes at age 8

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ABSTRACT

Objective: Wheeze has many underlying pathophysiologies in childhood, but is the main reason for anti-asthma drugs prescription. This study was conducted to describe asthma medication use patterns among children in their first eight years of life.
Methods: Longitudinal medication use data from 777 children participating in the PIAMA study were used. Medication patterns were described for four groups that started therapy before the third birthday, when the peak in prescriptions occurred in our cohort; Short-acting beta-agonist (SABA), inhaled corticosteroids (ICS), SABA+ICS or none of these.
Results: One third (n = 255) of the children received a first SABA or ICS prescription before age eight. Only three children (1.2%) used medication continuously during follow-up. Of the children who started SABA, 53.8% discontinued within 1-2 years. Of the children who started ICS before age 3, 42.1% discontinued within 1-2 years and 31.6% received additional SABA. 41.5% of the children who started SABA+ICS used this short-term (≤ 1-2 years) and 21.5% long-term (≥ 3 years). Fifteen percent of children who did not start asthma therapy in their first three years of life did receive prescriptions between age three and eight. Children prescribed SABA+ICS before age three had the highest prevalence of hyper responsiveness at age eight, and similar prevalence of atopy as the other groups.
Conclusion: Asthma medication is prescribed frequently in the first eight years of life, particularly before age three, and only few children use it continuously. ICS and SABA prescription occurs especially in who were more likely to develop signs of asthma at age eight.

INTRODUCTION

Anti-asthmatic drugs are the most widely chronically used drugs in children [1]. Asthma therapy is initiated to reduce respiratory symptoms and acute exacerbations and to minimize sleep disturbances and absences from school [2]. According to guidelines of the Dutch College of General Practitioners, treatment of childhood asthma follows a stepwise approach until control is achieved. Short-acting -agonists (SABA) are prescribed for symptomatic treatment. Inhaled corticosteroids (ICS) are prescribed to reduce airway inflammation and are part of the maintenance therapy for persistent asthma [2 3]. In young children, differentiating asthma from other wheezing disorders is very difficult. Therefore, treatment is often a therapeutic trial and regular reassessment of therapy effects is necessary [4]. This is expected to lead to specific treatment patterns in young children.
Apart from acute situations, most patients visit their physician only once or twice a year. During these consultations medication will be prescribed and discussed with the patients, who generally take care of their own medication. Low persistence, non-adherence and variable patterns of asthma medication use have been described [5-8]. Previous studies on medication were generally cross-sectional in design and did not describe patterns of medication use over years of follow-up, but only reported age-related differences in use [9-13]. We investigated the PIAMA birth cohort [14 15] and this provided us with the opportunity to study medication use in the same group of children at different ages in a longitudinal design. The objectives of this study were to describe patterns of asthma medication use in children who started at very young age and to compare usage patterns in children who started with SABA, ICS or the combination. Second, we investigated the association with the outcome at age eight, i.e. with respect to respiratory symptoms, atopy and bronchial hyper responsiveness in these different therapy starting groups.

METHODS

Study population

We studied children who participated in the PIAMA (Prevention and Incidence of Asthma and Mite Allergy) birth cohort study and for whom complete medication histories from birth until age eight were available. Details of the study design have been published previously [15]. Recruitment took place in 1996 -1997. A screening questionnaire was distributed to 10232 pregnant women. Based on this screening 7862 women were invited to participate in the study; 4146 agreed and gave written informed consent. 183 participants were lost to follow-up before any data on the child had been obtained, so that the study started with 3963 newborn children. Data on respiratory and allergic symptoms, potential risk factors for asthma and allergy and demographic factors were collected by postal questionnaires. Questionnaires were sent to the participating parents during the last trimester of pregnancy, at the child's age of three months, at the age of one year and annually thereafter. An extensive medical examination was carried out at age eight in a subset of the population. Children of atopic mothers were overrepresented in this subset. The medical examination included measurement of bronchial hyper responsiveness (BHR) and sensitization against common allergens.

Longitudinal data on medication retrieval have been collected at age eight through prescription data from community pharmacy records. In the questionnaire sent at age eight, parents were asked to sign written informed consent for retrieval of their child's medication history from their community pharmacy. The formation of this pharmacy dataset has been described elsewhere [14]. Briefly, informed consent was given in 2805 of the 3271 questionnaires returned at age eight. Pharmacy information was retrieved from community pharmacies for 2004 children. For 777 children these pharmacy data were complete from birth until age eight. In this study we only included children for whom the complete medication history was available.

Medication use

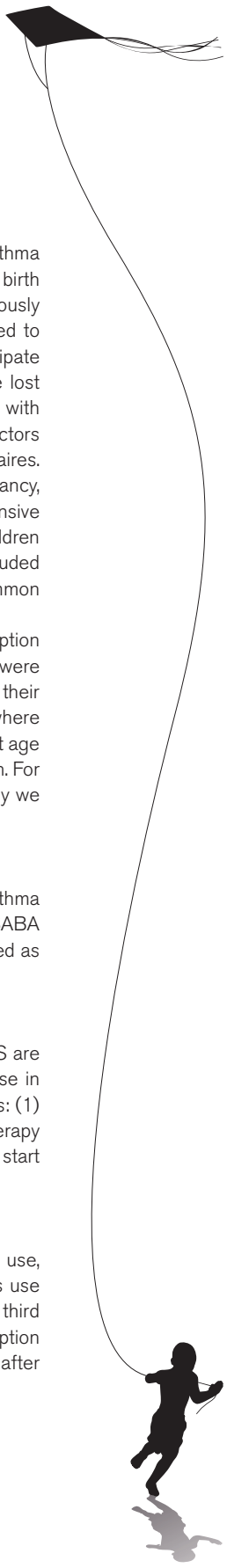
The Anatomical Therapeutical Chemical (ATC) code R03 was used to select asthma medication prescriptions [16]. The yearly prevalence was calculated for treatment with SABA and/or ICS. Incidence of asthma medication use was also calculated; a child was defined as incident user if he or she filled their first prescription in that specific year.

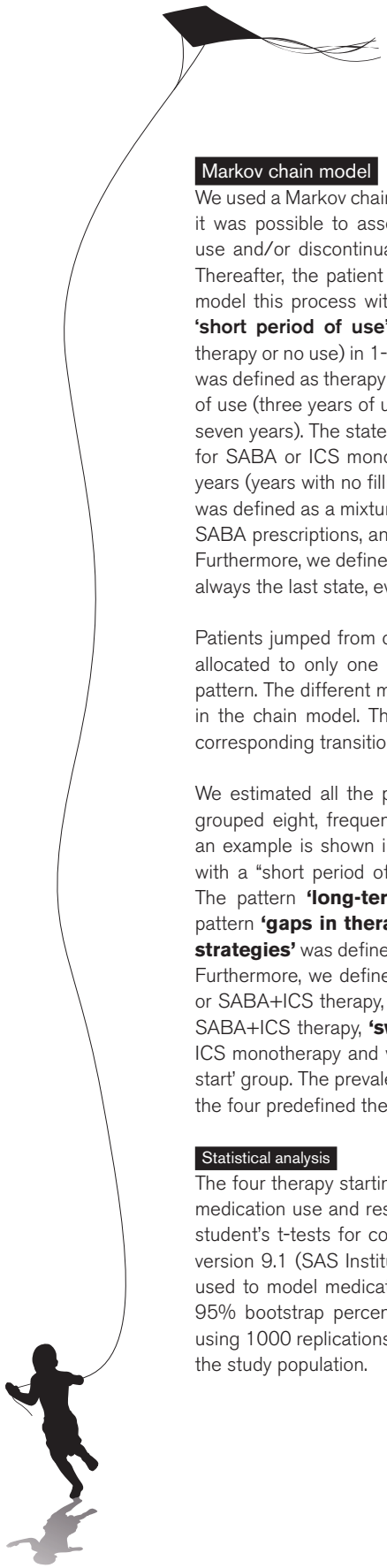
Definition of therapy starting groups

Based on the guidelines for treatment of children with asthma, in which SABA and ICS are recommended as standard treatment and the peak incidence of asthma medication use in children under age three in our cohort we defined four possible therapy starting groups: (1) start before the third birthday with SABA monotherapy ('SABA start'), (2) ICS monotherapy ('ICS start') or (3) SABA+ICS therapy ('SABA+ICS start') and (4) children who did not start with any of these therapies before their third birthday ('no start').

Refill patterns after the third birthday

We described different refill patterns for the four therapy starting groups: continuous use, never use and ever use after the third birthday. In the content of this study continuous use was defined as the filling of at least one prescription in each year of follow-up (after the third birthday). Never users after the third birthday were children who did not refill a prescription after the third birthday. Ever users were children who had at least one refill prescription after the third birthday.





Markov chain model

We used a Markov chain model to describe individual medication use patterns. With this model it was possible to assess the probabilities of various patterns, instead of just continuous use and/or discontinuation. At each time moment a patient uses medication or does not. Thereafter, the patient uses the same therapy, another therapy or discontinues therapy. To model this process with Markov chain modelling we defined the following state: the state **'short period of use'** was defined as therapy (SABA or ICS monotherapy, SABA+ICS therapy or no use) in 1-2 years within a period of three years. The state **'long period of use'** was defined as therapy in ≥ 3 years within a period that was smaller than two times the period of use (three years of use within a period of five years or four years of use within a period of seven years). The state **'gaps in use'** was defined as the filling of two or more prescriptions for SABA or ICS monotherapy, or SABA+ICS therapy with gaps of ≥ 3 years subsequent years (years with no filling of any prescriptions) between these prescriptions. The state **'mix'** was defined as a mixture of different treatment patterns, for example: the filling of one or two SABA prescriptions, an ICS prescription, SABA+ICS therapy and again SABA monotherapy. Furthermore, we defined the states **'no filling in the last ≥ 3 years'** and **'age 8'** which was always the last state, everybody had a transition into this state due to the end of follow-up.

Patients jumped from one state to another in time and at every time moment a patient was allocated to only one of the defined states. Together these subsequent states formed a pattern. The different medication use patterns could be distinguished by following the 'route' in the chain model. The probability of a certain pattern was calculated by multiplying the corresponding transition probabilities for this certain route.

We estimated all the patterns and based on the distribution of the different patterns, we grouped eight, frequently occurring, summarizing patterns. For each of the eight patterns an example is shown in Figure 1. The pattern **'short-term therapy'** was defined as start with a "short period of use" followed by "no filling of prescriptions in the last ≥ 3 years". The pattern **'long-term therapy'** was defined as start with "long period of use". The pattern **'gaps in therapy'** was the same as the state "gaps in use". **'Mixture of therapy strategies'** was defined as a mixture of the filling of prescriptions in different therapy groups. Furthermore, we defined **'switching to SABA monotherapy'** after a short period of ICS or SABA+ICS therapy, **'switching to ICS monotherapy'** after a short period of SABA or SABA+ICS therapy, **'switching to SABA+ICS therapy'** after a short period of SABA or ICS monotherapy and we defined **'never therapy'** which only concerns children in the 'no start' group. The prevalence of these eight defined medication use patterns was described in the four predefined therapy starting groups.

Statistical analysis

The four therapy starting groups were compared with respect to socio-demographic factors, medication use and respiratory symptoms by using Chi-square tests for frequency data and student's t-tests for comparison of means. Statistical analyses were performed using SAS, version 9.1 (SAS Institute, Cary, NC). The statistical software package R version 2.6.2 was used to model medication use patterns with the help of a Markov chain model. Two-sided 95% bootstrap percentile confidence intervals for transition probabilities were computed using 1000 replications. Bootstrap samples were obtained by random sampling patients from the study population.

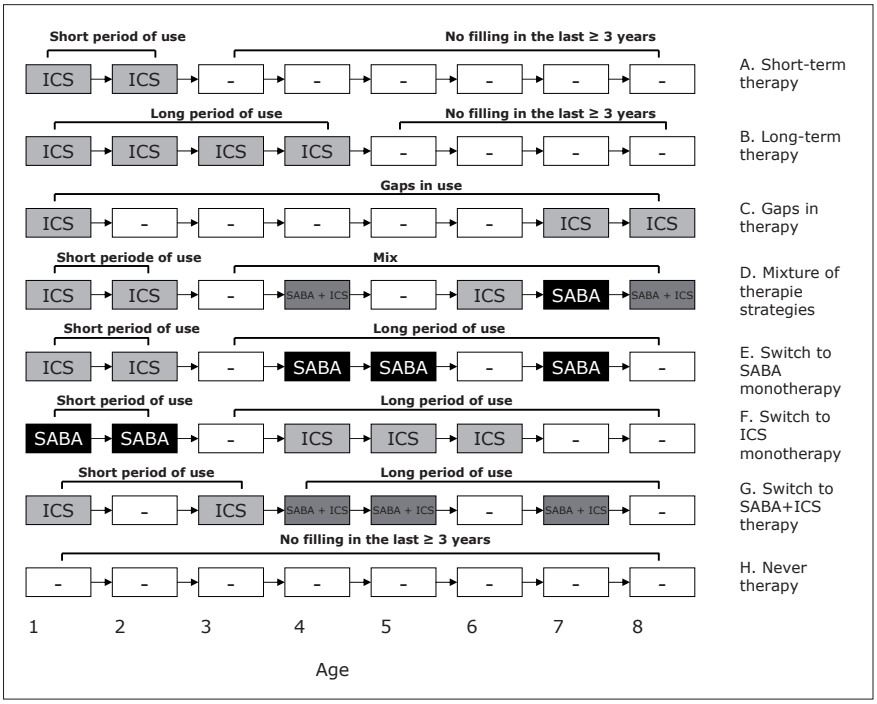


Figure 1. Examples of patterns of medication use during follow-up. This figure shows examples of the possible patterns of medication use during follow-up for children in the 'ICS start' group. Except pattern F (switch to ICS monotherapy) and pattern H (never therapy), they are an example for children in the 'SABA start' and 'no start' group respectively.

RESULTS

Study population

Only 19 (2.4%) children started with ICS monotherapy before their third birthday, 78 (10.0%) started with SABA monotherapy, 65 (8.4%) with SABA+ICS therapy and 615 children (79.2%) did not start with any of these therapies before the third birthday. The four therapy starting groups were very similar with respect to demographic factors, such as region, parental educational level and ethnicity and lifestyle factors, such as breastfeeding and smoking during pregnancy (Table 1). However, there were fewer boys in the 'no start' group compared to the other groups ($p < 0.05$). 32.8% ($n = 255$) of the children received a first prescription for SABA or ICS somewhere in time before age of eight. Only 3 (1.2%) children used medication continuously during their first 8 years of life. Figure 2 shows initiation of therapy; SABA and SABA+ICS therapy incidence decreased with age and the incidence of ICS use increased very slightly till age 4 where after it decreased. The majority of children started therapy before age 3; 63.4% ($n = 78$) of the children who started on SABA monotherapy during the first eight years started before the third birthday, 48.7% ($n = 19$) of the children who started ICS monotherapy started before the third birthday and 70.6% ($n = 65$) of the children who started with SABA+ICS therapy started on this therapy before the third birthday.

Table 1. General characteristics of study population

	SABA start (n = 78)	ICS start (n = 19)	SABA+ICS start (n = 65)	No start (n = 615)
Gender, % male	62.8	57.9	64.6	48.8*
Ethnicity, % Dutch [a]	96.2	89.5	92.3	95.8
Low maternal educational level, % [b]	22.1	26.3	23.1	20.7
Low paternal educational level, % [b]	23.4	21.1	29.2	25.7
Region in the Netherlands				
North, %	38.5	31.6	21.5	34.0
Central, %	43.6	52.6	58.5	46.7
Southwest, %	18.0	15.8	20.0	19.4
Smoking during pregnancy, %	15.4	5.3	16.9	12.8
Breastfeeding, %	83.1	89.5	73.0	82.3
Atopy [c]				
Mother, %	21.8	26.3	30.8	26.5
Father, %	26.9	31.6	35.4	32.7
Siblings, %	21.8	15.8	29.2	20.3

[a] Based on country of birth of the mother and self-reported maternal ethnicity, both Dutch, [b] Educational level low: primary, lower vocational and lower general education, [c] Questionnaire based, * $p < 0.05$ (tested against other 3 groups)

Respiratory symptoms and medication use

In the 'SABA+ICS start' group the prevalence of bronchial hyperresponsiveness at age eight was significantly higher than in the other groups (Table 2), i.e. 68.2% of the children showed bronchial hyperresponsiveness compared to 38.4% in the other groups combined ($p < 0.05$). The prevalence of atopy at age eight was not significantly different between the four therapy starting groups. Asthma medication use at age eight was significantly lower in the 'no start' group ($p < 0.05$) than in the other therapy starting groups.

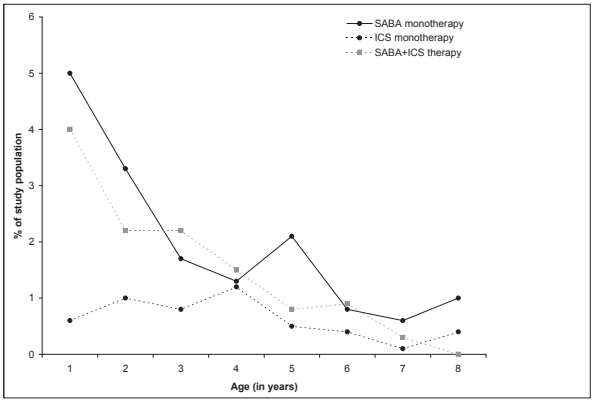


Figure 2. Initiation of asthma medication use within the PIAMA pharmacy cohort

Table 2. Prevalence of respiratory symptoms and medication use

	SABA start (n = 78)	ICS start (n = 19)	SABA+ICS start (n = 65)	No start (n = 615)
Respiratory symptoms				
Wheezing				
Before 3rd birthday, %	74.4 (58/78)	61.1 (11/18)	88.7 (55/62) *	23.4 (138/590) **
At age 3, %	39.7 (31/78)	27.8 (5/18)	52.4 (33/63)	8.9 (54/607) *
At age 8, %	9.0 (7/78)	5.3 (1/19)	12.3 (8/65)	5.2 (32/615)
Duration in first 8 years, mean (SD)	2.1 (2.1)	1.8 (1.5)	2.9 (2.2)	0.6 (1.2) **
Shortness of breath [a]				
At age 3, %	27.6 (21/76)	42.1 (8/19)	53.1 (30/64)	8.2 (50/610) **
At age 8, %	14.1 (11/78)	10.5 (2/19)	24.6 (16/65)	6.7 (41/614)
Duration of in first 8 years, mean (SD)	1.2 (1.9)	1.6 (1.9)	2.3 (2.1)	0.5 (1.0) **
Physical examination [b]				
Atopy at age 8 c, %	35.4 (17/48)	10.0 (1/10)	48.7 (19/39)	28.8 (100/347)
BHR at age 8 d, %	27.8 (5/18)	20.0 (1/5)	68.2 (15/22) *	40.0 (70/ 175)
Asthma medication use				
At age 8, %	16.7	21.1	23.1	6.5 *
Duration in first 8 years, mean (SD)	2.5 (1.8)	3.0 (1.9)	3.1 (2.0)	0.3 (0.8) **

[a] No information was available on shortness of breath symptoms before the 3rd birthday, [b] The physical examination was carried out in a subset of the population, [c] Defined as a specific IgE concentration of at least 0.7 IU/ml on at least one of the airborne allergens in blood sample collected at age eight, [d] Defined as a decrease of 20% in FEV1 at a cumulative dose of 0.61 mg metacholine bromide, * $p < 0.05$ (compared to the other 3 groups), ** $p < 0.0001$ (compared to the other 3 groups)

Asthma medication refill patterns after the third birthday

We found that 'starters' used asthma medication after the third birthday more often continuously than 'no starters' ($p < 0.0001$). In the 'SABA+ICS start' group more children were continuous users after the third birthday (9.2%) than in the other groups; 6.4% in the 'SABA start' group, 5.2% in the 'ICS start' group and 1.0% in the 'no start' group ($p < 0.0001$). Most children were irregular users or discontinued medication use after the third birthday. Only a small fraction of children (15.0%) in the 'no start' group started therapy after the third birthday (Figure 3). Only 2 children (3.1%) in the 'SABA+ICS start' group used SABA+ICS therapy continuously after the third birthday. None of the children in the 'SABA start' group were continuous users of SABA. The same was true for children in the 'ICS start' group; none of them had a yearly refill for an ICS. This indicates that children switched to other treatment regimes, e.g. a SABA was added or ICS monotherapy or SABA+ICS therapy was switched to monotherapy, because continuous users were present in all groups after the third birthday (Figure 3).

Probabilities of medication use patterns assessed by Markov analysis

Figure 4 shows the probabilities of different medication use patterns (birth up till age eight) for the four therapy starting groups. Short-term anti-asthma therapy was the most frequent pattern in all therapy starting groups (n=151). In the 'SABA start' group, the probability of short-term therapy was 53.8% (95% CI: 43.3 - 64.9), switching from SABA monotherapy to SABA+ICS therapy (12.8%, 95% CI: 5.8 - 16.1) and long-term therapy of SABA monotherapy (12.8%, 95% CI: 5.9 - 20.8) were less common. Children in the 'ICS start' group also had a high probability of short-term therapy (42.1%, 95% CI: 18.2 - 66.7) and adding of SABA to therapy (31.6%, 95% CI: 11.1 - 55.0), switching to SABA monotherapy was less common (10.5%, 95% CI: 0.0 - 27.8). In the 'SABA+ICS start' group, the probability of short-term therapy was 41.5% (95% CI: 29.6 - 54.2), long-term therapy (21.5%, 95% CI: 11.9 - 32.2), switching to SABA monotherapy (4.6%, 95% CI: 0.0 - 10.3) or ICS monotherapy (10.8%, 95%CI 3.4 - 18.5) and mixture (18.5%, 95% CI: 9.4 - 29.0) of therapies were less common. In the 'no start' group, children had a high probability (85.0%, 95% CI: 82.1 - 87.9) of never start using asthma medication; 7.2% started on SABA monotherapy, 3.3% on ICS monotherapy and 4.5% on SABA+ICS therapy.

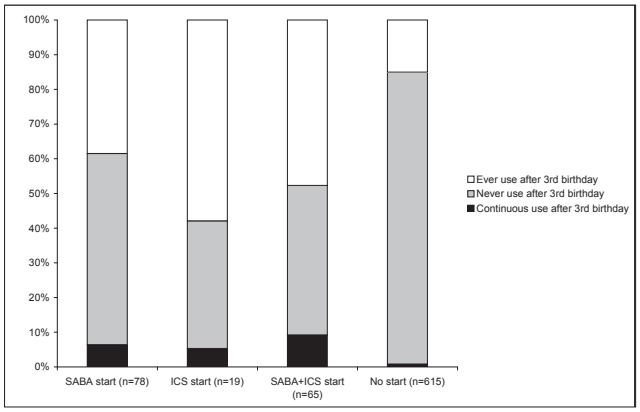


Figure 3. Asthma medication refill patterns after third birthday for SABA and/or ICS

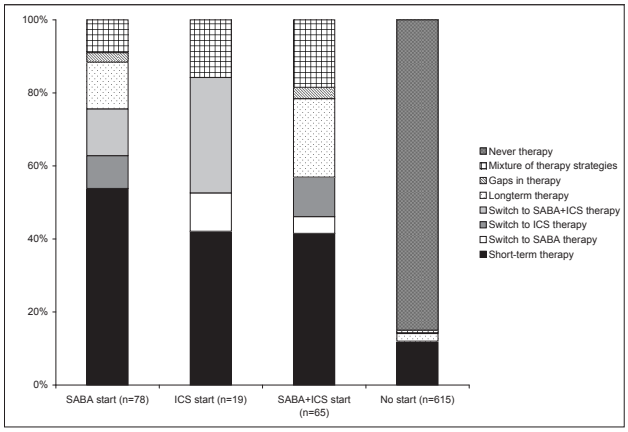


Figure 4. Patterns of asthma medication use for different therapy groups Probabilities for the different medication use patterns with their corresponding 95% bootstrap confidence intervals.

Table 3. Patterns of asthma medication use for different therapy groups

	SABA start (n = 78)	ICS start (n = 19)	SABA+ICS start (n = 65)	No start (n = 615)	Total (n = 777) N (%)
Short-term therapy	53.8 (43.3 – 64.9)	42.1 (18.2 – 66.7)	41.5 (29.6 – 54.2)	12.0 (9.5 – 14.7)	151 (19.4)
Switch to SABA monotherapy	-	10.5 (0.0 – 27.8)	4.6 (0.0 – 10.3)	-	5 (0.6)
Switch to ICS monotherapy	9.0 (2.8 – 16.1)	-	10.8 (3.4 – 18.5)	-	14 (1.8)
Switch to SABA+ICS therapy	12.8 (5.8 – 20.7)	31.6 (11.1 – 55.0)	-	-	16 (2.1)
Long-term therapy	12.8 (5.9 – 20.8)	-	21.5 (11.9 – 32.3)	2.1 (0.9 – 3.4)	37 (4.9)
Gaps in therapy	2.6 (0.0 – 6.7)	-	3.1 (0.0 – 7.2)	0.2 (0.0 – 0.5)	5 (0.6)
Mixture of therapy strategies	9.0 (3.3 – 15.6)	15.8 (0.0 – 32.0)	18.5 (9.4 – 29.0)	0.7 (0.2 – 1.3)	26 (3.3)
Never therapy	-	-	-	85.0 (82.1 – 87.9)	523 (67.3)

DISCUSSION

The longitudinal PIAMA birth cohort study provided us with the opportunity to study patterns of asthma medication use for children who started medication use at young age in a longitudinal design. Therapy was initiated at very young age: the majority of the children who started anti-asthma therapy started before age three. There were few continuous users, most children used medication only short-term, i.e. therapy continuation during the first eight years of life was low.

Four therapy starting groups were defined; most children were classified as 'no starters' before the third birthday, a part was classified as SABA starter or SABA+ICS starter and only a small percentage of study population started on ICS monotherapy. SABA monotherapy is generally used as try-out medication, if the child has no favourable effect from SABA therapy, the most probable conclusion is that the child does not have asthma. An ICS is frequently added later on when symptoms have shown to be persistent. Few children in our cohort started on ICS monotherapy since this is not recommended in the guidelines [6]. ICS could sometimes be prescribed for severe coughing (possibly unrelated to asthma). The four therapy starting groups were very similar with respect to socio-demographic factors such as ethnicity, parental educational level and region. There were more girls than boys in the 'no start' group. This is an expected finding because it is common knowledge that the prevalence and incidence of childhood wheezing and asthma is higher in boys, and boys are therefore more likely to use asthma medication [13 17 18].

Children in the 'SABA+ICS start' group showed more often bronchial hyper responsiveness and a trend towards a higher prevalence of wheezing symptoms at age eight suggesting that these children are the more severe (asthmatic) patients who are treated more extensively compared to the other groups. This finding might also be further evidence that (inhaled) steroids do not alter the natural history of asthma, which was also shown by Guilbert et al [19]. In all therapy starting groups, the prevalence of wheeze and shortness of breath was much higher at age three than at age eight. In addition, children who started therapy before the third birthday had a greater decline in symptoms than 'no starters'. This could indicate that initiation of therapy at young age reduces symptoms at later age. However, it is difficult to asses therapy effectiveness with these epidemiological data, because disease severity has not been taken into account. In asthma, especially in childhood, symptoms can also dissolve spontaneously.

Approximately 40% and 25% of the children who started with ICS monotherapy and SABA monotherapy respectively did not report wheeze before the third birthday. This could be due to several factors. First, in our study, wheeze was assessed by parental report. A study of Lowe et al. [20] showed that there exists poor parental perception of the term "wheeze". Another study showed that the congruence between parent and physician recorded wheeze is poor [21]. Thus, reporting bias may have contributed to our results. Second, the fact that part of the asthma medication users had no respiratory symptoms before the third birthday could be the result of good asthma control (good response to pharmacotherapy). Lastly, an important reason for medication use while no symptoms are being reported is that these children were prescribed medication for other symptoms than wheezing. Hoan et al. [22] showed that ICS were used for coughing symptoms. Furthermore, it has been reported that asthma medication is also used to treat respiratory virus infections in childhood [23].

Only a few children used continuously treatment after their third birthday, most children being irregular users or discontinuing medication use after the third birthday. The highest percentage of continuous users was found in the 'SABA+ICS start' group. In all therapy starting groups, children had the highest probability for short-term therapy. This could be explained by the fact that especially in young children asthmatic symptoms are often transient. Most children who only experience wheezing symptoms at very young age, as reported by Martinez et al. [24] are so called transient wheezers. Therefore, in young children, treatment with asthma medication is often a therapeutic trial which is supported by the finding of the high probabilities of short-term therapy. Not many children refilled prescriptions on the same therapy regime, i.e. they switched to different treatment strategies. Children switched from ICS or SABA monotherapy to SABA+ICS therapy or from SABA+ICS therapy to monotherapy. This is according to the guidelines which recommend SABA+ICS therapy for children with persistent asthmatic symptoms [2 3 11 25-27]. Finally, the probability for starting with anti-asthma drugs after the third birthday in children of the 'no start' group was low suggesting that indeed respiratory symptoms start early in life predominantly.

The strength of our study is that we have complete longitudinal data of medication histories of the children from birth up till age eight. In contrast to other studies with cross-sectional data, we were thus able to study initiation of therapy and the differences in medication use over the years between children who start with different treatment regimes. We estimated the probabilities of different medication use patterns for children who start on different treatment

strategies with a Markov model. These models are very useful for the study of medication use patterns and by defining specific states it was possible to describe and cluster all possible medication use patterns in our longitudinal cohort [5].
Limitation of our study was the relatively high number of drop-outs; pharmacy information was retrieved from about 2000 children whilst pharmacy records were complete for 777 children. However, we do not think this has caused any selection bias. As shown in the paper of Zuidgeest et al. [14] our study population, which is a subset of the PIAMA cohort corresponds very well to the original PIAMA study population; there were no differences in characteristics of children with complete medication history (n = 777), children for whom the parents gave informed consent to extract pharmacy data (n = 2805) and the total PIAMA study population at age 8 (n = 3271) [14]. Notwithstanding this, our results should be carefully interpreted because of our relatively small sample size (especially in the 'ICS start' group). Furthermore, children and parents were followed from birth up to age eight, which may have caused more awareness and a different perception of parents towards their child's respiratory symptoms compared to not in the study included children and parents. However, this limitation is shared with previous surveys. The prevalence of wheeze in our cohort is comparable with other Dutch birth cohorts [28]. Considering the large sample size of the PIAMA study, high follow-up and response rates, the population based PIAMA birth cohort is a reasonable reflection for Dutch children.

Several conclusions can be drawn from the work presented here. First, the incidence of asthma medication use is highest before the age of three years. Therapy is initiated at an age when proper diagnostic tools for asthma are lacking. Second, not many children used asthma medication continuously during their first eight years of life. Many children received therapy for a short period while others switched to a different therapy strategy. This observation is consistent with transient patterns of respiratory symptoms. Lastly, children who start on SABA+ICS therapy before the third birthday seem to have more severe disease.

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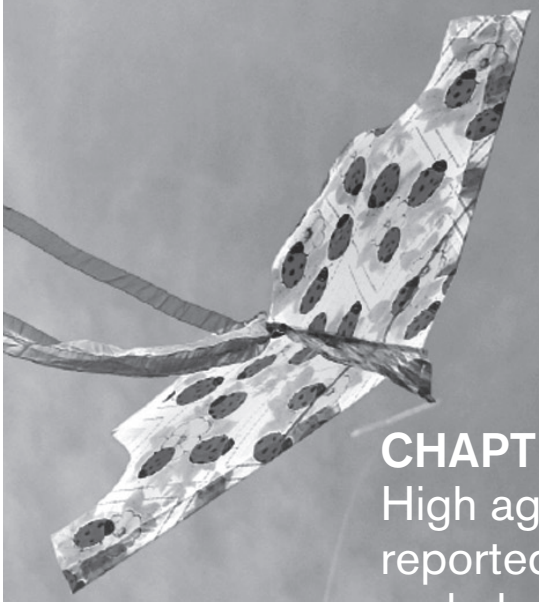

CHAPTER 4
High agreement between parental reported inhaled corticosteroid use and pharmacy prescription data

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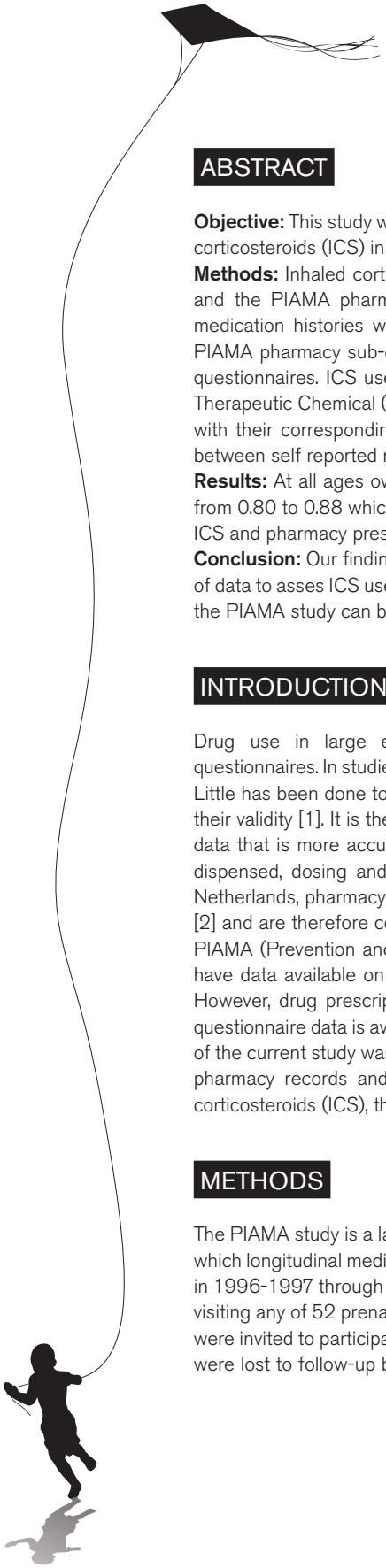


ABSTRACT

Objective: This study was conducted to assess the validity of parental reported use of inhaled corticosteroids (ICS) in children.
Methods: Inhaled corticosteroid users were identified within the PIAMA birth cohort study and the PIAMA pharmacy sub-cohort that is nested within the PIAMA study. Complete medication histories were available for the first eight years of life for children within the PIAMA pharmacy sub-cohort. Parental reported ICS use was measured by using data from questionnaires. ICS use in the pharmacy records was determined by using the Anatomical Therapeutic Chemical (ATC) codes. The proportion of overall agreement and kappa statistics with their corresponding 95% confidence intervals were calculated to quantify agreement between self reported medication use and pharmacy prescription data.
Results: At all ages overall agreement was very high (>97%) and Cohen's kappa's ranged from 0.80 to 0.88 which also reflects excellent agreement between parental reported use of ICS and pharmacy prescription data.
Conclusion: Our finding suggests that parental report of medication use is a reliable source of data to asses ICS use in children. The questionnaire based medication data collected within the PIAMA study can be used to study asthma medication use in a large group of children.

INTRODUCTION

Drug use in large epidemiological studies is often measured by self-administered questionnaires. In studies concerning children, drug use is measured by parental reported data. Little has been done to assess the accuracy of these data, though some studies questioned their validity [1]. It is therefore important to compare parental reported data with prescription data that is more accurate (these records also contain detailed information on the amount dispensed, dosing and prescriber), but more difficult to obtain in many situations. In the Netherlands, pharmacy records are virtually complete with regard to outpatient prescriptions [2] and are therefore considered as a gold standard in terms of prescription data. Within the PIAMA (Prevention and Incidence of Asthma and Mite Allergy) birth cohort study [3 4] we have data available on both parental reported medication use and pharmacy prescriptions. However, drug prescription data are only available for part of the study population whilst questionnaire data is available for the majority of the children within the PIAMA study. The aim of the current study was to assess the agreement between prescription data from community pharmacy records and parental reported medication usage in children who use inhaled corticosteroids (ICS), the cornerstone of asthma therapy.

METHODS

The PIAMA study is a large ongoing prospective birth cohort study of 3963 Dutch children in which longitudinal medication use can be studied [4]. Briefly, recruitment took place prenatally in 1996-1997 through a screening questionnaire distributed among 10232 pregnant women visiting any of 52 prenatal clinics enrolled in the study. Based on this screening 7862 women were invited to participate; 4146 agreed and gave written informed consent. 183 participants were lost to follow-up before any data on the child had been obtained, therefore the PIAMA

study started with 3963 newborn children. A sub-cohort of 2004 children has information on prescriptions from community pharmacies. For 777 children these pharmacy data were complete from birth until age eight. Details of the formation and design of this pharmacy cohort have been described elsewhere [3].

Data on respiratory symptoms, medication use, risk factors for asthma and allergy and demographic factors were collected by postal questionnaires sent to the parents during the last trimester of pregnancy, at the child's age of three months, at the age of one year and annually thereafter. An extensive medical examination was carried out at age eight in a subset of the population. By design of the study, children of atopic mothers were oversampled and invited for this medical examination. At age eight, longitudinal data on medication retrieval was collected through prescription data from community pharmacy records. These records contained detailed information on delivered prescriptions (amount dispensed, dosing and prescriber).

Parental reported ICS use was measured by using the following question (at the age of 3 to 8 annually): "Did your child use one of the following drugs in the past 12 months: Aerobec, Becloforte, Becotide, Beclomethason, Budesonide, Flixotide, Fluticason, Pulmicort, Qvar, Seretide, Symbicort?" The parents were asked to respond "yes or no" in relation to the drugs listed. These drugs were all the ICS available in the Netherlands during the study period. ICS use in the pharmacy records was determined by using the Anatomical Therapeutic Chemical (ATC) codes [5]. The ATC code 'R03BA' ('Glucocorticoids') was used to select ICS prescriptions.

The characteristics of children with parental reported ICS use (data of all children with questionnaire data) and children with ICS prescriptions at age 5 were compared by using Chi-square testing (age 5 was chosen as an example). The proportion of overall agreement and kappa statistics with their corresponding 95% confidence intervals were calculated to quantify agreement between parent reported medication use and prescription data. Kappa statistics were interpreted using the classification system developed by Landis and Koch [6]: $\kappa < 0.40$ indicates poor agreement, κ between 0.41 and 0.60 indicates moderate agreement, 0.61-0.80 substantial agreement and κ values of 0.81 to 1.00 indicate almost perfect to perfect agreement. All analyses were performed using SAS version 9.1 (SAS Institute, Cary, NC).

RESULTS

At age five, 278 children out of 3429 (8.1%) had parent-reported ICS use, while 61 out of 777 (7.9%) children with pharmacy data filled at least one ICS prescription. 54 of the 61 (88.5%) children were among the 278 children who used ICS at age five. The characteristics of children with parent-reported ICS use (all children with questionnaire data) and children with ICS prescriptions at age five are shown in Table 1. The two groups were similar with respect to gender, ethnicity, atopy, atopic parents, siblings, breastfeeding, passive smoking and pets.

Table 1. Characteristics of study population

	ICS use age 5 based on		
	Pharmacy prescription data (n = 61)	**Parental report (questionnaire) (n = 278)**	**p**
Male gender, % (n)	63.9 (39)	64.0 (178)	0.99
Caucasian ethnicity, % (n)	95.0 (57/60)	93.3 (251/269)	0.63
Atopic mother, % (n)	31.2 (19)	39.6 (110)	0.22
Atopic father, % (n)	34.4 (21)	36.3 (101)	0.78
Breastfeeding in first 3 months	70.0 (42)	75.0 (207)	0.37
Exposure to pets in first 3 months	52.5 (32)	54.0 (150)	0.83
Passive smoking in first 3 months	42.6 (26)	43.5 (121)	0.90
Atopy, % (n)	70.0 (14/20)	52.9 (46/87)	0.16
BHR*, % (n)	59.1 (13/22)	53.9 (42/78)	0.71
Wheezing at age 4, % (n)	55.7 (34)	52.0 (143)	0.54
Shortness of breath at age 4, % (n)	68.9 (42)	64.4 (177)	0.44

* BHR = Bronchial hyper responsiveness

Both pharmacy prescriptions and parental reports were available for 657 (84.6%) children aged 3-8. For the separate age categories data was available for a larger number of children (Table 2). According to the pharmacy records, 142 children (21.6%) received at least one prescription between ages 3 and 8. According to the parental reported data, 139 children (21.2%) received at least one ICS prescription between age 3 and 8. For 127 children (89%) who had ICS prescriptions, the parents also reported ICS use (sensitivity = 0.89). Among children without ICS prescriptions, 98% were reported as non-users by the parents (specificity = 0.98). The proportion of overall agreement between parental reported medication use and pharmacy prescriptions by age is shown in Table 2. At all ages overall agreement was very high (>97%), both agreement for use of ICS (98.6% tot 99.3% for all ages) and non-use of ICS (76.1% to 86.7% for all ages) was high. For children in whom parental reported use and prescriptions data did not correspond (< 3% showed no agreement (varying from 1.7% to 3.0% for the different age categories)), ICS use was most likely to be underreported by the parents compared to the pharmacy data. Parental underreporting in the questionnaire ranged from 13.3% to 23.9% for the different age classes and over-reporting ranged from 0.7% to 1.4% for all ages. Kappa statistics were 'almost perfect to perfect', ranging from 0.81 to 0.88 for all ages, except age 7. For age 7, kappa was 0.80 which also indicates substantial agreement between prescription and questionnaire data.

Table 2. Congruence between parental reported ICS use and prescription data

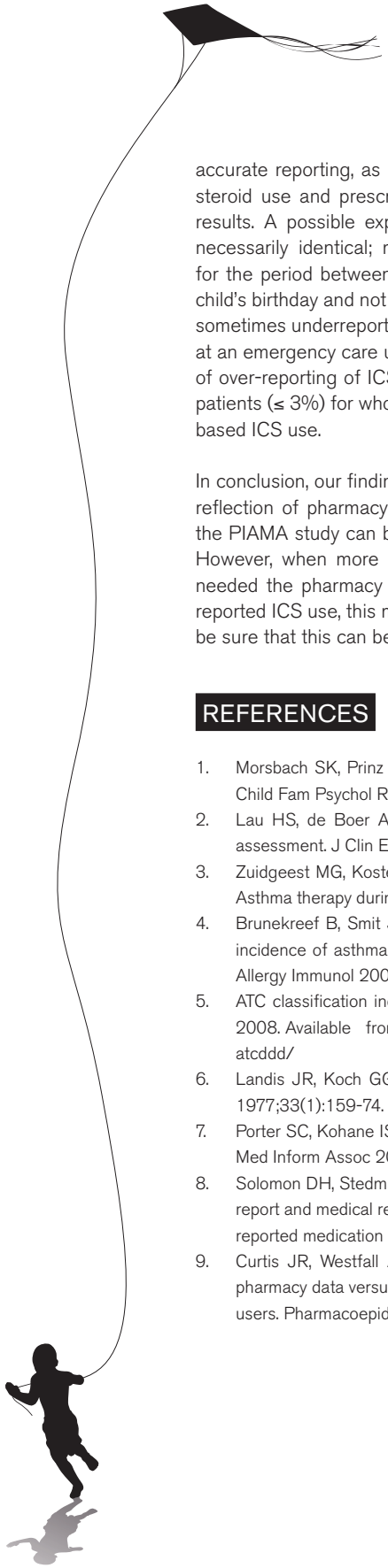
ICS use at age (years)	Overall agreement, % (n)	N_{Pharm} - N_{Ques}, % (n)	Y_{Pharm} - Y_{Ques}, % (n)	Y_{Pharm} - N_{Ques}, % (n)	N_{Pharm} - Y_{Ques}, % (n)	Kappa (95% CI)
Age 3	97.6 (737/755)	99.0 (691/698)	80.7 (46/57)	19.3 (11/57)	1.0 (7/698)	0.82 (0.74-0.91)
Age 4	97.4 (719/738)	98.7 (665/674)	84.4 (54/64)	15.6 (10/64)	1.3 (9/674)	0.84 (0.76 – 0.91)
Age 5	97.0 (735/758)	99.1 (681/687)	76.1 (54/71)	23.9 (17/71)	0.9 (6/687)	0.81 (0.73 – 0.88)
Age 6	98.3 (739/752)	99.3 (687/692)	86.7 (52/60)	13.3 (8/60)	0.7 (5/692)	0.88 (0.82 – 0.94)
Age 7	97.1 (729/751)	98.6 (680/690)	80.3 (49/61)	19.7 (12/61)	1.4 (10/690)	0.80 (0.72 – 0.88)
Age 8	97.6 (744/762)	99.0 (697/704)	81.0 (47/58)	19.0 (11/58)	1.0 (7/704)	0.83 (0.75 – 0.90)

N_{Pharm} = No-use according to prescription data, N_{Ques} = no-use according to parent reported data,
Y_{Pharm} = ICS use according to prescription data, Y_{Ques} = ICS use according to parent reported data.

DISCUSSION

In this study we showed strong agreement between parental reported and ICS prescriptions as reported in the pharmacy system: agreement was high for both use and non-use. Furthermore, characteristics of ICS users classified according to the prescription data or classified according to the parent-reported data were mainly the same.
Few studies evaluated the validity of parental reported medication use. Porter et al. [7] showed that parents were able to provide a reliable source of medication data at the emergency department for emergency asthma care. The validity of self-reported medication use was examined for other medications and these studies suggested that self-reported medication use in adults is a valid source of data. Solomon et al. [8] found strong agreement between self-reported drug use for rheumatoid arthritis and medical records. Curtis et al. [9] showed high agreement rates between self-reported use of osteoporosis medications and filled prescriptions. Both studies showed that agreement rates were highest for current drug use. Boudreau et al. [10] also showed accurate reporting of antihypertensive drug use. Banks et al. [11] showed excellent agreement in their study between general practitioner prescription data and self-reported use of hormone replacement therapy.

The PIAMA study is a large ongoing cohort study in which longitudinal medication use can be studied. The annual questionnaire data on respiratory symptoms and asthma medication use (combined with the physical examination at age eight) provides the opportunity to study medication usage patterns combined with respiratory symptoms in a longitudinal design. Furthermore, findings can be compared and combined with other (Dutch) cohorts, such as the KOALA [12] and PREVASC [13] study. This has been shown in the Allergenic study [14]. Difficulties in self-reported or parent-reported medication use is that information is collected over a long period of time, e.g. during the past 12 months. We think that this does not preclude



accurate reporting, as in our study we found strong agreement between parental reported steroid use and prescription data in the past year. There were however some discrepant results. A possible explanation is that the reported periods of medication use were not necessarily identical; medication use derived from the pharmacy records was extracted for the period between two birthdays and the questionnaire was sent in the month of the child's birthday and not always returned directly. Furthermore, community pharmacy data may sometimes underreport use of drugs received outside the community pharmacy (for example, at an emergency care unit or during hospitalisation). This could explain the small percentage of over-reporting of ICS use in the questionnaire. However, we only had a small number of patients (≤ 3%) for whom there was no agreement between parental reported and pharmacy based ICS use.

In conclusion, our findings show that parental reported medication use provided an excellent reflection of pharmacy records. The questionnaire based medication data collected within the PIAMA study can be used to study asthma medication use in a large group of children. However, when more detailed information on dosing or exact time of medication use is needed the pharmacy prescription data are essential. This study aimed to validate parent reported ICS use, this might be a proxy for other paediatric medications, however we can not be sure that this can be extrapolated.

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CHAPTER 5
Asthma medication use in infancy: determinants related to prescription of drug therapy

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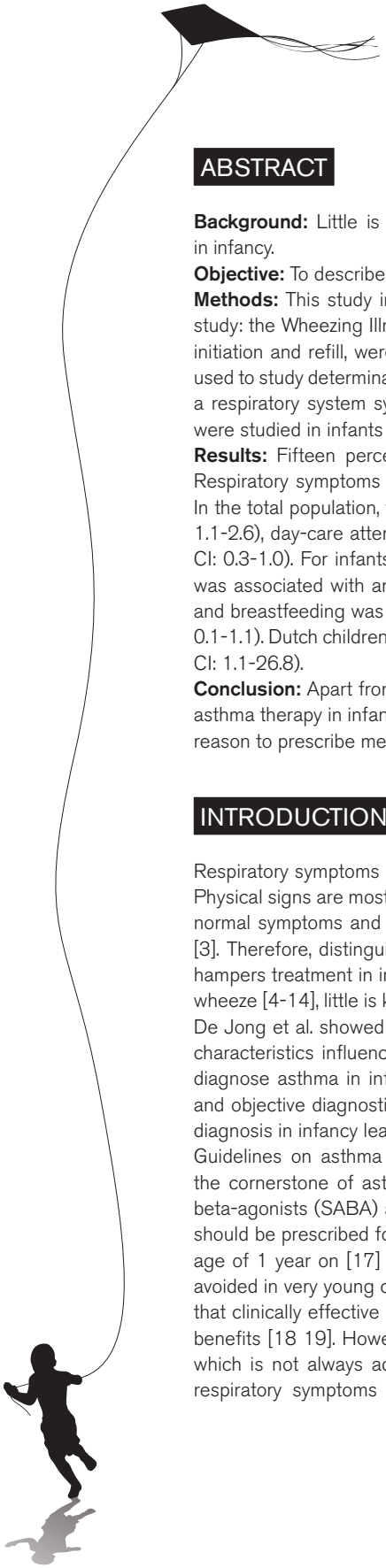


ABSTRACT

Background: Little is known about factors that determine prescribing of asthma therapy in infancy.
Objective: To describe factors related to the initiation and refill of asthma therapy in infancy.
Methods: This study included 1202 infants who participated in a prospective birth cohort study: the Wheezing Illnesses Study Leidsche Rijn (WHISTLER). Outcomes, asthma therapy initiation and refill, were assessed using prescription data. Logistic regression analysis was used to study determinants of therapy initiation in two groups: total population and infants with a respiratory system symptom diagnosis. In addition, determinants of refilling prescriptions were studied in infants who started therapy in their first year of life.
Results: Fifteen percent of all infants started asthma therapy in their first year of life. Respiratory symptoms were an important driver of both initiation and refill of prescriptions. In the total population, therapy initiation was associated with male gender (OR: 1.6, 95% CI: 1.1-2.6), day-care attendance (OR: 1.6, 95% CI: 1.0-2.5) and breastfeeding (OR: 0.6, 95% CI: 0.3-1.0). For infants with a respiratory system symptom diagnosis, day-care attendance was associated with an increased chance of therapy initiation (OR: 5.3, 95% CI: 1.8-16.2) and breastfeeding was associated with a lower chance of starting therapy (OR: 0.4, 95% CI: 0.1-1.1). Dutch children had a higher chance of refilling prescriptions in infancy (OR: 5.3, 95% CI: 1.1-26.8).
Conclusion: Apart from other factors involved, the principal reason for initiation and refill of asthma therapy in infancy was the presence of respiratory symptoms. This appeared the only reason to prescribe medication and physicians are not distracted by other factors.

INTRODUCTION

Respiratory symptoms are common in infancy and result in high health care utilization [1 2]. Physical signs are most often not present and parents find it difficult to differentiate between normal symptoms and abnormal respiratory symptoms such as wheeze and chronic cough [3]. Therefore, distinguishing asthma from other respiratory disorders is difficult which also hampers treatment in infancy. In contrast to studies that describe determinants of asthma or wheeze [4-14], little is known about factors that determine asthma medication use in infancy. De Jong et al. showed that besides the severity of symptoms, child, physician and maternal characteristics influence prescription behaviour [15]. It is very difficult, if not impossible to diagnose asthma in infancy, because most respiratory symptoms early in life are transient and objective diagnostic tools are lacking at this age. It is expected that the lack of a clear diagnosis in infancy leads to variability in general practitioner's (GP) prescription behaviour.
Guidelines on asthma treatment in young children state that inhaled therapy constitutes the cornerstone of asthma treatment in children aged under five years [16]. Short-acting beta-agonists (SABA) are the preferred reliever treatment and inhaled corticosteroids (ICS) should be prescribed for persistent symptoms. However, ICS are registered for use from the age of 1 year on [17] and prolonged use of inhaled or systemic corticosteroids should be avoided in very young children. Studies performed in children aged under five years, suggest that clinically effective doses of ICS are safe and potential risk are well balanced by clinical benefits [18 19]. However, many children start using asthma medication at very young age which is not always advised by treatment guidelines. Therefore, treatment of infants with respiratory symptoms is very difficult for general practitioners (GPs) and non-symptom

related factors are often stated to play a role in this. The objective of the present study was to obtain more insight into factors associated with both initiation and refill of asthma therapy in infancy. We expected that the influence of non-symptom related determinants (such as environmental factors) would decrease when a diagnosis of asthma is more likely, i.e. for children in the general population there is probably a wider range of factors that influence medication prescribing, whilst for children with physician diagnosed respiratory symptom there will be a smaller set of determinants that are associated with drug prescription.

METHODS

Study population and setting

All infants in the study were participants of the Wheezing Illnesses Study Leidsche Rijn (WHISTLER), a large prospective ongoing population-based birth cohort study on determinants of lower respiratory illnesses which started December 2001 [20]. Briefly, healthy infants born in Leidsche Rijn, a new residential area near the city of Utrecht, are enrolled in the study at age 2-3 weeks. Exclusion criteria were gestational age <36 weeks, major congenital abnormalities and neonatal respiratory disease. The Leidsche Rijn district is provided with a new health care infrastructure, which provides an excellent basis for epidemiological research. Currently, there are 4 health care centres with each approximately 5 GP's with supporting staff and a pharmacist. At this moment, the Leidsche Rijn area has around 41000 inhabitants. The expectation is that in 2023 there are over 80000 inhabitants.

In the Netherlands parents must register a new birth within 3 days at the city council. The city council provides information on these new births to WHISTLER and "Stichting Thuiszorg". During a visit of a nurse from "Stichting Thuiszorg", an organisation that routinely visits newborns in the first week after birth for their heel-prick test, the parents receive an information brochure about the WHISTLER study. Within 14 days after birth the WHISTLER staff contacts the households and eligible children are invited to participate. The WHISTLER study has been approved by the Medical Review Ethics Committee of the University Medical Centre Utrecht.

Data collection

At intake (second or third week after birth), the parents filled in a questionnaire on possible pre-and postnatal risk factors for respiratory illnesses. The infant's weight and length were measured. Data on parental demographics, socioeconomic status and disease history were obtained from the linked database of the Utrecht Health Project, a large health monitoring study in Leidsche Rijn [21]. Follow-up data on respiratory symptoms (wheeze and cough) were assessed daily by the parents who filled in diaries during the first year of life. Parents were instructed by research physicians on how to recognize various respiratory symptoms. Further questions were asked about anthropometrics and environmental factors, such as feeding pattern, passive smoking and day-care attendance. New questionnaires were sent on a monthly basis. If parents failed to return the questionnaires, they were contacted by phone. Furthermore, general practitioner and pharmacy records were extracted to collect additional health information. These records include information on prescription data, additional examinations, referral to other primary care staff or secondary care, contact dates and diagnosis.

Patient selection

We selected participating infants from the WHISTLER study. For this study we only selected children who had information available from both the monthly questionnaires and GP medical records. Furthermore, complete follow-up data (minimal 9 monthly questionnaires) was a necessary condition for this study. Figure 1 shows three groups in which we investigated determinants related to drug prescription. Determinants of therapy initiation were studied in: (1) the total WHISTLER population with complete follow-up during the first year of life and (2) infants with a respiratory system symptom diagnosis in their first year of life. Furthermore, determinants of refilling asthma prescriptions were studied in (3) infants who started asthma therapy in their first year of life. The second and the third group are a selection of the first group.

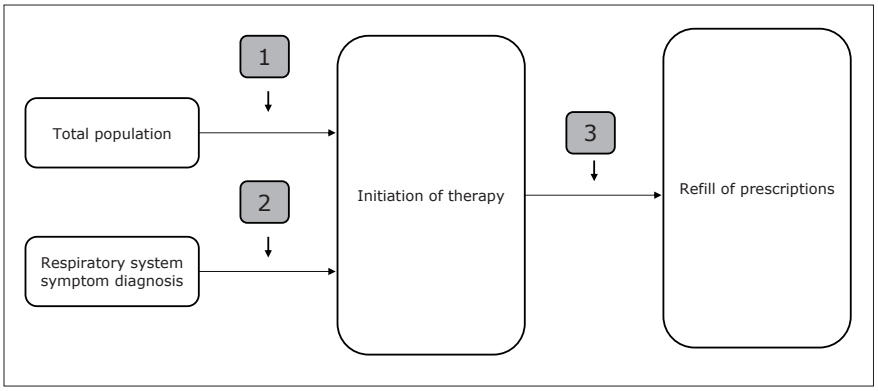


Figure 1. Three levels of determinants of asthma medication prescription in the first year of life Determinants of therapy initiation (1, 2) were studied in two groups during the first year of life: the total population and infants with a respiratory system symptom diagnosis. Determinants of refilling prescriptions (3) were studied in children who filled at least one prescription for any anti-asthma drug in the first year of life.

Definition of outcome

Asthma medication prescriptions were collected from medical records. The Anatomical Therapeutical Chemical (ATC) [22] code R03 (drugs for obstructive airway diseases) was used to select prescriptions. Outcome, initiation of therapy, was defined as at least one prescription for any anti-asthma drug in the first year of life.

Definition of main determinants

Four groups of determinants were studied: (1) general characteristics (the infant's gender and ethnicity), (2) parental factors (educational level, asthma or atopy), (3) environmental factors (feeding pattern, passive smoking, day-care attendance and exposure to pets during the first three months of life) and (4) respiratory symptoms (both parent reported and physician diagnosed). Respiratory system symptom diagnoses were classified in GP medical records using the International Classification of Primary Care (ICPC) codes [23]. We defined a respiratory system symptom diagnosis as the occurrence of a 'respiratory ICPC code'. Asthma was defined as the occurrence of a R96 code. Parent reported respiratory symptoms were defined as at least one day with symptoms in the first year of life. Above average days with parent reported symptoms in the first year of life was used as determinant

in the regression model (> 57 days with respiratory symptoms and > 5 days with wheezing symptoms in the first year of life).

Statistical analysis

Logistic regression analysis was used to investigate determinants of drug prescription. Only variables associated with drug prescription in the univariate model were included in the multivariate model ($p < 0.05$). The inclusion of potential confounding factors in the multivariate regression model was based on the assessment of the influence of each potential confounding factor on the odds ratio (OR) for the association between the univariate associated factors and the outcome drug prescription. The child's gender, ethnicity, parental educational level, having an atopic or asthmatic parent, environmental tobacco smoke exposure, pet exposure, day-care attendance, breastfeeding and respiratory symptoms were considered potential confounders. Potential confounders were included in the multivariate model if they induced a 10% change or more in the crude regression coefficient for the determinant of interest [24]. All analyses were performed using SAS version 9.1 (SAS Institute, Cary, NC).

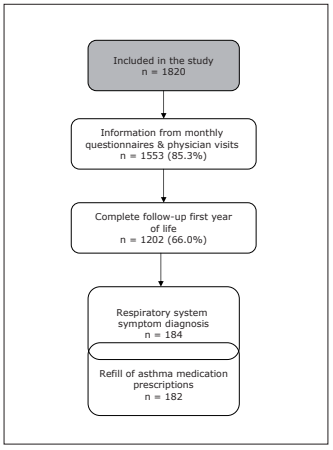


Figure 2. Flow-chart study population

RESULTS

Study population

Around two third (67.2%) of the contacted parents agreed to participate in the WHISTLER study and 11.8% did not fulfil the inclusion criteria (5.0 % health problems child, 0.7% health problems mother, 1.1% language difficulties, 2.0% older than 2 months, 1.8% moving out of area, < 2 months, 0.4% died, 0.8% other reason). Of the 1820 infants that were included in the WHISTLER study, 1553 (85.3%) had information available from both the monthly questionnaires and GP medical records. Complete follow-up data (minimal 9 monthly questionnaires) was available for 1202 infants (66.0%) (Figure 2). There were no differences in medication use or GP diagnosis between children who had complete follow-up data (n = 1202) and children who did not have complete follow-up data (n = 351) with respect to the monthly questionnaires (data not shown). The general characteristics of the study population are shown in Table 1. 16.8% was diagnosed with respiratory symptoms and only 2.4% had a doctor's diagnosis asthma.

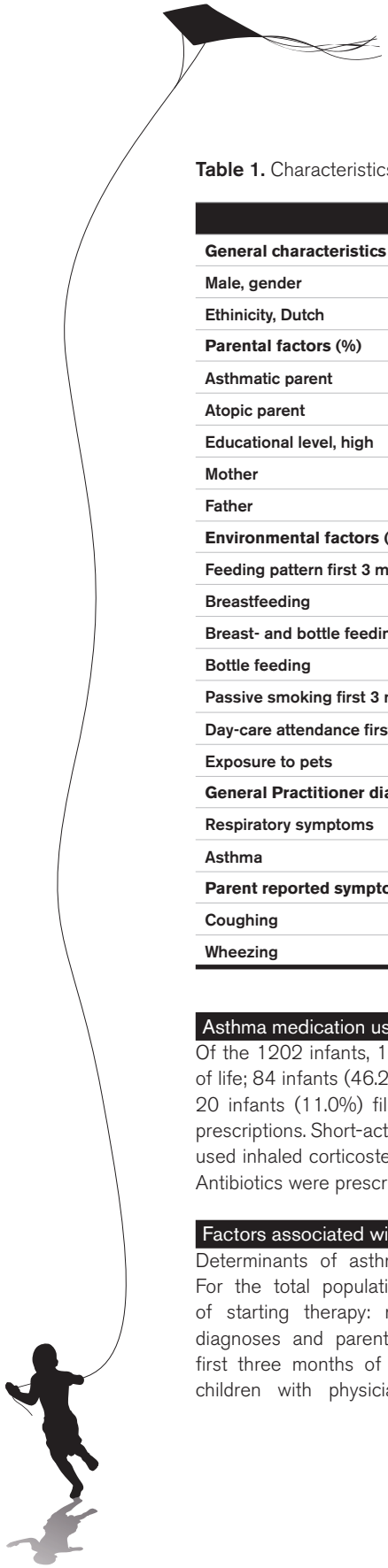


Table 1. Characteristics of study population

	Study population (n = 1202)
General characteristics (%)	
Male, gender	607 (50.5)
Ethinicity, Dutch	797/836 (95.3)
Parental factors (%)	
Asthmatic parent	146 (12.2)
Atopic parent	724 (60.2)
Educational level, high	
Mother	684/1031 (66.3)
Father	576/980 (58.8)
Environmental factors (%)	
Feeding pattern first 3 months of life	
Breastfeeding	356/1148 (31.0)
Breast- and bottle feeding	614/1148 (53.5)
Bottle feeding	178/1148 (15.5)
Passive smoking first 3 months of life	170/1147 (14.8)
Day-care attendance first 3 months of life	519/1151 (45.1)
Exposure to pets	500/1151 (43.4)
General Practitioner diagnosis (%)	
Respiratory symptoms	184/1093 (16.8)
Asthma	26/1093 (2.4)
Parent reported symptoms (%)	
Coughing	1151 (95.8)
Wheezing	506 (42.1)

Asthma medication use

Of the 1202 infants, 182 (15.1%) filled an asthma medication prescription in the first year of life; 84 infants (46.2%) filled one prescription, 54 infants (29.7%) filled two prescriptions, 20 infants (11.0%) filled three prescriptions and 24 infants (13.1%) filled at least four prescriptions. Short-acting β-agonists were the most frequently used therapy (95.1%), 24.2% used inhaled corticosteroids and less than 8% used other anti-asthma therapies (Figure 3). Antibiotics were prescribed in 53.8% of the infants.

Factors associated with therapy initiation and refill of therapy

Determinants of asthma therapy initiation were investigated in two groups (Table 2). For the total population the following factors were associated with a higher chance of starting therapy: male gender, day-care attendance, respiratory system symptom diagnoses and parent reported respiratory symptoms. Exclusively breastfeeding in the first three months of life was associated with a lower chance of starting therapy. For children with physician diagnosed respiratory symptoms, day-care attendance and

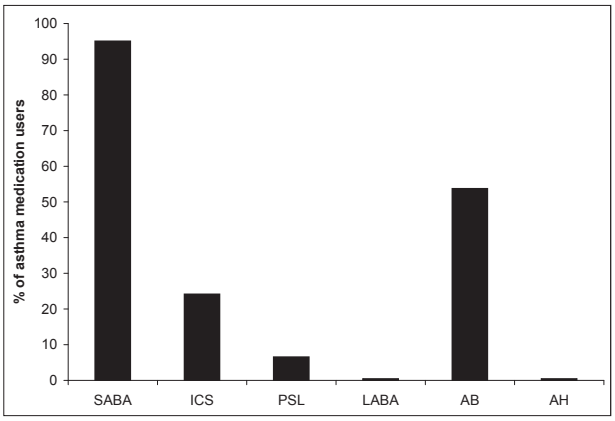


Figure 3. Medication use in children who started therapy in their first year of life (N = 182) SABA = short-acting β-agonists, ICS = inhaled corticosteroids, PSL = parasympathicolytics, LABA = long-acting β-agonists, AB = antibiotics, AH = anti histaminic drugs

Table 2. Determinants of asthma therapy initiation

	Total population (n = 1202)		Respiratory system symptom diagnosis (n = 184)	
	Univariate analysis	Multivariate analysis	Univariate analysis	Multivariate analysis
	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)
Male, gender	2.0 (1.4 - 2.7)*	1.6 (1.1 - 2.6)*	1.1 (0.6 - 2.1)	
Ethnicity, Dutch	0.6 (0.3 - 1.3)		1.2 (0.2 - 6.5)	
Educational level, high				
Mother	1.3 (0.9 - 1.9)		2.1 (1.0 - 4.3)*	1.5 (0.4 - 5.7)
Father	1.2 (0.8 - 1.7)		0.8 (0.4 - 1.8)	
Atopic parent	1.2 (0.8 - 1.6)		0.9 (0.5 - 1.8)	
Asthmatic parent	1.6 (1.0 - 2.4)*	1.1 (0.6 - 2.1)	0.7 (0.3 - 1.5)	
Exclusively breastfeeding	0.7 (0.5 - 1.0)*	0.6 (0.3 - 1.0)*	0.5 (0.2 - 0.9)*	0.4 (0.1 - 1.1)
Parental smoking	1.0 (0.6 - 1.6)		0.8 (0.3 - 2.0)	
Day-care attendance	1.9 (1.4 - 2.6)*	1.6 (1.0 - 2.5)*	2.7 (1.4-5.4)*	5.3 (1.8 - 16.2)*
Exposure to pets	0.9 (0.7 - 1.3)		1.3 (0.6 - 2.6)	
Respiratory system symptom diagnosis respiratory symptoms	50.5 (32.6 - 78.4)*	50.5 (32.6 - 78.4)*	-	
Parent reported respiratory symptoms [a]	5.2 (3.7 - 7.4)*	7.8 (4.2 - 14.5)*	6.8 (3.2 - 14.4)*	11.3 (4.1 - 31.3)*

[a] Above average days with respiratory symptoms (> 57 days with parent reported symptoms in the first year of life) Factors that caused a 10% change or more in the crude regression coefficient were included in the multivariate model., * $p < 0.05$

parent reported respiratory symptoms were independently associated with a higher chance of starting therapy. Exclusively breastfeeding during the first 3 months of life was borderline significantly associated with a lower chance of therapy initiation (p = 0.08). In the multivariate analysis, only maternal ethnicity and physician diagnosed symptoms were associated with a higher chance of refilling prescriptions (Table 3).

Table 3. Determinants of refilling asthma therapy in the first year of life

	Refilling asthma therapy (n = 182)	
	Univariate analysis	Multivariate analysis
	OR (95% CI)	OR (95% CI)
Male, gender	1.3 (0.7 - 2.5)	
Ethnicity, Dutch	5.3 (1.1 - 26.8)*	5.3 (1.1 - 26.8)*
Educational level, high		
Mother	1.2 (0.6 - 2.3)	
Father	1.2 (0.6 - 2.5)	
Atopic parent	1.6 (0.9 - 3.0)	
Asthmatic parent	0.8 (0.4 - 1.7)	
Exclusively breastfeeding first 3 months	1.2 (0.6 - 2.3)	
Parental smoking first 3 months	0.7 (0.3 - 1.6)	
Day-care attendance first 3 months	1.8 (1.0 - 3.3)*	1.1 (0.5 - 2.4)
Exposure to pets	1.2 (0.6 - 2.1)	
Respiratory system symptom diagnosis	1.8 (1.0 - 3.6)*	2.4 (1.1 - 5.3)*
Parent reported respiratory symptoms [a]	2.2 (1.2 - 4.3)*	1.8 (0.8 - 4.2)

[a] Above average days with respiratory symptoms (>57 days with symptoms in the first year of life). Factors that caused a 10% change or more in the crude regression coefficient were included in the multivariate model.

DISCUSSION

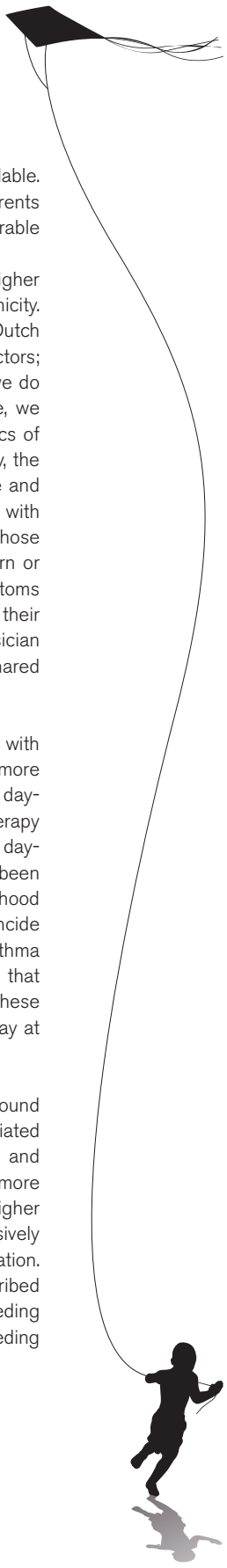
Fifteen percent of the infants in our cohort started asthma therapy in their first year of life and most of them filled only one prescription. Principal reason for both initiation and refill of therapy were the presence of respiratory symptoms: if a physician diagnosed respiratory symptoms or the parents reported symptoms then asthma therapy was much more likely to be prescribed. Furthermore, day-care attendance was associated with asthma medication prescription.

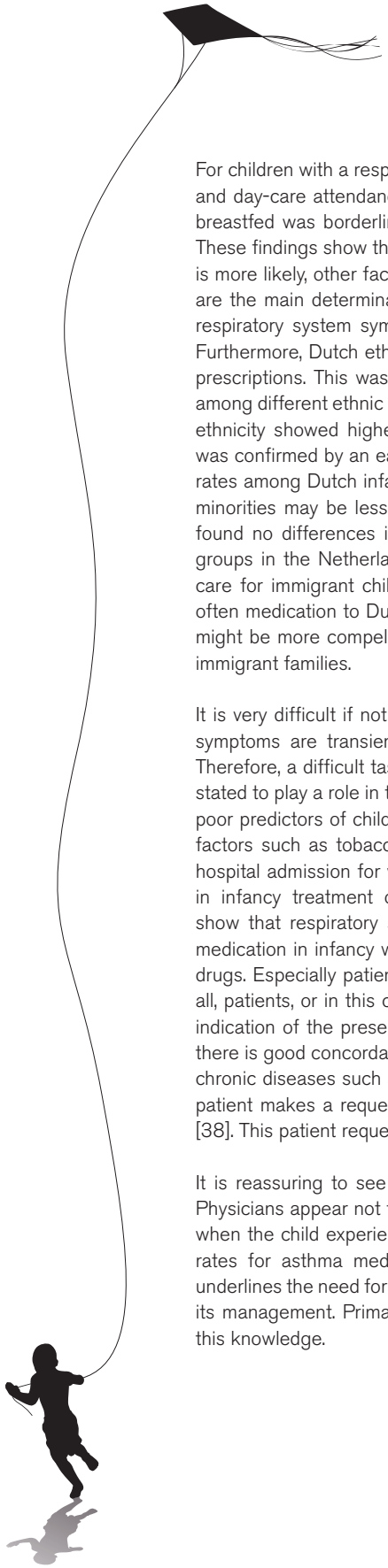
One of the strengths of this study was the availability of complete medication histories for the first year of life. Therefore, we were able to study initiation of asthma therapy and determinants of medication use early in life. Data on medication retrieval was collected through community pharmacy records. In the Netherlands, pharmacy records are virtually complete with respect to outpatient medication use [25]. Another strength of our study is the availability of GP medical records including the respiratory system symptom diagnoses. It might be difficult to retrieve reliable data on respiratory symptoms, especially in infants, from parents because parents might not always recognize symptoms such as “wheeze”. In the present study we had

both physician reported and parent reported data regarding respiratory symptoms available. An advantage of this study is the high response rate. About half of the contacted parents participated in the study. This is a high response rate for this kind of research and comparable with other Dutch birth cohort studies [8].
It should be noted that the inhabitants of the Leidsche Rijn area we studied had a higher average income and the majority of our study population (95%) was of Caucasian ethnicity. This percentage is slightly higher when compared to the composition of the general Dutch population (89% Caucasians) [26]. However, there was no active selection on these factors; they are a result of the construction process of the Leidsche Rijn district. Therefore we do not think that such selection hampers the interpretation of our findings. Furthermore, we do not think that loss to follow-up has biased our results as the baseline characteristics of those with complete follow-up did not differ from those lost to follow-up. Unfortunately, the monthly questionnaires (with daily questions on respiratory symptoms such as wheeze and cough) were not complete for the entire cohort. However, the characteristics of children with complete follow-up data with regard to the monthly questionnaires did not differ from those with incomplete follow-up data. Therefore, we do not think that non-response to return or fill in the monthly questionnaires has led to selection bias. The daily recording of symptoms by the parents may have caused more awareness and a different perception towards their child's respiratory symptoms. This could have influenced the parental visits to a physician compared to not in the study included parents and children. However, this limitation is shared with previous surveys.

Respiratory symptoms, both physician diagnosed and parent reported, were associated with a higher chance of drug prescriptions. This was as expected; children who experienced more respiratory symptoms were much more likely to get medication prescribed. Furthermore, day-care attendance in the first three months of life was associated with a higher risk of therapy initiation in both groups as well as with a higher risk of refilling prescriptions. Although, day-care attendance is supposed to be protective for childhood asthma [27 28], it has also been suggested that day-care attendance in the first year of life is associated with childhood wheezing [12]. This is probably due to more respiratory viruses and does not have to coincide with asthma. Another reason for the finding that the general practitioner prescribes asthma medication more often in children who attend day-care facilities might be the fact that mothers whose children attend day-care are mostly working mothers and therefore these mothers might be more compelling in getting a prescription. Otherwise they have to stay at home with their sick child.

In the general population, besides respiratory symptoms and day-care attendance, we found gender and being breastfed to play a role in therapy initiation. Male gender was associated with prescribing of asthma medication. It is common knowledge that the prevalence and incidence of childhood wheezing and asthma is higher in boys [29]. Boys are therefore more likely to use asthma medication. This was confirmed by Yuan et al. [30] who showed a higher prevalence of asthma medication use among male infants. Infants who were exclusively breastfed during the first three months of life showed a lower chance of therapy initiation. The protective effect of breastfeeding for asthma and allergic disorders has been described in many studies. Mother's milk is a source of immuno-modulating factors and breastfeeding reduces external antigen exposure [11]. Wright et al. [31] showed that exclusive breastfeeding in the first years of life was associated with lower rates of wheeze.





For children with a respiratory system symptom diagnosis, symptoms reported by the parents and day-care attendance were associated with a higher chance of therapy initiation. Being breastfed was borderline significantly associated with a lower chance of therapy initiation. These findings show that when a more solid diagnosis pointing towards asthmatic symptoms is more likely, other factors (such as male gender) are filtered out and respiratory symptoms are the main determinant of medication use. The same was true for refilling prescriptions: respiratory system symptom diagnoses were an important driver of refilling prescriptions. Furthermore, Dutch ethnicity was independently associated with a higher chance of refilling prescriptions. This was against our expectations as most studies on health care utilisation among different ethnic groups in the Netherlands demonstrated that children from non-Dutch ethnicity showed higher health care utilization for respiratory symptoms [14 32 33]. This was confirmed by an earlier WHISTLER study [14]. In contrast, our study shows higher refill rates among Dutch infants. There may be several reasons for this finding. First of all, ethnic minorities may be less adherent and do not refill prescriptions. However, van Dellen et al. found no differences in adherence to inhaled steroids among children in different ethnic groups in the Netherlands [34]. In contrast, another study did indicate suboptimal asthma care for immigrant children in the Netherlands [35]. Second, the GP may prescribe more often medication to Dutch patients. One possible explanation is that non-immigrant parents might be more compelling in getting another prescription for their child when compared to immigrant families.

It is very difficult if not impossible to diagnose asthma in infancy because most respiratory symptoms are transient and diagnostic tools only become useful after the age of four. Therefore, a difficult task for treating physicians and non-symptom related factors are often stated to play a role in this. Reijonen et al. [36] showed that non-symptom related factors are poor predictors of childhood asthma: a family history of atopy or asthma and environmental factors such as tobacco smoke exposure were no predictors of asthma three years after hospital admission for wheezing in infancy. As a consequence of the diagnosing difficulties in infancy treatment of respiratory symptoms may be hampered. However, our results show that respiratory symptoms are the most important factor in prescription of asthma medication in infancy which indicates that general practitioners are careful with prescribing drugs. Especially patient-reported symptoms are very important in drug prescribing. First of all, patients, or in this case the parents of asthmatic patients, themselves can give the best indication of the presence and severity of their disease. Previous research has shown that there is good concordance between patient self-report and medical records on diagnosis for chronic diseases such as asthma [37]. Furthermore, in about 10% of the physician visits, a patient makes a request for a drug prescription and most of these requests are honoured [38]. This patient requesting might influence physician's prescription behaviour [39].

It is reassuring to see that non-symptom related factors play a much less important role. Physicians appear not to be distracted by these other factors and only prescribe medication when the child experiences respiratory symptoms. The current study also showed that refill rates for asthma medication prescriptions are lower in immigrant children. This finding underlines the need for good education of immigrant parents about respiratory symptoms and its management. Primary care physicians and pharmacists should make efforts to improve this knowledge.

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CHAPTER 6
Inhaled corticosteroid adherence in paediatric patients: the PACMAN cohort study

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ABSTRACT

Objective: Poor adherence with inhaled corticosteroids has been reported frequently and may be associated with uncontrolled asthma. Better understanding of factors influencing adherence may help to achieve higher adherence rates for a larger part of the population, which will eventually lead to better asthma control. The aim of this study was to investigate factors associated with adherence in paediatric inhaled corticosteroid users.

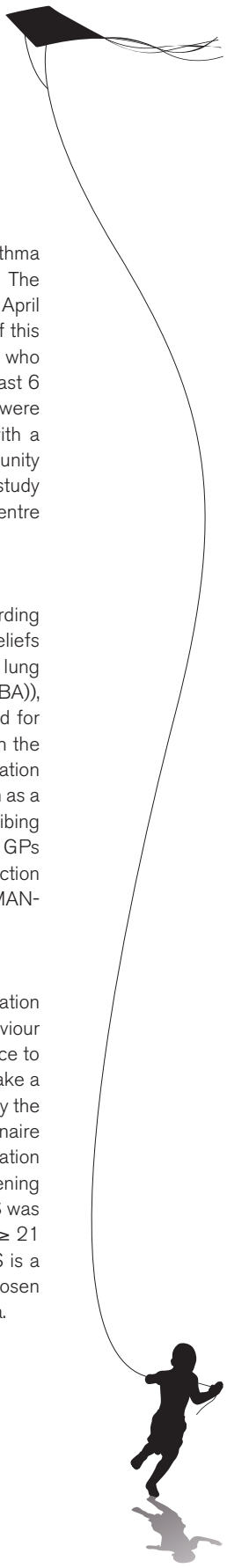
Methods: We included 527 children using inhaled corticosteroids (ICS) who participated in the PACMAN (Pharmacogenetics of Asthma medication in Children: Medication with ANti-inflammatory effects)-cohort study. Outcome, parental reported adherence was assessed by using the Medication Adherence Report Scale. Four categories of determinants were studied; child and family characteristics, medication use (parental beliefs towards medication (Beliefs about Medicines Questionnaire)) and environmental factors.

Results: Good adherence was observed in 302 children (57%). Increased fractional exhaled nitric oxide values (indication for airway inflammation) were associated with a lower chance of good adherence (OR: 0.25, 95% CI: 0.15-0.41). Parental necessity beliefs about medication were associated with higher adherence (OR: 2.32, 95% CI 1.59-3.39). Dutch origin was also associated with higher adherence rates (OR: 2.11, 95% CI: 1.09-4.07). Furthermore, younger age (<6 years) was associated with better adherence (OR: 1.62, 95% CI: 1.02-2.59).

Conclusion: Increased airway inflammation was associated with lower ICS adherence; this underlines the need of good adherence to reach disease control. Our results suggest that by improving knowledge, especially in ethnic minorities, and by stimulating positive parental perception towards the nature of disease, characteristics of prescribed drugs as well as medication use, better adherence and as a result better asthma control could be reached.

INTRODUCTION

Therapy adherence for chronic diseases is generally low [1]. In asthma, poor adherence with inhaled corticosteroids (ICS), which are used chronically to control airway inflammation, has been reported frequently [2-4]. Adams et al. [5] showed that use of asthma therapy was inadequate in a large part of the asthma patients. Low adherence rates are associated with uncontrolled asthma [6]. Therefore, it is important to study factors that drive adherence and identify factors which can be improved to increase therapy success. Better understanding of asthma therapy adherence determinants may help to achieve higher adherence rates for a larger part of the population which will eventually lead to better asthma control. Menckeberg et al. [7] showed that reasons for adult patients to discontinue medication use were: sufficient control of symptoms, low therapy effectiveness, or the occurrence of side effects. In children, maternal educational level, the child's age, parent beliefs, characteristics of the treatment regime and family functioning have been associated with ICS adherence [8 9]. However, many other determinants remain understudied. More insight in factors influencing adherence in paediatric asthma patients is important for the development of strategies to improve adherence rates. The aim of the present study was to investigate a wide range of factors that could influence therapy adherence in paediatric inhaled corticosteroid users. The emphasis of this study was on possible determinants of adherence that could be changed or improved to increase therapy success, such as factors related to medication use behaviour and environmental exposures.



METHODS

Study population and setting

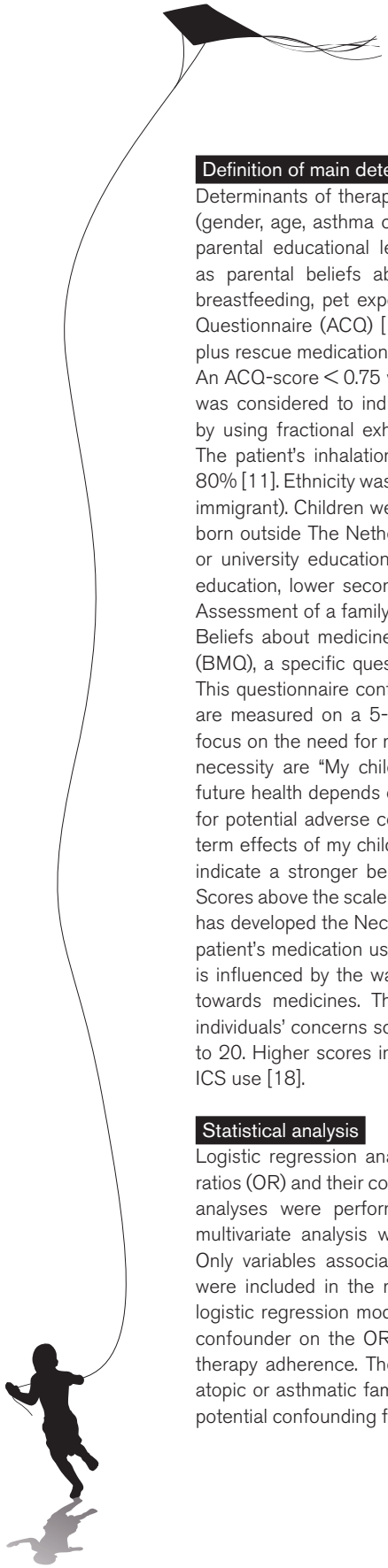
We studied children who participated in the PACMAN (Pharmacogenetics of Asthma medication in Children: Medication with ANti-inflammatory effects)-cohort study. The PACMAN-cohort study is an ongoing observational retrospective study that started in April 2009 and aims to include at least 1000 children who use asthma medication. Details of this study protocol have been described elsewhere [10]. Briefly, children aged 4-12 years who are regular users (≥ 3 prescriptions within the last 2 years and ≥ 1 prescription in the last 6 months) of asthma medication (Anatomical Therapeutical Chemical (ATC) code R03) were selected from pharmacies in the Netherlands. Selected children and their parents with a telephone number available in the pharmacy were invited for a visit to their own community pharmacy. Written informed consent was obtained from all participants. The PACMAN study has been approved by the Medical Review Ethics Committee of the University Medical Centre Utrecht.

Data collection

During the pharmacy visit, parents filled in a questionnaire to collect information regarding general health, asthma and respiratory symptoms, medication use, adherence, parent's beliefs about medicines, environmental and socio-demographic factors. Furthermore, the child's lung function was measured (both before and after use of a short-acting beta-agonist (SABA)), fractional exhaled nitric oxide (FeNO) was measured and saliva samples were collected for DNA extraction and future genotyping. The child's inhalation technique was scored with the help of an inhaler specific inhalation control checklist [11]. Longitudinal data on medication use were collected through prescription data from community pharmacy records. As soon as a child was included in the PACMAN-cohort study, an information letter was sent to prescribing general practitioner (GP). This letter was sent to collect additional information from the GPs records including letters for referral to a respiratory physician on asthma status, lung function and allergic sensitisation. For this study, we selected participating children from the PACMAN-cohort study who used inhaled corticosteroids in the past year.

Definition of outcome

Outcome, parental reported therapy adherence, was assessed by using the Medication Adherence Report Scale (MARS) comprising 5 questions on medication use behaviour [12]. We have previously shown that parental reported medication use is a reliable source to assess medication use in children [13]. The MARS contains questions on forgetting to take a dose, altering the dose, deciding to miss a dose, taking less medication then instructed by the physician and deciding not to take medication for a while. The 5-item MARS questionnaire has been used in various settings and various countries to assess self-report of medication adherence. Previous research showed that this questionnaire was also a satisfactory screening tool to identify non-adherent ICS users within Dutch community pharmacies. The MARS was dichotomised by using a cut-off point for the sum-score. Patients with a MARS score ≥ 21 were considered to be highly adherent. Menckeberg et al. [14] showed that the MARS is a satisfactory screening tool for non-adherence among ICS users, irrespective of the chosen cut-off point. Furthermore, MARS-scores correlated well with pharmacy dispensing data.



Definition of main determinants

Determinants of therapy adherence were divided into 4 categories: (1) child characteristics (gender, age, asthma control, airway inflammation), (2) family characteristics (ethnic group, parental educational level, family history of asthma or atopy), (3) medication use (such as parental beliefs about medicines) and (4) environmental factors (passive smoking, breastfeeding, pet exposure). Asthma control was assessed by using the Asthma Control Questionnaire (ACQ) [15]. In this study we used the 6-item shortened version (symptoms plus rescue medication use) of the ACQ, which has also been validated by Juniper et al. [16]. An ACQ-score < 0.75 was considered to indicate good asthma control, while a score ≥ 1.50 was considered to indicate poor asthma control [15]. Airway inflammation was measured by using fractional exhaled NO levels > 25 ppb as a proxy for airway inflammation [17]. The patient's inhalation technique was regarded to be good if the checklist score was $\geq$ 80% [11]. Ethnicity was defined as Dutch (native/non-immigrant) versus non-Dutch (foreign/immigrant). Children were classified as non-Dutch ethnicity if at least one of its parents was born outside The Netherlands. Educational level was categorized as high (higher vocational or university education) versus moderate (higher secondary education) or low (no formal education, lower secondary education or intermediate secondary education) as reference. Assessment of a family history of asthma or atopy was questionnaire-based.

Beliefs about medicines were assessed using the Beliefs about Medicines Questionnaire (BMQ), a specific questionnaire that measures general beliefs about the medication used. This questionnaire contains 10 questions (5 on necessity and 5 on concerns) and all items are measured on a 5-point Likert scale [12]. The questions on necessity or need beliefs focus on the need for medication use to maintain good health. Examples of questions about necessity are "My child's health, at present, depends on his/her inhaler" and "My child's future health depends on his/her inhaler". The concerns scale contains questions about fear for potential adverse consequences of medication use. "I sometimes worry about the long-term effects of my child's inhaler", is an example of the concerns scale. Higher BMQ scores indicate a stronger belief in the concepts represented by the scale (needs or concerns). Scores above the scale midpoint (BMQ-score > 15) were considered as strong beliefs. Horne has developed the Necessity-Concerns differential to describe the salient beliefs influencing patient's medication use behaviour. This framework suggests that medication use behaviour is influenced by the way a patient judges necessity of medication use relative to concerns towards medicines. The Necessity-Concern differential is calculated by subtracting the individuals' concerns score from the individuals' necessity score leading to a range from -20 to 20. Higher scores indicate stronger perceived necessity and/or lower concerns towards ICS use [18].

Statistical analysis

Logistic regression analysis was used to study determinants of therapy adherence. Odds ratios (OR) and their corresponding 95% confidence intervals (CI) were calculated. Univariate analyses were performed to study individual determinants of adherence and second, multivariate analysis was performed to assess independence of the associated factors. Only variables associated with therapy adherence ($p < 0.20$) in the univariate analyses were included in the multivariable analysis. The inclusion of potential confounders in the logistic regression model was based on the assessment of the influence of each potential confounder on the OR for the association between the univariate associated factors and therapy adherence. The child's age, gender, ethnicity, parental educational level, having an atopic or asthmatic family member, and parent's believes about medicines were considered potential confounding factors. Potential confounding factors were included in the multivariate

model if they induced a 10% change or more in the crude regression coefficient for the determinant of interest [19]. For the factors significantly associated with adherence in this multivariate model we also studied the relationship between determinants. All analyses were performed using SAS version 9.1 (SAS Institute, Cary, NC).

RESULTS

Response rate

In total, until February 2011, 2114 children were selected from the pharmacies. For, 595 (28.1%) parents and their children the telephone number was unknown in the pharmacy and could not be traced through Internet or a national telephone number service. Therefore, our eligible study population consisted of 1519 children. Furthermore, 393 (18.6%) parents, whose telephone numbers were known, could not be reached after at least five phone calls. Currently, 1126 parents could be contacted by phone and were asked to participate in the PACMAN-cohort study. Based on the telephonic invitation, 684 (60.7%) parents agreed to participate. For 82 (12.0%) children no data was obtained due to various reasons: the child was unwilling to participate, parents/children did not show up at the scheduled appointment, or the parent/children were willing to participate, though unable to come to the pharmacy in the period the patient inclusion took place in that specific pharmacy. As a result 602 children were included in the study indicating a response rate of 53.5% (Figure 1).

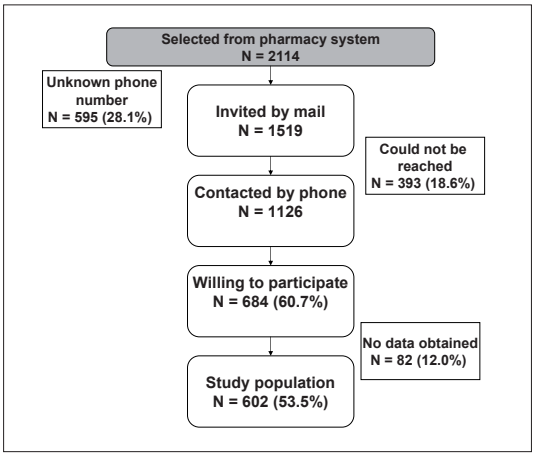


Figure 1. Flow diagram study population

Study population

Of the 602 children that were included in the study, 527 children (87.5%) used inhaled corticosteroids in the past year. The general characteristics of this population are shown in Table 1: 61% of the participants were males and the mean age was 8.4 years. The majority of the study population was of Dutch origin (90%) and non-Dutch children were mainly non-western foreigners (65%). Approximately 8% used oral steroids and 37% received at least one antibiotics course. Mean inhalation technique score was 92.8% (± 11.2%) and for the majority of the population (90.4%) inhalation technique was scored as good (score ≥ 80%). In total, 302 children (57.3%) showed good adherence (MARS score ≥ 21) to inhaled corticosteroids.

Table 1. Characteristics of study population

Characteristics	Study population (n=527) % (n)
Male gender	61.0
Dutch ethnicity a	89.6 (420/469)
Age, mean (SD)	8.4 (2.5)
Atopy	80.3
Asthma diagnosis	69.4
Medication use in past year	
Short-acting beta agonist use	84.6
Oral steroid use	8.0
Antibiotic use	37.1 (194/523)
Environmental factors	
Passive smoking	11.2 (59/525)
Pet exposure	39.4 (207/525)
Urban environment	62.6 (328/524)

[a] Both parents Dutch ethnicity

Medication use

As shown in Figure 2, prescribed asthma medication was generally the same for children with good and children with poor adherence. In both groups, around 85% of the children used SABA. Long-acting beta-agonists (LABA) were more frequent used in children with good adherence, 30.5% vs. 20.9% respectively ($p < 0.05$).

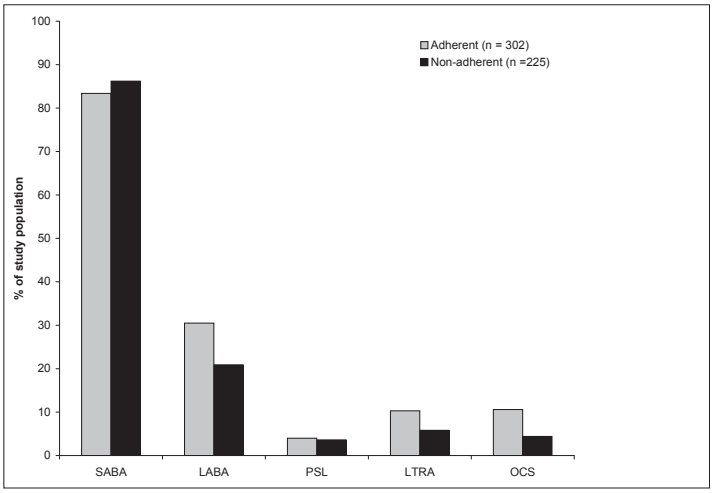


Figure 2. Asthma medication use. All patients used ICS in the past year. SABA = short-acting beta-agonist, LABA= long-acting beta agonist, PSL = parasympathicolytics, LTRA = leukotriene receptor antagonist, OCS = oral corticosteroids

Factors associated with therapy adherence

The following factors were associated with therapy adherence in the univariate analysis: young age (< 6 years old), airway inflammation, Dutch ethnicity, high paternal educational level, oral steroid use, strong parental necessity beliefs about medication use for maintaining present and future health and high parental concerns about medication use (Table 2). In the multivariate analysis, only age, airway inflammation, Dutch ethnicity and parental necessity beliefs about medication use remained significantly associated with therapy adherence (Table 3). Increased FeNO values (> 25 ppb), which were used as an indicator for airway inflammation, were independently associated with a lower chance of good adherence (adjusted OR: 0.25, 95% CI: 0.15-0.41). Younger children (aged < 6 years) were more often adherent (adjusted OR: 1.62, 95% CI: 1.02 - 2.59). Dutch ethnicity was associated with higher

Table 2. Factors associated with therapy adherence

	Good therapy adherence		Univariate analysis	
	n (%)		OR (95% CI)	p
Child characteristics				
Gender (male / female)	180 (56.1)	121 (59.0)	0.89 (0.62 - 1.26)	0.51
Ethnicity (Dutch / non-Dutch) [a]	242 (57.6)	24 (49.0)	1.42 (0.82 - 2.56)	0.19
Age, mean (SD)			0.95 (0.88 - 1.02)	0.16
Age (< 6 years / ≥ 6 years)	75 (65.8)	227 (55.0)	1.58 (1.02 - 2.43)	0.04
Asthma diagnosis (yes / no)	104 (54.2)	32 (53.3)	1.00 (0.72 - 1.40)	0.99
ACQ score, mean (SD)			1.09 (0.88 - 1.34)	0.61
Asthma control (yes / no) [b]	162 (57.9)	68 (54.4)	0.90 (0.69 - 1.16)	0.41
FeNO level, mean (SD)			0.98 (0.97 - 0.99)	< 0.0001
Airway inflammation (yes / no) [c]	38 (34.2)	210 (63.3)	0.30 (0.19 - 0.48)	< 0.0001
Family characteristics				
High educational level (yes / no)	131 (55.7)	102 (59.3)	0.86 (0.58 - 1.29)	0.47
Parental atopy [d] (yes / no)	256 (56.4)	46 (63.0)	0.76 (0.46 - 1.26)	0.29
Parental asthma e (yes / no)	131 (56.2)	142 (60.7)	0.83 (0.58 - 1.20)	0.33
Medication use				
SABA (yes / no)	252 (56.5)	50 (61.7)	0.81 (0.50 - 1.31)	0.38
Oral steroid (yes / no)	32 (76.2)	270 (55.7)	2.55 (1.23 - 5.30)	0.01
Antibiotic (yes / no)	117 (60.3)	184 (55.9)	1.20 (0.84 - 1.72)	0.33
High necessity beliefs (yes / no)	189 (66.6)	113 (46.5)	2.29 (1.61 - 3.26)	< 0.0001
High concerns beliefs (yes / no)	72 (63.2)	230 (55.7)	1.36 (0.89 - 2.09)	0.15
Environmental factors				
Passive smoking (yes / no)	33 (55.9)	268 (57.5)	0.94 (0.54 - 1.62)	0.82
Pet exposure (yes / no)	118 (57.0)	184 (57.9)	0.97 (0.68 - 1.38)	0.85
Urban environment (yes / no)	189 (57.6)	112 (57.1)	1.02 (0.71 - 1.46)	0.91

ACQ = Asthma Control Questionnaire, FeNO = fractional exhaled nitric oxide. [a] Both parents Dutch ethnicity, [b] ACQ-score < 0.75, [c] FeNO value > 25 ppb, [d] at least one atopic parent, e at least one asthmatic parent.

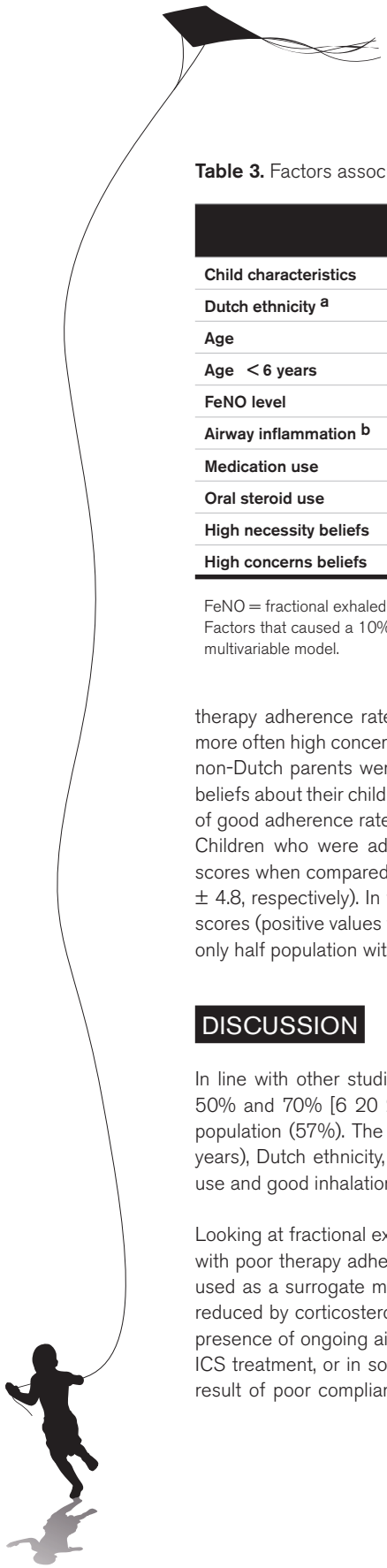


Table 3. Factors associated with therapy adherence (multivariate analysis)

	Multivariate analysis OR (95% CI)	p
Child characteristics		
Dutch ethnicity [a]	2.11 (1.09 - 4.07)	0.03
Age	0.97 (0.90 - 1.04)	0.38
Age < 6 years	1.62 (1.02 - 2.59)	0.04
FeNO level	0.97 (0.96 - 0.98)	< 0.0001
Airway inflammation [b]	0.25 (0.15 - 0.41)	< 0.0001
Medication use		
Oral steroid use	1.80 (0.84 - 3.86)	0.13
High necessity beliefs	2.32 (1.59 - 3.39)	< 0.0001
High concerns beliefs	1.06 (0.67 - 1.68)	0.81

FeNO = fractional exhaled nitric oxide. [a] Both parents Dutch ethnicity, [b] FeNO value > 25 ppb. Factors that caused a 10% change or more in the regression coefficient were included in the multivariable model.

therapy adherence rates (adjusted OR: 2.11, 95% CI: 1.09-4.07). Non-Dutch parents had more often high concerns about their child's medication use (55% vs. 17% ($p < 0.0001$)) and non-Dutch parents were more often low educated ($p < 0.0001$). Strong parental necessity beliefs about their child's medication use were independently associated with a higher chance of good adherence rates (adjusted OR: 2.32, 95% CI: 1.59-3.39).
Children who were adherent to therapy had higher mean Necessity-Concern differential scores when compared to children with good adherence rates ($p < 0.0001$, 4.4 ± 5.0 vs. 2.3 ± 4.8, respectively). In the high adherent group, necessity scores were higher than concern scores (positive values for Necessity-Concern differential) for 71% of the group compared to only half population with poor adherence (53.8%).

DISCUSSION

In line with other studies showing inhaled corticosteroid adherence rates varying between 50% and 70% [6 20 21], we found high adherence rates in more than half of the study population (57%). The main determinants of high adherence rates were younger age (< 6 years), Dutch ethnicity, strong parental beliefs towards the necessity of asthma medication use and good inhalation technique.

Looking at fractional exhaled nitric oxide (FeNO) we found increased levels to be associated with poor therapy adherence. Increased FeNO values are not unique for asthma, but can be used as a surrogate marker for eosinophilic airway inflammation [17] and are known to be reduced by corticosteroid therapy [22]. Therefore, increased FeNO levels could indicate the presence of ongoing airway inflammation. This inflammation might be caused by inadequate ICS treatment, or in some cases, steroid resistance. Inadequate treatment effects can be a result of poor compliance, poor inhaler technique or inadequate dosing. In this study, poor

inhaler technique has not biased the results, as inhalation technique was good in the majority (94%) of the population. Therefore, FeNO might be an additional indicator in monitoring asthma therapy adherence which was also indicated by Beck-Ripp et al. [23] who demonstrated that FeNO measurement has the potential to provide early warning of non-adherence.
We showed better therapy adherence rates for children aged six years or less. This has been previously shown by a study of Jonasson et al [24]. The observed higher adherence rates may be a result of more parental motivation in this younger age group.

In our study, non-immigrant children were more often adherent to therapy. Previous studies have shown that ethnic minority groups experience poorer health outcomes compared with majority groups for asthma [25 26]. Urbanus-van Laar et al. showed that this is also the case in The Netherlands: asthma care for immigrant children is suboptimal in the Netherlands [27]. There might be several explanations for the differences in adherence rates observed for different ethnic groups. First of all, different health beliefs or beliefs towards medication use may play an important role. For example, when patients believe that asthma is an acute rather than a chronic disorder, it would be less likely they use their preventive anti-inflammatory treatment regularly [28]. In our study, immigrant parents had more often high concerns towards their child's medication use than non-immigrant parents. These strong concerns may lead to lower adherence rates. Second, ethnic minorities may have difficulties in understanding the physician. We showed that immigrant parents were more often low educated, and therefore, they might have more often difficulties in understanding the physician. Van Dellen et al. [29] showed that asthma control was associated with insufficient comprehension of the Dutch language. Another study also observed that Turkish and Moroccan mothers experienced a language barrier [30].

Furthermore, we found good adherence to be associated with high perceived parental need (to maintain present and future health) for medication use. Our findings are consistent with other studies showing associations between beliefs on medication use and adherence. Menckeberg et al. [20] showed that in adult asthma patients, beliefs about ICS correlated with adherence rates. Another study showed parents' medication beliefs to be important drivers of paediatric therapy adherence: a positive attitude towards their child's asthma medication use was associated with better asthma management [31]. Van Dellen et al. [30] interviewed asthmatic children and their mothers and showed that there was much uncertainty about cause, consequences, symptoms and treatment of asthma in this group. Hence, many concerns and feelings on asthma and asthma medication use may be caused by a lack of knowledge. This may be very important in the explanation of adherence behaviour.

One of the strengths of the PACMAN-cohort study is that we only included children who use asthma medication on a regular basis. Occasional asthma medication users were excluded. This is possibly reflected by the relatively high number of oral steroid users, which might indicate more severe asthma, and the relatively high number of LABA users (which is also an indication for more persistent asthma symptoms). Existing population-based observational asthma studies have mainly focussed on determinants of paediatric asthma [32-34]. In these studies, only small numbers of participants regularly use medication which hampers medication use studies. Within the PACMAN-cohort, information is available on a wide range of variables related to medication use, which makes this dataset very suitable to study determinants of therapy adherence in more detail. Furthermore, overall response rate in our study was high and comparable with other cohort studies including a physical examination of

paediatric patients [35 36]. A previous pilot study showed that there were no differences in mean age, gender or asthma medication use between children from families that agreed to participate as compared to families that refused participation in the PACMAN-cohort study [10].

We have measured parental reported adherence which may not be the same as true adherence rates, as children, especially the older ones, may well be taking medication without supervision form their parents. Burgess and co-workers showed parents to overestimate adherence in their children [37]. In addition, Orrell-Valente et al. [38] showed indeed that asthma medication use responsibility increased with age, however, in their study by age 11 only 50% of the children took daily medicines on their own. Therefore, it is expected that in school-aged children, as included in the PACMAN-cohort study, parents are main drivers of medication use and adherence, and therefore able to give a reliable estimation of their child's adherence rates. In line with van Dellen and co-workers, we assessed adherence rates in children aged less then 12 years old, by parental reporting [39].

A potential flaw of the study was the high number of missing phone numbers in pharmacy information systems: more than a quarter of the patients could not be contacted due to these missing phone numbers. This finding of poor documentation of telephone numbers in pharmacy patient records was also described by Floor-Schreudering et al. [40]. Health care providers should focus on completeness of patient documentation which would facilitate good patient care in the first place, as well as research in health care settings. Only 14% of the population was of non-Dutch descent. However, selection bias seems unlikely in our study; the majority of our study population (88%) is of native Dutch ethnicity. As this is in line with the composition of the general Dutch population (89% non-immigants) [41] selection bias was not a major problem.

In conclusion, we showed that parental attitude and perception play an important role in achieving good adherence. Perceived parental need for medication use and Dutch ethnicity (non-immigrant status) appeared to be important drivers of adherence. We did not find an association between a family history of asthma or atopy and adherence rates. This highlights the importance of asthma disease and therapy education on behalf of health care providers even if the family has experience in coping with a chronic disease such as asthma. Besides the physician, the community pharmacist should play an active role in the process of medication counseling. By providing information on medication use and disease, adequately informing parents and patients about the need for medication use to reach sufficient control of their disease and taking away unfounded concerns (with special attention on ethnic minorities), during regular asthma checks in the pharmacy (for example every three months when a patient is filling his/her prescriptions) we believe that adherence rates and as a result treatment outcomes can be improved.

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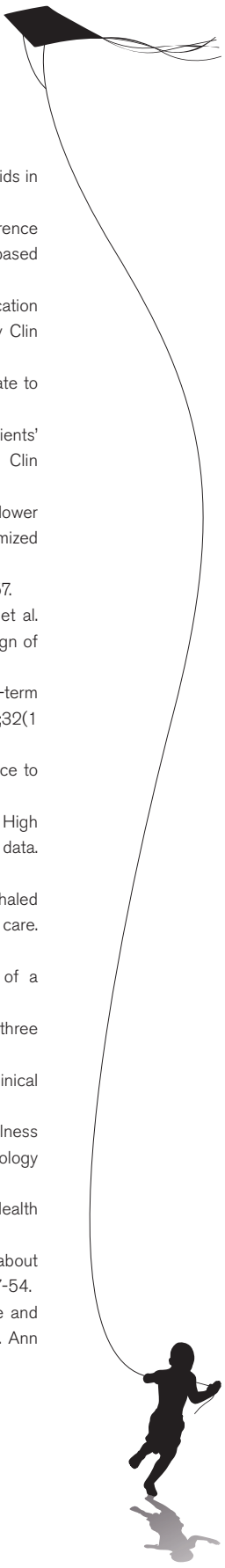

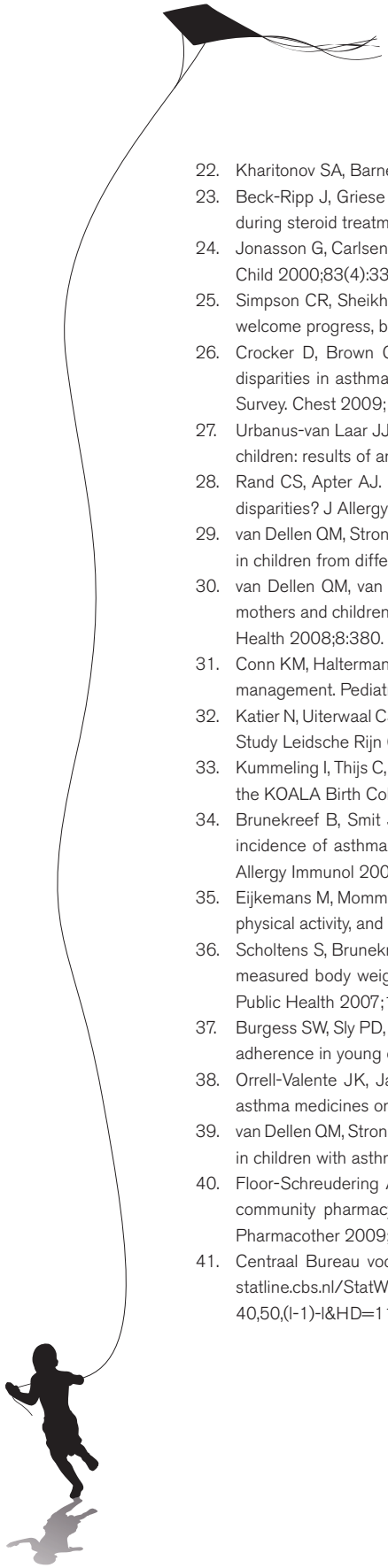

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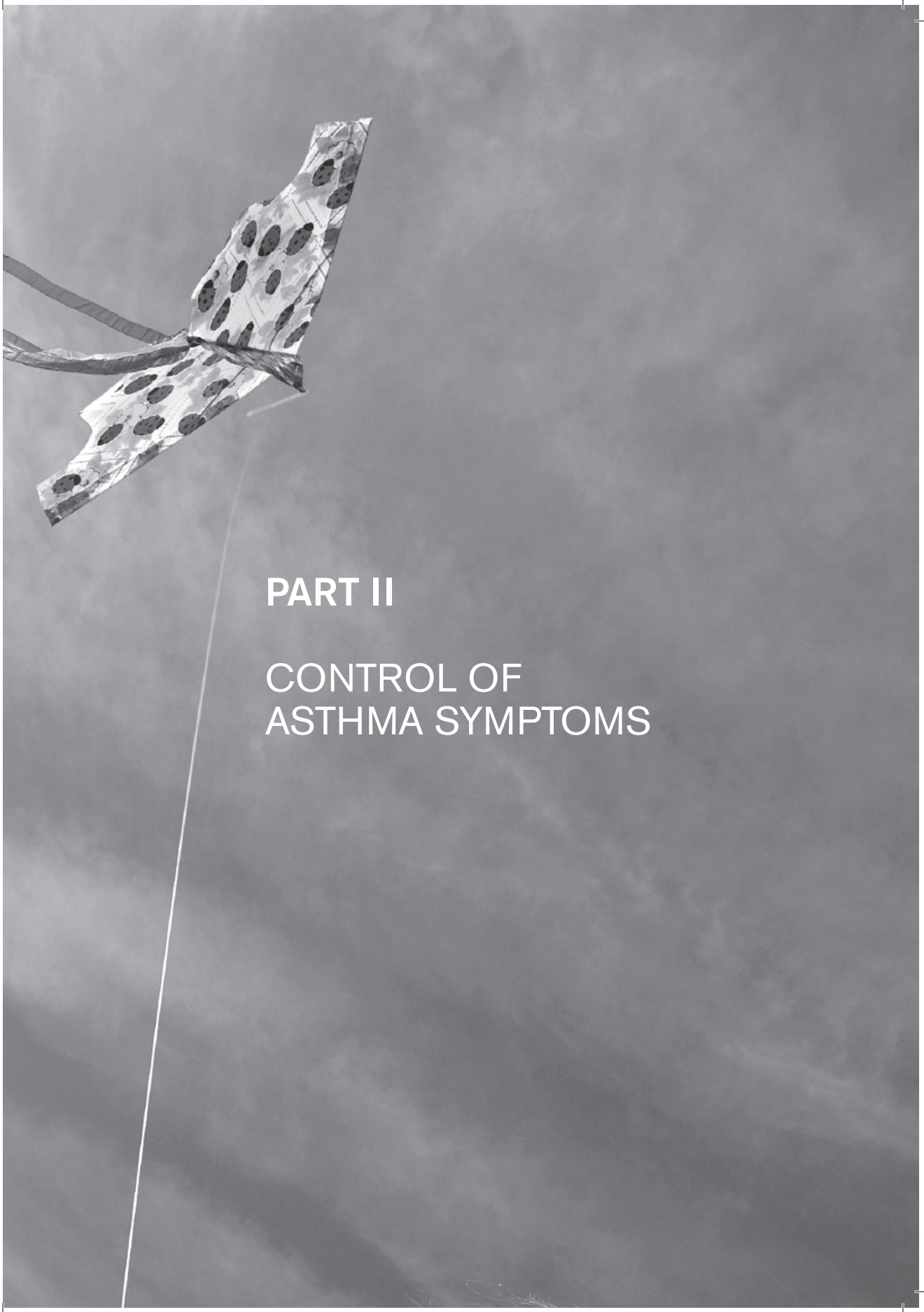

PART II

CONTROL OF ASTHMA SYMPTOMS

CHAPTER 7
Uncontrolled asthma at age 8: the importance of parental perception towards medication

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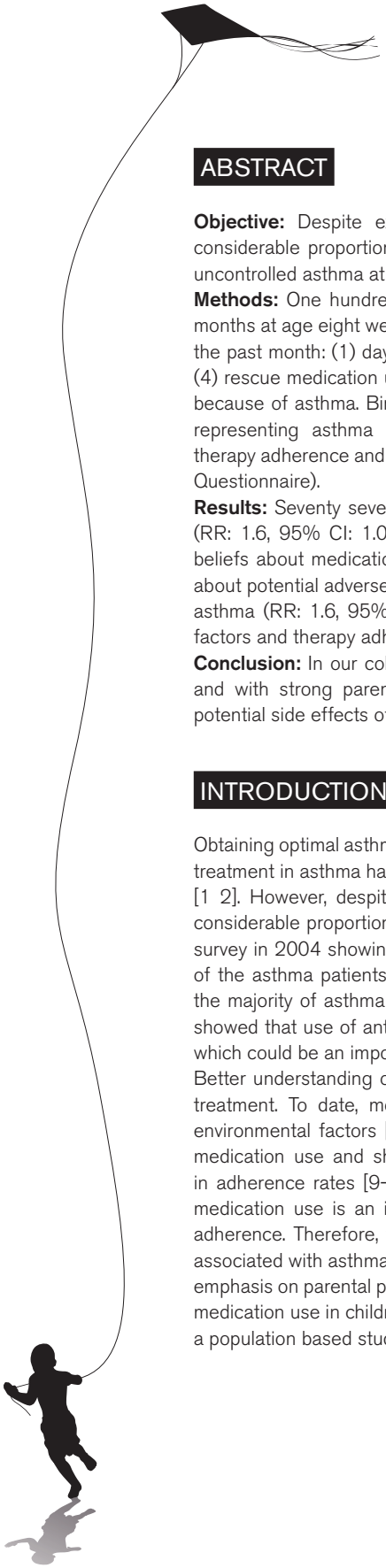


ABSTRACT

Objective: Despite existing effective treatment options, asthma is uncontrolled in a considerable proportion of patients. The aim of this study was to identify determinants of uncontrolled asthma at age 8 in children participating in the PIAMA birth cohort study.

Methods: One hundred seventy children using inhaled corticosteroids in the previous 12 months at age eight were included. Uncontrolled asthma was defined as: ≥3 items present in the past month: (1) day-time or (2) night-time asthma symptoms, (3) limitations in activities, (4) rescue medication use, (5) FEV1 < 80% predicted and (6) unscheduled physician visits because of asthma. Binomial regression was performed to study 5 groups of determinants representing asthma control: child and parental characteristics, environmental factors, therapy adherence and parental perception towards medication use (Beliefs about Medicines Questionnaire).

Results: Seventy seven children (45%) had uncontrolled asthma. Low maternal education (RR: 1.6, 95% CI: 1.0-2.4) was associated with uncontrolled asthma. Parental necessity beliefs about medication use to maintain present and future health and parental concerns about potential adverse consequences of medication were also associated with uncontrolled asthma (RR: 1.6, 95% CI: 1.1-2.2 and 1.6, 95% CI: 1.0-2.5, respectively). Environmental factors and therapy adherence were not associated with asthma control.

Conclusion: In our cohort, uncontrolled asthma is associated with low maternal education and with strong parental beliefs about medication necessity and higher concern about potential side effects of medication.

INTRODUCTION

Obtaining optimal asthma control is the major goal of asthma management. Anti-inflammatory treatment in asthma has been proven effective in both clinical trials and daily clinical practice [1 2]. However, despite existing effective treatment options, asthma is uncontrolled in a considerable proportion of patients. This was indicated in a large worldwide asthma control survey in 2004 showing evidence for suboptimal asthma control in many patients: 32-49% of the asthma patients experienced severe symptoms [3]. Chapman et al. [4] showed that the majority of asthma patients treated in general practice are uncontrolled. Another study showed that use of anti-inflammatory agents was low in a large number of asthma patients which could be an important cause of uncontrolled asthma [5].

Better understanding of determinants that influence asthma control could help to optimise treatment. To date, most studies on asthma control have focussed on phenotypic and environmental factors [6-8]. A few studies have focussed on parental perception towards medication use and showed that differences in medication beliefs resulted in disparities in adherence rates [9-12]. We hypothesized that parental perception towards their child's medication use is an important driver of asthma control, as it is associated with therapy adherence. Therefore, the aim of this study was to obtain more insight in factors that are associated with asthma control in asthmatic children using inhaled corticosteroids (ICS), with emphasis on parental perception towards medication use. In addition, we investigated asthma medication use in children with controlled vs. uncontrolled asthma at age eight selected from a population based study.

METHODS

Setting

We studied a subpopulation of children who participated in the PIAMA (Prevention and Incidence of Asthma and Mite Allergy) birth cohort study. Details of the study design have been published previously [13 14]. Recruitment took place in 1996 - 1997. A screening questionnaire was distributed to 10232 pregnant women visiting one of 52 prenatal clinics. Based on this screening 7862 women were invited to participate; 4146 agreed and gave written informed consent. 183 participants were lost to follow-up before any data on the child had been obtained, so that the study started with 3963 newborn children. At age eight 3271 parents returned the questionnaire.

Study population

At age eight, an additional questionnaire was sent to the parents of children with respiratory symptoms and who also used any asthma medication at this age (as reported in the annual questionnaire). The parents were asked to fill in this extra questionnaire to gather information regarding general health, respiratory symptoms, medication use, therapy adherence and parental beliefs about medicines.

Data collection

Data on respiratory and allergic symptoms, potential risk factors for asthma and allergy and demographic factors were collected by annual postal questionnaires. An extensive medical examination was carried out at age eight in a subset of the population. Children of atopic mothers were overrepresented in this subset. The medical examination included measurement of bronchial hyperresponsiveness (BHR), sensitization against common allergens and lung function. Forced expiratory volume in 1 second (FEV1) %predicted was used as lung function variable and FEV1 values $< 80\%$ predicted are an indication for airway obstruction [15 16].

Definition of uncontrolled asthma

Asthma control can be classified in various ways. A recent study [17] showed no differences between asthma control measured by Asthma Control Questionnaire (ACQ), the Global Initiative for Asthma (GINA) or Gaining Optimal Asthma Control (GOAL) criteria. We based uncontrolled asthma on the definition from the guideline “Paediatric Asthma” of the Dutch Pediatric Society (NVK) [18]. These guidelines follow the GINA guidelines which state that good asthma control consists of absence of asthma symptoms, night-time awakenings, doctor visits related to asthma, urgent care or hospitalisation related to asthma and no absences from school due to asthma, (near) normal lung function and normal activities [19]. Based on the data available within the PIAMA study, the outcome “uncontrolled asthma” at age eight, was defined as at least three of the following six items being present: (1) asthma symptoms during the day in the past month, (2) limitations in daily activities in the past month, (3) night-time asthma symptoms in the past month, (4) as-needed use of rescue medication (defined as short-acting beta-agonist (SABA) use) in the past month, (5) FEV1 $< 80\%$ predicted and (6) unscheduled physician visits related to any respiratory symptom (asthma, shortness of breath, wheezing, long-term coughing) in the past month (Table 1).

Table 1. Definition of uncontrolled asthma

	GINA [19]	NVK [18]	Items used for definition "uncontrolled asthma"
Absence of asthma symptoms	X	X	Day-time respiratory symptoms
Absence of night-time awakenings	X	X	Night-time respiratory symptoms
No doctor visits related to asthma	X		Doctor visits related to respiratory symptoms
No urgent care/hospitalisation related to asthma	X		
No school absences	X		
Normal lung function	X	X	FEV1 < 80% predicted
No limitations in daily activities	X	X	Limitations in daily activities
Rescue medication use		X	SABA use
Exacerbations		X	

FEV_1 = Forced Expiratory Volume in 1 second, SABA = short-acting beta-agonist

We performed a sensitivity analysis by using different cut-offs for uncontrolled asthma at age eight. A child was graded to have uncontrolled asthma if at least 3 of the above described items were present and controlled asthma if none of these items were present. Children with partially controlled asthma (presence of 1 or 2 of the above described items) were excluded for analysis. Excluding partially controlled patients did not change our results (data not shown). In addition, we classified uncontrolled asthma if at least 4 of the above described items were present which also did not influence our results (data not shown).

Determinants of uncontrolled asthma

Potential determinants of asthma control were divided into five groups: (1) child characteristics (gender, ethnicity, respiratory tract infections, overweight), (2) parental characteristics (educational level, atopy), (3) environmental factors (exposure to environmental tobacco smoke, pet exposure), (4) therapy adherence and (5) parental perception towards medication use. Most data was derived from the questionnaires.

Parental reported adherence was assessed by using the Medication Adherence Report Scale (MARS) comprising five questions on medication use behaviour [20]. "The 5-item MARS questionnaire has been used in various settings and various countries to assess self-report of medication adherence [21-24]. Previous research showed that this questionnaire was also a satisfactory screening tool to identify non-adherent ICS users within Dutch community pharmacies [25]. The MARS contains questions on forgetting to take a dose, altering the dose, deciding to miss a dose, taking less medication than instructed by the physician and stop taking medication for a while. The MARS was dichotomised by using a cut-off point for the sum-score: a score < 21 was considered as poor adherence. It has been shown that the MARS is a satisfactory tool to monitor adherence among ICS users irrespective of the chosen cut-off point [25].

Parental perception towards medication use

We focussed on parental perception towards medication use, including beliefs about medication (both parental necessity beliefs and concerns about their child's medication use) and knowledge about asthma medication. Beliefs about medicines were assessed using

the validated Beliefs about Medicines Questionnaire (BMQ) questionnaire. Originally, the BMQ was developed in the United Kingdom to measure patient's beliefs about medicines, however, a previous study showed that the BMQ can also be used in a Dutch setting [26]. This questionnaire comprises two scales with each 5 questions: the necessity scale and the concerns scale. All items are measured on a 5-point Likert scale [27]. The questions on necessity beliefs focus on the need for medication use to maintain present and future health. The questions on concerns focus on concerns about potential adverse consequences of medication use. Higher scores on the specific scales indicate stronger need beliefs or concerns. BMQ scores greater than scale midpoint (>15) were considered as stronger beliefs [26]. Horne has developed the Necessity-Concerns differential to describe the salient beliefs influencing patient's medication use behaviour [20]. This framework suggests that medication use behaviour is influenced by the way a patient judges necessity of medication use relative to concerns towards medicines. The Necessity-Concern differential is calculated by subtracting the individuals' concerns score from the individuals' necessity score leading to a range from -20 to 20. Higher scores indicate stronger perceived necessity and/or lower concerns towards ICS use.

Parental knowledge about asthma medication use was scored by using 10 questions on medication use based on previous used questionnaires [28 29] (such as "Antibiotics are important for the treatment of asthma" and "Prednison decrease acute shortness of breath symptoms within 15 minutes"), scores ≥ 70% were considered as good knowledge on asthma medication use.

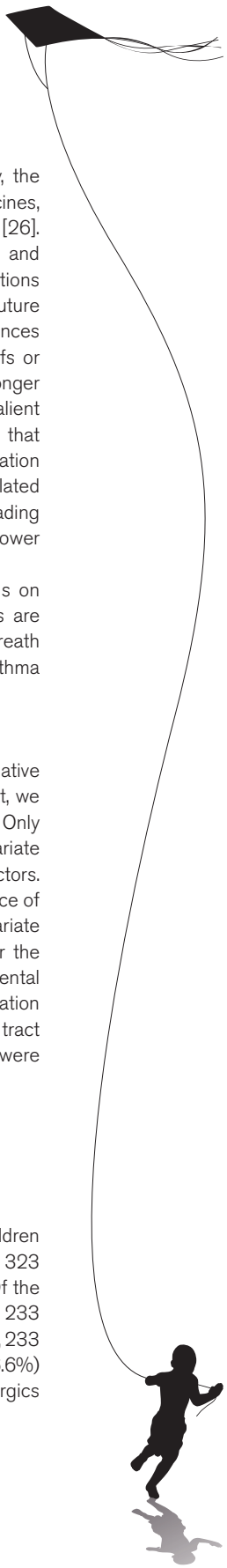
Statistical analysis

Binomial regression was used to investigate determinants of uncontrolled asthma [30]. Relative risks (RR) and their corresponding 95% confidence intervals (CI) were calculated. First, we performed univariate analysis to study individual determinants of uncontrolled asthma. Only variables associated with uncontrolled asthma ($p < 0.10$) were included in the multivariate model. Multivariate analysis was used to asses the independence of the associated factors. The inclusion of potential confounders in the regression model was based on the influence of each potential confounder on the RR. Confounding factors were included in the multivariate model if they induced a 10% change or more in the crude regression coefficient for the determinant of interest [31]. The child's gender, ethnicity, parental educational level, parental knowledge about asthma medication use, parental beliefs towards their child's medication use, pet exposure at age eight, tobacco smoke exposure at age eight and respiratory tract infections at age eight were considered potential confounding factors. All analyses were performed using SAS version 9.1 (SAS Institute, Cary, NC).

RESULTS

Study population

At age eight, 948 children (29.0%) experienced respiratory symptoms and 386 children (11.8%) used asthma medication. The 3271 screened 8-year questionnaires rendered 323 children (9.9%) with both respiratory symptoms and reported asthma medication use. Of the 323 extra questionnaires sent at age eight, 278 questionnaires were returned (86.1%); 233 (83.8%) children reported asthma medication use in the last 12 months. Of these children, 233 (83.8%) reported asthma medication use in the last 12 months. Sixty two children (26.6%) only used short-acting beta agonists (SABA), 1 child (0.4%) used exclusively anticholinergics



and 170 children (73.0%) used ICS in the past 12 months. Our study population consisted of 170 children who reported ICS use in this extra questionnaire at age eight (Figure 1). Children in our study population were primarily of Dutch origin (95.2%) and 61.8% was male (Table 2). Antibiotics were used by 34.7% of the study population in the preceding year and 25.3% used antihistaminic drugs in the past year. Eighty percent of the parents reported that a physician diagnosed their child with asthma. Almost one third (29.4%) of the population had a physician visit because of respiratory symptoms in the month prior to the questionnaire and 67.2% of the children showed bronchial hyperresponsiveness at age eight. Of the 170 children in our study population, 77 children (45.3%) met our definition of uncontrolled asthma. Table 3 shows the contribution of each of the six studied factors in this definition. SABA use in the past month, daytime respiratory symptoms and limitations in daily activities were the most prevalent factors.

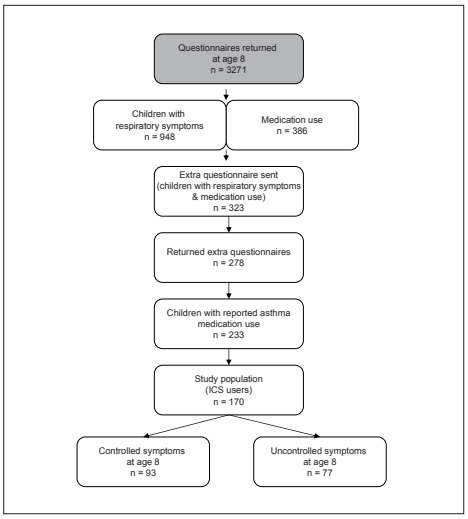


Figure 1. Definition of study population

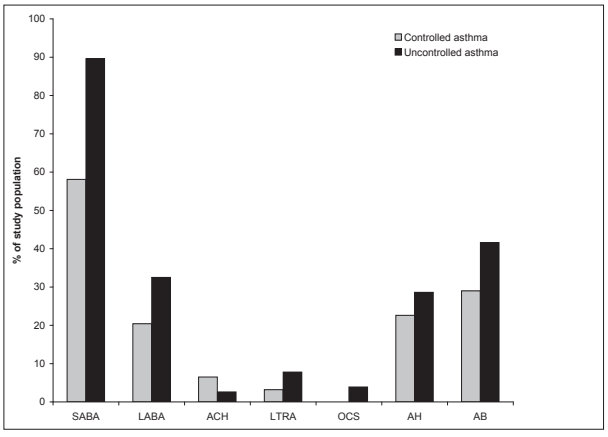


Figure 2. Asthma medication use in controlled vs. uncontrolled asthma at age 8. SABA = short-acting beta-agonist, LABA = long-acting beta-agonist, ACH = anticholinergic drugs, LTRA = leukotriene receptor antagonists, AH = antihistaminic drugs, AB = antibiotics

Table 2. General characteristics of study population

	n = 170
Child characteristics	
Male gender, %	61.8
Dutch ethnicity, % [a]	95.2 (159/167)
Atopy [b]	77.3 (51/66)
Asthma diagnosis ever, % [c]	79.8 (130/163)
Bronchial hyperresponsiveness age 8, % [d]	67.2 (41/61)
Overweight age 8, % [e]	18.3 (23/126)
Parental characteristics	
Low maternal educational level, % [f]	24.1
Low paternal educational level, % [f]	26.2 (44/168)
Atopic mother, % [c]	40.0
Atopic father, % [c]	37.7
Environmental factors	
ETS exposure age 8, %	21.4 (36/168)
Pet exposure age 8, %	40.0
Medication use	
Oral corticosteroids age 8, %	1.8
Antibiotics age 8, %	34.7
Antihistaminic drugs age 8, %	25.3
Good adherence [g]	45.3
Strong parental need beliefs for medication use	35.9
High concerns about medication use	19.4

[a] Based on both the country of birth of the mother and the self-reported ethnicity of the mother, if both Dutch, [b] Defined as a specific IgE concentration of at least 0.7 IU/mL on at least one of the airborne allergens in blood sample collected at age eight, [c] Questionnaire based, [d] Defined as a decrease of 20% in FEV_1 at a cumulative dose of 0.61 mg metacholine bromide, [e] Overweight definition is gender and age specific according to Cole et al. [37], [f] Educational level low: primary, lower vocational and lower general education, [g] MARS-score ≥ 21, ETS: environmental tobacco smoke.

Table 3. Uncontrolled asthma definition

Factor	N (%) of children
Day-time respiratory symptoms	98 (57.7)
Night-time respiratory symptoms	48 (28.2)
Doctor visits related to respiratory symptoms	50 (29.2)
FEV1 < 80% predicted	2/71 (2.8)
Limitations in daily activities	76 (44.7)
SABA use in the past month	123 (72.4)

Asthma medication use

Figure 2 shows asthma medication use in children with controlled vs. children with uncontrolled asthma at age 8. The prevalences of use of long-acting beta-agonist (LABA), leukotriene receptor antagonists, oral corticosteroids, antibiotics and antihistaminic drugs were higher in children with uncontrolled asthma compared to children with controlled asthma (although not statistically significant).

Factors associated with uncontrolled asthma

The univariate and multivariate associations between potential determinants and uncontrolled asthma at age eight are shown in Table 4. In the multivariate analysis, low maternal education and both high parental necessity beliefs and concerns about their child's medication use were independently associated with a greater risk for uncontrolled asthma. Phenotypic factors

Table 4. Determinants of uncontrolled asthma

	Univariate analysis	Multivariate analysis
Determinants	**RR (95% CI)**	**RR (95% CI)**
Child characteristics		
Male gender	1.1 (0.8 – 1.4)	
Dutch ethnicity	0.6 (0.3 – 1.4)	
Lower respiratory tract infections age 8	1.4 (0.9 – 2.1)	
Upper respiratory tract infections age 8	1.2 (0.9 – 1.6)	
Overweight age 8	1.2 (0.8 - 1.9)	
Parental characteristics **Low parental educational level**		
Mother	1.4 (1.0 – 2.1)*	1.6 (1.0 - 2.4)* a
Father	1.0 (0.7 – 1.4)	
Parental atopy		
Mother	0.9 (0.7 - 1.3)	
Father	1.0 (0.8 - 1.4)	
Environmental factors		
Pet exposure age 8	1.3 (0.9 – 1.7)**	1.2 (0.9 - 1.6) b
ETS exposure age 8	1.1 (0.8– 1.6)	
Medication use behaviour		
Poor adherence	1.1 (0.8 – 1.4)	
Parental perception towards medication use		
Strong need beliefs	1.5 (1.1 – 2.1)*	1.6 (1.1 - 2.2)* c
High concern beliefs	1.5 (1.0 – 2.3)*	1.6 (1.0 - 2.5)* c
Good knowledge about asthma medication	1.3 (0.8 – 2.2)	

ETS: Environmental tobacco smoke. Potential confounders that caused a 10% change or more in the crude regression coefficient were included in the multivariate model. [a] Adjusted for paternal educational level, exposure to pets, knowledge about asthma medication, [b] Adjusted for maternal educational level, [c] Adjusted for upper respiratory infections at age 8. * $p < 0.05$, ** $p < 0.10$

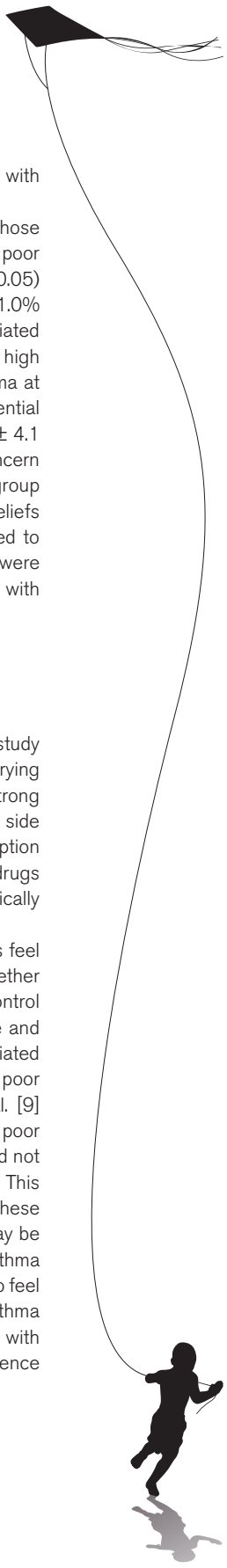
such as gender and ethnicity and therapy adherence were not significantly associated with uncontrolled asthma at age eight in this population.

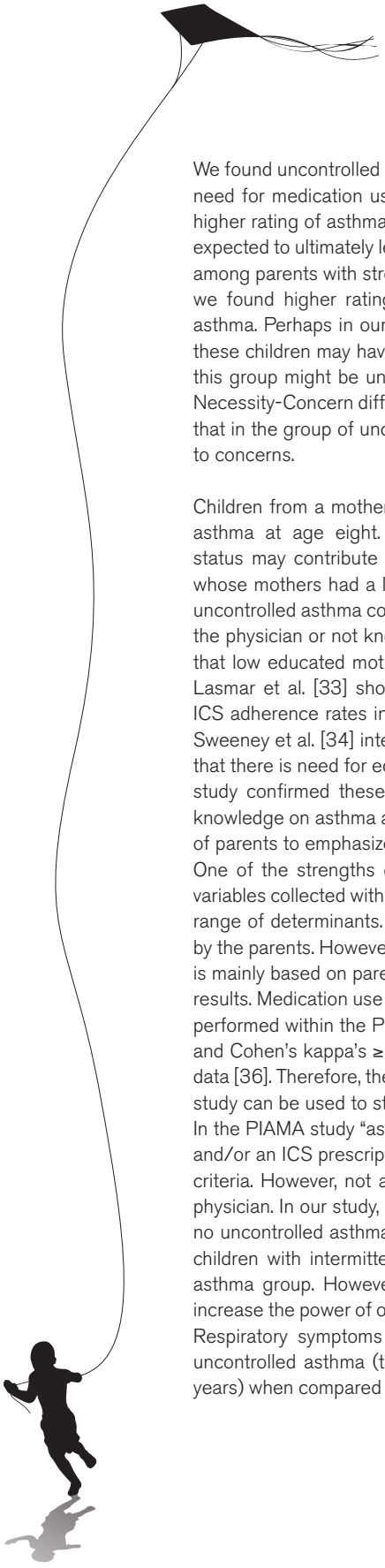
The risk for uncontrolled asthma at age eight was almost twice as high in children whose mother had a low education ($p < 0.05$). Mothers with low education had more often poor knowledge about asthma medication use compared with higher educated mothers ($p < 0.05$) and children whose mothers had a low education were more often exposed to pets (61.0% versus 33.3% ($p< 0.05$)). Strong parental need beliefs about medication use were associated with an almost two times higher risk of uncontrolled asthma. Children whose parents had high concerns about medication also showed a two times higher risk for uncontrolled asthma at age eight. Children with uncontrolled asthma had higher mean Necessity-Concern differential scores when compared to children with controlled asthma ($p = 0.03$), 2.0 ± 5.3 vs. 1.2 ± 4.1 respectively. In the uncontrolled asthma group, necessity scores where higher than concern scores (positive values for Necessity-Concern differential) for more than half of the group (56.2%) compared to 45.6% in the controlled asthma group. Parents with strong need beliefs towards their child's medication use showed higher therapy adherence rates compared to parents with low perceived need for medication use (60.6% vs. 36.7%, p=0.003). There were no differences observed in adherence rates between parents with strong and parents with minor concerns towards their child's medication use (51.5% vs. 44.5%, $p = 0.68$).

DISCUSSION

Uncontrolled asthma at age eight was present in approximately half (45%) of our study population. This is in line with other studies reporting uncontrolled asthma rates varying between 44% and 59% in asthmatic children [4 7]. Low maternal education and strong parental beliefs about the need for medication use and concerns about potential side effects of asthma medication were associated with uncontrolled asthma. With the exception of anticholinergic drugs, children with uncontrolled asthma also used other asthma drugs more frequently, but differences with children with controlled asthma were not statistically significant in this relatively small population.

An important factor involved in the level of paediatric asthma control is the way parents feel about the added value and/or danger of prescribed drugs. Therefore, we studied whether factors related to parental beliefs about medication use were associated with asthma control in childhood. In our study, storng parental concerns about e.g. medication dependence and adverse effects or diminished effectiveness with long-term medication use were associated with a higher risk of uncontrolled asthma at age eight. Severe concerns may lead to poor therapy adherence and as a consequence asthma may be uncontrolled. Conn et al. [9] showed that parental concerns about asthma medication use were associated with poor medication adherence in children with asthma. However, against our expectations we did not find an association between therapy adherence and uncontrolled asthma in our study. This might be due to the fact that there are children with only mild or intermittent symptoms (these children are classified as good controlled in our study) and therefore these children may be less adherent. Van Dellen et al. [32] showed in their study that children with the best asthma control were nondaily users of ICS. Their explanation for this finding was that children who feel their asthma is controlled stop their ICS or that these children have better controlled asthma and do not need ICS. In our study, this group of children with poor adherence combined with controlled asthma may be the cause that we do not find an association between adherence and uncontrolled asthma.





We found uncontrolled asthma also to be associated with the parent's attitude towards higher need for medication use. This was against our expectations, as another study showed that higher rating of asthma medication necessity was associated with better adherence which is expected to ultimately lead to better asthma control [10]. We did show higher adherence rates among parents with strong necessity beliefs for medication use, but against our expectations we found higher rating of necessity to be associated with a higher risk of uncontrolled asthma. Perhaps in our case, strong parental need beliefs may reflect more severe asthma; these children may have more severe disease which is difficult to control and a small part of this group might be unresponsive to therapy. Children with uncontrolled asthma had higher Necessity-Concern differential scores compared to children with controlled asthma, indicating that in the group of uncontrolled asthmatics, necessity of medication use was higher relative to concerns.

Children from a mother with a low educational level were at increased risk for uncontrolled asthma at age eight. Differential life style factors coherent with lower socioeconomic status may contribute to this increased risk of uncontrolled asthma. In our study, children whose mothers had a low educational level were more often exposed to pets. Furthermore, uncontrolled asthma could be a result of insufficient medication use due to misunderstanding the physician or not knowing how to use medication properly. We confirmed this by showing that low educated mothers had more often poor knowledge about asthma medication use. Lasmar et al. [33] showed that low maternal educational level was associated with lower ICS adherence rates in children, which could be a cause of poor asthma control. Peterson-Sweeney et al. [34] interviewed parents on medication use in childhood asthma and showed that there is need for education on medication use from the physician or pharmacist. Another study confirmed these findings by showing that more than half of the parents had poor knowledge on asthma and asthma management [35]. Therefore, good education and training of parents to emphasize the need for adequate medication use is of utmost importance.

One of the strengths of our study was the availability of information on a wide range of variables collected within the annual questionnaires. Therefore, we were able to assess a wide range of determinants. A limitation of this study is that respiratory symptoms were reported by the parents. However, this is also the case for clinical practice, where asthma management is mainly based on parental report of asthma control. Therefore, this likely has not biased our results. Medication use was also reported by the parents in the questionnaire. An earlier study performed within the PIAMA cohort showed excellent agreement (overall agreement $> 97\%$ and Cohen's kappa's ≥ 0.80) between parental reported ICS use and pharmacy prescription data [36]. Therefore, the questionnaire based medication use data collected within the PIAMA study can be used to study prevalences of asthma medication use in children.

In the PIAMA study "asthma" was defined as at least one attack of wheeze and/or dyspnoea and/or an ICS prescription in the last year. All the children in our study population met these criteria. However, not all parents reported that their child was diagnosed with asthma by a physician. In our study, good asthma control was regarded to be present in any situation that no uncontrolled asthma existed. This could have led to some degree of misclassification, as children with intermittent or partly-controlled asthma were also included in the controlled asthma group. However, a sensitivity analysis did not show any differences, therefore, to increase the power of our study, we combined these two groups (partially and fully controlled).

Respiratory symptoms in the previous years were much more common in children with uncontrolled asthma (the prevalence of wheeze varied from 61.0% to 66.2% for age 4-8 years) when compared to children with controlled asthma at age 8 (wheeze prevalence varied

between 25.3% and 41.9% for age 4-8 years). This strengthens the idea that we hence have selected children with more regular respiratory symptoms by using our definition and thereby more uncontrolled asthma.

In conclusion, the current study shows that uncontrolled asthma is associated with parental beliefs about the use and potential side effects of asthma medication and with a lower level of maternal education. These findings underline the need for good education of parents, and particularly mothers, about asthma and its management. Health care professionals such as physicians and pharmacists should make efforts to improve this knowledge and positively influence parental perception towards medication use. By adequately informing parents, unfounded concerns about medication use can be taken away. This could eventually contribute to better asthma control in paediatric patients.

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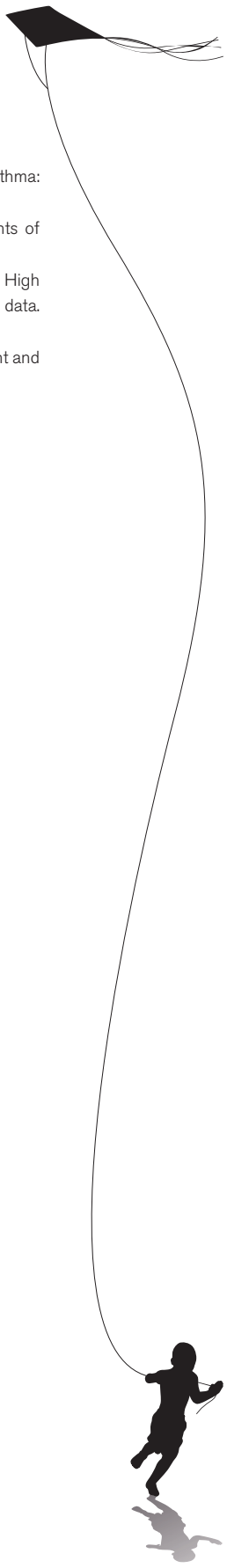

CHAPTER 8
Limited agreement between current and long-term asthma control in children: the PACMAN cohort study

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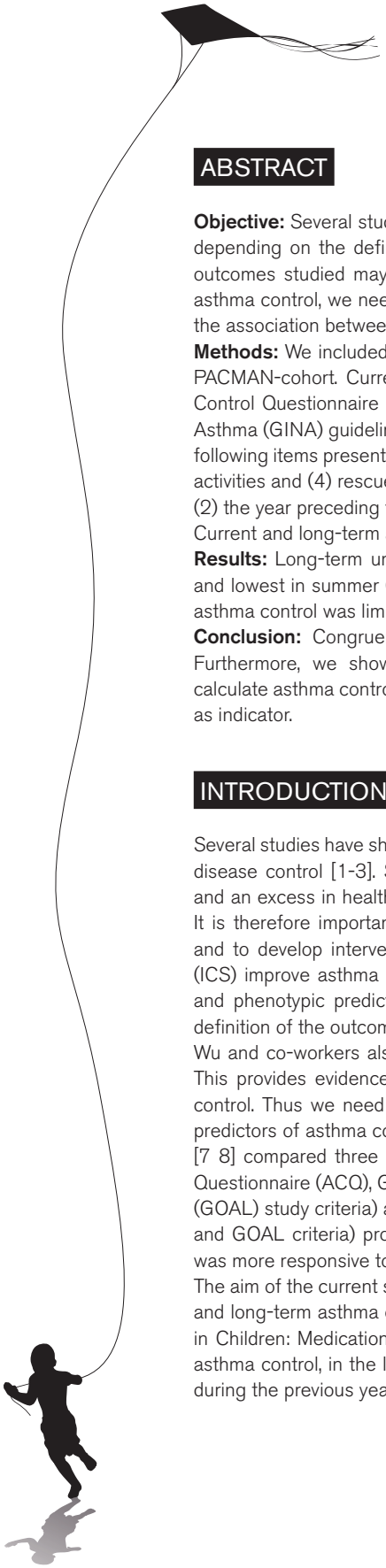


ABSTRACT

Objective: Several studies have shown that predictors of asthma treatment outcomes differ depending on the definition of the outcome chosen. This provides evidence that different outcomes studied may reflect distinct aspects of asthma control. To assess predictors of asthma control, we need firm outcome phenotypes. The aim of this study was to investigate the association between measurements of current and long-term asthma control.

Methods: We included 527 children using inhaled corticosteroids (ICS) participating in the PACMAN-cohort. Current asthma control (previous week) was defined using the Asthma Control Questionnaire (ACQ). Long-term asthma control was based on Global Initiative for Asthma (GINA) guidelines. Not well-controlled asthma in a season was defined as: ≥3 of the following items present in a season: (1) day-time or (2) night-time symptoms, (3) limitations in activities and (4) rescue medication use. Asthma control during: (1) the previous season and (2) the year preceding the pharmacy visit were used as long-term asthma control definitions. Current and long-term asthma control were compared in order to investigate agreement.

Results: Long-term uncontrolled asthma rates were highest in autumn and winter (50%) and lowest in summer (32%) ($p < 0.05$). Overall agreement between current and long-term asthma control was limited (66% for previous season and 68% for previous year).

Conclusion: Congruence between current and long-term asthma control was limited. Furthermore, we showed significant seasonal differences. It is therefore important to calculate asthma control over a longer period of time, instead of using current asthma control as indicator.

INTRODUCTION

Several studies have shown that a substantial proportion of asthmatics do not reach sufficient disease control [1-3]. Suboptimal asthma control is associated with a lower quality of life and an excess in health care use with consequently increased health care costs for society. It is therefore important to obtain in depth knowledge on determinants of asthma control and to develop interventions to improve this. It is well known that inhaled corticosteroids (ICS) improve asthma control [4]. A recent study of Rogers et al. [5] showed that genetic and phenotypic predictors of long-term ICS treatment response differ depending on the definition of the outcome chosen (lung function or exacerbations). In line with these findings, Wu and co-workers also reported different predictors for symptoms and exacerbations [6]. This provides evidence that the two outcomes studied reflect distinct aspects of asthma control. Thus we need firm asthma control phenotypes, to assess phenotypic and genetic predictors of asthma control. Asthma control can be defined in different ways. O'Byrne et al. [7 8] compared three different asthma control classification systems (the Asthma Control Questionnaire (ACQ), Global Initiative for Asthma (GINA) or Gaining Optimal Asthma Control (GOAL) study criteria) and showed that the two categorical scales used in their study (GINA and GOAL criteria) provided comparable results. The ACQ, an ordinal measurement scale, was more responsive to change over time than the categorical scales used in their study [8]. The aim of the current study was to investigate the agreement between definitions of current and long-term asthma control using data from the Pharmacogenetics of Asthma medication in Children: Medication with ANti-inflammatory effects (PACMAN)-cohort [9]. Both current asthma control, in the last week, and long-term asthma control, during the past season and during the previous year, were determined and compared.

METHODS

Study population and setting

Recruitment of the PACMAN-cohort started in April 2009 by means of selecting paediatric asthma medication users from community pharmacies. Details of this study protocol have been described elsewhere [9]. Briefly, children aged 4-12 years who are regular users (≥ 3 prescriptions within the last 2 years and ≥ 1 prescription in the last 6 months) of asthma medication (Anatomical Therapeutical Chemical (ATC) code R03) were selected from pharmacies in The Netherlands. Selected children and their parents were invited for a visit to their own community pharmacy. Recruitment for the PACMAN-cohort took place throughout the year. Nevertheless, most children were included in spring (46%) and autumn (30%) as a result of holiday seasons (summer and winter). Only 7% of the population was included during summer and 17% was included during winter. In order to access whether the studied selection was a representative to the general population, information was extracted from the pharmacy information system in four randomly chosen pharmacies participating in the PACMAN-cohort study. The following information was extracted: number of children registered to the pharmacy aged 4-12 years (gender and age), number of children aged 4-12 years who filled a prescription for asthma medication in the past 2 years (gender and age) and the number of children who fulfilled the PACMAN inclusion criteria (as described above). The PACMAN study has been approved by the Medical Ethics Committee of the University Medical Centre Utrecht.

During the pharmacy visit, parents filled in a questionnaire to collect information regarding general health, asthma and respiratory symptoms, medication use, adherence (the 5-item MARS questionnaire [10]), parent's beliefs about medicines, environmental and socio-demographic factors. This questionnaire also contained questions regarding the presence of asthma symptoms, limitations in activities, sleep disturbances and extra medication use during the different seasons (spring, summer, autumn, winter) in the past 12 months. In addition, a question on the frequency of the described symptoms during the different seasons was added: "How often did your child experience these symptoms during spring/summer/autumn/winter?" (Answers: daily, weekly, monthly or rarely symptoms).
Furthermore, the child's lung function was measured (both before and after use of a short-acting beta-agonist (SABA)) with a hand-held diagnostic spirometer (Microspirometer, Micro Medical, Kent, UK), fractional exhaled nitric oxide levels (FeNO) were measured with a hand-held analyzer (Niox Mino, Aerocrine, Solna, Sweden) and the child's inhalation technique was scored with an inhaler specific inhalation control checklist [9]. The children and parents were asked to withheld bronchodilating agents such as short-acting β-agonists and anti-cholinergic agents 4 hours before the pharmacy visit and long-acting β-agonists 36 hours before testing. For the present study, we only selected children who used ICS in the past year.

Definitions

We defined both current asthma control (control of symptoms during the last week) and long-term asthma control (asthma control during the previous season (3 months) and asthma control during the pervious year (12 months)).

Current asthma control

Current asthma control outcome (well-controlled, partially or not well-controlled asthma) was defined using the 6-item Asthma Control Questionnaire (ACQ). This questionnaire can be used to differentiate between well-controlled and not well-controlled asthma, an ACQ-score < 0.75 indicating good asthma control, a score between 0.75 and 1.50 indicating partially controlled asthma and an ACQ-score of ≥ 1.50 indicating not well-controlled asthma [11]. This questionnaire is validated in asthmatic patients aged $\geq$ 18 years, but has also been successfully used in paediatric populations [12].

Long-term asthma control

Long-term asthma control was based on the definitions from the Global Initiative for Asthma (GINA) guidelines [13]. First, asthma control was defined based on the presence of symptoms in a specific season. Not well-controlled asthma was defined as: ≥ 3 of the following items present in a specific season: (1) day-time asthma symptoms, (2) night-time asthma symptoms, (3) limitations in daily activities and (4) rescue medication use. Partially controlled asthma was defined as the presence of one or two of the above described items and well-controlled asthma was defined if none of the above described items were present. Second, we used a definition for asthma control that was adjusted for the frequency of symptoms during a season (as is also proposed according to the GINA criteria). Children with three or more features of frequent symptoms (daily or weekly symptoms during a season) were classified as not well-controlled, children with one or two features of monthly symptoms were classified as partially controlled and children who only experienced rarely symptoms (less than once a month) during a season were classified as well-controlled.

Long-term asthma control was defined as: (1) asthma control during the previous season (3 months) and (2) asthma control during the previous year (12 months). Overall long-term asthma control during the past year was defined as at least three seasons in which the symptoms were controlled and not well-controlled asthma was defined as at least three seasons with uncontrolled asthma. These two definitions of long-term asthma control were compared with current asthma control (based on the ACQ).

Statistical analysis

Chi-square testing was used to compare frequency data and Kruskal-Wallis testing was used for comparison of means. The proportion of overall agreement and kappa statistics with their 95% corresponding confidence (CI) intervals were calculated to quantify agreement between current and long-term asthma control. Kappa statistics were interpreted using the classification system developed by Landis and Koch [14]: $\kappa \leq 0$ poor agreement; κ 0 - 0.2 indicating slight agreement, κ 0.21-0.4 indicating fair agreement, κ 0.41-0.60 indicating moderate agreement, 0.61–0.80 substantial agreement and κ values 0.81 to 1.00 indicating almost perfect to perfect agreement. All analyses were performed using SAS version 9.1 (SAS Institute, Cary, NC).

RESULTS

Selected subjects vs. general population

Figure 1 shows the characteristics of a random sample of the PACMAN study population compared to the complete population (children aged 4-12 years) out of four randomly chosen pharmacies (data were pooled, because there were no significant differences between the pharmacies). Almost 8% of the children (aged 4-12 years) registered to these pharmacies filled a prescription for asthma medication in the past 2 years. Around one third of these asthma medication users met the PACMAN inclusion criteria.

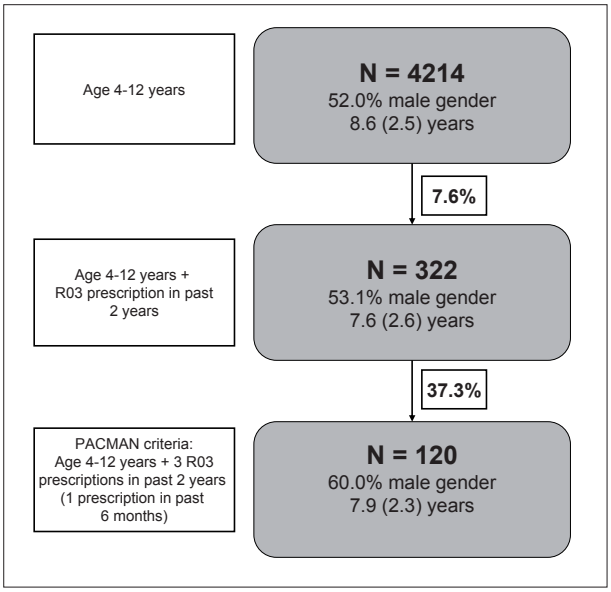


Figure 1. Selection of PACMAN study subjects in four randomly selected pharmacies. This flow scheme shows the selection of study participants for the PACMAN study from the complete population of children registered in one of the four randomly chosen community pharmacies.

Response rate

Until February 2011, 2114 children were selected from 67 Dutch pharmacies. For 595 (28.1%) parents and their children the telephone number was unknown in the pharmacy and could not be traced through Internet or a national telephone number service. Furthermore, 393 (18.6%) parents, whose telephone numbers were known, could not be reached after at least five phone calls during office hours. Therefore, in total, 1126 parents could be invited by telephone and of these, 684 parents (60.7%) agreed to participate. No data was obtained in 82 (12.0%) children due to various reasons (e.g. child was unwilling to participate, parents/children did not show up at the scheduled appointment, or the parent/children were willing to participate, though unable to come to the pharmacy in the period of patient inclusion). As a result, 602 children were included in the study indicating a response rate of 53.5%. For the present study, we only included children using ICS in the past year (n = 527). The general characteristics of this population are shown in Table 1: 61% of the participants were males and the mean age was 8.4 years.

Table 1. Characteristics of study population

	Study population (n = 527)
General characteristics	
Male gender, %	61.0
Age, mean ± SD	8.4 ± 2.5
Caucasian ethnicity, %	89.6
Clinical characteristics	
Hay fever, %	47.1
Eczema, %	66.2
Food allergy, %	52.5
Doctor-diagnosed asthma, %	76.2

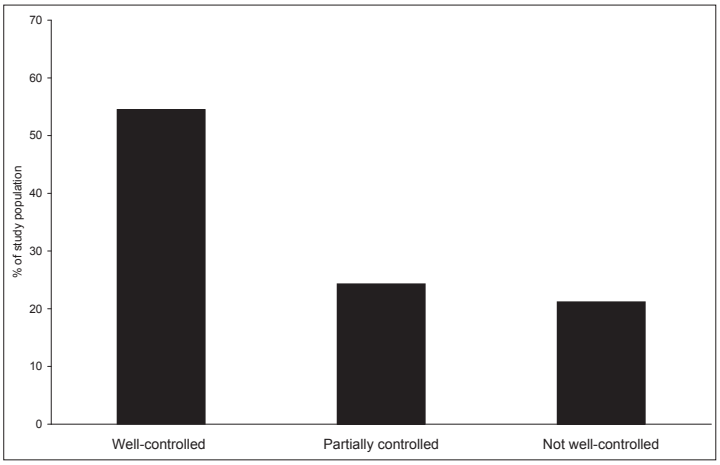


Figure 2. Definition of current asthma control status by ACQ. ACQ = Asthma Control Questionnaire

Current asthma control during the past week

Figure 2 shows current asthma control as defined by the ACQ-scores. Most patients are classified as well-controlled (54.5%). A quarter (24.3%) is classified as partially controlled and 21.2% of the children had not well-controlled asthma.

Long-term asthma control during the previous season

Figure 3A shows the level of asthma control during the different seasons (prevalence based, not adjusted for symptom frequency). Uncontrolled asthma rates were highest in autumn (62.2%) and lowest in summer (36.7%) ($p < 0.05$). Well-controlled asthma rates ranged from 8.4% (autumn) to 33.3% (summer). The prevalence of partially controlled asthma varied between 29.4% (autumn) and 33.7% (spring) for the different seasons.

Figure 3B shows asthma control, taking the symptom frequency into account. Uncontrolled asthma rates were highest in autumn and winter (49.8% and 49.9% respectively) and lowest in summer (31.6%) ($p < 0.05$). The prevalence of well-controlled asthma was highest in summer (28.7%) and lowest in autumn and winter (16.7% and 18.5% respectively). Asthma symptoms were not well-controlled during the previous season (Figure 3B) in 46.8% of the patients. Symptoms were well-controlled in 22.6% and partially controlled in 30.6% of the patients.

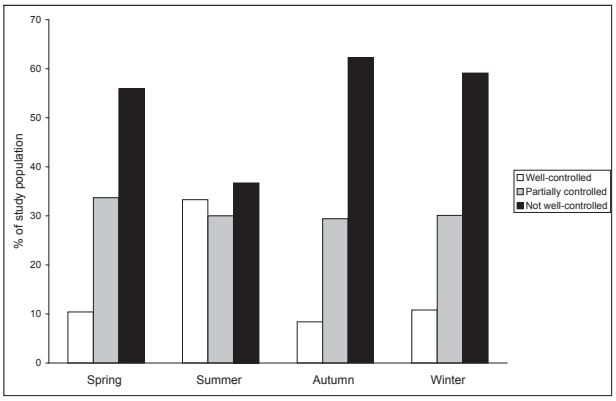


Figure 3A. Long-term asthma control, prevalence based definition

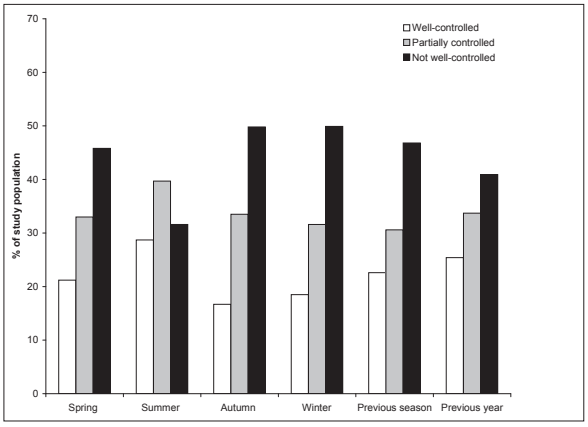


Figure 3B. Long-term asthma control, adjusted for symptom frequency

Table 2. Congruence between current and long-term asthma control

Current asthma control (ACQ) – Long-term asthma control	Overall agreement, % (n)	Kappa (95% CI)
Previous season (3 months)	65.8	0.38 (0.29 ; 0.47)
Previous year (12 months)	67.8	0.40 (0.31 ; 0.49)

ACQ = Asthma Control Questionnaire

Long-term asthma control during the previous year

Asthma symptoms were not well-controlled during the past year in 40.9% of the patients (symptoms in at least three seasons). Symptoms were well-controlled in 25.4% and partially controlled in 33.7% of the patients (Figure 3B).

Agreement between current and long-term asthma control

Overall agreement between current asthma control and asthma control during the previous season was 66%. Kappa statistics indicated 'fair agreement' (Table 2). A large proportion of the children with current well-controlled asthma, did not meet our definition of long-term asthma control (specificity 52.4% and sensitivity 95.9%). Overall agreement between current control and long-term control over the past year was also fair (overall agreement 68%, $\kappa = 0.40$).

Table 3. Description of study population

	Controlled n = 105	Partly controlled n = 142	Uncontrolled n = 217
General characteristics			
Gender, male	64.8	63.4	59.0
Age, mean (SD)	8.5 (2.4)	8.4 (2.4)	8.4 (2.5)
Dutch ethnicity	90.4	90.6	86.3
Doctor-diagnosed asthma	81.3	74.2	73.2
Medication use			
OCS	1.9	5.6	12.4*
SABA	76.2	87.3	88.0*
LABA	29.5	24.7	25.8
LTRA	5.7	6.3	12.4 **
AB	29.8	34.8	43.7*
Good adherence	59.1	54.2	58.5*
Good inhalation technique	86.7	83.8	81.2
Environmental factors			
Smoke exposure	10.5	8.5	7.0
Pet exposure	44.8	32.4	38.6
Urban environment	63.8	58.5	63.1
Current asthma control, ACQ			
Well-controlled	69.6	59.7	36.6*
Partially controlled	27.5	27.3	23.2*
Not well-controlled	2.9	13.0	40.3*
Mean (SD)	1.4 (0.5)	1.6 (0.7)	2.1 (0.9)*

Note: For the definition of long-term asthma control phenotypes (asthma control in previous 3 months), data were available for 88.0% of our study population (n=464). OCS = oral corticosteroids, SABA = short-acting beta-agonist, LABA = long-acting beta-agonist, LTRA = leukotriene antagonist, AB = antibiotics, ACQ = asthma control questionnaire. * $P < 0.05$, ** $P < 0.10$

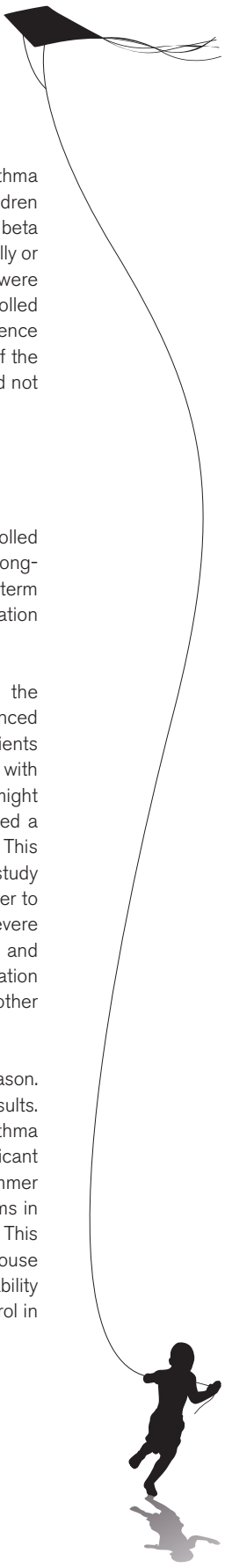
The characteristics of the study population when using the definition for long-term asthma control (previous season) are shown in Table 3. As a confirmation of this finding, children with long-term not well-controlled asthma used more often oral steroids, short-acting beta agonists, leukotriene receptor antagonist and antibiotics compared to children with partially or well-controlled asthma ($p < 0.05$). Current asthma control rates (based on ACQ scores) were significantly lower for children with asthma that was not well-controlled or partially controlled in the previous season compared to children with well-controlled asthma. Therapy adherence and inhalation technique were not associated with asthma control. The characteristics of the study population when using the definition for overall asthma control in the past year did not change our results (Appendix 1).

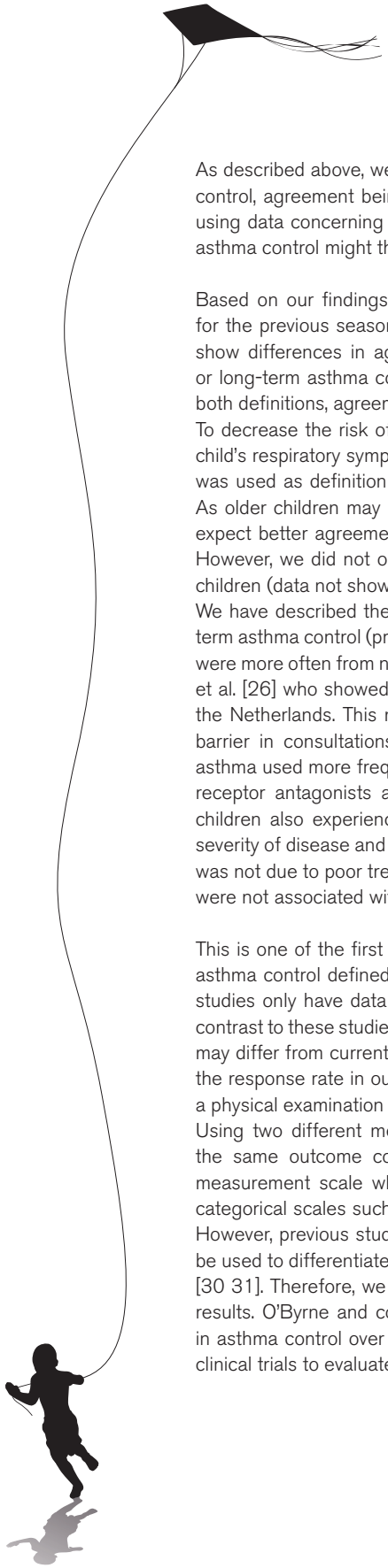
DISCUSSION

Our data show that there exists a relatively low prevalence of currently uncontrolled asthma (21%) in a cohort of pediatric asthma medication users. At the same time, long-term uncontrolled asthma rates were higher and agreement between current and long-term asthma control was limited. Of interest, we additionally showed significant seasonal variation in asthma control.

Currently uncontrolled asthma rates (asthma control during the week previous to the pharmacy visit) were low in our study. Less than a quarter of the population experienced symptoms in the past week. Previous cross-sectional studies in pediatric asthma patients showed much higher rates of uncontrolled asthma in the week previous to the survey with rates ranging from 37% to 76% [15-18]. A possible explanation for this discrepancy might be that parents who participated in the PACMAN-cohort study may not have scheduled a study visit in the pharmacy whilst their child experienced a period of uncontrolled asthma. This may be the case since one of the conditions for participation in the PACMAN-cohort study was that asthma medication should be withheld the day before the pharmacy visit in order to allow accurate lung function measurements. When a child experiences a period with severe symptoms and thus uncontrolled asthma, parents may not be willing to stop medication and therefore re-schedule the pharmacy appointment. This may have resulted in overestimation of current asthma control in our study population. This limitation is probably shared with other observational studies.

Almost half of the population experienced uncontrolled asthma during the previous season. When defining asthma control over the past year (12 months) this did not change our results. This is in line with other studies on long-term asthma control, showing uncontrolled asthma rates between 44% and 59% in asthmatic children [1 19-21]. Our study showed significant seasonal differences in asthma control, controlled asthma rates being highest in summer and lowest in autumn. Other studies also reported a low prevalence of asthma symptoms in summer and a peak in symptom frequency and severity beginning in autumn [22 23]. This seasonality in asthma may result from an increase in exposure to respiratory viruses, house dust mite, air pollution and to pollen in autumn and spring [24 25]. Because of the variability in asthma control over time, it is very important to stratify for seasonality in asthma control in stead of only evaluating current asthma control.





As described above, we observed limited agreement between current and long-term asthma control, agreement being especially low for not well-controlled asthma (low specificity). By using data concerning current asthma control (symptoms during the past week) a patient's asthma control might therefore be easily overestimated.

Based on our findings, we will use long-term asthma control based on the GINA criteria for the previous season as definition for future studies in the PACMAN-cohort. We did not show differences in agreement when using long-term asthma control over the past year or long-term asthma control defined as control during the previous season (3 months). For both definitions, agreement with current control measured by means of the ACQ was limited. To decrease the risk of recall bias, because parents were asked retrospectively about their child's respiratory symptoms, asthma control during the season preceding the pharmacy visit was used as definition for long-term asthma control in stead of control over the past year. As older children may better describe to their parents the symptoms that occur, one might expect better agreement between current and long-term asthma control for older children. However, we did not observe improved agreement between these two definitions for older children (data not shown).
We have described the characteristics of the study population using this definition of long-term asthma control (previous season) and show that children with not well-controlled asthma were more often from non-Dutch descent. This is in line with the study from Urbanus-van Laar et al. [26] who showed that ethnic minorities experience poorer asthma health outcomes in the Netherlands. This might be due to the presence of a cultural difference and language barrier in consultations between the physicians and patients. Children with uncontrolled asthma used more frequent additional respiratory medication such as antibiotics, leukotriene receptor antagonists and oral steroids in our study. Furthermore, long-term uncontrolled children also experienced more often poor current asthma control which may reflect the severity of disease and the persistence of respiratory symptoms. Our study suggests that this was not due to poor treatment adherence, since therapy adherence and inhalation technique were not associated with asthma control.

This is one of the first studies reporting on the comparison between current and long-term asthma control defined in an observational epidemiological study. Many other observational studies only have data on respiratory symptoms concerning the past one or two weeks. In contrast to these studies, we were able to define long-term control of asthma symptoms which may differ from current asthma control because of factors such as seasonality. Furthermore, the response rate in our study was high and comparable with other cohort studies including a physical examination in pediatric patients [27-29].
Using two different methods (ACQ vs. categorical scale such as GINA criteria) to asses the same outcome could have affected the presented results. The ACQ is an ordinal measurement scale which is based on intensity and impact of asthma symptoms, whilst categorical scales such as GOAL or GINA criteria are mainly based on symptom frequency. However, previous studies have shown that both methods of assessing asthma control can be used to differentiate between patients with controlled and uncontrolled asthma symptoms [30 31]. Therefore, we do not think the use of different instruments has affected our study results. O'Byrne and colleagues [7] showed that the ACQ was more sensitive to changed in asthma control over time, which may make this tool more valuable in a clinical setting or clinical trials to evaluate medication efficacy or improvement after treatment.

A possible limitation of our study is that symptoms were based on parental reporting. Parents may not always be able to give an accurate estimate of their child's health status. However, this limitation is shared with previous studies, and resembles daily clinical practice, where asthma management in young children is also based on parental reporting. Another potential limitation of the study is the large number of missing phone numbers in the pharmacies: more than a quarter of the patients selected from the pharmacies could not be invited for participation due to missing phone numbers. Floor-Schreudering et al. [32] also reported this poor documentation of contact information in pharmacies. We do not anticipate that selection bias was a problem, as a previous pilot study within the PACMAN-cohort showed that mean age and gender did not differ between children from families that agreed or refused to participate in the PACMAN-cohort study [9]. In addition, we compared the PACMAN study population with the general population (children aged 4-12 years) and found that approximately 8% of the children characterized in four randomly selected pharmacies filled a prescription for asthma medication in the past two years. The latter is in line with the prevalence of asthma and the prevalence of asthma medication use in childhood [33 34]. Approximately one third of the children who filled asthma medication prescriptions in the past two years were current users and selected for participation in the PACMAN-cohort study. This is also in line with previous studies which showed that discontinuation of asthma medication use in childhood is high [35 36].

In conclusion, we showed substantial seasonal variation in asthma control. Furthermore, we have demonstrated that the congruence between current and long-term asthma control is limited. This leads us to conclude that a patient's asthma control may be easily overestimated when using data concerning current asthma control (symptoms during the past week). Therefore, to properly define asthma control and treatment response in observational studies, it is important to assess asthma control over a longer period of time, or at least to stratify or adjust for the different seasons as we have shown in this study. Our findings may also be of relevance for daily clinical practice.

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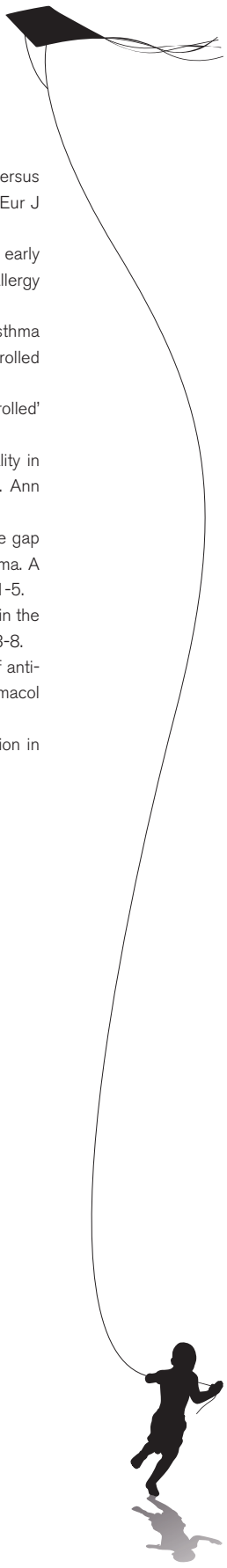
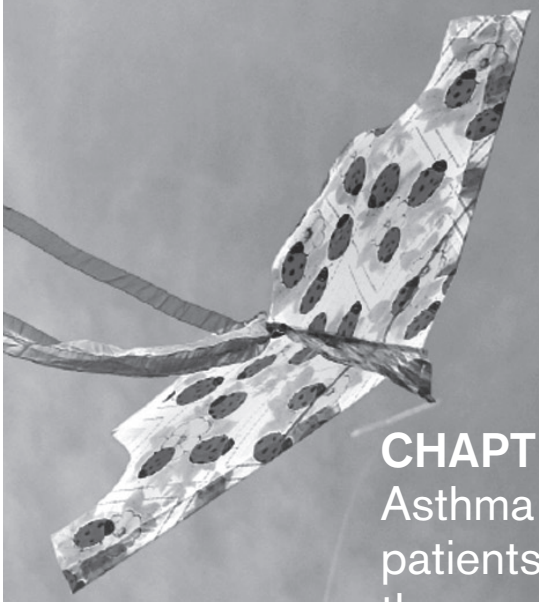


APPENDIX

Table 1. Description of study population (long-term asthma control previous year)

	Controlled n = 130	Partly controlled n = 172	Uncontrolled n = 209
General characteristics			
Gender, male	63.9	58.7	59.8
Age, mean (SD)	8.4 (2.5)	8.4 (2.6)	8.3 (2.4)
Dutch ethnicity	90.3	94.4	85.1
Doctor-diagnosed asthma	85.0	73.9	72.4
Medication use			
OCS	3.1	6.4	12.9*
SABA	76.9	85.5	89.5*
LABA	22.3	29.7	24.9
LTRA	2.3	8.1	12.4 **
AB	27.1	33.9	47.8*
Good adherence	61.5	48.7	59.8*
Good inhalation technique	86.9	82.0	82.3
Environmental factors			
Smoke exposure	11.5	7.0	6.7
Pet exposure	38.8	43.6	37.5
Urban environment	64.3	59.7	62.5
Current asthma control, ACQ			
Well-controlled	75.6	57.2	36.7*
Partially controlled	19.7	27.1	26.1*
Not well-controlled	4.7	15.7	37.2*
Mean (SD)	1.3 (0.6)	1.6 (0.8)	2.1 (0.8)*

Note: For the definition of long-term asthma control phenotypes (asthma control in previous year), data were available for 97.0% of our study population (n=511). OCS = oral corticosteroids, SABA = short-acting beta-agonist, LABA = long-acting beta-agonist, LTRA = leukotriene antagonist, AB = antibiotics, ACQ = asthma control questionnaire. * $P < 0.05$, ** $P < 0.10$



CHAPTER 9
Asthma symptoms in paediatric patients: differences throughout the seasons

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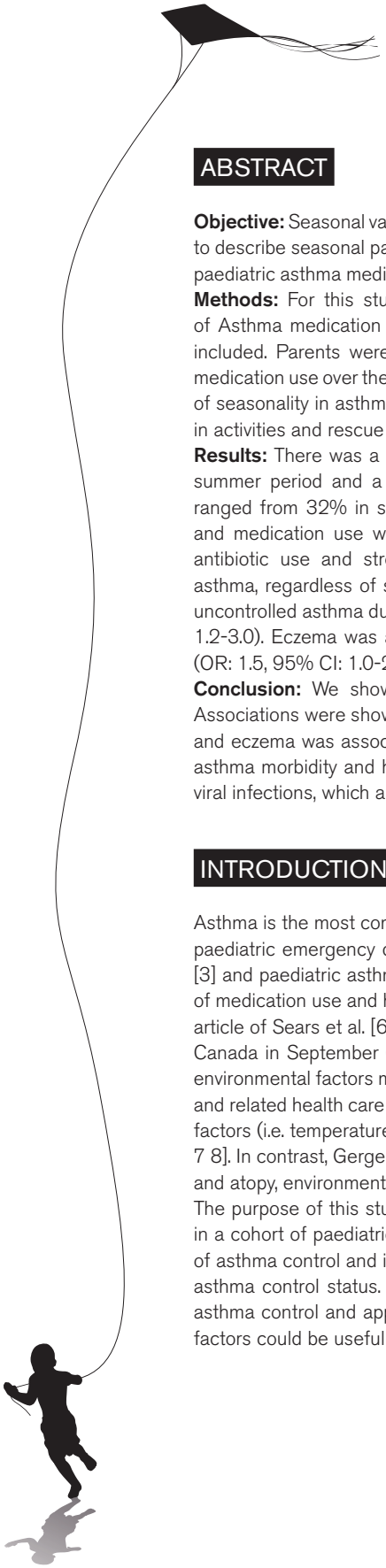


ABSTRACT

Objective: Seasonal variation in asthma has been widely recognized. The aim of this study was to describe seasonal patterns of asthma symptoms and asthma medication use in a cohort of paediatric asthma medication users and to study determinants of seasonal childhood asthma.
Methods: For this study, 602 children participating in the PACMAN (Pharmacogenetics of Asthma medication in Children: Medication with Anti-inflammatory effects)-cohort were included. Parents were asked about their child's respiratory symptoms and quick reliever medication use over the past year. Logistic regression analysis was used to study determinants of seasonality in asthma control (the level of disease control based on symptoms, limitations in activities and rescue medication use).
Results: There was a decline in asthma symptoms and asthma medication use during the summer period and a peak occurred from autumn to spring. The prevalence of wheeze ranged from 32% in summer to 56% in autumn. The prevalence of respiratory symptoms and medication use was significantly lower during summer ($p<0.0001$). Oral steroid and antibiotic use and strong parental necessity beliefs were associated with uncontrolled asthma, regardless of seasonality. Allergic rhinitis was associated with an increased risk of uncontrolled asthma during spring (OR: 1.9, 95% CI: 1.3-2.8) and summer (OR: 1.9, 95% CI: 1.2-3.0). Eczema was associated with a higher risk of uncontrolled asthma during autumn (OR: 1.5, 95% CI: 1.0-2.2) and winter (OR: 1.3, 95 %CI: 1.0-1.9).
Conclusion: We showed seasonal patterns in asthma symptoms and medication use. Associations were shown between allergic rhinitis and asthma control during spring/summer and eczema was associated with uncontrolled asthma during autumn/winter. Seasonality in asthma morbidity and health care use is most likely associated with atopic constitution and viral infections, which are common during fall, winter and spring.

INTRODUCTION

Asthma is the most common chronic disorder in childhood and one of the leading causes for paediatric emergency department visits [1 2]. Seasonal patterns in asthma medication use [3] and paediatric asthma hospital admissions have been described previously [4]. A decline of medication use and hospital admissions was found during the summer period [5]. A review article of Sears et al. [6], described a peak in hospitalization for paediatric asthma patients in Canada in September (during the period 1990-2004). It has been suggested that variable environmental factors might account for the observed seasonal patterns in asthma symptoms and related health care utilization. Previous studies have shown associations between climate factors (i.e. temperature, humidity, rainfall, barometric pressure) and asthma exacerbations [4 7 8]. In contrast, Gergen et al. found no correlation between the seasonal patterns of asthma and atopy, environmental tobacco smoke exposure and air pollutants [9].
The purpose of this study was to describe possible seasonal patterns of asthma symptoms in a cohort of paediatric asthma medication users. In addition, we investigated determinants of asthma control and investigated whether there were seasonal differences in predictors of asthma control status. This might be of interest for the treating physician when assessing asthma control and appropriate treatment strategies. Better understanding of seasonal risk factors could be useful for lifestyle changes and prevention of asthma (exacerbations).

METHODS

Study population and setting

Recruitment of the PACMAN-cohort started in April 2009 by means of selecting paediatric asthma medication users from community pharmacies. Details of this study protocol have been described elsewhere [10]. Briefly, children aged 4-12 years who are regular users (≥ 3 prescriptions within the last 2 years and ≥ 1 prescription in the last 6 months) of asthma medication (Anatomical Therapeutical Chemical (ATC) code R03) were selected from pharmacies in The Netherlands. Selected children and their parents were invited for a visit to their own community pharmacy. Recruitment for the PACMAN-cohort took place throughout the year. Nevertheless, most children were included in spring (46%) and autumn (30%) as a result of holiday seasons (summer and winter). Only 7% of the population was included during summer and 17% was included during winter. The PACMAN study has been approved by the Medical Ethics Committee of the University Medical Centre Utrecht.

During the pharmacy visit, parents filled in a questionnaire to collect information regarding general health, asthma and respiratory symptoms, medication use, adherence, parent's beliefs about medicines, environmental and socio-demographic factors. Furthermore, the child's inhalation technique was scored with an inhaler specific inhalation control checklist [10].

Seasonal differences in asthma symptoms and medication use

Parents were asked about their child's airway symptoms over the past 12 months. The questionnaire completed during the pharmacy visit contained questions regarding the presence of asthma symptoms, limitations in activities, sleep disturbances and extra medication use during the different seasons in the past 12 months (For example: "Has your child had tightness of the chest or shortness of breath during summer (June - August)?". In addition, a question on the frequency of the described symptoms during the different seasons was added: "How often did your child experience these symptoms during spring/summer/autumn/winter?" (Answers: daily, weekly, monthly or rarely symptoms). The prevalence of asthma symptoms (wheeze, cough and shortness of breath), sleep disturbances, limitations in daily activities and rescue medication use was compared for the four seasons.

Definition of asthma control

Asthma control was defined based on the international Global Initiative for Asthma (GINA) guidelines [11]. First, asthma control was defined based on the presence of symptoms in a specific season. Uncontrolled asthma was defined as: ≥ 3 of the following items present in a specific season: (1) day-time asthma symptoms, (2) night-time asthma symptoms, (3) limitations in daily activities and (4) rescue medication use. Subsequently, this initial definition of asthma control was adjusted for the frequency of symptoms during a season. Children with frequent symptoms (daily or weekly symptoms during a season) were classified as uncontrolled, children with monthly symptoms were classified as partially controlled and children who experienced rarely symptoms (less than once a month) during a season were classified as well-controlled.

Determinants of uncontrolled asthma throughout the seasons

We studied the influence of child characteristics (gender (male vs. female), ethnicity (Dutch (non-immigrant) vs. non-Dutch (immigrant)), presence of eczema and allergic rhinitis (yes/no)), medication use (adherence (good vs. poor), inhalation technique (good vs. poor), parental perception towards medication use, course of antibiotics and oral steroids during past year

(yes/no)) and environmental factors (environmental tobacco smoke exposure (yes/no), pet exposure (yes/no) and living environment (urban vs. rural environment)) on asthma control. Parental reported adherence was assessed by using the Medication Adherence Report Scale (MARS) comprising five questions on medication use behaviour [12]. The MARS was dichotomised using a cut-off point for the sum-score: a score of <21 was considered as poor adherence [13 14]. Inhalation technique was scored using a validated questionnaire developed by van der Palen et al. [15]. Parental perception towards medication use, including beliefs about medication (both parental necessity beliefs and concerns about their child's medication use) and knowledge about asthma medication. Beliefs about medicines were assessed using the validated Beliefs about Medicines Questionnaire (BMQ) questionnaire. This questionnaire comprises two scales with each five questions: the necessity scale and the concerns scale. All items are measured on a 5-point Likert scale [16]. The questions on necessity beliefs focus on the need for medication use to maintain present and future health. The questions on concerns focus on concerns about potential adverse consequences of medication use. Higher scores on the specific scales indicate stronger need beliefs or concerns. BMQ scores greater than scale midpoint (>15) were considered as stronger beliefs [14].

Statistical analysis

Chi-square testing was used to compare frequency data. Logistic regression analysis was used to study determinants of uncontrolled asthma. Odds ratios (OR) and their corresponding 95% confidence intervals (CI) were calculated. Univariate analyses were performed to study individual determinants of uncontrolled asthma and second, multivariate analysis was performed to assess independence of the associated factors. Only variables associated with uncontrolled asthma ($p < 0.10$) in the univariate analyses were included in the multivariable analysis. The inclusion of potential confounders in the logistic regression model was based on the assessment of the influence of each potential confounder on the OR for the association between the univariate associated factors and uncontrolled asthma. The child's gender, ethnicity, parental believes about medicines, exposure to pets and environmental tobacco smoke and living environment were all considered potential confounding factors. Age was included as covariate in the model. Potential confounding factors were included in the multivariate model if they induced a 10% change or more in the crude regression coefficient for the determinant of interest [17]. All analyses were performed using SAS version 9.1 (SAS Institute, Cary, NC).

RESULTS

Study population

Until February 2011, 2114 children were selected from 67 Dutch pharmacies. For, 595 (28.1%) of these children there was no contact telephone number registered in the pharmacy, nor could a telephone number be traced through Internet or a national telephone number service. Another 393 (18.6%) parents, whose telephone numbers were known, could not be reached after at least five phone calls during office hours. Therefore, in total, 1126 parents could be invited by telephone and of these, 684 parents (60.7%) agreed to participate. No data was obtained in 82 (12.0%) children due to various reasons (e.g. child was unwilling to participate, parents/children did not show up at the scheduled appointment, or the parent/children were willing to participate, though unable to come to the pharmacy in the period of patient inclusion). As a result, 602 children were included in the study indicating a response

rate of 53.5%. The general characteristics of this population are shown in Table 1: 62% of the participants were males and the mean age was 8.3 years. Almost 75% of the children had a doctor's diagnosis of asthma.

Table 1. Characteristics of study population

	Study population (n = 602)
General characteristics	
Male gender, %	61.6
Age, mean (SD)	8.3 ± 2.5
Caucasian ethnicity, %	87.4
Clinical characteristics	
Allergic rhinitis [a], %	46.1
Eczema [a], %	64.9
Food allergy [a], %	51.2
Doctor-diagnosed asthma, %	74.4
Medication use past 12 months	
SABA	82.7
ICS	87.5
Antibiotics	36.0
Oral steroids	7.0

[a] Based on parental reporting in the questionnaire. SABA = short-acting beta-agonist, ICS = inhaled corticosteroids

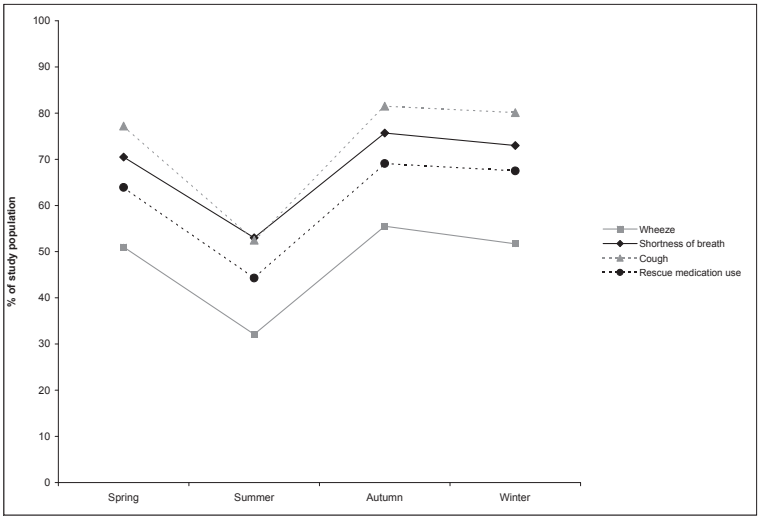
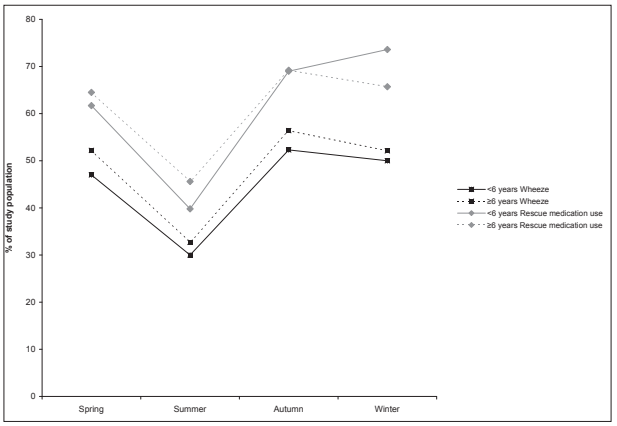


Figure 1. Overall seasonality of asthma symptoms and medication use



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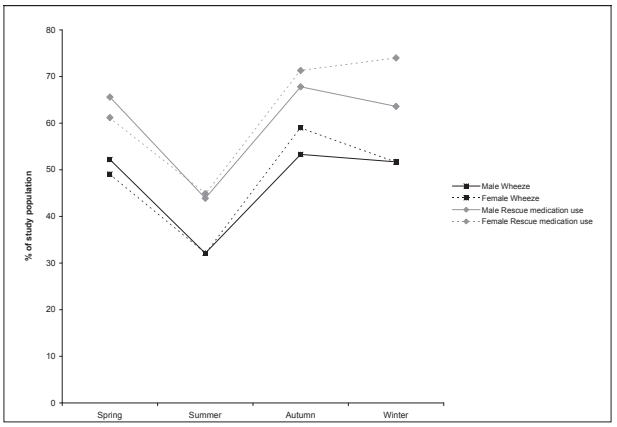

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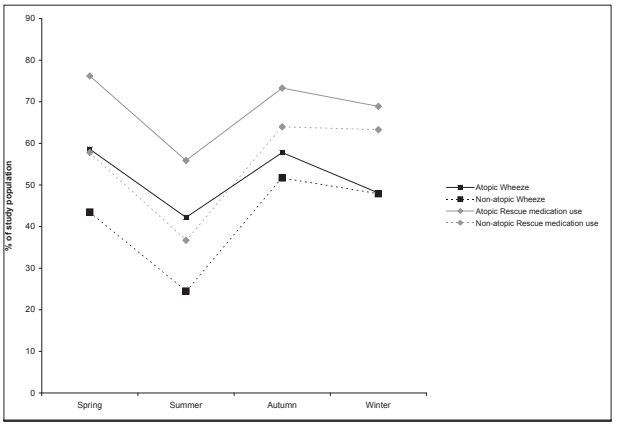

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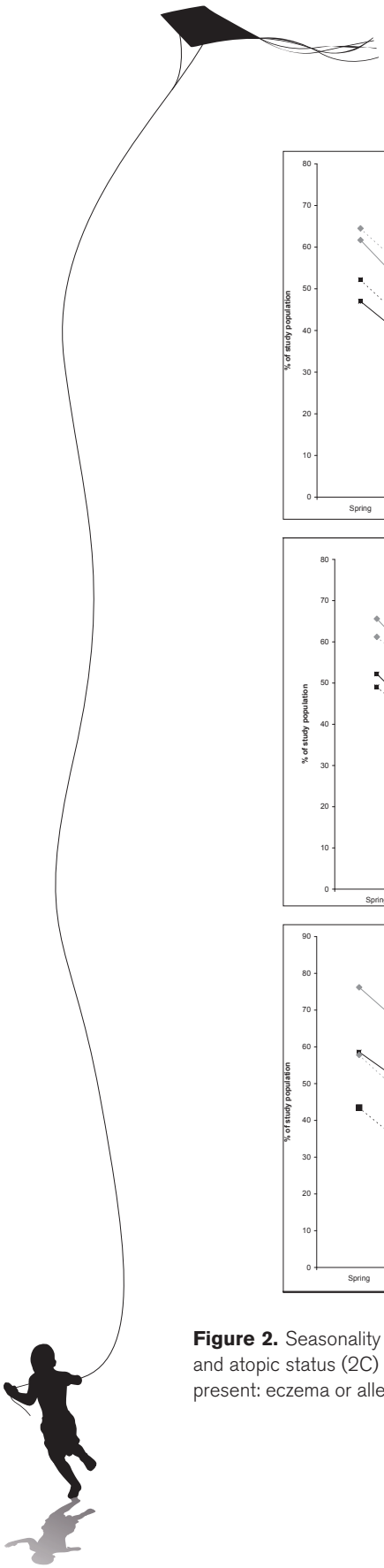


Figure 2. Seasonality of asthma symptoms and medication use by age (2A), gender (2B) and atopic status (2C) [a] A child was considered atopic if one of the following conditions was present: eczema or allergic rhinitis.

Seasonal differences in asthma symptoms and medication use

Figure 1 shows the prevalence of reported asthma symptoms and reliever medication use for the entire population over the past year (during the 12 months preceding the pharmacy visit). There was a decline in asthma symptoms (cough, shortness of breath and wheeze) and rescue medication use during the summer period and a peak occurred from autumn to

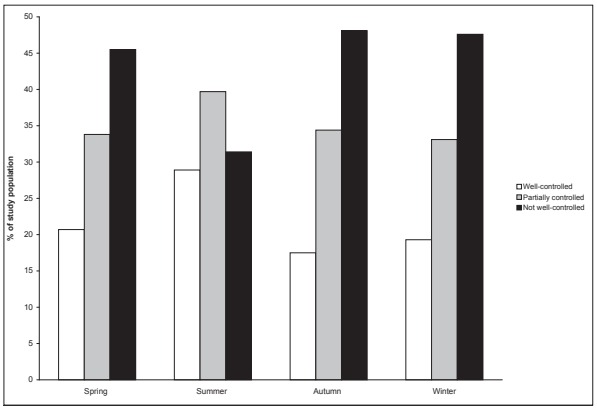


Figure 3. Seasonality of asthma control

Table 2. Determinants of uncontrolled asthma (univariate analysis)

	Spring OR (95% CI)	Summer OR (95% CI)	Autumn OR (95% CI)	Winter OR (95% CI)
Child characteristics				
Male gender	1.1 (0.7 - 1.5)	1.1 (0.7 - 1.7)	0.7 (0.5 - 1.0)	0.7 (0.5 - 1.0)
Dutch ethnicity (non-immigrant)	0.5 (0.3 – 1.0)	0.5 (0.3 - 1.1)	0.9 (0.5 - 1.6)	0.6 (0.3 - 1.1)
Eczema [a]	1.5 (1.1 – 2.2)	1.4 (1.0- 2.3)	1.7 (1.1 – 2.4)	1.4 (1.0 – 2.0)
Allergic rhinitis [a]	2.0 (1.4 – 2.8)	2.0 (1.3 – 3.1)	1.1 (0.8 - 1.6)	1.3 (0.9 - 1.9)
Medication use				
Good adherence	1.0 (0.7 - 1.4)	1.2 (0.8 – 1.8)	1.4 (1.0 – 2.0)	1.2 (0.8 - 1.7)
Poor inhalation technique	0.8 (0.5 - 1.2)	1.0 (0.6 - 1.8)	1.1 (0.7 - 1.7)	0.9 (0.6 - 1.4)
Strong need beliefs [a]	1.9 (1.3 – 2.7)	1.5 (1.0 - 2.3)	2.0 (1.4 – 2.8)	2.0 (1.4 – 2.9)
Strong concern beliefs [a]	1.5 (1.0 – 2.3)	1.3 (0.8 – 2.2)	1.5 (1.0 – 2.3)	1.8 (1.2 – 2.8)
Antibiotics	2.0 (1.4 – 2.9)	1.8 (1.2 – 2.9)	1.9 (1.3 – 2.7)	2.7 (1.9 – 3.9)
Oral steroids	3.7 (1.8 – 7.7)	3.6 (1.7 – 7.5)	4.5 (2.0 – 9.9)	2.2 (1.1 - 4.5)
Environmental factors				
Environmental tobacco smoke exposure	1.0 (0.6 – 2.0)	1.5 (0.7 – 3.2)	0.5 (0.3 – 1.0)	0.8 (0.4 - 1.6)
Pets	1.0 (0.7 - 1.4)	1.0 (0.7 - 1.6)	1.2 (0.9 - 1.7)	1.3 (0.9 - 1.9)
Urban environment	0.9 (0.6 - 1.2)	1.5 (0.7 - 3.2)	1.0 (0.7 - 1.4)	1.5 (1.0 – 2.1)

[a] Based on parental reporting in the questionnaire

spring. The prevalence of respiratory symptoms and rescue medication use was significantly lower during the summer period compared to the other seasons ($p < 0.0001$). Cough was the most frequent reported respiratory symptom with a prevalence ranging from 53% in summer to 82% during autumn. Wheeze, more specific for asthma, was reported for 32% in summer and ranged to 56% during autumn. Seasonal variation in wheeze and asthma medication use was evident across both age groups (children aged under six years and children aged six years and older) (Figure 2A). Figure 2B shows that seasonal variation in both genders is evident and nearly identical. For atopic children, wheeze and use of rescue medication was significantly higher ($p < 0.05$) during spring and summer (Figure 2C).

Table 3. Determinants of uncontrolled asthma (uncontrolled vs. controlled): multivariate

	Spring OR (95% CI)	Summer OR (95% CI)	Autumn OR (95% CI)	Winter OR (95% CI)
Child characteristics				
Male gender	-	-	0.7 (0.5 - 1.1)	0.7 (0.5 - 1.0)
Dutch ethnicity (non-immigrant)	0.7 (0.4 - 1.3)	-	-	-
Eczema [a]	1.2 (0.8 - 1.8)	1.1 (0.7- 1.9)	1.5 (1.0 – 2.1)*	1.3 (1.0 - 1.9)*
Allergic rhinitis [a]	1.9 (1.3 – 2.8)*	1.9 (1.2 – 3.0)*	-	-
Medication use				
Good adherence	-	-	-	-
Poor inhalation technique	-	-	-	-
Strong need beliefs [a]	1.7 (1.1 - 2.5)*	1.1 (0.7 - 1.9)	1.8 (1.3 - 2.6)*	1.8 (1.2 – 2.6)*
Strong concern beliefs [a]	1.1 (0.7 - 1.7)	-	1.4 (0.9 – 2.1)	1.4 (0.9 – 2.2)
Antibiotics	2.2 (1.4 – 3.3)*	1.8 (1.1 – 3.1)*	1.8 (1.2 - 2.6)*	2.8 (1.9 - 4.0)*
Oral steroids	2.8 (1.3 - 6.1)*	3.4 (1.5 – 7.9)*	4.4 (2.0 – 9.9)*	1.6 (0.8 – 3.3)
Environmental factors				
Passive smoking	-	-	0.4 (0.2 - 1.1)	-
Pets	-	-	-	-
Urban environment	-	-	-	1.5 (1.0 – 2.1)

[a] Based on parental reporting in the questionnaire, * $p < 0.05$

Determinants of uncontrolled asthma

Figure 3 shows asthma control during the seasons. Uncontrolled asthma rates are lowest in summer (32%) and highest in winter (49%).

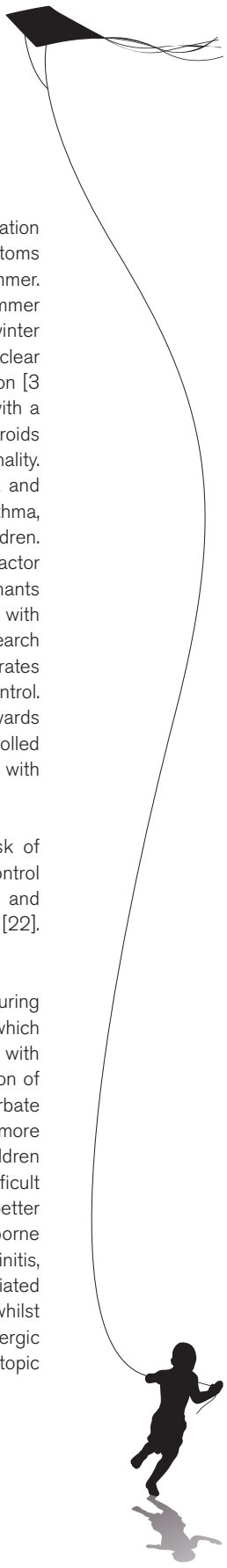
The univariate and multivariate associations between potential determinants and uncontrolled asthma in the different seasons are shown in Tables 2 and 3. Both oral steroid use and antibiotics during the past year use were associated with increased risk of uncontrolled asthma in all seasons. The same was true for parental necessity beliefs towards their child's medication use. Allergic rhinitis was associated with an increased risk of uncontrolled asthma during spring (OR: 1.9, 95% CI: 1.3-2.8) and summer (OR: 1.9, 95% CI: 1.2-3.0). Eczema was associated with a higher risk of uncontrolled asthma during autumn (OR: 1.5, 95% CI: 1.0-2.2) and winter (OR: 1.3, 95% CI: 1.0-1.9).

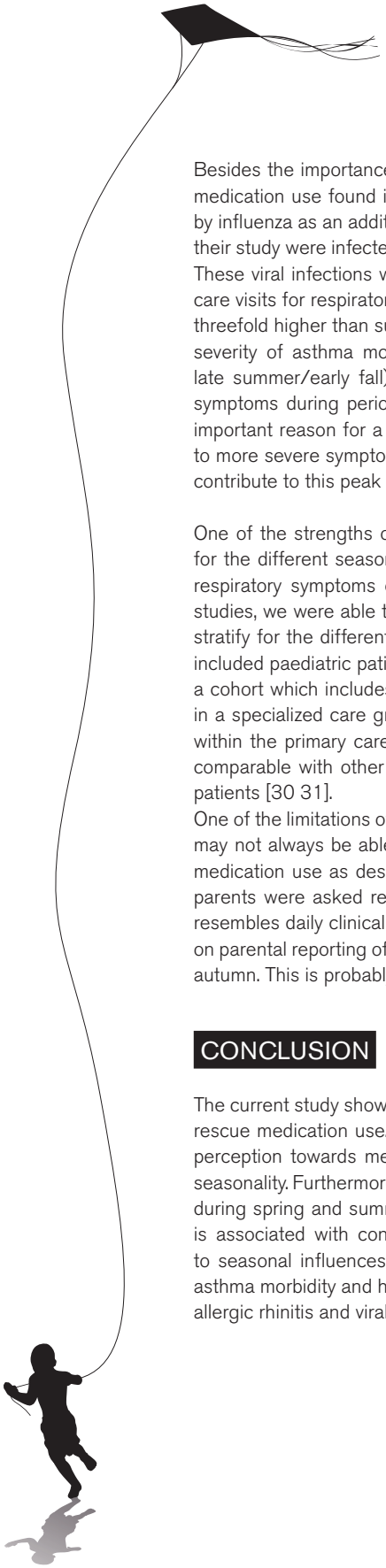
DISCUSSION

We showed the existence of seasonal variation in respiratory symptoms and medication use in a cohort of pediatric asthma medication users. The prevalence of asthma symptoms (wheeze, shortness of breath and cough) and rescue medication use was lowest in summer. Uncontrolled asthma was present in approximately one third of the children during summer (31%), compared to up to half of the population in spring (46%), autumn (48%) and winter period (48%). This is in line with other studies reporting a summer improvement and a clear pattern of fall-winter worsening for asthma symptoms and related health care utilization [3 6 9]. Strong parental beliefs towards the need for medication use were associated with a higher risk of uncontrolled asthma in all seasons except summer. Antibiotics and oral steroids use were also associated with uncontrolled asthma symptoms, regardless of seasonality. During spring and summer, allergic rhinitis was associated with uncontrolled asthma and during autumn and winter eczema was associated with a higher risk of uncontrolled asthma, suggesting that atopic constitution plays an important role in asthma control in these children. We have shown that parental perception towards medication use is a very important factor in pediatric asthma control. Within the PIAMA cohort we have also studied determinants of uncontrolled asthma and showed parental necessity beliefs to be associated with uncontrolled asthma [18]. This finding was against our expectation, as previous research has shown an association between strong necessity beliefs and higher adherence rates for asthma medication [19] which is in turn expected to lead to improved asthma control. However, also in the present study, regardless of seasonality, strong parental beliefs towards the necessity of their child's medication use were associated with higher risk of uncontrolled asthma. Therefore, we believe that these strong need beliefs may reflect a population with more severe asthma.

Children with reported oral steroid use and antibiotic use were at an increased risk of uncontrolled asthma. Oral steroid use has been associated with inadequate asthma control [20]. Oral steroids are prescribed for treatment of severe exacerbations [11 21 22] and antibiotics are used in the treatment of respiratory symptoms such as acute coughing [22]. Use of these drugs may reflect more severe, uncontrolled disease.

We found allergic rhinitis to be associated with a higher risk of uncontrolled asthma during spring and summer period. During these months, exposure to grass pollen is highest which may trigger the allergic reaction observed in allergic rhinitis. The majority of children with asthma also have allergic rhinitis [23]. Both conditions are characterized by inflammation of the respiratory mucosa and due to their mechanistic intertwine; one condition can exacerbate the other [24]. It has been shown that asthmatic children with allergic rhinitis have more hospitalizations and more frequent emergency departments visits compared to children without allergic rhinitis [25]. Allergic rhinitis might therefore be a marker of more difficult to control asthma and poorer asthma outcomes, treating allergic rhinitis may result in better asthma outcomes [26]. Furthermore, Erbas et al. [27] showed that high levels of airborne grass pollen, which may cause the allergic reaction observed in patients with allergic rhinitis, influenced hospital admissions for asthma. Eczema, also an allergic disorder, was associated with uncontrolled asthma during autumn and winter. Rhinitis is more common in spring, whilst eczema exacerbations are more common in fall and winter. This finding, together with allergic rhinitis associations with uncontrolled asthma during spring/summer, suggests that an atopic constitution plays an important role in asthma control.





Besides the importance of atopy, the seasonal pattern of respiratory symptoms and asthma medication use found in the present study may also be attributed to viral infections caused by influenza as an additional factor. Olenec et al. [28] showed that most asthmatic children in their study were infected with viruses during the peak common cold seasons (spring and fall). These viral infections were associated with loss of asthma control. It is known that primary care visits for respiratory disorders are primarily driven by viral circulations. Winter visits were threefold higher than summer visits as shown in the study of Moineddin et al. [29]. Increased severity of asthma morbidity may be primarily driven by respiratory viruses (rhinovirus in late summer/early fall) and factors related to holiday season such as undertreatment of symptoms during periods with lack of daily routine. Furthermore, school return may be an important reason for a peak after summer: school return may induce stress which can lead to more severe symptoms and re-exposure to viral infections in the school environment may contribute to this peak [6].

One of the strengths of our study was the availability of information on asthma symptoms for the different seasons over the past year. Many observational studies only have data on respiratory symptoms concerning the past month or the past week. In contrast to these studies, we were able to study the presence of asthma symptoms over the past year and to stratify for the different seasons. Another strength of the PACMAN cohort study is that we included paediatric patients in the primary care setting. This has the advantage that we have a cohort which includes a very broad range of asthmatics and not only focuses on children in a specialized care group (lung physician/paediatrician) as most children are also treated within the primary care setting. Furthermore, the response rate in our study was high and comparable with other Dutch cohort studies including a physical examination in paediatric patients [30 31].
One of the limitations of our study was that symptoms were based on parental report. Parents may not always be able to give an accurate estimate of their child's asthma symptoms and medication use as described by Dell et al. [32]. Recall bias might have been a problem as parents were asked retrospectively about their child's respiratory symptoms. However, this resembles daily clinical practice, where asthma management in young children is also based on parental reporting of symptoms. Furthermore, most children were included during spring or autumn. This is probably due to the holiday seasons in both summer and winter.

CONCLUSION

The current study showed seasonal patterns in both the prevalence of asthma symptoms and rescue medication use. We have found uncontrolled asthma to be associated with parental perception towards medication use and use of oral steroids and antibiotics, regardless of seasonality. Furthermore, we showed associations between allergic rhinitis and asthma control during spring and summer and eczema during autumn and winter. Thus, atopic constitution is associated with control of asthma symptoms. Atopic children might be more sensitive to seasonal influences, such as increased allergen exposure during spring. Seasonality in asthma morbidity and health care use is most likely associated with exacerbations caused by allergic rhinitis and viral infections, which are common during fall, winter and spring.

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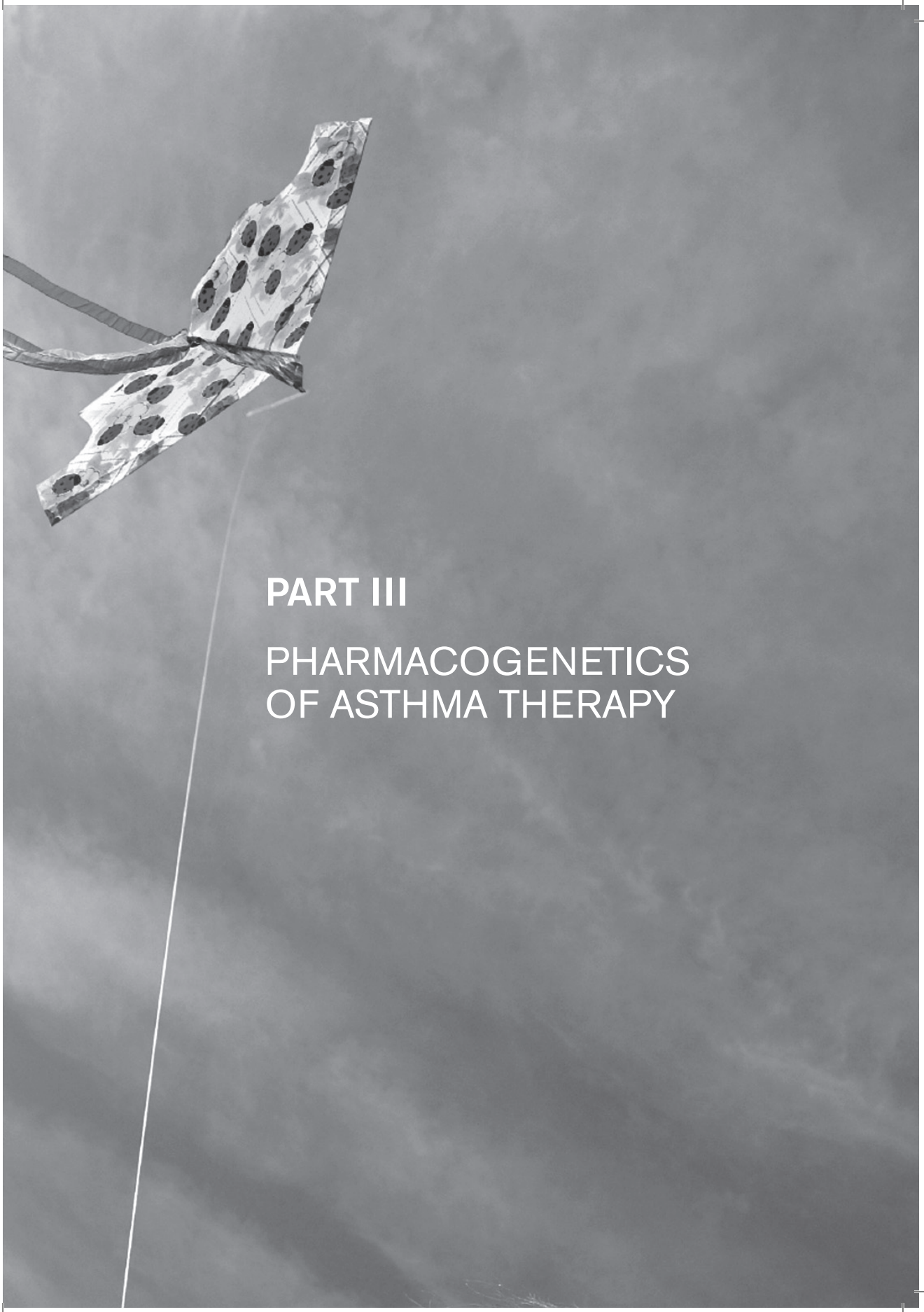

PART III

PHARMACOGENETICS OF ASTHMA THERAPY

CHAPTER 10
Systems biology in pharmacogenomic research: the way to personalized prescribing?

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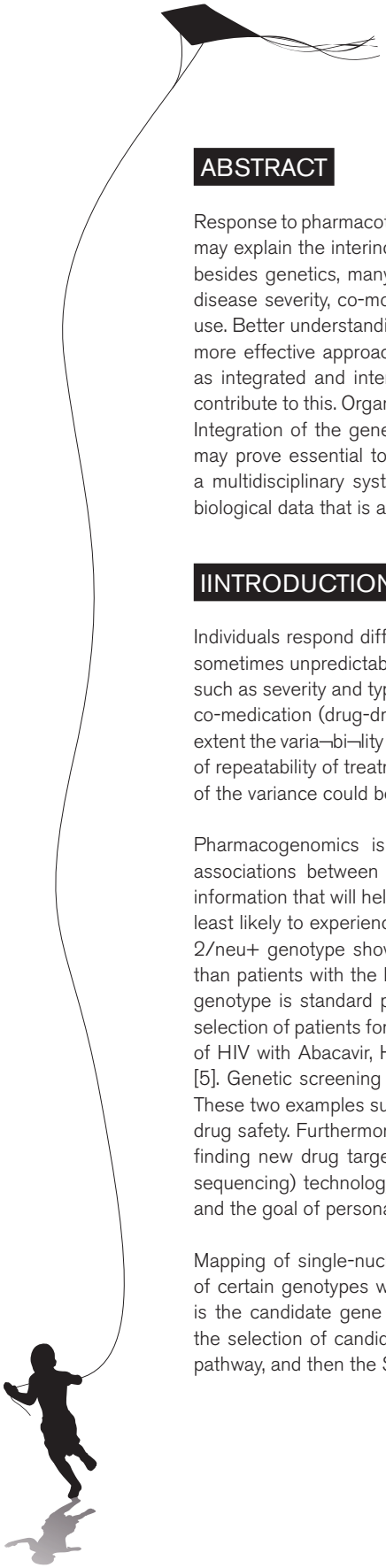
Pharmacogenomics 2009;10(6):971-81



ABSTRACT

Response to pharmacotherapy can be highly variable amongst individuals. Pharmacogenomics may explain the interindividual variability in drug response due to genetic variation. However, besides genetics, many other factors can play a role in the response to pharmacotherapy; disease severity, co-morbidity, environmental factors, therapy adherence and co-medication use. Better understanding of these factors and inter-relationships should bring about a much more effective approach to disease management. Systems biology, that studies organisms as integrated and interacting networks of genes, proteins and biochemical reactions, can contribute to this. Organisms are no longer studied part by part, but in a more integral manner. Integration of the genetic data with intermediate and endpoint phenotypic characterization may prove essential to define the inherent nature of drug effects. Therefore, in the future a multidisciplinary system-based approach will be necessary to deal with the bulk of the biological data that is available and, ultimately, to reach the goal of personalised prescribing.

IINTRODUCTION

Individuals respond differently to pharmacological therapy and effects of drug treatment are sometimes unpredictable [1-3]. Variability in treatment response may be due to many factors, such as severity and type of disease (phenotype), age, compliance with therapy, co-morbidity, co-medication (drug-drug interactions), and other environmental factors (e.g. diet). To which extent the varia–bi–lity is genetic in each particular case is not clear yet. However, calculations of repeatability of treatment response amongst individuals suggest that a substantial fraction of the variance could be genetic [2].

Pharmacogenomics is an emerging research field that offers the opportunity to find associations between genetic variability and response to a variety of drugs. It provides information that will help to administer therapies to patients who can benefit most or to those least likely to experience adverse effects. For example, breast cancer patients with the Her-2/neu+ genotype show more effect of Trastuzumab (also known as Herceptin) treatment than patients with the Her-2/neu- genotype. In the United States, screening for Her-2/neu genotype is standard practice in newly diagnosed breast cancer patients [4]. Furthermore, selection of patients for certain drug therapies could lead to less adverse effects: in treatment of HIV with Abacavir, HLA-B*5701 expression is associated with hypersensitivity reactions [5]. Genetic screening before start of Abacavir therapy is used to prevent these reactions. These two examples support the role for personalized medicine to improve drug efficacy and drug safety. Furthermore, pharmacogenomics may help in the development of new drugs by finding new drug targets. Despite relatively cheap and easy genotyping (and, increasingly, sequencing) technology, pharmacogenomics is still relatively uncommon in clinical practice and the goal of personalised prescribing is far from being reached.

Mapping of single-nucleotide polymorphisms (SNPs) can be used to study the correlation of certain genotypes with the efficacy of drug therapy [6, 7]. The classic way to study this is the candidate gene approach. If a candidate gene approach is chosen the first step is the selection of candidate genes. Genes of interest can be selected relevant to a specific pathway, and then the SNPs in these genes of interest should be selected.

There is a growing list of SNPs in genes encoding drug targets, transporters, enzymes and disease related genes that have been related to drug response [8-10]. Most of these response traits on this list are monogenic and highly penetrant, with a clear association between genotype and phenotype. For the majority of the drug response phenotypes, however, this is not the case and it seems that multiple genes are involved. Therefore, we are currently moving from the single gene to the multiple gene systems. A good example of this trend is the application of genome-wide approaches (GWA) or large multi-candidate gene studies. A major advantage of GWA is that it allows identification of genes (and non-gene SNPs) not previously known to be important in drug response. However, there is also a disadvantage: the large amount of SNPs (500000 and more) makes it impossible to study epistatic interactions using traditional statistical techniques.

There are at least three general ways in which genetic variation may lead to variability in treatment response [11]. First, genetic variants can be associated with altered uptake or metabolism of the drug (pharmacokinetics). Especially, variation in enzymes involved in the uptake, catabolism or excretion of a drug can alter treatment response. One very important example is the highly genetically diverse cytochrome P-450 system, known for its many pharmacogenetic effects. The cytochrome P450 enzymes are involved in the oxidative metabolism of drugs and play a major role in their activation or elimination. Genetic variation in these enzymes results in altered drug responses [12]. Second, genetic variation can result in actions of a drug outside its therapeutic indication (idiosyncratic). This is variance among individuals that can lead to adverse effects that are not based on the drug's primary therapeutic indication. Drug induced liver injury (DILI) is such an idiosyncratic adverse drug reaction. Most drugs responsible for severe DILI are not predictable hepatotoxic, they are completely safe over a wide range of doses for the majority of patients, however, in a small subset of patients they are severely toxic. Several polymorphisms in genes in the major histocompatibility locus and genes that encode drug metabolizing enzymes have been associated with susceptibility to DILI [13]. And third, genetic variation in the drug target or components of the drug pathway can result in altered drug efficacy (pharmacodynamics). These are polymorphisms in receptors for drugs or other proteins potentially modifying the drug's actions. One well described example, is variation in response to treatment with β-agonists in asthma due to genetic variation in the adrenergic β2-receptor [14, 15]. A great amount of the currently available pharmacogenomic data falls into this last category, in which individuals are categorized as responders or non-responders to pharmacotherapy and analysis of specific genetic variants is used to distinguish these groups.

CHALLENGES IN PHARMACOGENOMIC RESEARCH

One of the main problems in pharmacogenomic research is the fact that many described drug-gene interactions have not been replicated in other populations [1]. This is in part probably due to the fact that for many drugs not one gene but many genes contribute to the variation in the efficacy or side effects of drug therapies [16]. Downstream of those genes, many proteins and metabolites are also involved in a specific drug or disease pathway. And in addition to this, in common diseases with heterogeneous phenotypes, such as asthma and cardiovascular diseases, many genes are involved in both the drug response and the disease pathways [17-19]. All these factors together make it difficult to find causal variants. Therefore, it is very

important to get deeper insight in the overall mechanisms of action. If the involved biological pathways are uncovered, multiple genes involved in these pathways can be considered. This can be done with the help of different techniques: as such, text mining approaches [20-22] can be used to select (candidate) genes, proteins, enzymes or metabolites that are described to be important in a pathway. Furthermore, proteomics and mRNA experiments can help in the search for the genes that play a role in drug response pathways. By profiling gene expression patterns across diverse conditions we might gain insight in biological function. Despite the differences in platforms (mRNA, protein expression, SNPs) they all share the same underlying process; each consists of unique positions at which there are distinct probes representing individual genes [23]. Only by generating results using different techniques and by reconciling and integrating these results we will be able to overcome the current challenges in pharmacogenomic research.

In many examples, there is only a modest clinical effect with respect to genotype. This could also be a reason for lack of consensus between different studies. To detect small clinical effects, large time-consuming clinical trials have to be carried out. Surrogate and intermediate phenotypes, such as minimal residual disease or pharmacodynamic endpoints, may result in the earlier data acquisition (compared to waiting for the conclusion of large clinical trials) and allow us to focus on the single drugs within complex treatment strategies [24]. On the other hand, the use of surrogate clinical endpoints could add to the lack of consensus between different studies because of different definition and interpretation of outcomes.

Another increasing challenge in pharmacogenomic research is data analysis. Interpretation of pharmacogenomic data increases in its complexity when more than one gene or non-genetic contributors to variable treatment response are considered, and traditional statistical methods are not well suited for the analysis and interpretation of these higher-order relationships and interactions [25, 26]. The possible model/hypothesis space grows exponentially, and potentially important gene-gene interactions can be easily overlooked. In pharmacogenetic research there is yet an extra challenge because not only the relationships between genes and phenotypes are relevant, our primary goal is actually the higher-order gene-drug interaction term per se!

INTRODUCTION TO SYSTEMS BIOLOGY

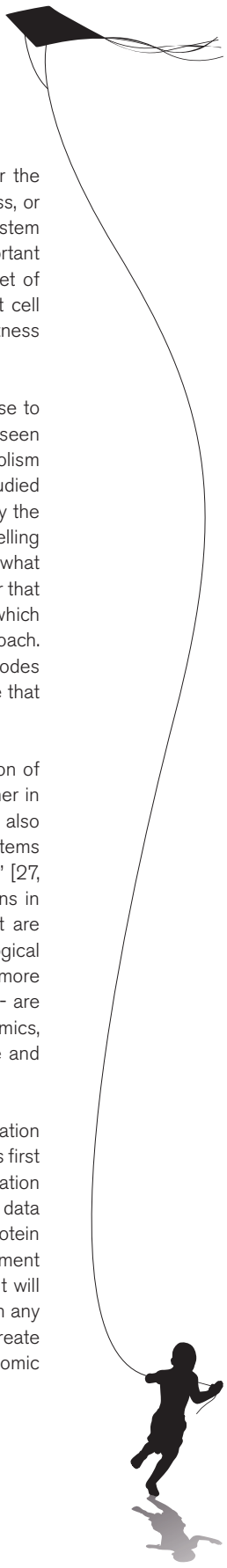
When we put together three things --- a small metal cup, a glass bowl in the shape of a small balloon and a wire pinched up into a small coil --- we have a light bulb. On their own, the individual parts are rather pointless in terms of providing light. However, if we put them all together we have a light bulb that spreads light [101]. This light bulb is a good example of a system --- three parts relatively useless on their own work together concertedly. The individual parts of the light bulb can be studied one at a time, but this would not reveal to an investigator any information on the properties of the system. Three basic concepts are very important for the understanding of complex biological systems [27]. First, complex systems often display emergent properties: these properties are not demonstrated by their individual parts, and cannot be predicted by the full knowledge of the individual parts. As in the example of the light bulb, the combination of the metal cup sealing the glass bulb and passing the current to the wire coil, of the glass bulb maintaining a vacuum and still allowing light to radiate

out, and of the wire coil glowing hot enough to give off light without melting itself or the glass balloon is what makes a light bulb such an “effective” system. Second, robustness, or phenotypic stability, is an inherent property of all biological systems. This protects the system from fluctuations imposed on it by the environment [27]. Third, modularity is another important characteristic of complex systems. Modules in a specific pathway or network are a set of nodes that have strong interactions (cell-cell interactions or signaling among different cell types) and a common function. Modularity and overlap of functions contribute to robustness of the system [27].

Systems are comprised of individual parts which interact and these interactions give rise to new properties and functions which are the key of the system. Organisms can also be seen as complex systems that function in an integrated manner, our senses, muscles, metabolism and mind work together seamlessly [101]. However, biologists have historically studied organisms part by part, molecule by molecule and gene by gene, thereby observing only the partial components of the biological system. This classical reductionist approach - unravelling complex systems by stabilizing as many factors as possible and alter just one to see what happens - is not very useful in pharmacogenomics, because there is not one single factor that is a major contributor to the variance in treatment response. An alternative approach which could be very rewarding in pharmacogenomic research, is the systems biology approach. Pathways and control systems that influence disease state are studied to identify nodes suitable for intervention. This data is then used to build mathematical models of disease that will require successive iterations between model and experiment to test it [28].

The systems biology approach is relatively new and seeks to understand the integration of pieces from biological systems. It can be defined as a systematic analysis of the manner in which all components of a biological system interact with each other. Systems biology is also referred to as pathway, network or integrative biology. The main question that the systems biology aims to address is ‘How can the phenotype be generated from the genotype?’ [27, 29] Since the objective of systems biology is creating a good model of the interactions in a system, the experimental techniques that most suit systems biology are those that are system-wide and attempt to be as complete as possible. Therefore, all kinds of biological information --- on DNA, RNA, proteins, signaling networks, cells and organs as well as more descriptive data, such as clinical diagnosis and pharmacological treatment response --- are needed [31]. In a nutshell, systems biology involves the integration of genomics, proteomics, and bioinformatics to build computational cellular or organ models of both descriptive and predictive value.

The general principle of a systems biology approach is based on the collection and integration of sets of biological data on as many hierarchical levels as possible [27, 29, 30]. The data is first collected in an extensive database, and is then subjected to various automated visualization and modeling algorithms, because it is impossible to meaningfully interpret thousands of data points manually. Eventually, phenotypic features of the systems are directly linked to protein and gene regulatory networks. Repeated cycles of data acquisition and model refinement will result in a more accurate model. If the model is sufficiently accurate and detailed, it will provide us with two deliverables. First, it is possible to predict behavior of a system given any disturbance in the system. Second, gene regulatory networks can be redesigned to create new systems properties. This second possibility could be very important in pharmacogenomic research for the development of new drugs.



After decades of biological research on molecules, cells, genes and organs many things still remain unclear. Nowadays systems biologists can choose between two approaches to make their computational models. In one, they reduce the number of biological pathways being modeled to a few well known ones, thus creating a model that is based on reliable data, yet not comprehensive. In the second approach, they try to model "everything" by making allowance for unobserved (and possibly unobservable) variables, thus creating a more complete model. It depends largely on the research question whether the latter or the former is to be adopted. If the question is narrow - 'which surface proteins are affected when insulin activates receptors on the pancreas cell' - the former approach might be preferable. But if the question is sufficiently broad - 'what will be the overall effect of a new drug X on disease' - a more detailed, comprehensive approach is necessary and this is almost always the case in the realm of pharmacogenomic research.

DATA ANALYSIS METHODOLOGY IN PHARMACOGENOMICS

As described above, interpretation of pharmacogenomic data is a real challenge, because not only gene-gene interactions and their effect on phenotype are of interest, but it is actually the gene-drug interactions and their association with the phenotype that are of the primary interest in pharmacogenomic research. This only exacerbates the "dimensionality curse" problem intrinsic to modern large-scale genetic epidemiology research.
To deal with this, a three-step data analysis strategy can be used. The first step is variable selection. In it, variables (primarily SNPs, but also gene alleles, protein expression measures and other genetic and environmental factors) are ranked in order of their relevance to the

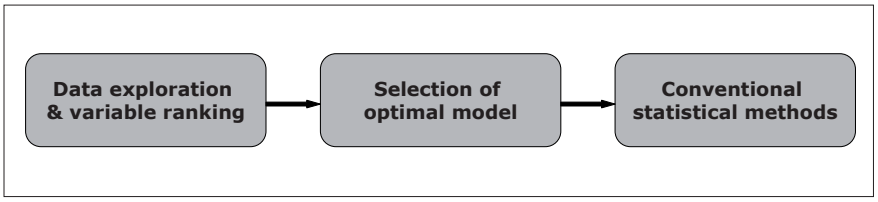


Figure 1. Proposed analysis strategy for a pharmacogenomics study

phenotype (or effect) of interest, and the irrelevant variables are removed from the dataset. This can be followed by the modelling (graphical, descriptive, predictive etc.) step. Finally, the models and their components (such as associations between SNPs or alleles, clinical endpoints, exposure and other relevant factors) can be evaluated using conventional statistical testing and estimation techniques (Figure 1). We should clarify here that in this context model implies all the relevant variables (genetic, environmental, outcomes, metabolites etc.) taken in conjunction with all their interrelationships. We should emphasize here that in addition to SNPs (primarily generated by genetic epidemiology studies), a variety of "-omics" data can be used in this variable selection step; Shankkavaram et al. [31] described how the integrated microarray and protein expression data from cancer cell lines can be used to predict drug responses; Riedel et al. [32] used microarray data to hypothesize pathways involved in drug resistance and Potti et al. [33] combined microarray data with in vitro drug sensitivity data to predict drug responses.

For ranking of variables one could use simple univariate ranking metrics, such as chi-square, information gain, Gini index, student's t-test or analysis of variance. With the use of these statistics, p-values are assigned to the potentially predictive variables on the basis of how well they distinguish the groups of samples. However, these statistic tools suffer from the multiple testing problem: too many variables are tested in too few samples, which makes it difficult to restrict the selection of variables to true positives. A p-value of 0.05 is usually considered to be statistically significant in biological research: however, in an array of 100000 elements 5000 statistically significant genes may be found purely by chance. Therefore, greater stringency is needed for gene selection [34]. To deal with the multiple testing problem there are several approaches [35]; Bonferroni correction adjusts for the manner in which p-values are calculated (the significance cut off is calculated by dividing the cut off p-value for one test by the number of tests). Westphal and Young step down p-value calculation relies on permutation testing for the selection of significance cut off values. Significance Analysis of Microarrays (SAM) uses an adjusted t-statistic along with permutation testing to estimate the False Discovery Rate (FDR) in a gene set [23]. The FDR is the ratio between the number of false positives and the number of positives and widely used in genomics and proteomics studies to deal with the significance analysis problem [36]. The p-values gained by use of any of the above described methods are useful for prioritizing genes for further study --- they provide a (ranked) subset of genes that can be used to search for biological patterns [23].

The described univariate ranking methods are, however, unable to account for non-additive variable interactions. More complex strategies, such as using the variable ranking embedded in high-performance classifiers, are often used instead. For example, Random Forests (RF) classifier can be applied to detect and remove irrelevant SNPs from the primary dataset by ranking the SNPs or proteins based on the strength of their association with the phenotype of interest. RF is widely used in bioinformatics and is capable of addressing (to some extent) variable interaction issues because many possible variable combinations are encountered repeatedly when RF uses bootstrapping and other randomization techniques to construct a large "forest" of single decision tree classifiers. The RF method is attractive for large-scale studies because it is more computationally efficient than comparable classifiers when the number of variables in a dataset is large [37]. The RF classifier is an effective tool in prediction and produces good results in classification [38]. Of course, there are other classification methods that can be useful in this context, such as Support Vector Machine (SVM) and "boosted" classifier methods derived from Adaboost [39, 40].
Another way of ranking the data is by using prior knowledge on biological pathways. Text-mining approaches can be used to select all relevant candidate genes/proteins or even the SNPs that are involved in a disease process or drug response pathway [21, 22, 41, 42]. This approach is similar to a candidate gene approach. The main goal is to retrieve knowledge that is "buried" in a text and to present it to users in an easy-to-interpret form. Text mining is a strategy that is used to process enormous amounts of texts efficiently and systematically. This text mining strategy will be followed by the selection of relevant SNPs in the candidate genes of interest and different tools have been developed and made available for the selection of SNPs [43-47]. Different algorithms can be used in a text mining strategy (classification, clustering, extraction etc.). One very simple example of a text mining algorithm, which is the core of any search engine, is keyword extraction: this is done by assigning a certain score to each word [48].

In the second step of our data analysis strategy, relationships between SNPs which have survived the selection procedure in step one and other relevant variables (in the case of pharmacogenomic research, treatment response and other factors which may influence the association between the SNP and treatment response) will be ascertained. In this step, we attempt to find the most optimal model that fits the data. Key challenges in network approaches in pharmacology are identification of node(s) in biological networks whose perturbation results in a desired therapeutic response and discovery of agents with the desired profile to perturb those nodes [49].

There are, of course, many methods that can be used for the selection of variables and descriptive/predictive modelling. Two examples of methods which can be used for constructing a useful biological network (or model) are Bayesian Networks (BN) and Multifactor Dimensionality Reduction (MDR). Besides these methods, there are many more available --- a useful internet source for the sophisticated data analysis tools can be found at www.kdnuggets.com [102], which is a web repository for the data mining resources and software (while it leans towards machine learning – derived methodology, it also provides a comprehensive coverage of the more "traditional" statistical tools. BN [50] are a popular tool for biological network and pathway reconstruction and have been used for gene function prediction, gene clustering, gene expression analysis, in linkage analysis and for reconstruction of cellular networks [51-53]. Friedman et al. [54] were the first to use the BN approach to recover gene interactions from microarray data. They applied the BN approach to a gene expression dataset and used it in the analysis of the yeast cell cycle, extracting a predictive model of the yeast cell cycle machinery from this data.
A BN is a probabilistic graphical network that represents the joint probability distribution between the variables (nodes) as a set of dependencies (edges) and conditional independences (absence of an edge between the two nodes indicates the conditional independencies between two corresponding variables). BNs are typically used to visualize interactions between genetic, physiological and environmental factors, including the outcome of interest. Their most important application in biology arguably is the modelling of networks and pathways. BN modelling comes with all the typical features of the Bayesian approach: the ability to deal with incomplete noisy data, the ability to combine expert knowledge and data from earlier studies to impose a suitable prior assumptions on the model before learning it from the data, and the ability (with the certain caveats) to express causal relationships [52].

The resulting networks can be tested for robustness and replicability using re-sampling techniques, and by comparing the results with current understanding of signal transduction pathways, possibly using that knowledge to modify the derived networks for further testing. It should be noted, however, that in its standard form BN does not make any prior assumptions and it is therefore strictly data-driven. Therefore, although called "Bayesian", it might be more natural to think of them rather as "marginal likelihood" networks. However, Djebbari et al. [55] demonstrated that inclusion of prior knowledge can improve the (Bayesian) networks that are produced. They showed better recovery of known pathways and relationships between genes from gene expression data from several leukaemia datasets by combining microarray data with prior network structures from the literature. The scalability of BN approach is only fair (up to several hundred variables), and less than that of, for example, RF.

As mentioned before, with the increasing number of genetic and environmental factors and therefore the increasing numbers of possible non-additive interactions, a major multiple testing

problem arises due to the large number of statistical tests that are performed simultaneously [35]. Hence, there is need for analytical methods that can both perform variable selection along with statistical modelling of the data. MDR is one of the approaches that can be useful in this step [56]. MDR was designed to detect gene-gene interactions and gene-environment interactions in human genetics [57]. MDR can be used to discriminate between clinical endpoints and identify interactions in the presence of many types of noise.

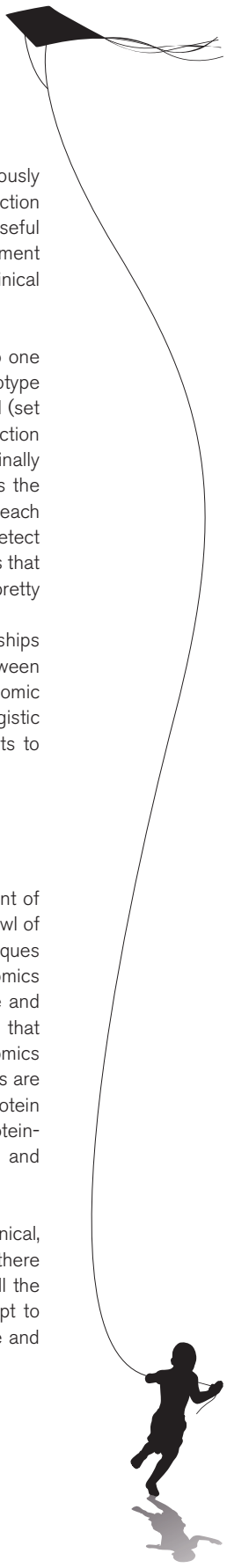
The basis of the MDR method is to convert multidimensional genotype predictors into one dimension with two classes: "high" and "low" risk groups. In the MDR method, each genotype is labelled as "high" or "low" risk for the phenotype of interest. Subsequently, the model (set of predicting variables) with the best misclassification error is selected and the prediction error of the model is calculated. This is repeated for all possible combinations and finally the combination of the risk profiles for each of the genotype combinations represents the final MDR model. In other words, a one dimensional genotype variable is computed for each set of predicting variables. The MDR method has been shown to have high power to detect interactions in a wide range of simulated data [56, 58]. However, the number of variables that can be taken into account in the model is limited and therefore its scalability is, again, pretty low.

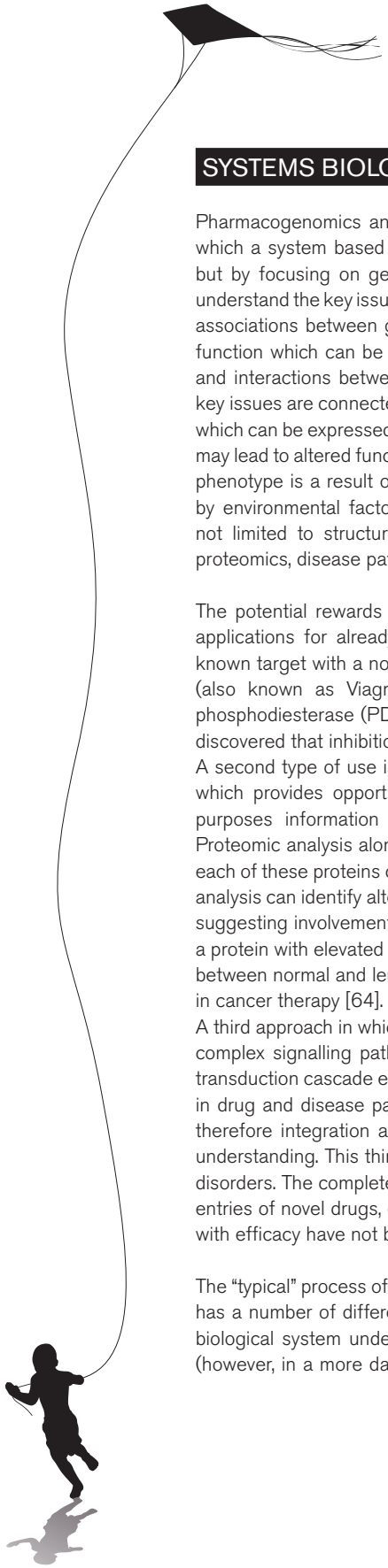
Once we have the models and use human expertise to manually identify the relationships and interactions of interest (e.g., previously unknown, and unsuspected, association between a SNP and a metabolite suggested by the BN), the final step in analysing pharmacogenomic data completes the analyses by using conventional statistical methods (such as logistic regression) to calculate odds ratios or relative risk ratios, etc and translate the results to clinical practice.

NEED FOR SYSTEMS BIOLOGY APPROACH

The past decades a true "omics revolution" has been brought about by the development of novel high-throughput technological platforms. There are different areas in which a sprawl of biological information is happening, and the above revolution has offered tools and techniques that may help in elucidating complex biological pathways and processes [30, 59]. Genomics focuses on DNA sequencing and genotyping, offering us different means to measure and quantify genetic variation. Pharmacogenomics is a more specialized research area that concentrates on the genes that define response to pharmacological therapy. Transcriptomics focuses on the expression of individual genes at mRNA level. These expression patterns are measured by RNA microarrays. Proteomics is about the determination of individual protein concentrations and protein expression patterns of a cell or tissue, protein-protein, protein-DNA and protein-RNA interactions. Finally, metabolomics focuses on characterization and quantification of small organic molecules within a cell or tissue.

The increasing amount of biological data, both -omics measurements and data on clinical, physiological and imaging measurements, open up new ways, but as a consequence there is need for a different approach in which we study the associations and relations of all the elements in a particular pathway or system as a whole. Systems biology is an attempt to create order and make sense of the enormous amount of data that becomes available and find, like in the example of the light bulb, the synergy in the various components.





SYSTEMS BIOLOGY APPLICATIONS IN PHARMACOGENOMICS

Pharmacogenomics and drug discovery research are good examples of research areas in which a system based approach can be applied, not only for the study of individual genes, but by focusing on genetic variation on the genomic scale. Systems biology can help to understand the key issues in pharmacogenomic and drug discovery research at different levels; associations between gene structure and function to assess how genetic structures affect function which can be used in therapeutics, correlations between genotype and phenotype and interactions between gene, drug and environmental factors [60, 61]. These described key issues are connected and interlinked; altered genetic structures may lead to malfunctions which can be expressed as different disease phenotypes. In addition, varied genetic structures may lead to altered functions that can affect the drug-response phenotype. The drug response phenotype is a result of gene-gene and gene-drug interactions and can also be influenced by environmental factors. It should be re-emphasized that pharmacogenomics is certainly not limited to structural genomics, but rather needs integration of functional genomics, proteomics, disease pathogenesis, environmental factors, pharmacology and toxicology.

The potential rewards are many [62]. First, systems biology may be used to identify new applications for already existing molecular targets. A successful example of exploiting a known target with a novel connection to a therapeutic endpoint is development of Sildenafil (also known as Viagra). The drug discovery program originally focussed on selective phosphodiesterase (PDE) type 5 inhibitors for use in cardiovascular disease. However, they discovered that inhibition of type 5 PDE was a treatment for male erectile dysfunction [63].
A second type of use is the identification of new molecular targets associated with disease which provides opportunities for development of new effective therapies. For these two purposes information from proteomic and metabolomic analysis should be combined. Proteomic analysis alone might identify proteins associated with a certain disease state and each of these proteins could be causative elements of the disease. Alternatively, metabolomic analysis can identify alteration in protein phosphorylation levels associated with disease state, suggesting involvement in the disease pathway. Discovery of the BCR-ABL gene coding for a protein with elevated tyrosine-kinase activity, provided the first selective (differential activity between normal and leukemic cells) tyrosine-kinase inhibitor Imatinib (also known as Glivec) in cancer therapy [64].
A third approach in which systems biology could play a role in drug discovery is by unravelling complex signalling pathways, which enables targeting of appropriate regions in the signal transduction cascade enabling the search for more efficient and safe drugs. Signalling events in drug and disease pathways are well known to be very complex and dynamic processes, therefore integration and combination of different biological data is necessary to improve understanding. This third approach can be exemplified by research in the field of depressive disorders. The complete pharmacological basis remains poorly understood, despite the many entries of novel drugs, elimination of adverse effects leads to non-compliance and problems with efficacy have not been solved yet [65].

The "typical" process of systems biology analysis in application to pharmacogenomic research has a number of different input modules [30, 66]. Figure 2 shows the logistics. Initially, the biological system under study needs to be selected along with an appropriate hypothesis (however, in a more data-driven study, such as a GWS study, the hypothesis could be fairly

"open-ended"). During this first step, the outcomes (in the case of pharmacogenomic research treatment responses), and samples sizes have to be determined. Subsequently, biological data has to be collected for the organism or cell type of interest; genomic, proteomic, physiological and clinical data are acquired on a variety of analytic platforms. These data sets have to be merged into composite files. Before this step can be carried out, data sets should be screened for missing and default data, and quality control issues have to be considered. The merged data files are compared, and correlation and causal models and networks are produced in the third step. This is carried out by comparing differences between responders and non-responders to treatment. The next step will be interrogation of correlation and causal models and networks against existing knowledge. Biological validation is very important to ensure the newly obtained results are biologically relevant. Once a system has been modelled, the pathway or network can be remodelled to produce a better outcome or to identify the optimal therapeutic agent. This network analysis is a starting point for further research.

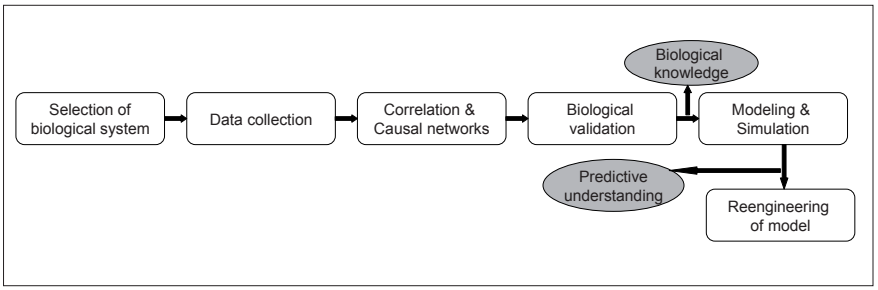


Figure 2. Different parts of the systems biology based analysis process

There are at least two "output" modules in the process of systems biology analysis. First, relevant biological knowledge is produced to answer the original research question. Second result of this systems biology approach is predictive understanding of the studied biological system. Based on modelling and simulation studies of the investigated biological system it should be possible to predict the outcome of specific changes in the pathway or network. This is especially important in pharmacogenomics research where we are studying the influence of genetic variation on treatment response and eventually hope to predict response to a certain drug based on knowledge of the genotype. One should always keep in mind that our overall goal is personalised prescribing of drugs based on prior knowledge on genetic variance, patient characteristics (age and gender), disease state or phenotype and environmental factors and systems biology paradigm can help us with not losing our focus on the big picture.

CONCLUSION

To reach the goal of individualized prescribing, a broad and detailed knowledge of genetic influences on drug response is necessary. Correct diagnosing and phenotyping are a prerequisite and multiple gene-drug interactions have to be taken into account. Therefore, we need to expand our research area with the use of genome-wide-association (GWA) scans, and with focussing not only on genes, but also on the molecular level: differences in protein expression (proteomics), transcription activity (transcriptomics). This increased amount of

data asks for the development of new analysis strategies aimed at large amounts of data, automated knowledge discovery and integration of traditional hypothesis-(user-) driven approaches with data-driven ones. The systems biology framework fits well with the above goals. In the future, a systems biology approach will be increasingly useful (and necessary) to deal with the bulk of biological data in order to achieve our ultimate goal, that of individualised therapy.

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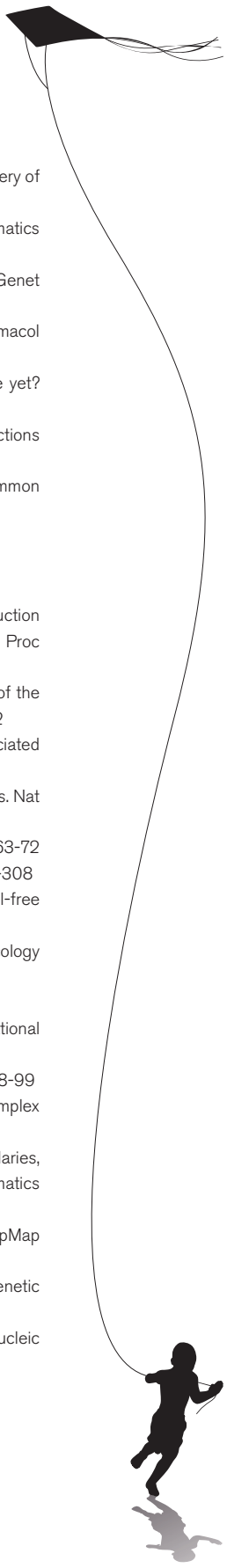

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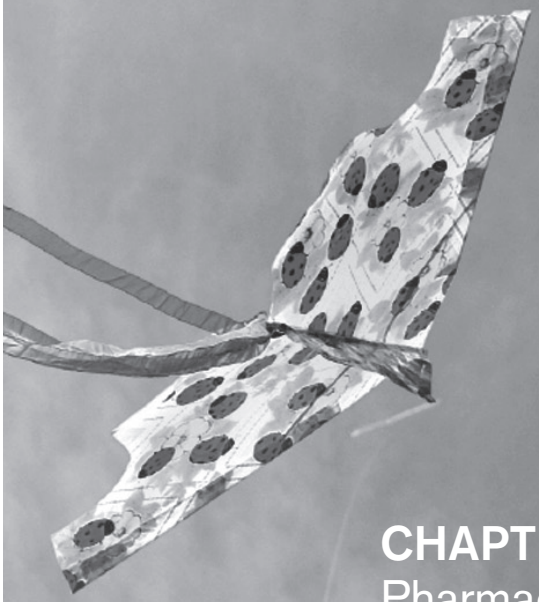

CHAPTER 11
Pharmacogenetics of anti-inflammatory therapy in asthma: corticosteroids and leukotriene antagonists

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ABSTRACT

Asthma is a chronic airway disease with a relatively high prevalence. Although the etiology of asthma is still not fully clear, there are effective treatments available. The treatment strategy for asthma consists of two steps: maintenance therapy with anti-inflammatory agents (inhaled corticosteroids and/or leukotriene antagonists) to reduce airway inflammation and retain proper lung function and secondly, the use of beta-agonists for quick symptomatic relief. Furthermore, oral steroids are used to treat acute exacerbations.

There are large differences in response to drug therapy. This may be due to many factors, such as severity and type of disease, compliance, co-morbidity, co-medication (drug-drug interactions), environmental exposures and age. However, calculations of repeatability of treatment response suggest that part of this variance in response to pharmacotherapy could be due to genetic factors. Pharmacogenetics may explain the inter-individual variability in drug response due to genetic variation.

Pharmacogenetics is a relatively new emerging research field that provides the opportunity to discover associations between genetic variation and response to a variety of drugs. This review will discuss the pharmacogenetics of anti-inflammatory agents (corticosteroids and leukotriene antagonists) used in the treatment of asthma.

INTRODUCTION

Asthma is among the most common chronic disorders in the world. In the United States, it is the most common reason for paediatric hospital admission [1]. This high prevalence induces a great economic and social burden [2 3]. Fortunately, effective therapy strategies for asthma are available. Standard treatment is based on regular use of inhaled corticosteroids (ICS) combined with as-need use of beta-agonists to prevent asthma exacerbations, retain proper lung function and reduce the inflammatory response. In some cases, leukotriene antagonists are prescribed as anti-inflammatory therapy. Furthermore, oral steroids are used to treat acute severe exacerbations [4-8]. Despite these effective therapies asthma control in children is still far from optimal worldwide [9].

Treatment response can be highly variable amongst patients. Currently available anti-inflammatory drugs (inhaled corticosteroids and leukotriene antagonists) are effective in most asthmatic patients, however there remains a significant group of patients who do not respond well to existing therapies [9 10]. These suboptimal treatment effects hamper a person's well being; many of these therapy non-responsive patients have frequent exacerbations and chronically impaired lung function. Therefore, further research for innovative and more effective therapies for those that cannot be treated effectively with current available drugs is needed. Pharmacogenetics/genomics, with personalized prescribing as ultimate goal could contribute to this.

DEFINITION OF PHARMACOGENETICS

The terms pharmacogenetics and pharmacogenomics are used interchangeably in literature. A precise, distinctive definition of these two terms remains difficult. In this review paper we will use the definitions of the European Medicine Agency (EMEA) concerning 'pharmacogenetics'

and 'pharmacogenomics'. According to the EMEA terminology, pharmacogenetics is 'the study of variations in DNA sequences as related to drug response'. The definition of pharmacogenomics is 'the study of variations of DNA and RNA characteristics as related to drug response'. Pharmacogenomics is a much broader research field which includes pharmacogenetics [11]. In this paper we will mainly focus on pharmacogenetic mechanisms in treatment response to anti-inflammatory asthma drugs.

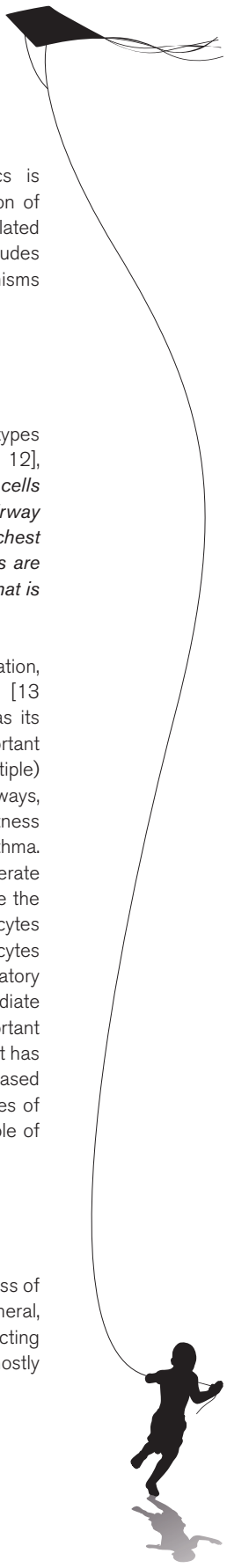
ASTHMA AND AIRWAY INFLAMMATION

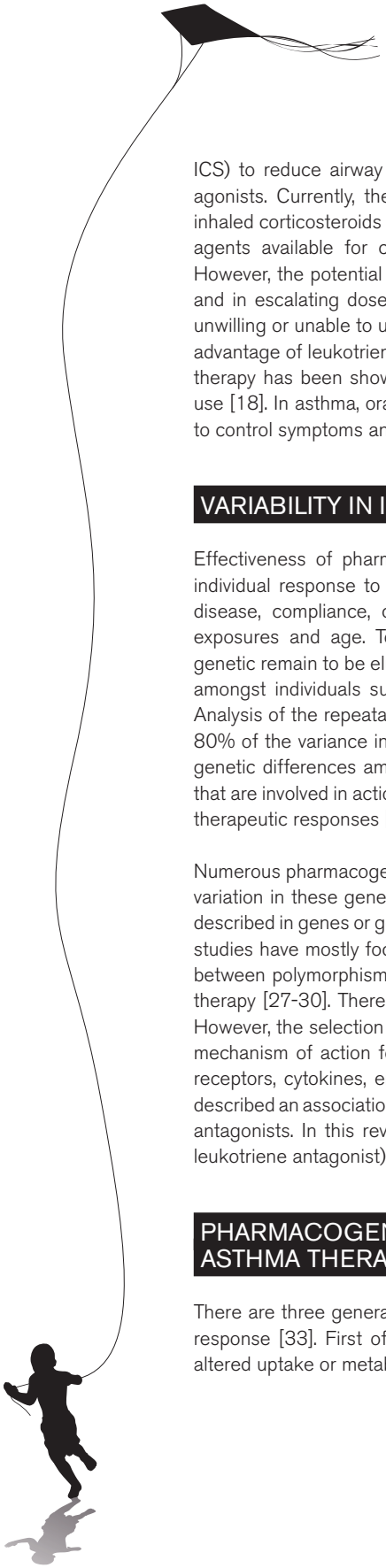
Allergic asthma is a complex inflammatory airway disease with many clinical phenotypes in both adults and children. According to the Global Initiative for Asthma (GINA) [2 12], asthma is defined as: *'A chronic inflammatory disorder of the airways in which many cells and cellular elements play a role. The chronic inflammation is associated with airway hyperresponsiveness that leads to recurrent episodes of wheezing, breathlessness, chest tightness and coughing, particularly at night or in the early morning. These episodes are usually associated with widespread, but variable, airflow obstruction within the lung that is often reversible either spontaneously or with treatment.'*

The major disease characteristics are reversible (with or without therapy) airflow limitation, airway hyperresponsiveness to a variety of stimuli and inflammation of the airways [13 14]. Asthma symptoms can occur at all ages, but for many patients the disease has its roots in infancy [15]. Inhaled allergens, such as pollen and tobacco smoke, are important environmental factors involved in the asthma pathogenesis. The interaction between (multiple) genetic factors and (multiple) environmental factors can result in inflammation of the airways, altered pulmonary physiology and asthmatic symptoms such as wheezing and shortness of breath [13 14]. Various cell types are involved in the inflammatory response in asthma. Mast cells are increased in both the epithelium and surface secretions. These cells generate and release mediators that act on smooth muscle and small blood vessels which cause the immediate reaction: airflow limitation. Furthermore, eosinophils and CD4+ lymphocytes are found in increased numbers in asthmatic patients. Both eosinophils and lymphocytes are mediators of the inflammatory response in asthma; eosinophils release inflammatory mediators such as leukotrienes to injure the airway tissues and lymphocytes also mediate the inflammatory response by the production of cytokines [13]. In recent years, an important subgroup of asthma patients with severe disease that is relatively corticosteroid resistant has been indentified. In this subgroup of asthmatic patients, neutrophils were found in increased numbers. Understanding of the mechanism of neutrophilia in asthma and consequences of decreasing airway neutrophils is limited. More research has to be done to clarify the role of neutrophils in asthma for the development of clinical trials that target neutrophils [16].

ANTI-INFLAMMATORY THERAPY IN ASTHMA

The main objective of asthma therapy is reduction of clinical symptoms (wheeze, shortness of breath and cough), severe exacerbations and to minimize sleep disturbances [6]. In general, the treatment strategy for asthma consists of three steps: (1) treatment with short-acting beta-agonists for quick symptomatic relief, (2) treatment with anti-inflammatory drugs (mostly





ICS) to reduce airway inflammation and, (3) use of ICS combined with long-acting beta-agonists. Currently, there are two main anti-inflammatory drug therapy classes available: inhaled corticosteroids (ICS) and leukotriene inhibitors [4 6-8 17]. ICS are the most effective agents available for control of airway inflammation and improvement of lung function. However, the potential side effects of ICS (such as growth inhibition) when used long-term and in escalating doses have led to the use of adjunctive therapies. For patients who are unwilling or unable to use ICS treatment with leukotriene antagonists can be considered. An advantage of leukotriene antagonist is that they can be taken orally. Leukotriene antagonists therapy has been shown to help control asthma symptoms while sometimes reducing ICS use [18]. In asthma, oral corticosteroids may be prescribed when inhaled corticosteroids fail to control symptoms and are used to treat acute exacerbations.

VARIABILITY IN INDIVIDUAL TREATMENT RESPONSE IN ASTHMA

Effectiveness of pharmacotherapy can be variable amongst individuals. Variability in this individual response to therapy may be due to many factors, such as severity and type of disease, compliance, co-morbidity, co-medication (drug-drug interactions), environmental exposures and age. To which extend inter-individual variability in treatment response is genetic remain to be elucidated. However, calculations of repeatability of treatment response amongst individuals suggest that a substantial fraction of the variance could be genetic. Analysis of the repeatability of treatment responses to anti-asthma drugs showed that up to 80% of the variance in treatment response in Caucasian asthmatic patients may be due to genetic differences among individuals [4]. Genetic variants have been discovered in genes that are involved in action or metabolism of anti-asthma drugs and are associated with altered therapeutic responses [10].

Numerous pharmacogenetic studies have been performed to asses the relationship between variation in these genes and response to treatment and several genetic variants have been described in genes or gene pathways of anti-asthma drugs [19-26]. Asthma pharmacogenetic studies have mostly focussed on response to beta-agonists. Associations have been shown between polymorphisms in the beta-receptor gene (*ADRB2*) and response to beta-agonist therapy [27-30]. There have also been pharmacogenetic studies of inhaled corticosteroids. However, the selection of candidate genes in these studies is hampered by the fact that the mechanism of action for corticosteroids is very complex. This mechanism involves multiple receptors, cytokines, enzymes, genes and other actors [31 32]. Several studies have also described an association between genetic polymorphisms and variable response to leukotriene antagonists. In this review we discuss anti-inflammatory asthma agent (corticosteroid and leukotriene antagonist) pharmacogenetic studies that have been performed.

PHARMACOGENETICS MECHANISMS WITH IMPLICATIONS FOR ASTHMA THERAPY

There are three general ways by which genetic variation may lead to variability in treatment response [33]. First of all, pharmacokinetic mechanisms, genetic variants associated with altered uptake or metabolism of a drug may play a role. Treatment response can be modified

by variation in enzymes involved in the catabolism or excretion of a drug. The highly genetically diverse cytochrome P (CYP) 450 system which is known for its many pharmacogenetic effects is a good example of such a mechanism. In asthma, CYP3A4, CYP2C9 and CYP1A2 are suggested to be involved in glucocorticoid and leukotriene receptor antagonist metabolism [34-38]. Secondly, idiosyncratic mechanisms, genetic variants resulting in actions of a drug outside its therapeutic indication, could be a pharmacogenetic mechanism for altered drug response. This is variance among individuals that can lead to adverse effects that are not based on the drug's action. Third, pharmacodynamic mechanisms, genetic variations in the drug target or components of the drug pathway resulting in altered drug efficacy, could be an important mechanism for altered drug responses. For example, polymorphisms have been described in the glucocorticoid receptor gene which can lead to modified receptor function [39 40]. Most of the currently available asthma pharmacogenetics data falls into this latter category, in which individuals are categorized as responders or non-responders and analysis of specific genetic variants is used to distinguish these groups.

PHARMACEGENETICS OF (INHALED) CORTICOSTEROIDS

Inhaled corticosteroids are the first-line maintenance therapy for all asthma patients and have been used for a long time to reduce airway inflammation and to improve lung function [5 6]. The most important effect of corticosteroids is to switch off inflammatory genes (encoding for cytokines, chemokines, inflammatory enzymes, receptors and proteins) that have been activated during the inflammation process [32 41]. Furthermore, they induce gene transcription, resulting in secretion of anti-inflammatory proteins. Corticosteroids may also interact with recognition sites of activated inflammatory genes to inhibit transcription and they also may influence signal transduction pathways through increased transcription of inhibitors of these pathways or repression of critical enzymes [32 41 42].

Glucocorticosteroids are a class of corticosteroids. These liposoluble hormones can diffuse across the cell membranes and bind to the cytoplasmic glucocorticoid receptors (GRs). These cytoplasmic GRs are bound to chaperone proteins, which protect the receptor. After binding of the corticosteroids to GRs, changes in structure of the receptor result in dissociation of the chaperone proteins and exposition of nuclear localisation signals on the GR. This results in transport of the GR-corticosteroid complex into the nucleus, where it binds to DNA of corticosteroid responsive genes (glucocorticoid response elements (GREs)), which on its turn causes changes in gene transcriptions [32 41]. Between 10 and 100 genes are supposed to be directly regulated by the glucocorticoid receptor and many other genes are indirectly regulated. Corticosteroids switch on genes with anti-inflammatory effects and thereby increase the expression of anti-inflammatory proteins. The effective control of airway inflammation by corticosteroids used in asthma therapy is mediated by inhibition of the synthesis of inflammatory cells and proteins by suppressing the genes that encode them [32 41]. Furthermore, corticosteroids interfere with multiple signal transduction pathways involved in regulation of transcription factors. Thereby corticosteroids cause gene repression [42].

The release of glucocorticoids is regulated by corticotrophin releasing hormone (CRH) via the hypothalamic pituitary adrenal (HPA) axis. This axis regulates the level of circulating glucocorticoid in the human body. CRH regulates adrenocorticotropin (ACTH) secretion, which

stimulates adrenal glucocorticoid secretion. Two receptors mediate the actions in this pathway: corticotropin releasing hormone receptor type 1 (*CRHR1*) and the homologous receptor type 2 (*CRHR2*). *CRHR1* mediates ACTH release and thereby exerts anti-inflammatory effects. Polymorphisms in this gene might therefore affect airway inflammation and treatment effects of corticosteroids. *CRHR1* has also been implicated in the pathogenesis of asthma. Absence of CRH causes a decrease in the production of endogenous glucocorticoids and an increase in airway inflammation and lung dysfunction [10]. Defects in *CRHR1* could cause a similar increase because of insufficient glucocorticoid production, this results in an increased response to exogenous glucocorticoids.

Many pharmacogenetic studies have been performed to study differences in treatment response after corticosteroid use (Table 1). Although corticosteroids are effective in most asthma patients, a few patients fail to respond well to these drugs [43]. This decreased treatment response may be due to several molecular mechanisms. Most studies focussed on polymorphisms that alter the glucocorticoid receptor itself or on variations in the *CRHR1* gene. The human glucocorticoid receptor (*NR3C1*) gene is located on chromosome 5q31 and several polymorphisms have been described in this gene [40 44-46].

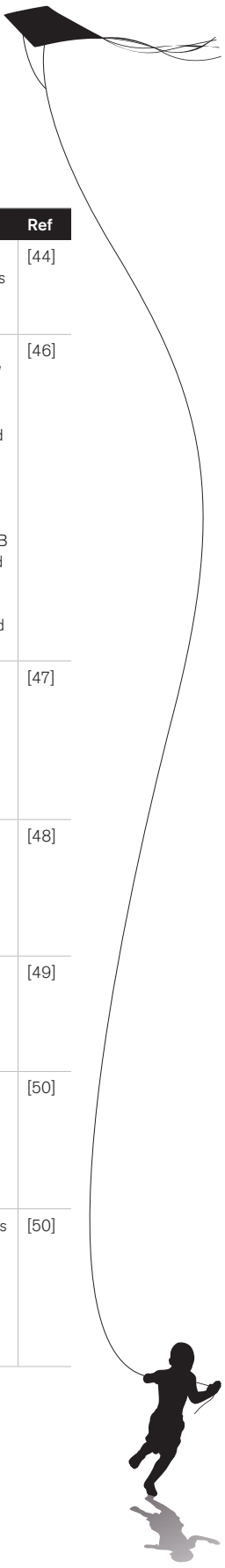
Associations have been found between polymorphisms in the glucocorticoid receptor gene (*NR3C1*) and altered response to steroid therapy, although results are not always consistent [45]. Within intron B (a part of the DNA sequence that is not translated into proteins) a three-point haplotype is associated with enhanced sensitivity to corticosteroids [47]. In contrast, the Ile729Val substitution impairs receptor function and is likely a cause of cortisol resistance [48]. Furthermore, a substitution at codon 641 results in lower binding affinity of the receptor and therefore steroid resistance [49]. There is also evidence that the BclI polymorphism is associated with response to corticosteroids [50]. However, more recent studies also identified other loci that contribute to heterogeneity in corticosteroid treatment response [51-53].

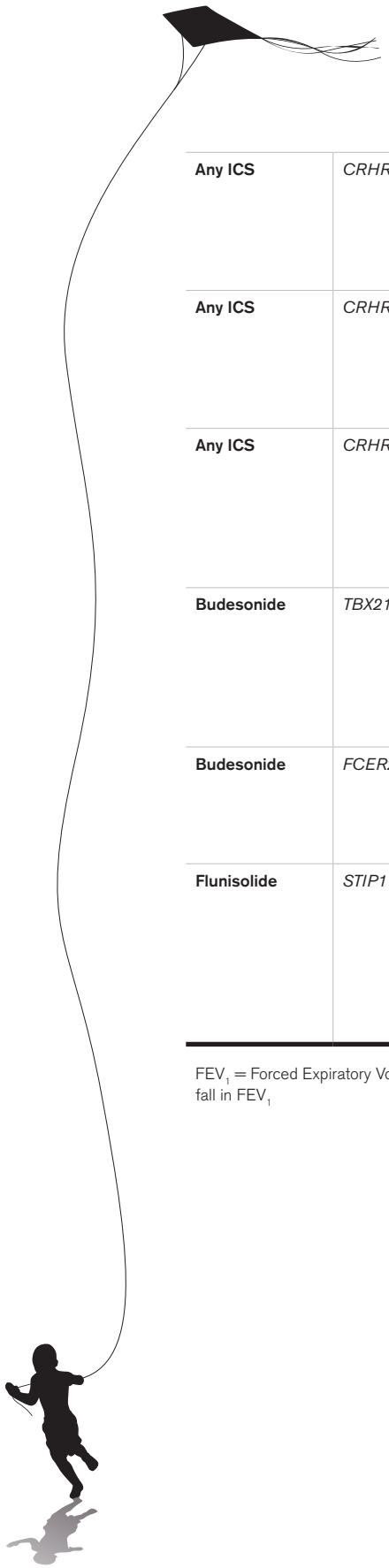
Variation in the *CRHR1* gene is associated with response to corticosteroid therapy. Individuals in three large clinical trial populations who were carriers of the variant gene manifested an enhanced response to treatment (improvement in FEV_1) compared to wild type carriers [54 55]. However, results are not consistent, as another study in an asthma cohort followed for 22 years did not find an association between *CRHR1* variants and treatment response [56].

Variation in the *TBX21* gene, which encodes for T-bet, an essential transcription factors for T lymphocyte development, has been associated with improvement in airway responsiveness after corticosteroid therapy in a large clinical trial with asthmatic children [52]. Furthermore, variation in the *FCER2* gene, encoding for the low-affinity IgE receptor, is also associated with response to steroid therapy. Patients with the variant *FCER2* gene were at higher risk of exacerbation while on ICSs [53]. Recently, genetic variation in *STIP1*, a glucocorticoidreceptor-complex gene, has been associated with improved lung function in asthmatic patients treated with ICS [51].

Table 1. Pharmacogenetic studies performed on (inhaled) corticosteroids

Drug	Gene	Variant	Population	Phenotype	Outcome	Ref
Dexamethasone	*NR3C1*	R477H and G679S	12 patients with primary cortisol resistance	Transactivating capacity, ligand binding capacity	Both variant *NR3C1* genotypes caused impaired receptor function.	[44]
	NR3C1	S651F, T504S and 231insA	DNA from 88 allergic patients and cell lines from 73 different individuals	mRNA/protein expression levels, transcriptional activity and inhibition of NF-kB transactivation	S651F and 2314insA *NR3C1* variant were associated with altered protein and mRNA expression levels, reduced transcriptional activity and reduction of NF-kB transactivation and were suggested to influence glucocorticosteroid response.	[46]
Dexamethasone	*NR3C1*	3-point haplotype in intron B	40 psoriasis patients and 76 controls	Dexamethasone suppression	The *NR3C1* haplotype within intron B was associated with enhanced sensitivity to glucocorticoids.	[47]
Dexamethasone	*NR3C1*	Ile729Val	DNA from patient with primary cortisol resistance and 2 healthy controls	Dexamathasone binding affinity	*NR3C1* variant was associated with impaired receptor function.	[48]
Dexamethasone	*NR3C1*	Val641Asp	DNA from 3 patients with familial glucocorticoid resistance	Dexamathasone binding affinity	*NR3C1* variant was associated with impaired receptor function.	[49]
	NR3C1	BclI, N363S and ER22/23EK	119 IBD patients	Corticosteroid withdrawal without the need for steroids for at least 1 year	*NR3C1* variant genotype was associated with better response to corticosteroid treatment	[50]
Prednisone	*MDR1*	C3435T and G2677T	119 IBD patients	Corticosteroid withdrawal without the need for steroids for at least 1 year	No association was found between variant *MDR1* genotypes and response to corticosteroid treatment.	[50]





Any ICS	*CRHR1*	Rs242941 and haplotypes	470 asthmatics, 311 asthmatic children and 336 asthmatics	Change in FEV_1	*CRHR1* variant genotypes were associated with an enhanced response to corticosteroid treatment.	[54]
Any ICS	*CRHR1*	Rs1876828, rs242939 and rs242941	470 asthmatics, 311 asthmatic children and 336 asthmatics	Change in FEV_1	*CRHR1* variant genotypes were associated with an enhanced response to corticosteroid treatment.	[55]
Any ICS	*CRHR1*	Rs1876828, rs242939 and rs242941	281 asthmatics	Change in FEV_1	*CRHR1* polymorphisms were not associated with immediate or long-term improvement in lung function.	[56]
Budesonide	*TBX21*	His33Glu	701 asthmatics	Improvement in PC20	*TBX21* Glu33 carriers showed improvement in airway responsiveness after budesonide use.	[52]
Budesonide	*FCER2*	T2206C	311 asthmatics	IgE levels and exacerbation rate	Variant *FCER2* genotype was associated with severe exacerbations.	[53]
Flunisolide	*STIP1*	Rs4980524, rs6591838 and rs2236647	382 asthmatics	Baseline FEV_1, % predicted FEV_1 and change inFEV_1	*STIP1* polymorphisms were associated with FEV_1 change after 4 and 8 weeks, baseline FEV_1 and baseline % predicted FEV_1.	[51]

FEV_1 = Forced Expiratory Volume in 1 Second, PC20 = Provocative concentration of metacholine causing a 20% fall in FEV_1

In summary, pharmacogenetic studies of corticosteroid response have shown associations with lung function, exacerbations and hyperresponsiveness. Earlier studies have described genetic variation in the glucocorticoid receptor gene. However, few clinical relevant endpoints have been studied. The glucocorticoid receptor is part of a multi-protein complex which may be involved in the glucocorticoid resistance phenotype. Several more recent studies focussed on genes in the glucocorticoid-receptor complex, focus in future studies should be on these genes coding for proteins in the entire complex.

PHARMACOGENETICS OF LEUKOTRIENE ANTAGONISTS

Leukotriene antagonists used in asthma therapy interfere with the action or synthesis of leukotrienes, lipid mediators that take part in the inflammatory response. There are a number of observations that suggest a role for leukotrienes in the pathogenesis of asthma. Leukotriene synthesis is initiated after trauma, infection and inflammation. Leukotrienes are important mediators in the pathophysiology of asthma; they are responsible for a variety of effects such as activation of inflammatory cells, increased mucus release, increased smooth muscle cell contractility and increased vascular endothelial cell permeability. At first, leukotrienes can induce many abnormalities seen in asthma, such as obstruction of the airways, mucus secretion and granulocyte chemotaxis. Secondly, leukotrienes are potent effector molecules that cause airway obstruction. Thirdly, one of the end products of the leukotriene pathway (leukotriene E4) has been detected in increased amounts in the urine of asthmatic patients after antigen challenge [57].

Leukotrienes are products of the 5-lipoxygenase pathway of arachidonic acid metabolism and act by binding to specific receptors located on structural and inflammatory cells [58]. They are synthesized from arachidonic acid via the action of 5-lipoxygenase (5-LO) and 5-lipoxygenase activating protein (FLAP). The first step is the conformation of arachidonic acid to 5-hydroperoxyeicosatetraenoic acid and subsequently to leukotriene A4 (LTA4) by a catalytic complex consisting of 5-LO and FLAP. LTA4 is unstable and may be transformed to leukotriene B4 (LTB4), which is involved in eosinophil and neutrophil chemotaxis. In the presence of leukotriene C4 (LTC4) synthase, LTA4 is converted to LTC4. LTC4 is subsequently cleaved to form the active entity, leukotriene D4 (LTD4). Cleavage of LTD4 results in the formation of leukotriene E4 (LTE4). LTC4, LTD4 and LTE4 are all known as cysteinyl leukotrienes, because they all contain a cysteine group.

Leukotrienes act by binding to specific receptors. LTB4 binds to the leukotriene B4 receptor that transduces chemotaxis and cellular activation. The cysteinyl leukotrienes (LTC4, LTD4 and LTE4) bind to two receptors: cysteinyl leukotriene receptor 1 (CYSLT1) and 2 (CYSLT2). Stimulation of the CYSLT1 receptors leads to smooth muscle contraction and stimulation of CYSLT2 leads to smooth muscle contraction and chemotaxis. Based on these two pathways, there are two main pharmacological treatment strategies to inhibit leukotriene activity. First of all, inhibition of 5-lipoxygenase which results in decreased leukotriene synthesis and secondly antagonism of the CYSLT2 receptor to prevent binding from cysteinyl leukotrienes to the receptor [59]. Variation in treatment response to both of these drug classes has been described. Table 2 gives an overview of leukotriene antagonist pharmacogenetic studies, showing that variation in genes in the leukotriene pathway can alter response to leukotriene antagonists.

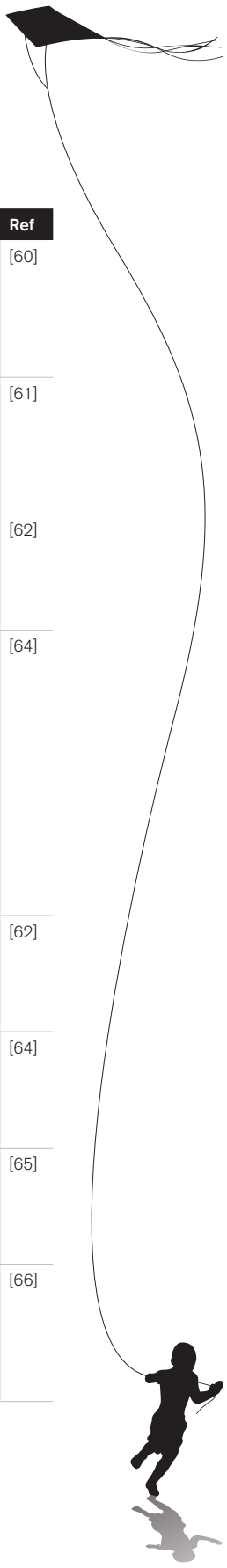
ALOX5 and *LTC4S* are the most frequent studied genes. *ALOX5* codes for 5-lipoxygenase and this gene has a tandem repeat polymorphism within the transcription factor-binding region of its promoter region, the region that regulates gene transcription. Individuals that do not carry the five tandem repeats allele show a decreased response to leukotriene antagonist therapy [60-63]. However, a study of Lima et al. [64] showed an increased response to leukotriene inhibitors in carriers of the mutant allele. These data suggest that the *ALOX5* gene may be an interesting pharmacogenetic locus. Polymorphisms in the *LTC4S* gene are also associated with response to leukotriene inhibitors. Several studies showed that the *LTC4S* A-444C polymorphism is associated with improved treatment response [64-67]. Furthermore, Tantisira et al. [68] showed that treatment response to two classes of leukotriene antagonists (a cys-leukotriene receptor antagonists and a 5-lipoxygenase inhibitor) that work by inhibiting different parts of the same pathway were modulated by the same loci.

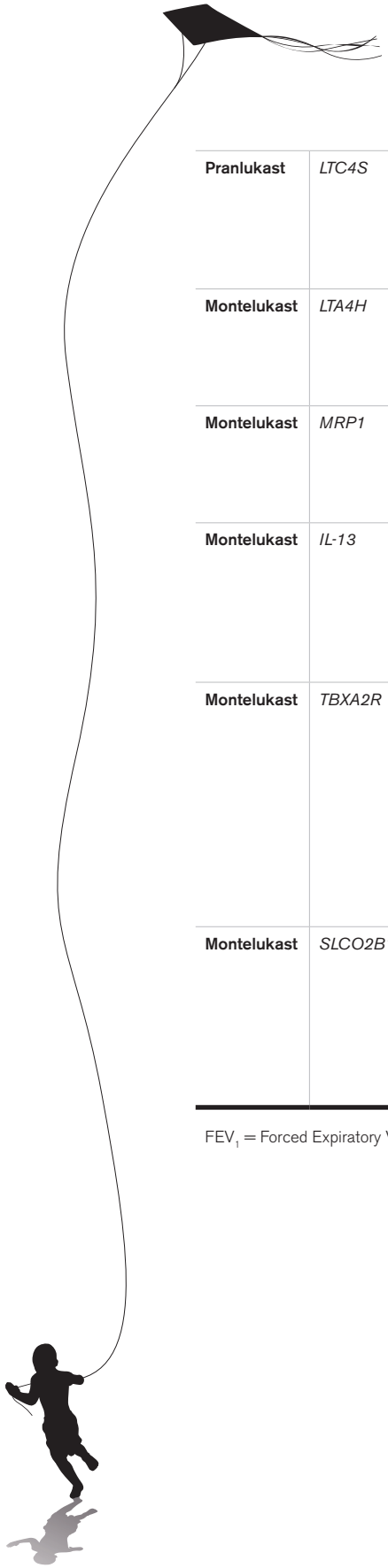
Besides variation in the *ALOX5* and *LTC4S* gene there are other potential interesting pharmacogenetic loci that can influence leukotriene antagonist treatment response. Polymorphisms in the *MRP1* gene (coding for the multidrug resistance protein 1) were associated with treatment response; patients with the variant genotype showed an improvement in lung function after montelukast therapy [64]. The *MRP1* gene is highly polymorphic and a mutation in the last transmembrane part influences LTC4 transport and could therefore alter treatment response. Furthermore, *CYSLT2* could be a candidate gene in the leukotriene pathway. *CYSLT2* variant genotypes may enhance response to leukotriene inhibitors [62]. Patients with a polymorphism in the *LTA4H* gene, that codes for a catalyzing enzyme in the leukotriene pathway, showed a higher risk for exacerbations while on leukotriene antagonist therapy [64].
Genes other then the ones involved in the leukotriene pathway may also influence treatment response. There have been studies that showed associations for *IL-13* polymorphisms and polymorphisms in the *TBXA2R* gene (coding for a negative regulator of LTC4) [69 70]. Mougey et al. [71] showed that genetic variation in the *SLCO2B1* gene could be a cause of poor response to montelukast in carriers of this polymorphism.

In summary, pharmacogenetic studies of response to leukotriene antagonists showed associations with lung function, exacerbations and clinical symptoms. To date, *ALOX5* and *LTC4S* are the most studied genes. More recent studies also focussed on other genes in the pathway and showed associations with treatment response. However, pharmacogenetic studies into this pathway remain in their early phases. Future studies are needed to focus also on other genes involved in leukotriene metabolism.

Table 2. Pharmacogenetic studies performed on leukotriene antagonists

Drug	Gene	Variant	Population	Phenotype	Outcome	Ref
ABT-761	*ALOX5*	Number of tandem Sp1 repeats	221 asthmatics	Change in FEV_1	Variant ***ALOX5*** genotypes were associated with decreased response to treatment.	[60]
Montelukast	*ALOX5*	Number of tandem Sp1 repeats	61 asthmatics	Exacerbation rate, SABA use, change in FEV_1	5/5 and 5/4 ***ALOX5*** genotypes were associated with improved treatment response.	[61]
Montelukast	*ALOX5*	Rs4987105 and rs4986832	174 asthmatics	Change in PEF and FEV_1	Variant ***ALOX5*** genotypes were associated with improved treatment response.	[62]
Montelukast	*ALOX5*	Number of tandem Sp1 repeats, rs2115819 and haplotypes	252 asthmatics	Exacerbation rate and change in FEV_1	Variant ***ALOX5*** tandem repeat genotypes were associated with reduced risk of exacerbations. SNP rs2115819 was associated with increased FEV_1. Haplotypes were associated with risk of exacerbations.	[64]
Montelukast	*CYSLT2*	Rs91227 and rs192278	174 asthmatics	Change in PEF and FEV_1	Variant ***CYSLT2*** genotypes were associated with improved treatment response.	[62]
Montelukast	*LTC4S*	A-444C polymorphism (Rs730012)	252 asthmatics	Exacerbation rate and change in FEV_1	Variant ***LTC4S*** genotype was associated with reduced risk of exacerbations.	[64]
Montelukast	*LTC4S*	A-444C polymorphism	59 asthmatics	Asthma symptoms, SABA use, change in PEF	Variant ***LTC4S*** genotype was associated with improved response to montelukast.	[65]
Zafirlukast	*LTC4S*	A-444C polymorphism	23 asthmatics	Change in FEV_1, FVC and PEF	Variant ***LTC4S*** genotypes were associated with increased treatment response.	[66]





Pranlukast	*LTC4S*	A-444C polymorphism	50 asthmatics	Change in FEV_1	Variant ***LTC4S*** genotypes were associated with increased treatment response.	[67]
Montelukast	*LTA4H*	Rs2660845	252 asthmatics	Exacerbation rate and change in FEV_1	Variant ***LTA4H*** genotype was associated with increased risk of exacerbations.	[64]
Montelukast	*MRP1*	Rs119774	252 asthmatics	Exacerbation rate and change in FEV_1	Variant ***MRP1*** genotype was associated with increased response to treatment.	[64]
Montelukast	*IL-13*	-1512A/C, -1112C/T, +2044G/A and haplotypes	374 asthmatics and 242 controls	Change in FEV_1	Variant ***IL-13*** genotype and IL-13 haplotypes were associated with improved response to treatment.	[69]
Montelukast	*TBXA2R*	+795T/C and +924T/C	695 asthmatics and 159 controls	Change in FEV_1	Variant ***TBXA2R*** +795T/C genotype and combined effect of +795T/C and +924T/C genotypes were associated with decreased response to treatment.	[70]
Montelukast	*SLCO2B1*	rs12422149	489 asthmatics	Plasma concentration and asthma symptom utility index score	Variant ***SLCO2B1*** was associated with reduced montelukast plasma concentrations and poor response to treatment.	[71]

FEV_1 = Forced Expiratory Volume in 1 Second, SABA = short-acting beta-agonist, PEF = Peak Expiratory Flow

CHALLENGES IN ASTHMA PHARMACOGENETICS

To perform a pharmacogenetic study the definition of a solid endpoint is of utmost importance. Differences in response phenotypes or endpoints between studies may be a reason for inconclusive results. This was recently studied by Rogers et al. [72] who showed that both genetic and phenotypic predictors of poor response to ICS differ depending on the definition of outcome. To improve comparability of studies objective phenotyping is essential. This can be difficult in asthma pharmacogenetic studies, because a correct diagnosis is sometimes difficult, especially in paediatric asthma patients. Children are not always able to perform lung function testing and symptoms are often transient [5]. Furthermore, asthma is a very heterogeneous disease with recurrent periods of symptoms which makes it difficult to distinguish disease phenotypes and to define true endpoints. Asthma control is a frequently used endpoint for treatment response. However, this is mostly defined as surrogate endpoint determined by clinical parameters, such as lung function measurement, clinical symptoms (symptom-free days, number of severe exacerbations and awakenings at night) and rescue medication use (short-acting beta-agonist use). This hampers the definition of true responders and non-responders to treatment. Using objective measurements to assess asthma severity and asthma control could solve this problem. Lung function is one of the few available objective measurements. Furthermore, fractional exhaled nitric oxide (FeNO) can be used as a marker for airway inflammation and thus response to anti-inflammatory therapy [73 74].

Another big challenge in asthma pharmacogenetics are the multiple genes involved in the asthma anti-inflammatory drug pathways. It is unlikely that variation in only one gene causes an altered treatment response. In most cases, multiple genes are involved and the eventual pharmacogenetic effect will be an addition of all these separate polymorphisms in different genes. Besides variation in genes in the anti-asthma pathway, polymorphisms in drug metabolizing enzymes such as CYP-enzymes may contribute to the differential response of patients to anti-asthma drugs. The multiple gene-drug interactions that play a role in pharmacogenetics research ask for a different statistical approach. Conventional methods are not sufficient to perform statistical analyses in large genetic databases with a variety of factors that have to be taken into account. In the future, there is need for a more system-based approach [75].

CONCLUSION

The increasing asthma incidence in the past decades has led to an increase in medication use. The development of new and faster genotyping techniques has offered us measures to quantify genetic variation. Pharmacogenetics is a specialized research area that tries to unravel genetic variants associated with response to pharmacotherapy. Its ultimate goal is to prescribe drugs only to patients in whom there is a high probability of therapy efficacy and a very low risk of adverse events. To date, many asthma pharmacogenetic studies (as discussed in this paper) have been carried out. However, we are far from the clinical implication of genetic data in daily clinical practice for treatment of asthmatic patients. More studies have to be carried out for replications of previous findings. Furthermore, whole genome scans may be promising for the discovery of new drug targets. In addition there is need for the definition of response phenotypes which can be used as an objective endpoint. Furthermore, challenges

such as ethical issues including patient confidentiality and storing of DNA samples need to be overcome.

In other therapeutic areas genotyping before start of therapy and personalised prescribing is more common. For example, in the treatment of HIV with abacavir genotyping is used to select appropriate patients for therapy (patients with the variant genotype show adverse reaction to this therapy) [76]. Furthermore, genotype defined dosing could be used to find the optimal dose in anticoagulation therapy. A large European trial will investigate the added value of genotyping patients before starting anticoagulation therapy [77].

Part of the variation in response to pharmacotherapy is explained by genetic variation and with reducing cost and time of genotyping, it may be cost-effective to screen asthma patient before starting therapy in the future. However, for the reduction of airway inflammation in asthma there are not many alternatives when a patient is not responding to (inhaled) corticosteroids. Leukotriene antagonists could be used instead, but these drugs have not been proven as effective as corticosteroids. Therefore, it is very important to perform more pharmacogenomic studies to search for new drug targets and the development of new drugs to improve asthma therapy. The research field of pharmacogenetics is expanding and it is possible when new data on genetic variation in for example the corticosteroid pathway becomes available and novel therapies are developed, that knowledge of a patient's genotype will help to guide which patients will react best from innovative therapies.

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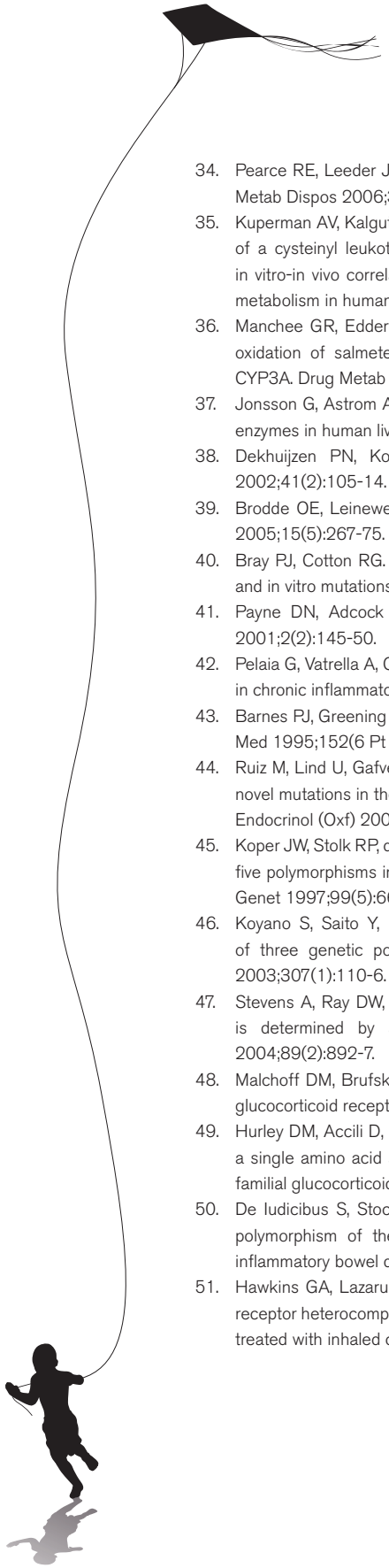

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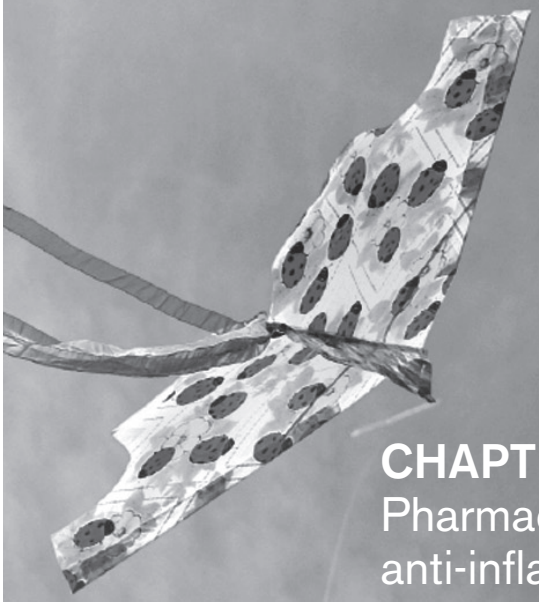

CHAPTER 12
Pharmacogenetics of anti-inflammatory treatment in children with asthma
- Rationale and design of the PACMAN cohort -

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ABSTRACT

Objective: To investigate effects of genetic variation on treatment response to asthma medication in children and to identify (profiles of) SNPs that characterize response phenotypes.
Methods: The Pharmacogenetics of Asthma medication in Children: Medication with ANti-inflammatory effects (PACMAN) study will be initiated in April 2009 as an observational retrospective pharmacy based study, including at least 1000 children with asthma medication (age 4-12 years). Data on respiratory symptoms and medication use behaviour will be collected using a questionnaire; complete medication histories will be extracted from the pharmacy information system; additional health information will be requested from the general practitioner; quality of inhalation technique and lung function measurements will be performed and saliva samples for DNA extraction and genotyping will be collected. Two groups of patients will be defined based on questionnaire data and lung function measurements: responders and non-responders to anti-inflammatory asthma treatment. These two groups will be compared with respect to genetic variation. Corrections will be made for potential confounding factors.
Results: The main study endpoint is treatment response, including asthma control, medication use, and fractional exhaled NO as a measure of airway inflammation. Whilst our focus is on genetic factors, this study allows us to also investigate other treatment response determinants, such as inhalation technique and therapy adherence.
Conclusion: Results from the PACMAN study could eventually lead to a more individualised therapy approach. PACMAN will focus on pharmacogenetics of asthma medication in children, while knowledge will be gained of relevant interest to the treatment of the asthma population at large.

INTRODUCTION

Allergic asthma is one of the most common chronic diseases in childhood [1]. It is an inflammatory disease of the airways, characterised physiologically by recurrent airway obstruction that resolves spontaneously or as a result of treatment [2 3]. Although the aetiology of asthma is still not fully clear, there are effective treatments to diminish the burden of disease. Standard treatment is based on regular use of inhaled corticosteroids (ICS) combined with short- or long-acting beta-agonists [4-8]. However, there is large variability in response to therapy, an inter-individual variability that has not been fully explained so far [5].
Asthma is a complex disease in which environmental and genetic factors contribute to its development, severity and progression. Likewise, genetic, environmental, disease associated and personal factors may contribute to an individual's response to asthma treatment. Indeed studies have suggested that a substantial fraction of the variation could be genetic [5 9] and several genes have been proposed to be associated with treatment response in asthma [10]. Pharmacogenetics holds potential for improving asthma management, since suboptimal effects of asthma therapy can hamper a person's wellbeing and instable asthma contributes disproportionately to healthcare costs through increased medical care and medication consumption. Thus there is need for further pharmacogenetic research to identify genetic variants that cause differences in treatment response which will then help to individualize therapy for asthma patients [11]. Mapping of single-nucleotide polymorphisms (SNPs) can be used to study the correlation between certain genotypes and the efficacy of drug therapy

[12 13]. Since chronic inflammation, airway remodelling and long-term treatment may affect treatment response we decided to focus on childhood asthma in particular. This has the advantage that the relation between treatment response and genetic factors is less biased than in adults and it also minimizes the influence of environmental factors such as smoking and occupational exposures. Within the Pharmacogenetics of Asthma medication in Children: Medication with ANti-inflammatory effects (PACMAN) study we focus on pharmacogenetics of asthma medication in children. This paper describes the design of the PACMAN cohort.

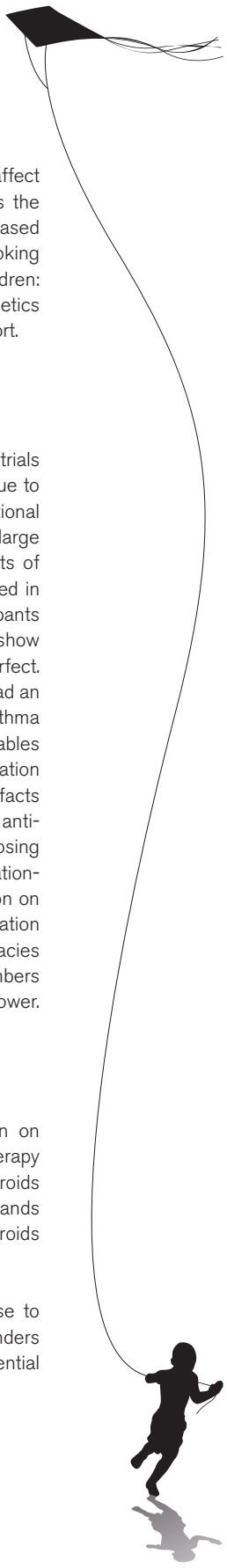
RATIONALE

Several asthma pharmacogenetic studies have been described, being mostly (clinical) trials [14-19]. These trials are limited in their ability to identify long-term treatment effects due to their relatively small sample size and short duration. Therefore well-designed observational studies may constitute a better approach to study long-term treatment efficacy. Several large population based observational studies have been published focusing on determinants of pediatric asthma or respiratory symptoms [19-22]. It is difficult to use the data collected in these studies for pharmacogenetic research, because only small numbers of participants regularly use asthma medication, especially in childhood. Besides this, previous studies show that the relationship between a diagnosis of asthma and asthma medication is far from perfect. Zuidgeest et al. showed that only 49% of the children who receive asthma medication had an asthma diagnosis [23]. This may also be due to the fact that it is difficult to diagnose asthma in pre-school children, because they are not able to perform a lung function test that enables to diagnose asthma with more certainty. However, concordance between asthma medication use and doctor-diagnosed asthma does not increase with age [23]. Based on these facts as observed in the literature, the focus of the PACMAN study will be the response to anti-asthma drugs in children, separated from all past clinical circumstantial criteria for diagnosing childhood asthma. The added value of our PACMAN study compared to existing (population-based observational) studies is that we will include children who use asthma medication on a regular basis instead of asthmatic children who only have one or two asthma medication prescription as a sort of trial medication. Inclusion of children will take place in pharmacies and therefore we are able to collect extensive medication dispense data and large numbers of treated children and therefore study response to asthma medication with increased power.

STUDY OBJECTIVE

The main objective of the PACMAN study is to define effects of genetic variation on pharmacological treatment response in children who use anti-inflammatory asthma therapy (inhaled corticosteroids and leukotriene antagonists). We will focus on inhaled corticosteroids (ICS), since leukotriene antagonists are not frequently prescribed in children in the Netherlands [24]. We will also include severe, difficult-to-treat patients who occasionally use oral steroids for asthma control in the analysis.

We aim to find SNPs (or profiles of SNPs) which are characteristic for the response to corticosteroid (or leukotriene antagonist) treatment. Children will be classified as responders or non-responders to therapy (see below) and adjustment will be made for potential



confounders. One very important factor that will be taken into account is therapy adherence. This can be measured by three elements: first, refilling of prescriptions (did parents pick up a prescription for their child at the pharmacy), second, adequate inhalation technique and third, questions on medication behaviour in the questionnaire (scoring for therapy adherence).
Primary objectives of the study are:
To describe differences in characteristics of children responding and non-responding to treatment

- To find pharmacogenetic associations between polymorphisms in genes involved in the corticosteroid (or leukotriene) pharmacological pathways and treatment response

Secondary objectives of the study are:

- To describe age-related patterns of asthma medication use
- To study the quality of therapy

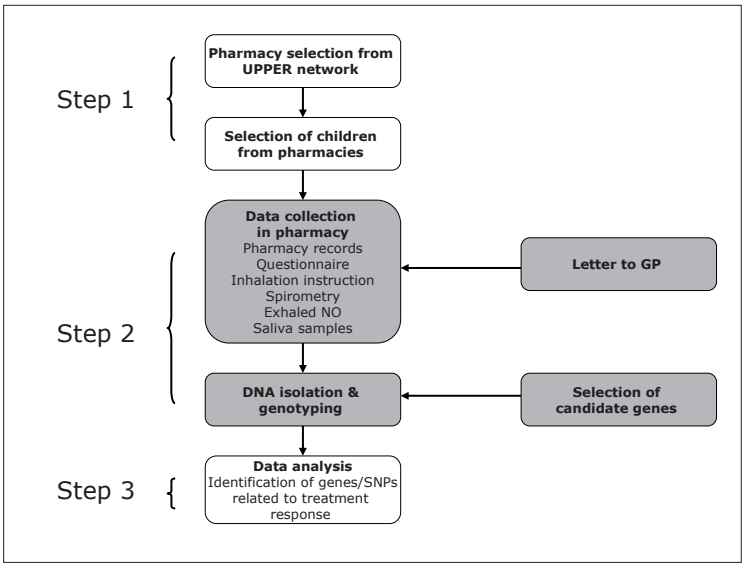


Figure 1. Enrolment and data collection

STUDY DESIGN

The PACMAN study is a cross-sectional cohort study and will include children who use asthma medication. Figure 1 presents an overview of enrolment and data collection. In the first step, we will define our study population, in the second step we will collect the data and as a third step we will perform data analysis.

SAMPLE SIZE CALCULATION

To detect a relative risk of 1.5 for non-response to therapy with a power of 80% and confidence level of 95%, 1000 subjects should be included in the study. This estimation is

based on the observation that approximately 10% of all asthma patients have difficult to treat or difficult to control asthma, i.e. non-responders to treatment [25 26] and the minor allele frequency of the SNP included in the model should be ≥ 0.15.

SELECTION OF STUDY SUBJECTS

Pharmacies will be selected from the Utrecht Pharmacy Panel for Education & Research (UPPER). UPPER is an electronic network of pharmacists (> 1400) who are interested in cooperating in epidemiologic or pharmaceutical research. UPPER also coordinates internships for the Pharmacy education. Children will be selected from pharmacies in different regions (urban, rural, city centre) in the Netherlands according the following inclusion criteria:

- Age 4-12 years
- At least 2 years of medication history available from the pharmacy information system
- At least 3 prescriptions for any asthma drug (ATC code R03) within the last 2 years and at least 1 prescription in the last 6 months

In this way we will recruit all categories of asthma patients, ranging from mild patients usually seen by a general practitioner (GP) to severe, hospital attending patients, and we will exclude patients with an occasional anti-asthma drug prescription (trial medication users). The selected children and their parents will be invited for participation in the PACMAN study. Written informed consent will be obtained from all participants.

DATA COLLECTION

Questionnaire

The parents are asked to fill in a questionnaire to gather information about general health, allergies, asthma and respiratory symptoms, asthma control, healthcare utilization for respiratory symptoms, medication use and compliance, believes on medicines, environmental (pets, environmental tobacco smoke exposure etc.) and socio-demographic factors. The questionnaire is similar to the respiratory questionnaire used in the PIAMA birth cohort study [20] that investigated the incidence of asthma and risk factors for development of asthma in childhood. The questions pertain to respiratory symptoms (wheezing, shortness of breath etc), limitations in daily activities and additional medication use because of respiratory symptoms during the different seasons in the past year. There are few additional questions on respiratory symptoms throughout the year (seasonal complaints).

Inhalation technique

Patients will be asked to demonstrate their inhalation technique. The efficient delivery of the inhaled drug remains highly technique dependent [27]. By scoring inhalation technique of all participants, stratification for inhalation technique will be possible in the analysis. We will make use of an inhaler specific inhalation control checklist developed by van der Palen et al. [28 29]. Most young children will use a medication chamber to abridge inhalation of medication. We will check if the chamber fits properly to the inhaler and score inhalation technique the same way as for children who do not use a medication chamber. The mean checklist score can be calculated and the patient's inhalation technique can be divided into good (≥ 80%), adequate (60-79%) and inadequate (< 60%) inhalation technique.

Lung function measurements

The child's lung function will be measured with the help of a hand-held diagnostic spirometer (Easy-One, NDD Medizintechnik, Zurich, Switzerland). This kind of spirometer meets American Thoracic Society (ATS) recommendation for diagnostic spirometry and has shown to be a reliable device which can be used in the field [30]. Spirometry is a non-invasive test which measures the volume of exhaled air after a maximal inhalation. We will use FEV_1, forced expiratory volume in 1 second, values to classify airway obstruction and will be expressed as percentage of predicted. Severity of lung function impairment will be categorised according ATS criteria [31]. Bronchodilating agents such as short-acting β-agonists and anti-cholinergic agents should be withheld 4 hours before testing and long-acting β-agonists 36 hours before testing.

Fractional exhaled nitric oxide (FeNO) will be measured with a hand-held analyzer (Niox Mino, Aerocrine, Solna, Sweden). This analyzer is suitable for measuring FeNO in children from approximately 4 years old. FeNO is easy to measure, higher levels correlate with airway inflammation in asthma, and occur during periods of uncontrolled asthma, while anti-inflammatory agents (inhaled corticosteroids) reduce FeNO levels. Therefore, FeNO levels can be used as an indicator of asthma control and severity [32 33]. For children, FeNo values can be classified in four categories: low (< 5 ppb), normal (5-15 ppb), high normal/increased (15-25 ppb) and high (> 25 ppb) [34]. Before performing these measurements, questions are asked about common cold and medication use in the past week. In a proportion of children, especially those aged under six years, there could be difficulties in performing lung function testing. Young children may have difficulties in performing controlled expiratory manoeuvres which hampers FeNO measurement with the Niox Mino. However, an earlier study among 71 subjects (aged 6-60 years) showed a success rate of >84% for both adults and children. However, younger children failed more frequently [35].

Medication history

Complete pharmacy records will be extracted from the pharmacy information system. In the Netherlands, the vast majority of the people registers only to one community pharmacy, independently of prescriber, and obtains all medication from that pharmacy [36]. Therefore, a complete medication history is available in the pharmacy database. Drugs used during hospital admissions and over-the-counter medication are not included in this database.

DNA isolation and genotyping

Saliva samples (2 mL) are collected for DNA extraction with the help of the Oragene DNA Self Collection kit (DNA Genotek, Inc. Ottawa, Ontario, Canada), according to the manufacturer's instructions, which provides samples with high DNA quality [37]. DNA will be isolated from the saliva samples and genotyped with a 384-SNP chip. 384 haplotype tagging SNPs will be determined in approximately 30 genes suggested to be involved in anti-asthma drug pathways. Main focus lies on genes in the corticosteroid pathway. Earlier studies have shown variation in the glucocorticoid receptor gene and other genes in this pathway such as *CRHR1*, *TBX21* and *FCER2* [9 14 38-43]. Furthermore, when there are sufficient numbers of children who use leukotriene antagonists, we will also focus on genes (such as *ALOX5*, *LTC4S* and *CYSLTR2*) in the leukotriene pathway, because they are potent inhibitors of the inflammatory response [44-49]. Although, a large population based study (n = 46371 children) in the Netherlands showed that only 0.1% of the children aged 1-17 years received a prescription for montelukast (a leukotriene antagonist) during a one year period (year 2001) [24].

Letter to general practitioner

As soon as a child is included a letter to the child's GP will be sent to collect additional information in his record or letters from a referral to a respiratory physician on asthma status (yes/no/uncertain), lung function in the past 12 months (spirometry and bronchial hyperresponsiveness) and allergic sensitisation (skin tests, serum total and specific IgE). This information will be used to establish whether a child is diagnosed with asthma or not.

DATA ANALYSIS

Definition of response phenotypes

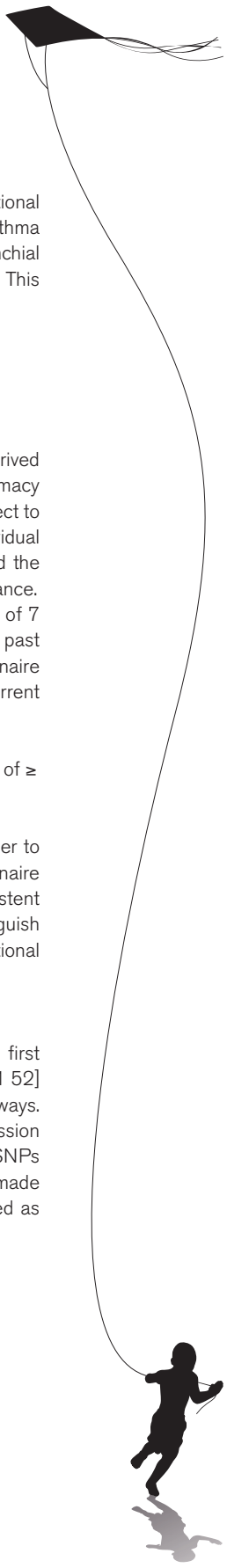
We will define two groups of patients: responders and non-responders based on data derived from the questionnaire, additional health information from the general practitioner, pharmacy records and lung function measurements. These two groups will be compared with respect to genetic variation. Non-compliant subjects will be excluded from further analysis. An individual is defined to be non-compliant when inhalation technique is scored as inadequate and the questions on therapy adherence and compliance in the questionnaire show non-compliance. Current asthma control (responder to anti-inflammatory therapy) is measured with help of 7 questions: 5 questions on respiratory symptoms, one on rescue bronchodilator use in the past month combined with FEV_1% predicted (based on Juniper's Asthma Control Questionnaire (ACQ) [50]) and FeNO (high FeNO levels are an indication of airway inflammation). Current asthma control is defined to be present if a patient shows:

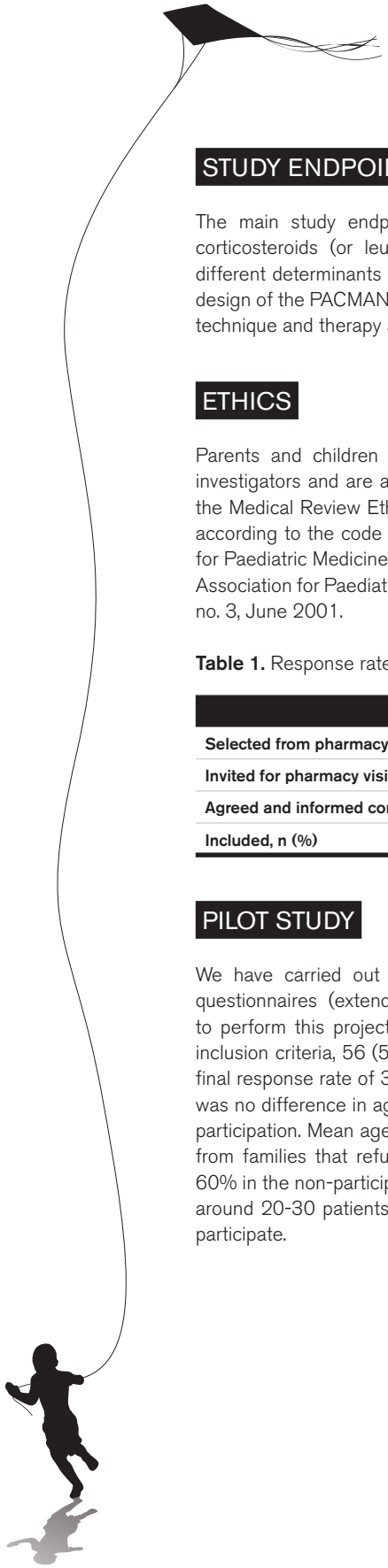
- Persistent use of inhaled corticosteroids (or leukotriene antagonists)
- Questionnaire based asthma control (ACQ): mean score on 7 questions ≤ 0.75 (score of ≥ 1.50 is an indication for not adequately controlled asthma)
- FeNO values < 25 ppb

If all of the above described criteria are present a child is currently defined as responder to therapy. Children with poor present asthma control are children who have poor questionnaire based asthma control (ACQ score > 1.50) and/or FeNO values > 25 ppb despite persistent medication use. These children will be defined as non-responders to therapy. To distinguish between asthma control (treatment response) and disease severity we will use the additional health information from the general practitioner.

Statistical analysis

In order to analyse all genetic variables (SNPs) we will use a two-step strategy. The first step will be graphical modelling. We will use approaches such as Bayesian Networks [51 52] and Multifactor Dimensionality Reduction [53 54] to build networks and visualize pathways. Finally, variables found important in this step will be used in conventional statistical regression methods. Multivariate analyse techniques will be used to study the association between SNPs or patterns of SNPs and response to treatment. Adjustments or stratifications will be made for potential disturbing confounding variables, such as atopy. Gender will be considered as an effectmodifier.





STUDY ENDPOINTS

The main study endpoint of the PACMAN cohort is treatment response to (inhaled) corticosteroids (or leukotriene antagonists). This endpoint enables the investigation of different determinants of treatment response. Genetic factors are our main interest, but the design of the PACMAN study allows us also to include other determinants, such as inhalation technique and therapy adherence.

ETHICS

Parents and children will be both orally and written informed about the study by the investigators and are asked to sign an informed consent. The study has been approved by the Medical Review Ethics Committee of the University Medical Centre Utrecht. We will act according to the code of conduct of objection by minors from the Netherlands Association for Paediatric Medicine. This code of conduct was approved by the Board of the Netherlands Association for Paediatric Medicine (NVK) on 21 May 2001 and published in NVK Newsletter no. 3, June 2001.

Table 1. Response rate pilot study

	Pharmacy 1	Pharmacy 2	Total
Selected from pharmacy information system, n (%)	63 (100)	47 (100)	110 (100)
Invited for pharmacy visit, n (%)	59 (94)	42 (89)	101 (92)
Agreed and informed consent, n (%)	28 (44)	28 (60)	56 (51)
Included, n (%)	18 (29)	19 (40)	37 (34)

PILOT STUDY

We have carried out a pilot study in two pharmacies in the Netherlands, testing the questionnaires (extended version of the PIAMA [20] questionnaire) and the possibility to perform this project in Dutch community pharmacies. A total of 110 children met the inclusion criteria, 56 (51%) children and parents agreed to participate in the study, giving a final response rate of 34% (Table 1) a reassuring result for this kind of research [55]. There was no difference in age or gender between children from families that agreed and refused participation. Mean age for participants was 7.6 ± 2.3 years and 7.3 ± 2.4 years for children from families that refused participation. 70% of the participants were boys, compared to 60% in the non-participating group. Based on the results of this pilot study, we estimate that around 20-30 patients per average sized pharmacy will fulfil our criteria and are willing to participate.

DISCUSSION

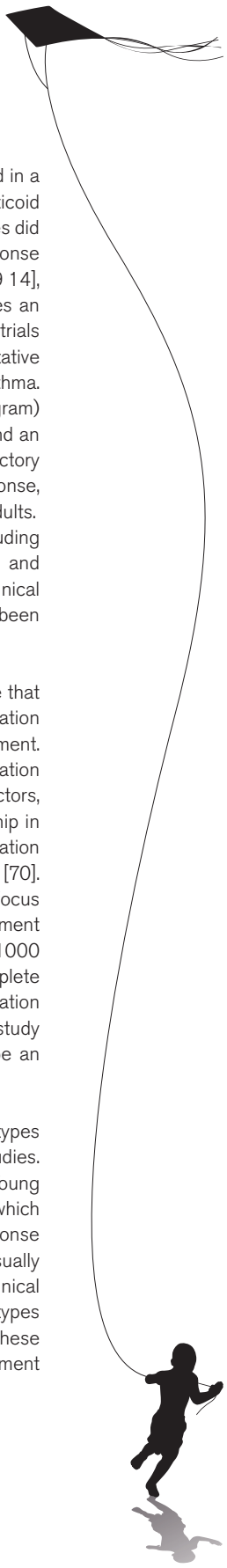
A considerable number of asthma pharmacogenetic studies have been designed as clinical trials so far. In these trials effects of polymorphisms in genes in specific drug pathways have been investigated with respect to treatment response, as measured by changes in lung function (FEV_1, FVC or PEF) or prevalence of clinical symptoms (number of exacerbations, days with respiratory symptoms etc.) after beta-agonist, corticosteroid or leukotriene antagonist use. Thus far, most studies on asthma pharmacogenetics have focussed on response to beta-agonists, because these drugs are widely used and have a direct therapeutic effect ('quick-relief medication'). The beta-adrenergic receptor gene was the first asthma pharmacogenetic locus to be studied and a lot of sequencing work has been done. Variation in the beta-adrenergic receptor (*ADRB2*) gene has been associated with altered response to beta-agonist [15-17 56-58]. In contrast to beta-agonists, which have only short-term effects, anti-inflammatory therapy is being used as long-term maintenance treatment in asthma. Corticosteroids taken by inhalation are the most effective and commonly used drugs for treatment of asthma. However in a subgroup of patients they may be associated with adverse effects or are unable to obtain sufficient asthma control. Therefore in some cases, leukotriene antagonists can be used. Leukotriene antagonist are orally administered and therefore generally preferred among paediatric patients and therefore indicated in children that are not able to inhale [59]. Since anti-inflammatory therapy is nowadays the mainstay of treatment in asthma, we focus in the PACMAN study on pharmacogenetics of anti-inflammatory therapy (inhaled corticosteroids and leukotriene antagonists).

Table 2. Pharmacogenetics of (inhaled) corticosteroids

Drug	Asthma population	Follow-up	Gene	Phenotype	Outcome	Ref
Budesonide	701 children	4 years	*TBX21*	PC20 change	Variant *TBX21* genotype associated with increased treatment response	[42]
Budesonide	311 children	4 years	*FCER2*	Exacerbation rate	Variant *FCER2* genotype associated with decreased treatment response	[43]
Flunisolide	382 adults	8 weeks	*STIP1*	FEV_1 change	Variant *STIP1* genotype associated with increased treatment response	[63]
Budesonide	311 children	8 weeks	*CRHR1*	FEV_1 change	Variant *CRHR1* genotype associated with increased treatment response	[14]
Flunisolide	470 adults	8 weeks	*CRHR1*	FEV_1 change	Variant *CRHR1* genotype associated with increased treatment response	[14]
Triamcinolone	336 adults	6 weeks	*CRHR1*	FEV_1 change	Variant *CRHR1* genotype associated with increased treatment response	[14]
Any ICS	164 adults	22 years	*CRHR1*	FEV_1 change	Variant *CRHR1* genotypes not associated with treatment response	[64]

Table 3. Pharmacogenetics of leukotriene antagonists

Drug	Asthma population	Follow-up	Gene	Phenotype	Outcome	Ref
Zileuton	577 adults	12 weeks	*ABCC1* *ALOX5* *LTC4S*	FEV_1 change	Variant *ALOX5* genotype associated with decreased treatment response. Variant *ABCC1* and *LTC4S* genotypes associated with increased treatment response	[65]
ABT-761	221 adults	2 weeks	*ALOX5*	FEV_1 change	Variant *ALOX5* genotype associated with decreased treatment response	[45]
Zafirlukast	23 adults	2 weeks	*LTC4S*	FEV_1, FVC, PEF change	Variant *LTC4S* genotype associated with increased treatment response	[66]
Pranlukast	50 Japanese adults	4 weeks	*LTC4S*	FEV_1 change	Variant *LTC4S* genotype associated with increased treatment response	[67]
Montelukast	61 adults	6 months	*ALOX5*	Exacerbation rate, FEV_1 change, SABA use	Variant *ALOX5* genotype associated with decreased treatment response	[49]
Montelukast	174 adults	12 weeks	*ALOX5* *CYSLTR2*	PEF, FEV_1 change	Variant *ALOX5* and *CYSLTR2* genotypes associated with increased treatment response	[48]
Montelukast	59 adults	8 weeks	*LTC4S*	PEF, FEV_1 change	Variant *LTC4S* genotype associated with increased treatment response	[47]
Montelukast	61 adults	6 months	*ALOX5* *LTA4H* *LTC4S* *MRP1*	Exacerbation rate, FEV_1 change	Variant *ALOX5, LTC4S* and *MRP1* genotypes associated with increased treatment response. Variant *LTA4H* genotype associated with decreased treatment response.	[46]
Montelukast	80 Korean children	8 weeks	*Il13*	FEV_1 change	Variant *IL13* genotype seems to be associated with increased treatment response	[68]
Montelukast	695 Korean children	8 weeks	*TBXA2R*	FEV_1 change	Variant *TBXA2R* may be a marker of treatment response	[69]



Differences in ICS or leukotriene antagonist treatment response have been investigated in a considerable number of pharmacogenetic studies. Several polymorphisms in the glucocorticoid receptor (*NR3C1*) gene have been described [38 60-62]. However, most of these studies did not address relevant clinical endpoints for asthma. Pharmacogenetic studies of ICS response in asthma have shown significant associations between polymorphisms in the *CRHR1* [9 14], *TBX21* [42], *FCER2* [43] and *STIP1* [63] gene and treatment response. Table 2 gives an overview of corticosteroid pharmacogenetic studies in asthma. Most studies are clinical trials with only short-term medication use or observation period and therefore not representative for daily clinical practice in which continuous use of ICS is prescribed for treatment of asthma. Two studies have longer follow-up; the CAMP (Childhood Asthma Management Program) study [14 42 43], a 4-year clinical trial of an inhaled steroid (budesonide) in children and an asthma cohort study in adults with 22 years follow-up [64]. These studies show contradictory results with respect to genetic variation in the *CRHR1* gene and ICS treatment response, which may be due to the fact that the first one investigated children an the other one adults. Table 3 gives an overview of leukotriene antagonist pharmacogenetic studies, including studies showing that variation in genes in the leukotriene pathway, such as *ALOX5* and *LTC4S* can alter response to leukotriene antagonists. Again, most of these studies are clinical trials with only a very short therapy period (mostly < 8 weeks) and only few studies have been performed in children.

Observational studies assessing pharmacogenetic profiles of drugs have the advantage that they can investigate considerable large numbers of asthmatics and they allow a longer duration of follow-up, thereby allowing not only short-term, but also long-term effects of treatment. Most pharmacogenetic studies have been performed in adult patients. In adults, the relation between genetic variation and treatment response may be biased by environmental factors, such as (passive) smoking, airway pollution and occupational exposures. The relationship in adults may also be biased (to a larger extent than in children) by years of suboptimal medication use which may lead to progressive loss of lung function as a result of airway remodelling [70]. The added value of our new cohort, PACMAN, to currently existing studies is that it will focus on children thereby increasing the power to detect associations between a specific treatment and genetic factors. Within PACMAN, complete medication histories will be collected of 1000 children who use asthma medication on a regular basis. The collection of these complete medication histories provides the opportunity to correct for additional respiratory medication use such as antibiotics, antihistamines or oral corticosteroids. Furthermore, we can study the initiation of therapy and monitor switches to other therapy regimes (which may be an indication for non-response).

There is need for a correct diagnosis and a solid definition of different response phenotypes in order to develop better pharmacogenetic research strategies in observational studies. Diagnostic dilemmas exist especially in paediatric asthma patients below the age of six. Young children are not able to perform lung function testing and symptoms are often transient which makes it difficult to make a valid diagnosis. Second, it is difficult to define treatment response as a true endpoint as it is intertwined with disease severity. Asthma control [50 71] is usually defined as a surrogate endpoint determined by parameters such as lung function, and clinical symptoms (exacerbations and night-time awakenings). The definition of response phenotypes is a big challenge in asthma pharmacogenetics and of utmost importance, because these phenotypes are used as clinical endpoint. Within the PACMAN study, we define treatment

response by as much information as possible: questionnaire data on asthma control combined with lung function and exhaled nitric oxide measurements.

At present, the effect of currently known genetic variation in asthma pharmacogenetic study results suggests that routine genotyping of certain subsets of patients before initiating therapy is not clinically relevant and cost-effective. However with improved diagnosis and better definitions of phenotypes, as well as increased knowledge which genes are responsible for variations in treatment response, combined with the reduction of time and costs of genotyping, it may be cost-effective to screen all patients before initiating therapy in the future. To reach this goal of personalized prescribing, more knowledge of genetic influences on treatment response and better phenotype definitions are necessary. PACMAN aims to contribute to this and will provide sufficient numbers of treated children to perform pharmacogenetic analyses, an important limitation of existing population-based asthma cohorts that are not focussing on pharmacogenetics. Finally, PACMAN includes real life observations that will ultimately give insight into the relevance of pharmacogenetic effects in asthma. Furthermore, the PACMAN-cohort is additive to the existing sets in focusing primarily on medication use.

Asthma is a disease that affects millions of children and adults worldwide, it is associated with decreased quality of life for the patient as well as high health care costs for society. Treatment is based on reduction of airway inflammation by use of anti-inflammatory drugs (mainly ICS and in some cases leukotriene antagonists) and use of short-acting beta-agonist use for symptomatic relief. Several studies have shown that, despite their efficacy to control asthma a substantial proportion of asthmatics do not reach sufficient control of their asthma with current therapeutic regime. Uncontrolled asthma causing recurrent asthma exacerbations contributes disproportionally to asthma health care costs through increased need of medical care and medication consumption. It is therefore of utmost importance to treat patients with efficacious drugs. Results from the PACMAN study could eventually lead to a more individualised therapy approach, not only for children, but also for adults.

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CHAPTER 13
FCER2 T2206C variant associated with chronic symptoms and exacerbations in steroid-treated asthmatic children

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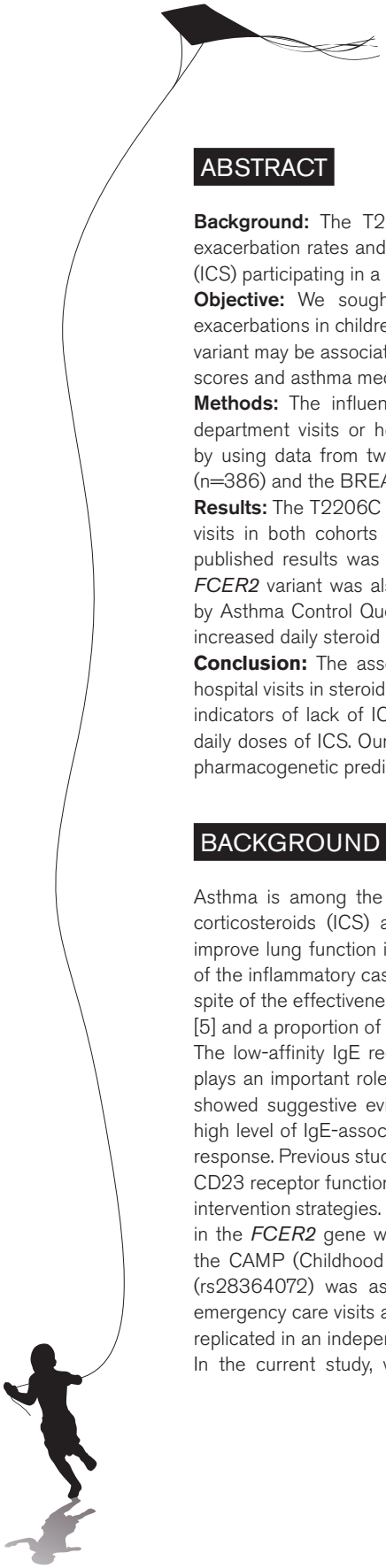


ABSTRACT

Background: The T2206C *FCER2* variant was previously associated with IgE levels, exacerbation rates and decreased *FCER2* expression in children on inhaled corticosteroids (ICS) participating in a clinical trial. This finding has not been replicated.

Objective: We sought to replicate the association between the *FCER2* gene and exacerbations in children with asthma. In addition, we tested the hypothesis that the T2206C variant may be associated with other markers of steroid resistance such as asthma symptom scores and asthma medication use.

Methods: The influence of the T2206C variant on asthma exacerbations (emergency department visits or hospitalisation), symptoms scores and medication use was explored by using data from two populations of asthmatic children using ICS: the PACMAN study (n=386) and the BREATHE study (n=939).

Results: The T2206C variant was associated with increased risk of asthma-related hospital visits in both cohorts (OR: 1.91, 95% CI: 1.08-3.40), and meta-analysis with previously published results was highly significant (OR: 2.38, 95% CI: 1.47-3.85, p = 0.0004). The *FCER2* variant was also associated with increased risk of uncontrolled asthma measured by Asthma Control Questionnaire (OR: 2.64, 95% CI: 1.00-6.98) and was associated with increased daily steroid dose (OR: 2.46, 95% CI: 1.38-4.39).

Conclusion: The association between the *FCER2* T2206C variant and asthma related hospital visits in steroid-treated asthma appears robus and may also be associated with other indicators of lack of ICS efficacy such as asthma symptoms and a requirement for higher daily doses of ICS. Our results suggest that the *FCER2* T2206C variant might be a useful pharmacogenetic predictor of steroid refractory patients.

BACKGROUND

Asthma is among the most common chronic diseases in children worldwide [1]. Inhaled corticosteroids (ICS) are first-line controller therapy to control airway inflammation and improve lung function in childhood asthma [2]. Corticosteroids suppress virtually every step of the inflammatory cascade and are therefore the cornerstone of asthma treatment [3 4]. In spite of the effectiveness of ICS in most asthmatic patients, individual response varies widely [5] and a proportion of patients suffers from uncontrolled asthma while on ICS [6 7].

The low-affinity IgE receptor (CD23), encoded by the Fc-fragment of IgE (*FCER2* gene) plays an important role in the regulation of IgE responses in asthma [8]. Laitinen et al. [9] showed suggestive evidence for an association between the *FCER2* gene region and a high level of IgE-associated traits. Receptor activation results in down-regulation of the IgE response. Previous studies have shown that corticosteroids influence *FCER2* expression and CD23 receptor function [10]. The *FCER2* receptor may therefore be a target for therapeutic intervention strategies. Previously, Tantisira et al. [11] showed associations of genetic variation in the *FCER2* gene with treatment response in asthmatic children on ICS participating in the CAMP (Childhood Asthma Management Program) clinical trial. The *FCER2* T2206C (rs28364072) was associated with IgE levels and severe exacerbations (measured by emergency care visits and/or hospitalisation) over a 4-year period. This finding has not been replicated in an independent population.

In the current study, we also tested the hypothesis that variation in the *FCER2* gene

contributes to variation in ICS treatment response in asthmatics. We sought to replicate the previous described association with severe asthma exacerbations and in addition, we studied the association between asthma symptoms and asthma medication use and genetic variation in the *FCER2* gene.

METHODS

For this study we used data from two cohorts of asthmatic children. We included 386 children (4-12 years) participating in the on-going PACMAN (Pharmacogenetics of Asthma medication in Children: Medication with ANti-inflammatory effects)-cohort study. Details of this study have been described elsewhere [12]. Briefly, children who were regular users of asthma medication (≥ 3 prescriptions within the last 2 years and ≥ 1 prescription in the last 6 months) were selected from community pharmacies in the Netherlands. Selected children and their parents were invited for a visit to their own community pharmacy. Written informed consent was obtained from all participants. During the pharmacy visit, information was collected on general health, asthma symptoms and medication use over the preceding 12 months. The questionnaire filled in during the pharmacy visit contained detailed questions regarding the presence of asthma symptoms, limitations in activities, sleep disturbances and extra medication use during the different seasons (spring, summer, autumn, winter). Saliva samples were collected for DNA extraction (Oragene DNA Self Collection kit, DNA Genotek, Inc., Ontaria, Canada). The PACMAN-cohort study was approved by the Medical Ethics Committee of the University Medical Centre Utrecht. We defined a population using ICS on a regular basis according to the British Thoracic Society (BTS) guidelines [13] as follows: step 0, no use of inhaled albuterol on demand in the past month, step 1, inhaled albuterol as-needed, step 2, step 1 plus regular ICS, step 3, step 2 plus regular inhaled salmeterol, step 4, step 3 plus oral montelukast. All selected children were on BTS treatment step 2, 3 or 4 and of Northern European origin.

The second population we used to study genetic variation related to ICS treatment outcome were children and young adults (3-22 years) with physician-diagnosed asthma (n = 939) recruited into the BREATHE study [14 15]. Participants attended primary or secondary clinics in either Tayside or Dumfries (Scotland, United Kingdom). A detailed history was obtained, including information on treatment and asthma exacerbations over the preceding 6 months. A DNA sample was collected by using mouthwash and prepared with the Qiagen DNeasy 96 kit (Qiagen GmbH, Hilden, Germany). The Tayside Committee on Medical Research Ethics approved the BREATHE study. For the present study we defined a subpopulation using ICS on a regular basis according to the British Thoracic Society (BTS) guidelines (BTS treatment steps 2, 3 or 4) [13]. In both studies all participants were of Northern European origin.

Definition of outcome

Within the PACMAN-cohort study, the following indicators regarding asthma exacerbations during the preceding 12 months were available: (1) emergency department visits related to asthma or (2) prescribed courses of oral steroids. Within the BREATHE study, an asthma exacerbation was defined as the presence of one or more of the following indicators during the preceding 6 months: (1) asthma-related hospital admission or (2) prescribed course of oral steroids. Because we were analyzing a relatively rare outcome, severe asthma exacerbations, we combined the data from the two cohorts and used the following outcome definitions as

measure for severe exacerbations: (1) asthma related hospital visits (emergency department visits or hospitalisations), (2) oral steroid courses and (3) any exacerbation (asthma related hospital visits and/or prescribed courses of oral steroids).
Respiratory symptoms (wheeze, shortness of breath and cough), asthma-related sleep disturbances, asthma-related limitations in daily activities and additional (airway) medication use during the preceding 12 months were defined as measures of asthma control in the PACMAN cohort. Furthermore, we defined current asthma control by means of the validated Asthma Control Questionnaire (ACQ) [16 17]. Within the BREATHE study, we defined daily bronchodilator use (SABA) and high daily doses of ICS (defined as a daily dose >400 mcg/day, which is more than advised in BTS treatment guidelines) as indicators of poor treatment response.

Genotyping

DNA extraction was performed according to the protocol provided by the manufacturer [18]. Genotyping for rs28364072 (*FCER2* T2206C variant) was done by using a TaqMan-based allelic discrimination assay with a 7700 Sequence Detection System (Applied Biosystems, Foster City, California, US). The genotype distribution of the *FCER2* SNP was in Hardy Weinberg equilibrium (>0.05) and genotype call rates were > 97%.

Statistical analysis

Our primary analysis involved testing the association between the genotype of the *FCER2* gene and severe asthma exacerbations in all asthma patients using ICS. Because of the relatively low frequency of severe exacerbations in our two cohorts, data were pooled and analyzed together. Logistic regression analysis, controlling for age, sex and BTS treatment steps, was used to calculate odds ratios (OR) for asthma treatment outcomes with their corresponding 95% confidence intervals (CI) and P-values. All analyses show the OR for those homozygous for the T2206C mutant allele compared with all other T2206C genotypes. Statistical analysis was carried out with SPSS for Macintosh version 18 (SPSS, Inc, Chicago, Ill, US). We pooled the ORs from our study with the association estimates found in the CAMP trial. For this meta-analysis we used the methods described for meta-analysis by Fleiss [19].

RESULTS

The general characteristics of both study populations are shown in Table 1. In both cohorts, around 60% of the participants were males. Children included within the PACMAN-cohort study were slightly younger (as a result of design of the study) compared to the BREATHE study. Furthermore, children participating in the PACMAN-cohort had a higher prevalence of atopic disorders, such as atopic eczema (69.3% vs. 53.1%) and allergic rhinitis (48.4% vs. 22.6%) when compared to children recruited into the BREATHE study. Most children were on BTS treatment step 2 (as-needed short-acting beta-agonist use and regular ICS). The genotype distribution was similar for both cohorts ($p > 0.05$, Figure 1); in both cohorts approximately 8% of the participants were homozygous carriers of the *FCER2* T2206C variant allele.

Table 1. Baseline characteristics study population

	PACMAN cohort (n = 386)	BREATHE cohort (n = 939)
Child characteristics		
Age, mean (SD)	8.4 (2.5)	10.0 (3.9)
Male gender, %	57.9	60.6
Atopic eczema, %	69.3	53.1
Allergic rhinitis,%	48.4	22.6
BMI, mean (SD)	16.9 (2.7)	18.9 (4.4)
Family history		
Paternal asthma	27.8	20.4
Paternal eczema	28.4	7.1
Maternal asthma	31.2	24.6
Maternal eczema	47.6	14.0
Asthma exacerbation preceding 12 months / 6 months		
ER visit / Hospital admission	7.6	12.3
Oral steroid use	9.1	23.1
Any asthma exacerbations	13.3	24.1
BTS treatment step		
2	78.8	71.0
3	18.4	17.1
4	2.8	11.8

Table 2. *FCER2* variant risk of exacerbations

Outcome	Crude OR (95% CI)	p	Adjusted OR (95% CI)*	p
Oral steroid use	1.17 (0.70 – 1.97)	0.54	1.04 (0.61 – 1.76)	0.89
Hospital visit**	2.00 (1.14 – 3.50)	0.02	1.91 (1.08 – 3.40)	0.03
Any exacerbation	1.46 (0.90 – 2.35)	0.13	1.31 (0.80 – 2.14)	0.28

* All analyses show the OR for those homozygous for the T2206C mutant allele compared with all other T2206C genotypes, controlling for age, gender and BTS treatment step. ** Asthma-related ER visit or hospitalisation

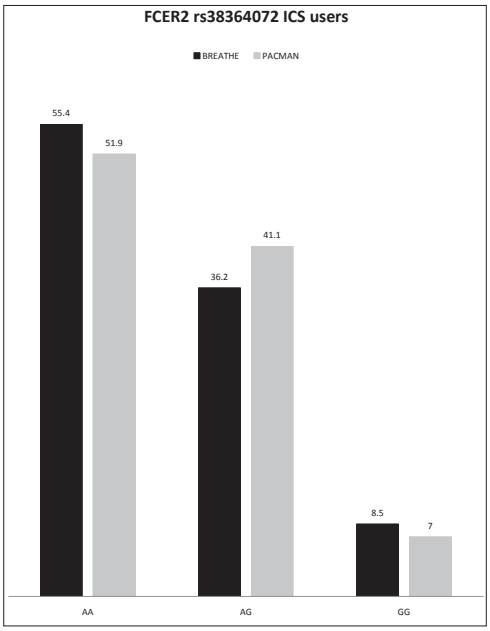


Figure 1. Genotype distributions

Table 3. *FCER2* variant risk of uncontrolled asthma symptoms PACMAN

Outcome	Crude OR (95% CI)	p	Adjusted OR (95% CI)*	p
Previous week				
Uncontrolled asthma (ACQ)	**2.30 (0.89 – 5.95)**	**0.09**	**2.64 (1.00 – 6.98)**	**0.05**
Previous year				
Wheeze	**3.32 (1.37 – 8.05)**	**0.008**	**3.43 (1.39 – 8.44)**	**0.007**
Shortness of breath	**2.50 (1.02 – 6.15)**	**0.05**	**2.64 (1.07 – 6.53)**	**0.04**
Cough	**3.29 (1.30 – 8.31)**	**0.01**	**3.22 (1.26 – 8.24)**	**0.02**
Sleep disturbances	**2.94 (1.20 – 7.23)**	**0.02**	**2.96 (1.19 – 7.38)**	**0.02**
Limitation in daily activities	1.94 (0.78 – 4.81)	0.16	1.98 (0.79 – 5.00)	0.15
Additional medication use	2.33 (0.97 – 5.58)	0.06	2.37 (0.98 – 5.73)	0.06

* All analyses show the OR for those homozygous for the T2206C mutant allele compared with all other T2206C genotypes, controlling for age, gender and BTS treatment step

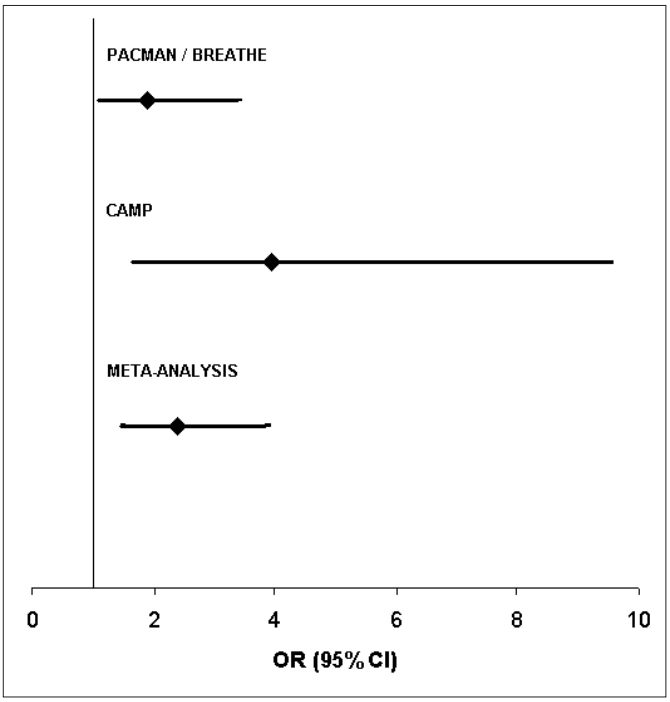


Figure 2. *FCER2* variant and severe exacerbations: meta-analysis

Association of *FCER2* variant with exacerbations

The multivariable association of the *FCER2* T2206 variant with exacerbations, defined by either prescribed courses of oral steroids or asthma related hospital visits are shown in Table 2. For both the PACMAN and BREATHE study we showed a trend towards a higher risk of hospital visits for carriers of the variant allele. By combining data from the two cohorts, we found the T2206C variant to be associated with an increased risk of severe exacerbations defined by asthma-related hospital visits (OR: 1.91, 95%CI: 1.08-3.40). Overall, we did not observe an association with prescribed oral steroid courses ($p = 0.89$).

We pooled the results from the CAMP study and our study for white subjects (Figure 2) and showed a combined effect estimate. Homozygous carriers of the variant allele had over twice the risk of severe exacerbations compared to heterozygous and homozygous wild type carriers (OR: 2.38, 95%CI: 1.47 – 3.85, $p = 0.0004$).

Association of *FCER2* variant with asthma symptoms

Within the PACMAN cohort study we also tested the association between the *FCER2* variant and asthma control outcomes (Table 3). The T2206C *FCER2* variant was associated with a higher risk of uncontrolled asthma (OR: 2.64, 95% CI: 1.00 – 6.98). Homozygous carriers of the variant allele had an increased risk of wheeze (OR: 3.43, 95% CI: 1.39-8.44), shortness of breath (OR: 2.64, 95% CI: 1.07 – 6.53) coughing in the preceding 12 months (OR: 3.22, 95% CI: 1.26-8.24) and asthma-related sleep disturbances (OR: 2.96 95% CI: 1.19-7.38).

Association of *FCER2* variant with steroid dosing

The T2206C variant was associated with increased daily dose of ICS in the BREATHE study (OR: 2.46, 95% CI: 1.38-4.39) (Table 4).

Table 4. *FCER2* variant and asthma medication use BREATHE

Outcome*	Crude OR (95% CI)	P	Adjusted OR (95% CI)*	P
Daily SABA use	1.61 (0.93 – 2.67)	0.11	1.61 (0.93 – 2.76)	0.09
High daily ICS dose***	2.27 (1.29 – 4.00)	0.005	2.46 (1.38 – 4.39)	0.002

* During the preceding 6 months, ** All analyses show the OR for those homozygous for the T2206C mutant allele compared with all other T2206C genotypes, controlling for age and gender. *** More than advised in guidelines

DISCUSSION

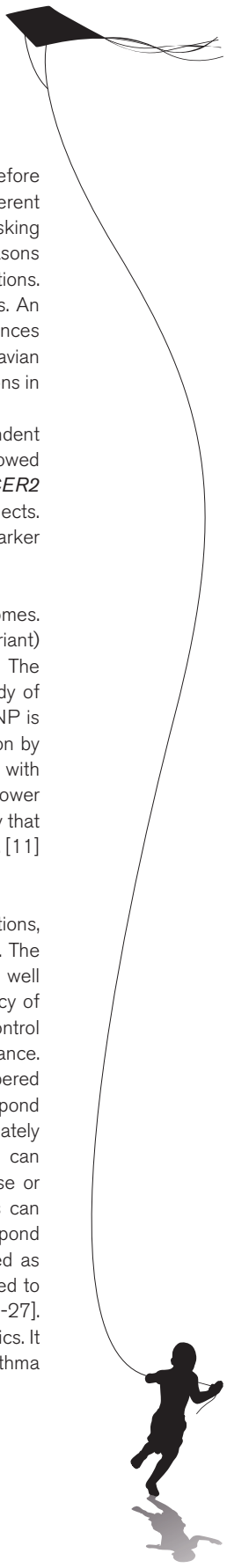
We have shown the ***FCER2*** T2206C variant to be associated with an increased risk of asthma-related hospital visits (asthma-related hospital admission or emergency department visits) in asthmatic children on ICS. Previously, Tantisira and co-workers [11] also described the association of the T2206C ***FCER2*** genotype with severe exacerbations over a 4-year period in children participating in the Childhood Asthma Management Program (CAMP). This finding had however not been replicated in a second population, presumably due to the lack of availability of suitable study populations at that time. Within the present study, we were able to replicate this previous association with severe exacerbations in an independent cohort of children with asthma on ICS. In addition, we showed that homozygous carriers of the variant allele are at a higher risk of experiencing asthma symptoms (wheeze, shortness of breath and cough) and asthma-related sleep disturbances compared to carriers of all other genotypic variants. These data regarding asthma symptoms were only available for participants in the PACMAN-cohort study. Asthma symptoms are one of the most important clinical parameters used for asthma management by the (general) physician and may therefore also be useful as endpoint for pharmacogenetics [13 20]. This data was not available for the BREATHE study. However, we did show an association between the T2206C variant allele and need for increasing daily ICS dose in the BREATHE study.

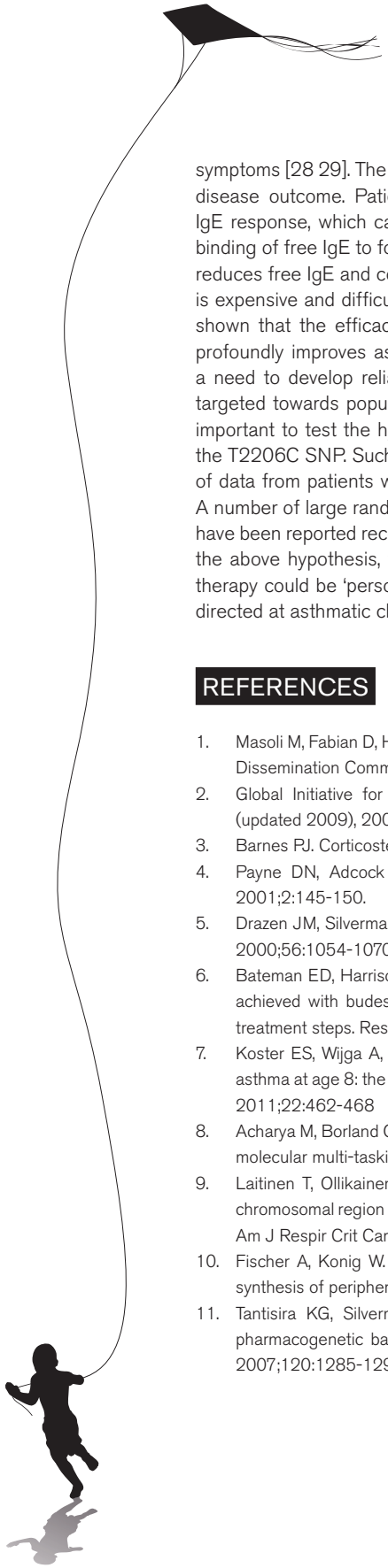
Within our final study population, we only had data available on the preceding 6 months for the BREATHE study and preceding 12 months for the PACMAN cohort study whilst the CAMP trial had information on exacerbations for the previous 4 years, which makes the occurrence of an exacerbation more likely. Uijen et al. [21] reported very low hospital admission rates for children in the general population with respiratory diseases, such as asthma, which makes this outcome measure less suitable for effectiveness studies in a primary care setting. This was also true in our two cohorts and therefore, in the present study, we had to combine data from the two different studies. The characteristics of the two study populations were very similar; we only included participants of Northern European origin (participants from a single region in Scotland and a population from the Netherlands) and we did not observe a difference in allele

frequency for the *FCER2* variant under study between the two populations. We therefore believe the data could be analysed in combination. We did however observe a different prevalence of atopic disorders. Atopy was measured in the BREATHE study by asking questions similar to the protocol used for the PACMAN study. We do not know the reasons for the differences in prevalence of eczema and allergic rhinitis between the two populations. However, atopic diseases are likely to result from major gene-environment interactions. An example is filaggrin-related allergic disease in childhood. There are significant differences in the prevalence of filaggrin gene defects between the Scottish, English and Scandinavian populations [22-24]. A more complete picture of the relative influence of these interactions in different populations is likely to emerge in subsequent years.
Data from participants not of Northern European origin were not sufficient for independent analysis in our study. The previous study of Tantisira and co-workers [11] also showed associations between asthma-related hospital admissions and carrying the T2206C *FCER2* variant in children of African American origin. The effect sizes were similar for white subjects. In African Americans, the T2206C variant was more common, which might make this marker even more important for this population.

There are not many studies on *FCER2* gene function in relation to asthma treatment outcomes. In the current study, we only investigated a single SNP in the *FCER2* gene (T2206C variant) as this variant had the strongest association in a previous pharmacogenetic study. The T2206C variant might be the causal variant or a strongly associated marker. The study of Tantisira indicates however that the T2206C variant might be the causal SNP. This SNP is located close to an exonic region and may therefore influence *FCER2* gene expression by altered splicing. Furthermore, it has been shown that the T2206C variant is associated with altered gene expression in lymphoblastoid cell lines. Gene expression was significantly lower in those homozygous for the mutant allele compared to the other genotypes. It is unlikely that another *FCER2* SNP with a MAF of >0.15 is the causal variant given that Tantisira et al. [11] found the strongest association with the T2206C polymorphism.

In conclusion, the *FCER2* T2206C variant is a marker associated with severe exacerbations, asthma symptoms and need for increased daily ICS dose in asthmatic children on ICS. The association with exacerbations was found in children participating in a clinical trial as well as in children participating in observational research. The relatively high allele frequency of the T2206C variant combined with the associations with several features of asthma control (symptoms, exacerbations and medication use) could make this marker of clinical importance. To date, implementation of asthma pharmacogenetics in daily clinical practice is hampered by the fact that there are not many treatment alternatives for patients who do not respond adequately to standard treatment. Early identification of patients who do not adequately respond to (ICS) treatment may reduce asthma-related mortality, as these patients can be referred to specialised care at an early disease stage and increasing the ICS dose or additional medication, such as add-on therapy with LABA or leukotriene antagonists can be started. *FCER2* polymorphism screening could predict patients more likely to respond to IgE-mediated therapies. Recently, anti-IgE therapy (omalizumab) has been approved as new anti-inflammatory treatment strategy in asthma. Treatment with omalizumab showed to improve asthma outcomes in both children and adult patients with severe asthma [25-27]. The IgE receptor plays an important role in the regulation of the IgE response in asthmatics. It has been shown that higher IgE levels correlate with more severe asthma and clinical asthma





symptoms [28 29]. The T2206C SNP may alter *FCER2* gene expression to influence asthma disease outcome. Patients who are carriers of the T2206C variant may have increased IgE response, which cannot be adequately managed by ICS. Anti-IgE therapy leads to the binding of free IgE to form IgG-IgE complexes that are not able to bind to IgE receptors. This reduces free IgE and consequently reduces asthma-related symptoms [30]. Anti-IgE therapy is expensive and difficult to administer (e.g. requiring regular injections). Recent studies has shown that the efficacy of anti-IgE therapy is variable [26 31 32], although this therapy profoundly improves asthma control and quality-of-life in a proportion of patients. There is a need to develop reliable biomarkers that predict efficacy so that therapy can be better targeted towards populations that are most likely to benefit. Our findings suggest that it is important to test the hypothesis that anti-IgE therapy is more effective in patients carrying the T2206C SNP. Such testing might be possible through post-hoc genotyping and analysis of data from patients who have participated in recent reported randomized controlled trials. A number of large randomized controlled trials testing the efficacy and safety of omalizumab have been reported recently [26 31 32]. If such analyses is performed and is found to support the above hypothesis, there will be a need to perform studies to explore whether anti-IgE therapy could be 'personalised', through further prospective clinical trials of anti-IgE therapy directed at asthmatic children stratified according to T2206C SNP genotype.

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APPENDIX

Table 1. *FCER2* variant exacerbation risk: PACMAN (n = 386)

Outcome*	Crude OR (95% CI)	p	Adjusted OR (95% CI)**	p
Oral steroid use	2.74 (0.86 – 8.83)	0.09	2.75 (0.83 – 9.12)	0.10
ER visit	3.40 (1.04 – 11.09)	0.04	2.94 (0.86 – 10.06)	0.09
Any exacerbation	2.32 (0.80 – 6.69)	0.12	2.21 (0.75 – 6.57)	0.15

* During the preceding 12 months, ** All analyses show the OR for those homozygous for the T2206C mutant allele compared with all other T2206C genotypes, controlling for age and gender.

Table 2. *FCER2* variant exacerbation risk: BREATHE (n = 939)

Outcome*	Crude OR (95% CI)	p	Adjusted OR (95% CI)**	p
Oral steroid use	1.09 (0.60 – 1.96)	0.79	0.91 (0.50 – 1.68)	0.78
ER visit	1.79 (0.92 – 3.50)	0.09	1.72 (0.86 – 3.44)	0.12
Any exacerbation	1.37 (0.78 – 2.41)	0.28	1.17 (0.65 – 2.09)	0.61

* During the preceding 6 months, ** All analyses show the OR for those homozygous for the T2206C mutant allele compared with all other T2206C genotypes, controlling for age and gender.

CHAPTER 14
The role of corticosteroid receptor complex genes in steroid response

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ABSTRACT

Background: Inhaled corticosteroids (ICS) have become first-line controller therapy for asthma. Individual response to ICS varies widely. Part of this observed variation may be due to genetic variation.
Objective: To study genetic variation in genes involved in the corticosteroid receptor complex in relation to ICS treatment response in paediatric asthmatics.
Methods: We conducted an analysis to screen 58 single nucleotide polymorphisms (SNPs) from 9 candidate genes for association with ICS treatment response in children participating in the PACMAN-cohort study. A total of 48 SNPs (82.8%) passed quality control. Logistic regression analysis was used to calculate odds ratios (OR) for asthma treatment outcomes with their corresponding 95% confidence intervals (CI) and p-values. The Bonferroni corrected p-value for statistical significance was set at 2.08 * 10-4.
Results: None of the pharmacogenetic associations met the Bonferroni corrected p-value threshold. We showed suggestive p-values for the *NR3C1*, *TBP* and *CREBBP* gene. The most significant association was found between exacerbations (defined as prescribed courses of oral steroids) and *NR3C1* rs10477211 (OR: 2.1, 95% CI: 1.3 - 3.3, p = 0.002).
Conclusion: We did not identify any SNPs significantly associated with steroid response. However, future meta-analysis of the data should reveal whether the most significant genes found in this study are truly associated or false positive findings.

BACKGROUND

Inhaled corticosteroids (ICS) have become first-line controller therapy to control airway inflammation and to improve lung function [1-5]. They suppress virtually every step of the inflammatory cascade and are therefore the cornerstone of asthma treatment. In spite of the effectiveness of corticosteroids in most asthmatic patients, individual response to ICS varies widely [6-8]. It has been suggested that a large part of this observed variation in treatment response may be due to genetic variation [9].
Glucocorticosteroids are liposoluble hormones that diffuse across the cell membranes to bind to the cytoplasmic glucocorticoid receptors (GR). The GRs are protected by chaperone proteins, after binding of the corticosteroids to the GRs, changes in structure of the receptor result in dissociation of the chaperon proteins and transport of the receptor-corticosteroid complex into the nucleus, where it binds to DNA of corticosteroid responsive genes [6-8]. Between 10 and 100 genes are supposed to be directly regulated by the glucocorticocoid receptor and many other genes are indirectly regulated. Variation in genes involved in the corticosteroid receptor complex, which are involved in pharmacodynamic actions may therefore contribute to the individual variation in ICS treatment response.
Genetic variation in the glucocorticosteroid receptor gene (*NR3C1*) has previously been associated with response to steroid therapy [10-15], although results were not always consistent. Not many previous studies have assessed the role of variation in the genes involved in the receptor-complex related to steroid treatment response. Hawkins et al. [16] evaluated 8 genes who are part of the glucocorticoid receptor complex and only showed positive correlations for one gene (*STIP1*). Variation in *SERPINA6*, one of the genes involved in the corticosteroid receptor complex has been previously associated with lack steroid binding activity [17].

In this study we hypothesize that variation in genes involved in the corticosteroid receptor complex may contribute to the variation in ICS treatment response in asthmatic children. We conducted an analysis to screen 58 single nucleotide polymorphisms (SNPs) from 9 candidate genes for association with treatment response to inhaled corticosteroids (ICS) in a population including (paediatric) asthma patients.

METHODS

Design and setting

The PACMAN-cohort study was used to study genetic variants related to treatment outcome [18]. Children (aged 4 - 12 years) who were regular users of asthma medication participated in this study. During a pharmacy visit, information was collected on general health, asthma symptoms, therapy adherence and medication use. Inhalation technique was scored and fractional exhaled nitric oxide levels were measured. Saliva samples were collected for DNA extraction (Oragene DNA Self Collection kit, DNA Genotek, Inc., Ontaria, Canada). For the present study we defined a population using ICS on a regular basis in the previous 12 months according to the British Thoracic Society (BTS) treatment guidelines (which correspond with the Dutch treatment guidelines) [3-5]. The Medical Ethics Committee of the University Medical Centre Utrecht has approved the PACMAN cohort study.

Selection of genes and SNPs

DNA extraction and genotyping were performed according to the manufacturers protocol [19]. In total, we selected 58 SNPs in 9 candidate genes (*SERPINA6, HSPCA, HSPA4, KFBP4, ST13, CREBBP, TBP, NCOA3, NR3C1*) involved in the corticosteroid-receptor complex. Genotyping for 52 SNPs was performed using the Seqeunom Mass Array platform (Sequenom, San Diego, California, US). For the remaining 6 SNPs we used a TaqMan-based allelic discrimination assay with a 7700 Sequence Detection System (Applied Biosystems, Foster City, California, US). Genotype calls of all SNPs were examined for their quality. SNPs with a less than 95% call rate were excluded and for the final analysis, we only included SNPs in Hardy-Weinberg equilibrium ($p > 0.05$). A total of 48 SNPs (82.8%) passed this quality control.

Definition of outcome

As outcome measure for this pharmacogenetic study we used the following three definitions: (1) asthma exacerbations during the preceding 12 months defined as emergency department visits related to asthma or prescribed courses of oral steroids, (2) uncontrolled asthma defined by means of the Asthma Control Questionnaire (ACQ) and (3) asthma symptoms (wheeze or shortness of breath) during the preceding 12 months.

Statistical analysis

Our primary analysis involved testing the association between the genotype of the above described genes and asthma treatment outcomes in all asthma patients using ICS. Logistic regression analysis was used to calculate odds ratios (OR) for asthma treatment outcomes with their corresponding 95% confidence intervals (CI) and p-values. Corrections were made for age and gender. Statistical analysis was carried out with SPSS for Macintosh version 18 (SPSS, Inc, Chicago, Ill, US). The Bonferroni corrected (48 tests * 5 outcomes = 240 tests) p-value for statistical significance was set at 2.08 * 10-4.

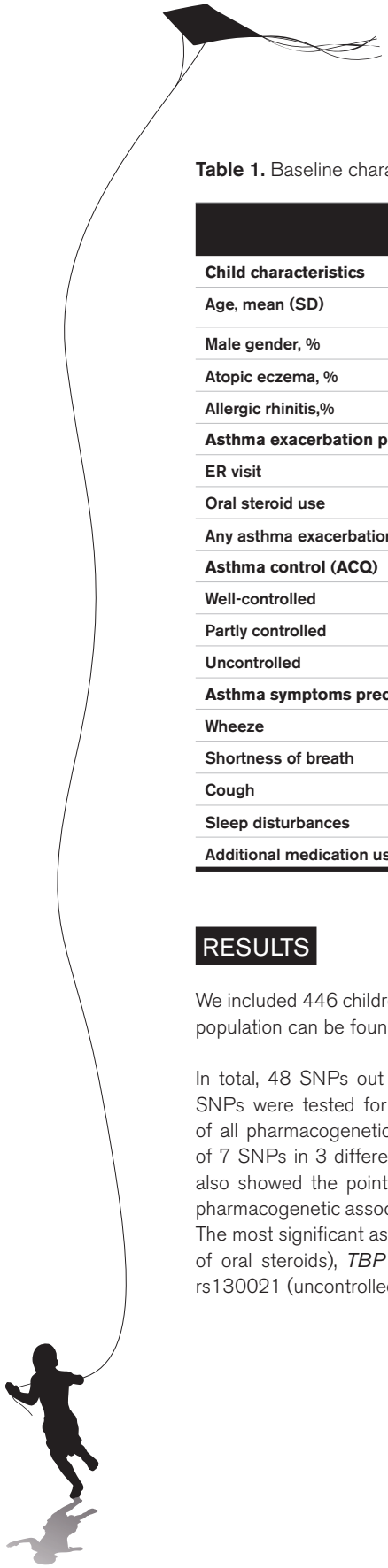

Table 1. Baseline characteristics study population

	Study population (n = 446)
Child characteristics	
Age, mean (SD)	8.4 (2.5)
Male gender, %	60.0
Atopic eczema, %	68.3
Allergic rhinitis,%	50.3
Asthma exacerbation preceding 12 months	
ER visit	7.4
Oral steroid use	9.0
Any asthma exacerbation	12.9
Asthma control (ACQ)	
Well-controlled	53.2
Partly controlled	24.4
Uncontrolled	22.4
Asthma symptoms preceding year	
Wheeze	22.9
Shortness of breath	43.0
Cough	43.7
Sleep disturbances	23.8
Additional medication use	35.4

RESULTS

We included 446 children who used ICS during the preceding year. The characteristics of this population can be found in Table 1.

In total, 48 SNPs out of the 58 genotyped SNPs (82.8%) passed quality control. These SNPs were tested for their association with steroid response. Table 2 gives an overview of all pharmacogenetic associations with a p-value of less than 0.05, representing a total of 7 SNPs in 3 different genes. For genetic associations with a p-value less than 0.05 we also showed the point estimates for the other asthma outcomes we tested. None of the pharmacogenetic associations met the Bonferroni corrected p-value threshold of 2.08 * 10-4. The most significant associations were found with *NR3C1* rs10477211 (prescribed courses of oral steroids), *TBP* rs12717 (wheeze), *CREBBP* rs129968 (wheeze) and *CREBBP* rs130021 (uncontrolled asthma).

Table 2. Pharmacogenetic associations with p-value smaller than 0.05

Gene	SNP	Tested outcome	Adjusted OR (95% CI)	P
CREBBP	**Rs129968**	**ER visit**	0.9 (0.4 - 1.9)	0.81
		Oral steroid course	0.8 (0.5 - 1.9)	0.92
		Uncontrolled asthma	1.5 (1.0 - 2.4)	0.05
		Wheeze	1.8 (1.1 - 2.7)	0.009
		Shortness of breath	1.2 (0.8 - 1.7)	0.34
CREBBP	**Rs130021**	**ER visit**	1.3 (0.7 - 2.4)	0.43
		Oral steroid course	1.2 (0.7 - 2.0)	0.52
		Uncontrolled asthma	0.6 (0.4 - 0.9)	0.007
		Wheeze	0.9 (0.6 - 1.3)	0.93
		Shortness of breath	1.1 (0.8 - 1.5)	0.51
TBP	**Rs3800235**	**ER visit**	1.1 (0.6 - 2.0)	0.84
		Oral steroid course	1.2 (0.7 - 2.1)	0.49
		Uncontrolled asthma	0.8 (0.5 - 1.2)	0.27
		Wheeze	0.7 (0.5 - 1.0)	0.05
		Shortness of breath	1.2 (0.9 - 1.6)	0.30
TBP	**Rs12717**	**ER visit**	0.9 (0.4 - 1.8)	0.76
		Oral steroid course	1.2 (0.6 -2.3)	0.61
		Uncontrolled asthma	0.8 (0.5 - 1.2)	0.20
		Wheeze	0.5 (0.3 - 0.8)	0.005
		Shortness of breath	1.3 (0.9 - 1.9)	0.13
NR3C1	**Rs2963155**	**ER visits**	0.3 (0.1 - 0.9)	0.03
		Oral steroid course	1.3 (0.7 - 2.5)	0.44
		Uncontrolled asthma	0.6 (0.4 - 1.0)	0.05
		Wheeze	0.6 (0.4 - 1.0)	0.06
		Shortness of breath	0.9 (0.6 -1.3)	0.46
NR3C1	**Rs10477211**	**ER visits**	1.1 (0.7 - 2.0)	0.64
		Oral steroid course	2.1 (1.3 - 3.3)	0.002
		Uncontrolled asthma	0.9 (0.7 - 1.3)	0.71
		Wheeze	0.9 (0.6 - 1.2)	0.85
		Shortness of breath	0.9 (0.6 - 1.1)	0.30
NR3C1	**Rs9324924**	**ER visits**	0.4 (0.2 - 0.9)	0.04
		Oral steroid course	0.9 (0.5 - 1.6)	0.76
		Uncontrolled asthma	0.8 (0.6 -1.3)	0.38
		Wheeze	0.8 (0.5 -1.1)	0.18
		Shortness of breath	0.9 (0.7 - 1.3)	0.61

DISCUSSION

In this study, out of 48 tested SNPs in genes involved in the corticosteroid receptor complex, we did not identify any SNPs that reached the statistical significant Bonferroni corrected p-value threshold of 2.08 * 10-4. We showed suggestive p-values smaller than 0.01 for the *NR3C1*, *TBP* and *CREBBP* gene. The most significant association was found for exacerbations (measured by means of prescribed courses of oral steroids during the preceding year) and NR3C1 rs10477211 (OR: 2.1, 95% CI: 1.3 - 3.3, p = 0.002). Genetic variation in the NR3C1 gene, coding for the glucocorticosteroid receptor itself, may result in altered binding capacity, which in turn may result in altered treatment response. Genetic variation in the *NR3C1* gene has previously been associated with altered response to steroids [10-14]. Furthermore, *CREBBP* (cyclic AMP response element binding protein) and TBP (TATA box binding protein) may be interesting candidate genes, as they play an important role in the inflammatory pathway and corticosteroids act by suppressing the inflammatory genes [6 7].

The most obvious explanation for not finding any significant associations is the fact that the present study was underpowered. Our results should therefore be considered preliminary. To increase the statistical power, analysis will be repeated when the inclusion of the ongoing PACMAN cohort (we aim at inclusion of at least 1000 pediatric asthma medication users) is finished. Furthermore, efforts are currently ongoing to genotype the studied SNPs in another cohort including pediatric asthmatics (the BREATHE study) [20 21].

An important strength of the PACMAN-cohort study is the fact that we have data available on many different asthma-related outcomes. In our dataset, treatment response can be defined in several ways: asthma exacerbations, asthma control or asthma symptoms. Therefore, the PACMAN-cohort is very suitable to be used for meta-analysis as we can define treatment response several ways. For the present study we used a candidate gene approach, this has as important limitation that we could have missed potentially important SNPs because we only selected tagging SNPs in previous selected genes with a minor allele frequency (MAF) of 0.20. By applying this method, rare variants will be missed.

In conclusion, we did not identify any SNPs significantly associated with steroid response. However, future meta-analysis of the data should reveal whether the most significant genes found in this study are truly associated or false positive findings.

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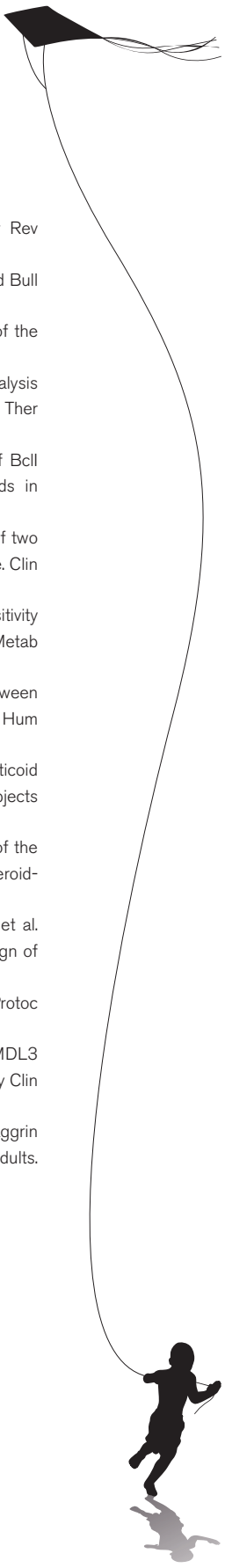


CHAPTER 15

GENERAL DISCUSSION

SCOPE OF THIS THESIS

Childhood asthma is a leading cause of missed school days, limitations in normal activities and hospital visits [1]. Inhaled corticosteroids (ICS) have become first-line maintenance therapy for asthma. Despite their effectiveness in most patients, a proportion of the asthmatics that receive steroid treatment still suffers from uncontrolled symptoms. This individual variation in treatment response is an important issue in daily clinical practice. Several factors may contribute to the observed heterogeneity in treatment response, such as disease severity, co-morbidities, continued exposure to allergens and triggers and therapy adherence. Besides environmental and medication-use related factors, genetic factors may influence effectiveness of ICS [2].

Both international and national guidelines describe instructions for diagnosing, treating and monitoring childhood asthma [1 3-8]. We summarised these instructions in a flow chart that describes the stepwise process from first physician visit towards an effective treatment strategy in childhood asthma (Figure 1). In general, this treatment process includes four steps: (1) start of therapy after first general practitioner (GP) visit, (2) first-time monitoring of treatment effectiveness and physician decision on discontinuation, continuation or adjustment of therapy, (3) evaluation of therapy effectiveness and (4) continuous monitoring. Several factors, such as environmental, genetic and medication use related factors can influence

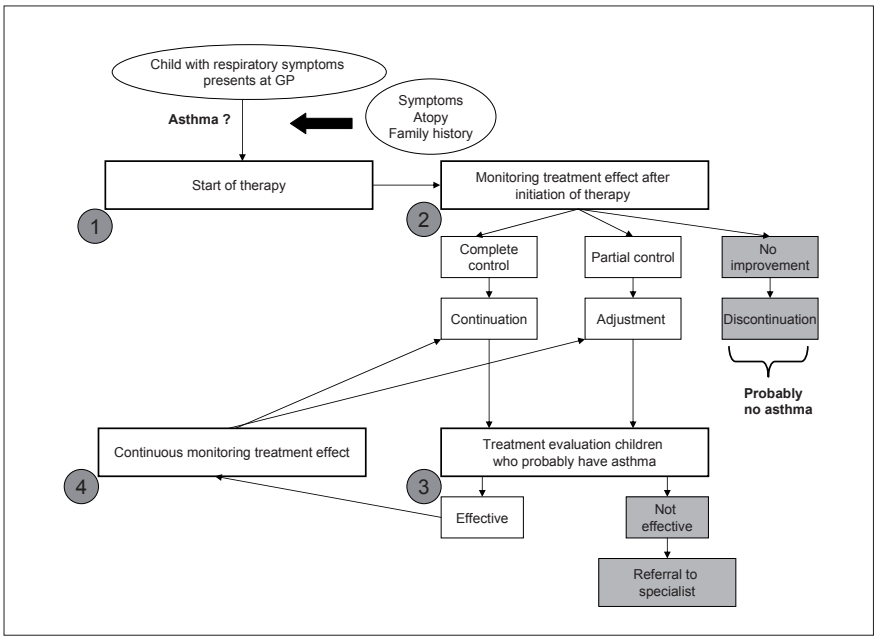


Figure 1. Treatment of childhood asthma
The process towards effective treatment of childhood asthma includes several steps; step 1: asthma therapy initiation, step 2: first time treatment monitoring and decision on continuation, step 3: treatment evaluation for children who probably have asthma (no effect: referral to specialist) and step 4: continuous monitoring of therapy effectiveness.

these different steps towards effective treatment. In this thesis we aim to contribute to the knowledge on a broad spectrum of factors related to medication use and treatment outcomes in paediatric asthma. First, we discuss our most important findings. Second, we describe the strengths and limitations of studies presented in this thesis and finally, we elaborate on implications for clinical practice and future research and present the conclusions of this thesis.

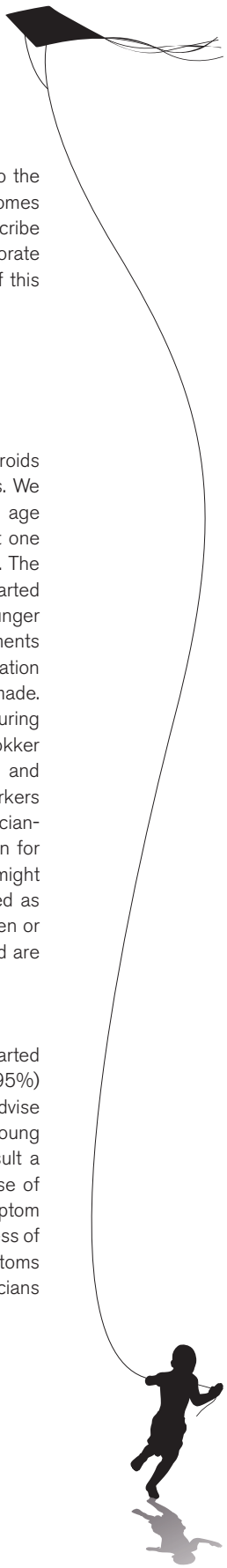
MAIN FINDINGS

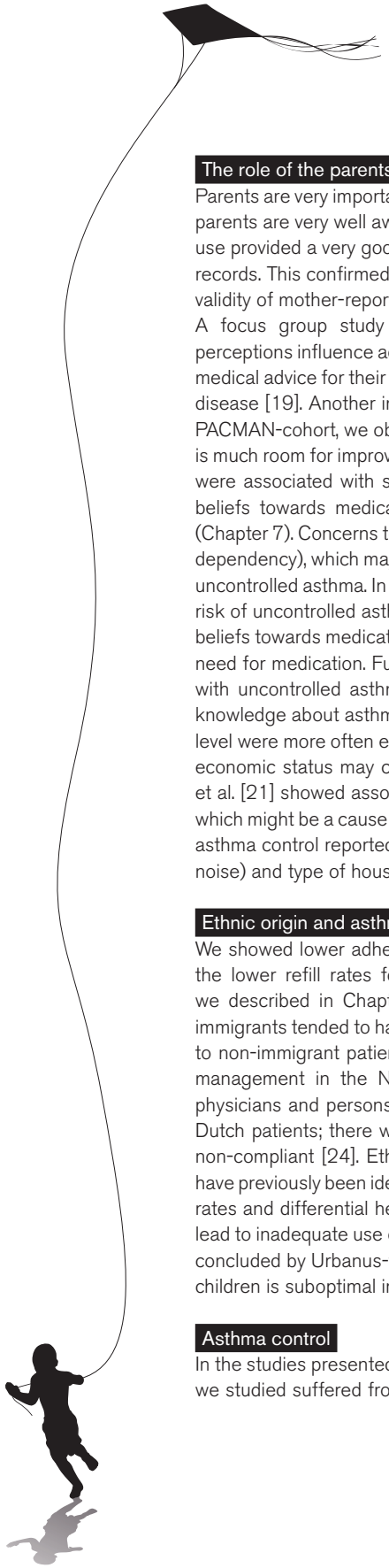
Asthma medication use in childhood

In line with treatment guidelines [1 4], short-acting beta-agonists (SABA) and inhaled steroids were the most frequently prescribed drugs among the children included in our studies. We evaluated longitudinal medication use patterns in children followed from birth up till age eight (PIAMA study), and found that more than one third of the children filled at least one prescription for any asthma medication during their first eight years of life (Chapter 2). The majority of the children who started first-line anti-asthma therapy (SABA and/or ICS), started before their third birthday (Chapter 3). It is known that in children aged five years and younger the clinical symptoms are often variable and non-specific and lung function measurements can not be assessed routinely in this age group [9 10]. Thus, in our study, asthma medication was frequently initiated at an age at which an asthma diagnosis cannot yet be firmly made. Furthermore, few children used medication continuously; most were irregular users during the first eight years of life or discontinued medication use after the third birthday. Schokker et al. [11] showed that prescribing of asthma medication in children was common and continuation of asthma therapy from preschool into school-age was low. Uijen and co-workers described low numbers of annual prescriptions per child (aged 0-17 years) with physician-diagnosed asthma [12]. The large proportion of children filling at least one prescription for asthma medication at young age combined with the low persistence of medication use might indicate that therapy initiation is used as a diagnostic tool. As such, therapy is initiated as trial management and the response to asthma medication is used as a tool to strengthen or reject the possible diagnosis of asthma. Furthermore, respiratory symptoms in childhood are transient which may also result in transient medication use patterns [13].

Asthma medication use in infancy

We showed that 15% of all infants participating in the WHISTLER birth-cohort study started asthma therapy during the first year of life. SABA were the most frequently used (>95%) and ICS were initiated in almost 25% of the infants (Chapter 5). Treatment guidelines advise regular use of ICS to control respiratory symptoms and to improve lung function in young children [9 14]. The Dutch general practitioner guidelines do however advise to consult a specialist before prescribing ICS for children aged younger than one year [4]. Because of diagnostic difficulties and the transiency of respiratory symptoms early in life, non-symptom related factors such patient or family characteristics are thought to play a role in the process of drug prescribing [15 16]. It was reassuring to see that parental reported respiratory symptoms were the most important factor in prescription of medication in infants (Chapter 5). Physicians appeared not to be distracted by non-symptom related factors, such as gender.





The role of the parents in paediatric asthma management

Parents are very important actors in paediatric disease management [17]. We have shown that parents are very well aware of their child's medication use (Chapter 4). Parental reported ICS use provided a very good reflection of ICS use measured by means of pharmacy prescription records. This confirmed the observations of a Norwegian cohort study that also showed high validity of mother-reported use of anti-asthmatic drugs [18].

A focus group study conducted in the Netherlands illustrated how strongly parental perceptions influence adherence to physician's advice. Parents decide whether they will follow medical advice for their child's asthma based on their own perceptions towards medicines and disease [19]. Another important factor in the outcome of treatment is adherence. Within the PACMAN-cohort, we observed good adherence rates in only 57% of the population, so there is much room for improvement. We found that higher adherence rates in paediatric asthmatics were associated with stronger parental necessity beliefs towards medication use. Parental beliefs towards medication use were also associated with control of asthma in children (Chapter 7). Concerns towards medication use (e.g. about potential side-effects or medication dependency), which may result in lower adherence rates, were associated with a higher risk of uncontrolled asthma. In addition, stronger necessity beliefs were also associated with a higher risk of uncontrolled asthma, which seems difficult to explain. Perhaps these strong necessity beliefs towards medication use may reflect more severe asthma and therefore an even higher need for medication. Furthermore, we found low maternal educational level to be associated with uncontrolled asthma. In our study, mothers with low education had more often poor knowledge about asthma medication use and children whose mothers had a low educational level were more often exposed to pets. Differential lifestyle factors coherent with lower socio-economic status may contribute to this increased risk of uncontrolled asthma [20]. Lasmar et al. [21] showed associations between low maternal education and lower adherence rates, which might be a cause of poor asthma control. Other studies on factors influencing paediatric asthma control reported associations with urban environment (exposure to air pollutants and noise) and type of housing [20 22].

Ethnic origin and asthma management

We showed lower adherence rates for immigrant children (Chapter 6). This was in line with the lower refill rates for asthma medication during infancy for immigrant children, which we described in Chapter 5. Our findings are consistent with other studies showing that immigrants tended to have less prescriptions of asthma controller medication when compared to non-immigrant patients [23]. A study on the influence of cultural differences on disease management in the Netherlands showed that communication in consultations between physicians and persons from ethnic minorities was less effective than in consultations with Dutch patients; there was more misunderstanding and immigrant patients were more often non-compliant [24]. Ethnicity as well as insufficient comprehension of the Dutch language have previously been identified as risk factors for uncontrolled asthma [25]. The low adherence rates and differential health perspectives related to ethnic or cultural background may often lead to inadequate use of medication and as a result more uncontrolled asthma. This was also concluded by Urbanus-van Laar and colleagues who showed that asthma care for immigrant children is suboptimal in the Netherlands [26].

Asthma control

In the studies presented in Chapter 7 and 8, we have shown that up to half of the populations we studied suffered from uncontrolled asthma. These high rates of uncontrolled asthma are

in line with the study of Chapman and co-workers who suggested that the majority of the asthmatics treated in primary care are uncontrolled [27] and other studies reporting on asthma control [28-30].

To study (genetic) predictors of treatment outcome, the definition of solid outcome phenotypes is of utmost importance. Therefore, we first evaluated the agreement between current and long-term asthma control (Chapter 8) and showed agreement between these two parameters to be limited. Current asthma control rates (control of symptoms during the previous week) were much higher than long-term asthma control rates reported for the previous season or preceding year. A previous study on reporting of current symptoms in paediatric asthmatics showed the absence of current asthma-related symptoms whilst the participants did experience airway hyperresponsiveness [31]. Second, we showed seasonal variations in asthma control (Chapter 8 and 9). Uncontrolled asthma rates were lowest in summer and peaked in spring and fall. This seasonality in asthma control has been reported before [32-35]. It seems that current asthma control rates might be overestimated in observational studies, as patients might not schedule study visits during periods with severely uncontrolled symptoms. Therefore, monitoring symptoms over a longer period of time seems more adequate. In addition, the observed seasonal variation in asthma control requires assessment of asthma control per season.

Pharmacogenetics of anti-inflammatory asthma therapy: initiation of the PACMAN study

To date, several asthma pharmacogenetic studies have been performed (Chapter 11), being mostly clinical trials [36-39]. These trials are limited in their ability to identify long-term treatment effects due to their relatively short duration and mostly small sample sizes. Well-designed observational studies might therefore be a better approach to study long-term treatment effectiveness and genetic factors influencing treatment effectiveness. Data from several large cohorts focusing on paediatric asthma have been published [40-43]. However, it is difficult to use the data collected within these studies for pharmacogenetic purposes, because only small numbers of children regularly used asthma medication. Besides this, the congruence between a diagnosis of asthma and asthma medication use is far from perfect [15]. Based on these facts, we initiated a new paediatric cohort for pharmacogenetic studies (Chapter 12): the PACMAN-cohort study (Pharmacogenetics of Asthma medication in Children: Medication with ANti-inflammatory effects) [44]. Within the PACMAN-cohort we included regular users of asthma medication aged 4-12 years in their own community pharmacy and collected a wide range of data. This resulted in a cohort that is a cross-section of paediatric asthma medication users, ranging from well controlled to more severe uncontrolled patients. The PACMAN-cohort provides a good basis to study different aspects of asthma medication use and pharmacogenetics of asthma medication of which the first results are described here.

Pharmacogenetics of inhaled corticosteroids

We used data from the BREATHE study (United Kingdom) [45] and our own PACMAN-cohort to investigate genetic variants that may influence response to steroids (Chapter 13). We tested the association between genetic variation in the *FCER2* gene, encoding the low affinity IgE receptor, and ICS treatment outcomes in paediatric asthmatics. The *FCER2* T2206C variant has been previously associated with increased risk of severe asthma exacerbations in the CAMP (Childhood Asthma Management Program) trial [37]. We not only

replicated the association with exacerbations (OR: 1.91, 95%CI: 1.08-3.40) but also showed an association with current asthma control, asthma symptoms during the previous year and increased daily steroid dose. Furthermore, we used our PACMAN-cohort to explore genetic variation in candidate genes that were all part of the corticosteroid receptor complex (Chapter 14). This study revealed several genes that might play a role in ICS treatment effectiveness. However, these findings need to be confirmed in a larger population.

STRENGTHS AND LIMITATIONS

Different data sources

Within the studies described in this thesis we used data from four different studies: the PACMAN study, the PIAMA study, the WHISTLER study and the BREATHE study. The different designs (cross-sectional vs. longitudinal), study aims (pharmacogenetics of asthma medication vs. prevalence and incidence of asthma in childhood vs. respiratory symptoms in infancy), study populations (paediatric asthma medication users vs. healthy newborns) and type of data collected (questionnaires, pharmacy prescription records, general physician medical records) allowed us to study a broad spectrum of factors related to asthma medication use in childhood. We had data available on many different aspects and factors that allowed us to investigate these as possible risk factors, but also to include these factors in the analysis as possible confounders.

Observational research

All studies were observational and may therefore be hampered by some degree of selection bias due to selective non-response. However, analysis of demographics such as age and gender and medication use did not reveal any differences between responders and non-responders in the PACMAN study. Compared to the general Dutch population, children participating in one of these three Dutch cohort studies had more often high-educated parents and both the PIAMA and the WHISTLER study contained more parents of native Dutch origin when compared to the general Dutch population.

Parental reporting of symptoms

In all studies, asthma symptoms were parental reported. Parents might not always be capable of optimally recognising clinical asthma symptoms (such as wheezing) and defining their child's asthma control [46]. On the other hand, this situation of parental reporting, especially in younger children, resembles daily clinical practice. In older children, ideally, asthma control should also be assessed by asking the child itself as a study of Davis et al. [47] showed underestimation of asthma burden by parents of 10-15 years olds. In future studies it might therefore be valuable to include a questionnaire such as the validated Childhood Asthma Control Test, which can be filled in by the child itself and has been shown to be a reliable tool to identify children with inadequately controlled asthma [48].

Definition of outcome phenotypes

To study determinants of therapy effectiveness, the definition of solid phenotypes is of utmost importance. This is a major issue in especially childhood asthma. Asthma is a very heterogeneous disease; with seasonal variations in disease severity. Many factors are stated to play a role in the process towards sufficient disease control. Several markers, such as, lung

function measurements, symptoms scores and hospital admissions have been suggested as markers for therapy effectiveness. However, previous studies showed that predictors of treatment response differed depending on the definition of the outcome [49 50]. This is a problem in asthma pharmacogenetics, especially for the comparison and meta-analysis of results from different studies, as the definition of outcome phenotypes may differ between them. Within the pharmacogenetic studies described in this thesis we pooled data from the BREATHE study with our own PACMAN study. The characteristics of the study participants were very similar and for both study populations we could define asthma exacerbations as outcome measure.

PACMAN cohort study

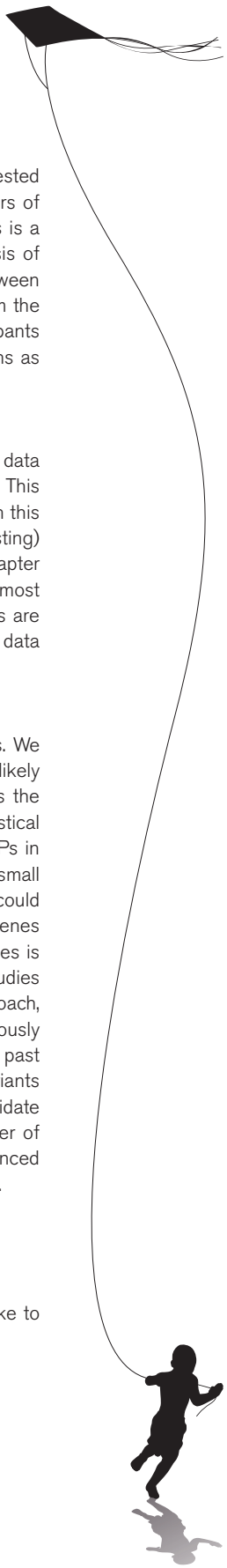
For many of the studies described in this thesis (Chapter 6, 8, 9, 13 and 14) we used data from our ongoing PACMAN study. To date, almost 750 children participated in this study. This is an adequate amount to answer most epidemiological research questions described in this thesis, however not sufficient to study large amounts of SNPs (correction for multiple testing) or to study rare SNPs and/or markers with only small effects. The study described in Chapter 14 was underpowered and to increase statistical power, a larger sample size is of utmost importance. We continue patient inclusion for the PACMAN study and currently efforts are being made to genotype the SNPs in another population (BREATHE study), so that the data can be analysed together.

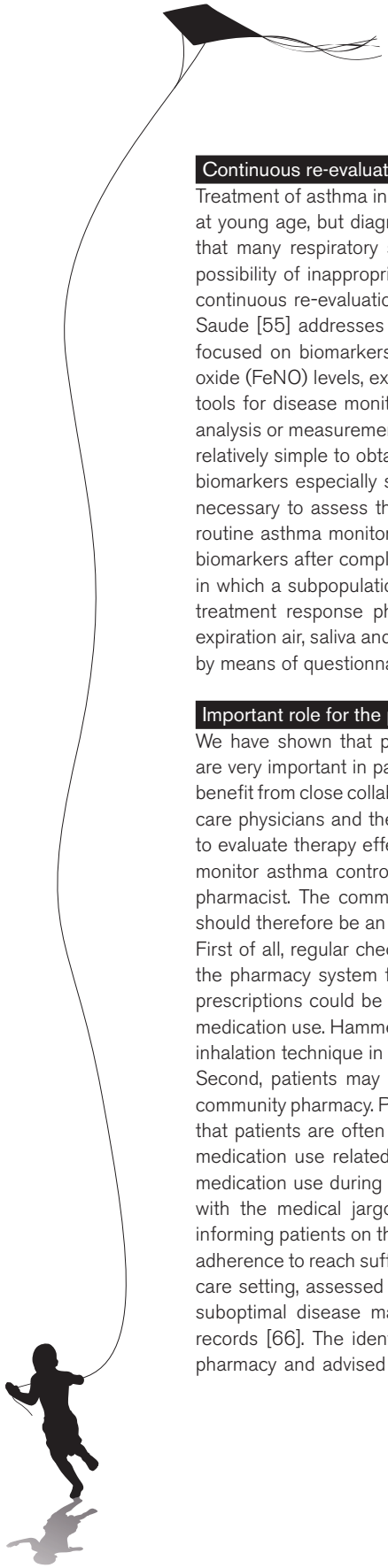
Candidate gene approach vs. GWAS

In this thesis, we used a candidate gene approach to investigate genetic associations. We focused on genes that were part of the corticosteroid receptor complex and therefore likely to be involved in corticosteroid treatment response. An advantage of this approach is the limited number of genes and SNPs that are tested and therefore keeping sufficient statistical power to detect associations in a relatively small population. We selected tagging SNPs in each candidate gene with information from the HapMap project [51]. In this way, with a small number of genetic tests, a large part of the genetic variation in the selected genes could be analysed. A limitation of this strategy is that by focussing on a limited number of genes (a selection based on prior knowledge) the detection of new previously unrelated genes is hampered. In contrast to the candidate gene approach, genome wide association studies (GWAS) are a more comprehensive method to study genetic associations. This approach, which is an examination of the whole genome, is not limited by selection of SNPs in previously selected genes, which makes this strategy very useful to discover new genes. During the past decade, GWAS have become the default study design for discovery of new genetic variants associated with a specific phenotype. Many of the considerations are common to a candidate gene study, although the greater costs and multiple testing problem (the large number of statistical tests leads to an increased risk of false positive results and asks for more advanced statistical techniques) have to be taken into account when performing a GWAS [52-54].

IMPLICATIONS FOR CLINICAL PRACTICE

Based on the results presented in this thesis and on findings in literature we would like to make some recommendations for clinical practice.





Continuous re-evaluation of therapy

Treatment of asthma in childhood is a complex process. Many children start using medication at young age, but diagnostic difficulties and the low persistence of medication use indicate that many respiratory symptoms in childhood might not be asthma. So there might be a possibility of inappropriate treatment in childhood. This asks for better diagnostic tools and continuous re-evaluation of the need for treatment. A recent review article of Skappak and Saude [55] addresses new developments in the monitoring of asthma. Many studies have focused on biomarkers to be used in asthma monitoring, such as fractional exhaled nitric oxide (FeNO) levels, exhaled breath condensate, saliva and sputum. Currently the most used tools for disease monitoring and evaluating treatment effects in clinical practice are FeNO analysis or measurement of other biomarkers in exhaled breath. They are totally non-invasive, relatively simple to obtain and measurements can easily be repeated [56]. This makes these biomarkers especially suitable for use in paediatric populations. However, more research is necessary to assess the validity of these biomarkers before they can be implemented into routine asthma monitoring. Hopefully, we are able to shed more light on the value of these biomarkers after completion of the PACMAN study and initiation of PACMAN study phase II in which a subpopulation of patients will be re-invited for further characterization of asthma treatment response phenotypes. Within PACMAN phase II, (inflammatory) biomarkers in expiration air, saliva and blood will be measured as well as lung function and symptom scores by means of questionnaires.

Important role for the pharmacist as provider of information

We have shown that parental disease knowledge and perception towards medication use are very important in paediatric asthma management. Paediatric asthma management would benefit from close collaboration of healthcare providers within the primary care setting (primary care physicians and the pharmacist). Regular follow-up visits to monitor asthma control and to evaluate therapy effects are helpful [57 58]. Besides the general practitioner who should monitor asthma control regularly, we believe there is an important role for the community pharmacist. The community pharmacy is the primary place for medication dispensing and should therefore be an important provider of information on topics related to medication use. First of all, regular checks in the pharmacy (e.g. for correct inhaler technique and checks in the pharmacy system for adequate filling of prescriptions) when a patient is filling his/her prescriptions could be helpful to increase awareness on good adherence rates and correct medication use. Hammerlein et al. [59] showed that a pharmacy intervention study to improve inhalation technique in patients with asthma was very successful.

Second, patients may not be aware of the possibilities of medication counselling at their community pharmacy. Previous studies carried out in the community pharmacy setting showed that patients are often unfamiliar with the counselling role of the pharmacist as provider of medication use related information [60-62]. This is especially important as information on medication use during GP consultations is often very brief en patients may have difficulties with the medical jargon [63]. Pharmacists should therefore have a more active role in informing patients on the mode of action, correct medication use and the importance of good adherence to reach sufficient disease control. A study carried out within the Australian primary care setting, assessed the use of a software application to identify patients with potentially suboptimal disease management (e.g. high rates of reliever medication) from pharmacy records [66]. The identified patients were sent educational material from their community pharmacy and advised to contact their general practitioner, which resulted in improvement

in medication use patterns. Burgess et al. [67] also showed that by measuring a patient's adherence and providing feedback use of asthma controller medication increased and the patient's clinical outcomes improved.
Third, good communication between the pharmacist and primary care physician may also help to optimise an individual patient's disease management strategy [64]. Berry and co-workers described the successful implementation of the "Asthma Friendly Pharmacy (AFP)" interventions in community pharmacies in the United States (US). The AFP model focused on: (1) pharmacist communication/intervening with the primary care provider (e.g. in case of medication overuse, non-adherence or for example LABA monotherapy prescriptions in stead of use of a combination with ICS) and (2) patient education provided by the pharmacist (e.g. explain the role of medication, assess respiratory device technique or provide smoking cessation counselling) [65]. The above-mentioned studies combined with our own findings suggest that a more active role of the pharmacist, as information source for medication related topics, should be incorporated more actively in the treatment process.

Ethnical differences and asthma management

Health care providers should also pay attention to cultural and ethnical differences as we have shown lower adherence and refill rates for medication in immigrant children. To date, in the Netherlands around 20% of the population are immigrants and approximately 11% of the population are non-western immigrants, most being of Moroccan and Turkish origin [68]. A recent Cochrane review found that cultural specific programs were better in improving quality of life and asthma knowledge in asthmatics [69]. Differential health perspectives may exist and the Dutch treatment guidelines contain few ethnic specific statements so far [70]. However, with a considerable proportion of immigrants, more attention to ethnic and cultural differences towards disease management and medication use is necessary to decrease asthma morbidity.

Pharmacogenetics of ICS

With the work described in this thesis we aimed to address many issues that are important to consider in pharmacogenetic research in paediatric asthma. Ultimately, pharmacogenetics aims to predict an individual's response to medication. Many papers have discussed how and when pharmacogenetics should be applied in clinical practice [71 72]. In asthma care, there is no role for pharmacogenetics yet.
We found the T2206C *FCER2* variant to be associated with ICS treatment outcomes. The T2206C variant is relatively common and may therefore be a relevant marker for clinical practice. Early identification of patients who do not sufficiently respond to ICS treatment may reduce asthma-related morbidity and perhaps even mortality, as these patients can be referred to specialized care at an early stage of disease and therapy can be adjusted. However, to date, implementation of pharmacogenetics in daily asthma care is hampered by the fact that there are not many treatment alternatives for patients not (sufficiently) responding to standard maintenance treatment with inhaled steroids. Apart from LTRA, which have shown to control asthma symptoms [73], but are not as effective as steroids in most patients, there are no real therapy alternatives. Possibly, anti-IgE therapy (omalizumab) may be an alternative in a subpopulation of patients, as it reduces the asthmatic response to allergen challenge in patients with allergic asthma [74-76].

There are other areas in which genotyping before start of treatment is more common, especially in the prevention of severe adverse events. For example, HIV patients are genotyped before start of abacavir treatment to prevent severe, sometimes fatal hypersensitivity reactions [77]. Pharmacogenetics can also provide information on which patients will benefit most from a certain therapy. As such, screening for Her-2/neu genotype is becoming standard practice as breast cancer patients with the Her-2/neu+ genotype have a greater response to trastuzumab treatment [78]. However, before we may expect implementation of routine genotyping for asthma care, we need genes that have strong effects and/or are common and account for a substantial part of the observed variation in treatment response. Important for implementation is thereafter, information on cost effectiveness and cost consequences.

RECOMMENDATIONS FOR FUTURE RESEARCH

The pharmacy as research setting

The successful initiation of the PACMAN-cohort study within Dutch community pharmacies demonstrates that the community pharmacy is a useful and valuable setting to carry out observational research related to medication use. In the Netherlands, most people are registered to only one community pharmacy independently of prescriber and obtain all their prescription medication from that pharmacy [79]. Information within the electronic pharmacy information system (medication dispensing records) is therefore very useful to study long-term medication use patterns. Furthermore, the information in the pharmacy system can often be linked with primary care medical records that contain diagnosis codes and other patient data. Another advantage of including patients in the community pharmacy setting, rather than via their GP or in a hospital setting, is the possibility to recruit all categories of patients (cross-section of the patient population), ranging from mild patients usually seen by a GP to severe, hospital attending patients. Pharmacy practice research is of importance to evaluate and further improve pharmaceutical patient care. For successful research in pharmacies cooperation of the pharmacist and his/her team is of utmost importance. This is also the result of a cross-sectional survey among Australian pharmacists showing the recognition of the value of research within the pharmacy setting [80].

Pharmacogenetics: definition of uniform outcome phenotypes

To date, the field of asthma pharmacogenetics is still relatively new. Most studies performed so far, are candidate gene association studies. There are many hurdles to tackle before personalised medicine in the field of asthma will be a fact [81]. First of all, future research on asthma medication efficacy and pharmacogenetics should take into account the problems of defining asthma control and treatment outcome phenotypes. Researchers should confirm the definition of one or more solid uniform reproducible asthma treatment outcome phenotypes, which can be used in future studies. In 2009, an expert panel organised by the World Health Organisation discussed the topic of different terminologies used to define asthma severity, control and exacerbations [82]. This panel proposed a definition for severe asthma upon which decisions can be made in clinical practice. However in epidemiological studies, it is often difficult to asses asthma severity due to a lack of data and differences in data collection between studies. Therefore, initiatives such as the European Network of Pharmacogenetics/ Pharmacogenomics (part of The European Federation for Pharmaceutical Sciences (EUFEPS)) and the NIH Pharmacogenomics Research Network could contribute to this by discussing this problem in scientific panels, symposia and their disease specific task forces.

Pharmacogenetics: genotyping approach

Many known pharmacogenetic associations only account for a small proportion of the individual variability in treatment response. But the application of GWAS to pharmacogenetics is just beginning. Genes found in GWAS should be further explored to get to better understanding of these genes, gene products and causal pathways. This may lead to the discovery of SNPs or panels of SNPs with larger effects. With projects such as the 1000 Genomes project [83], which aims at sequencing the entire genome of many individuals of different ethnic backgrounds, it is expected that pharmacogenetics and genetics of asthma will develop more rapidly the next decades. Within GWAS studies, researchers aim to discover genome regions associated with a specific disease or treatment response. Sequencing will allow researchers to localise functionally associated and rare variants more precisely because all the genetic variation in the genome will be determined.

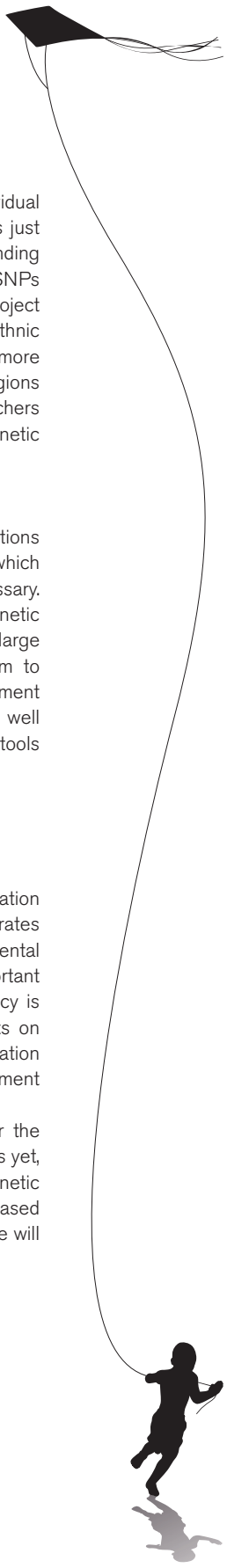
Systems biology approach

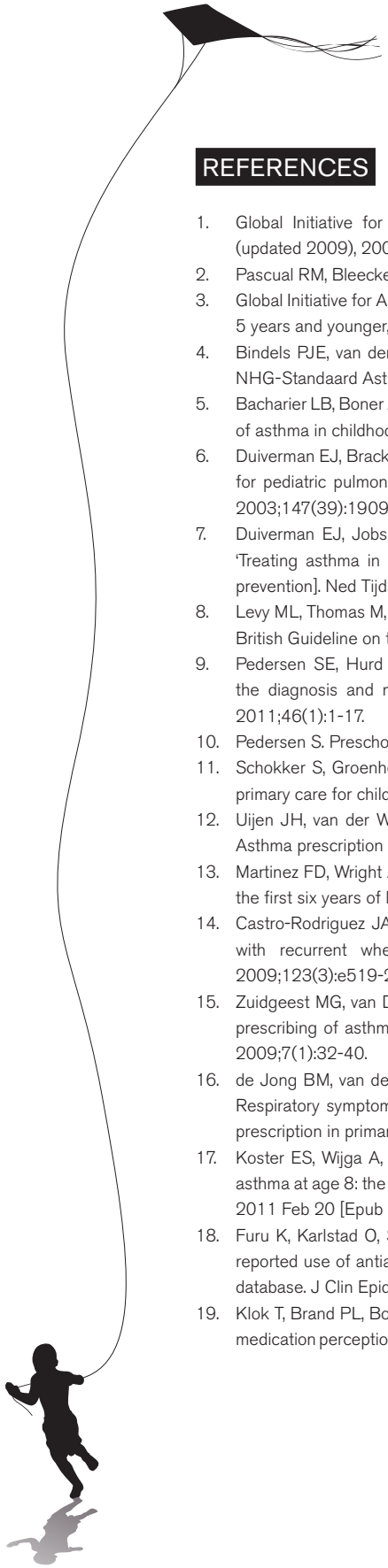
Not one single gene is expected to determine treatment outcome. Gene-gene interactions are likely to be important, as well as other actors. To elucidate the exact mechanism by which genetics influences drug response, integration of all kinds of biological data is necessary. In Chapter 10, we described the use of a "systems biology approach" in pharmacogenetic research [84]. For this approach, large datasets are needed with many patients or large collaborating consortia, such as the GABRIEL consortium, a large-scale consortium to study genetics of asthma [85]. Assessment of multiple gene-gene and gene-environment interactions requires sophisticated analytical methods as traditional statistics are not well suited [86]. Future research should also focus on the development of easy-to-work-with-tools and statistics for analysis of large datasets with many contributing factors.

CONCLUSIONS

The studies presented in this thesis focused on different aspects related to asthma medication use and treatment outcomes in paediatric asthma. We have shown that adherence rates were generally low and many patients suffered from suboptimal controlled disease. Parental perception towards medication use, parental educational level and ethnicity were important factors associated with adherence rates and asthma control. The community pharmacy is an important place for medication counselling and education of patients and parents on adequate asthma management. By carrying out a more active role as provider of medication information, the pharmacist can become a key player in the process towards better treatment outcomes in childhood asthma.

Furthermore, we have shown several SNPs to have the potential to be markers for the effectiveness of steroid treatment. In asthma care, there is no role for pharmacogenetics yet, however, the definition of solid outcome phenotypes and extensive investigation of genetic variation in genes involved in the inflammatory asthma response may lead to genotype based personalised treatment and/or discovery of new drug targets in the future. Hopefully, we will be able to shed more light on this upon completion of the PACMAN study.





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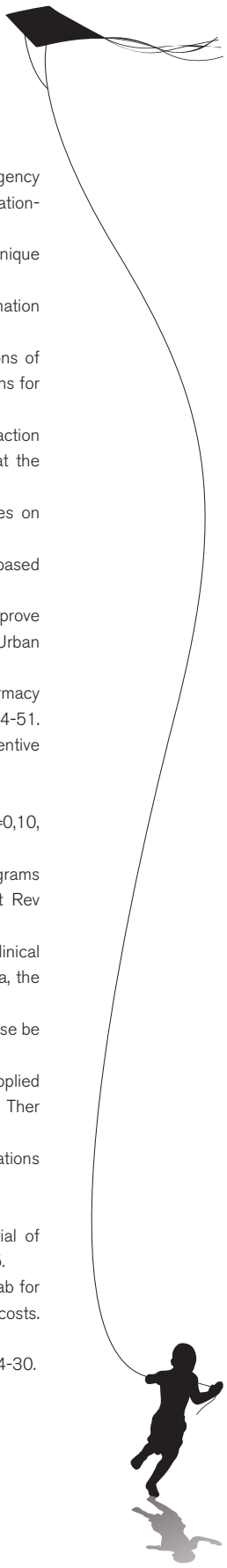

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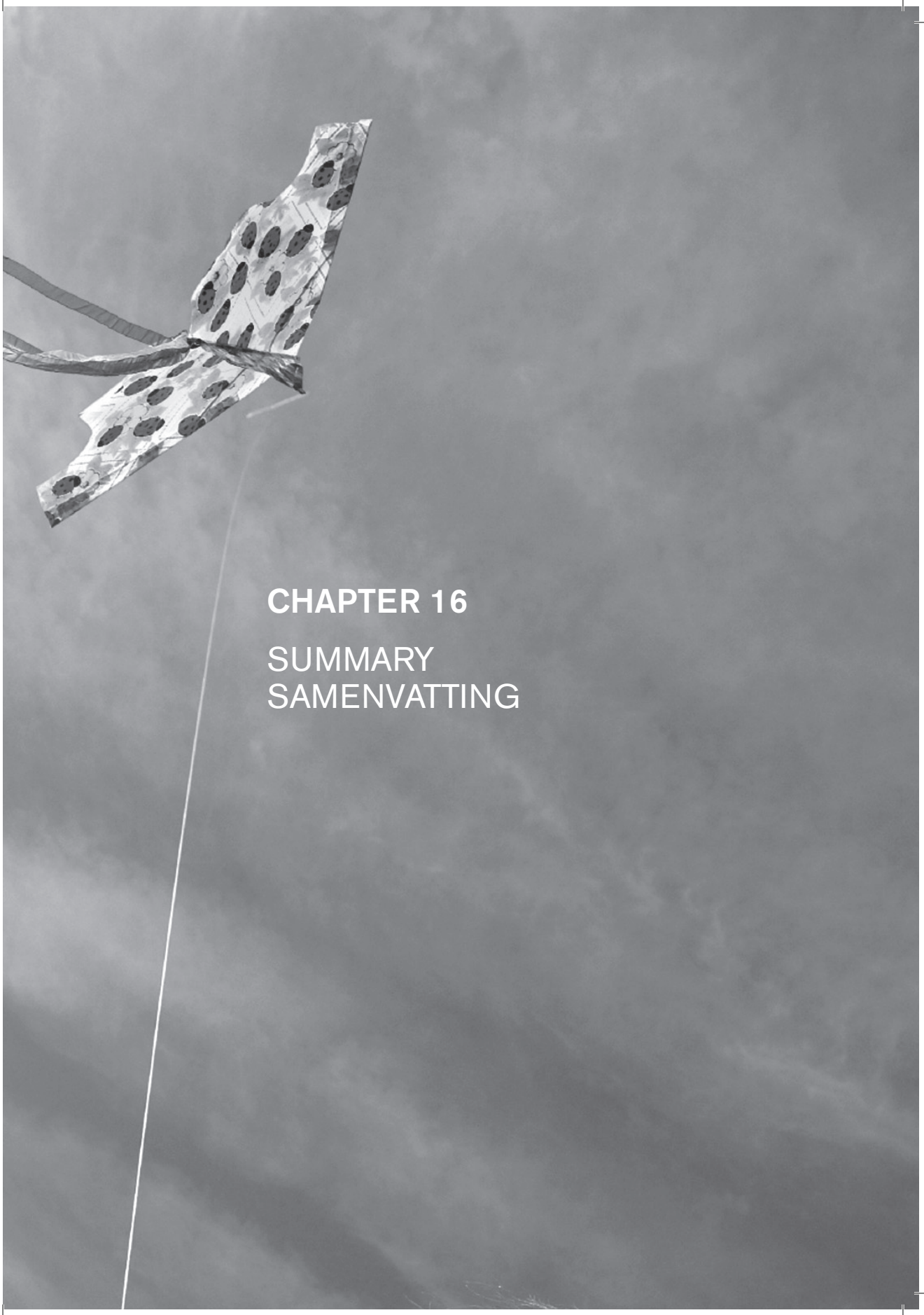
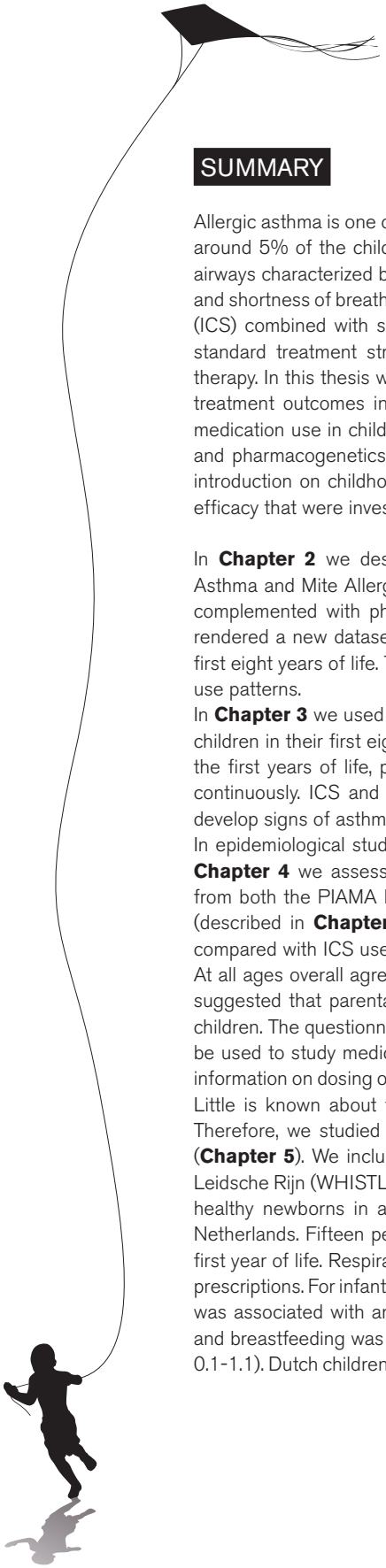


CHAPTER 16

SUMMARY
SAMENVATTING



SUMMARY

Allergic asthma is one of the most common chronic diseases in childhood. In the Netherlands, around 5% of the children suffers from asthma. Asthma is an inflammatory disease of the airways characterized by recurrent airway obstruction and symptoms such as wheeze, cough and shortness of breath. Standard treatment is based on regular use of inhaled corticosteroids (ICS) combined with short-acting beta agonists (SABA). Despite the effectiveness of this standard treatment strategy in most asthmatics, there is large variability in response to therapy. In this thesis we aim to contribute to the knowledge on asthma medication use and treatment outcomes in childhood asthma. This thesis is divided into three topics: asthma medication use in childhood (**Chapter 2 - 6**), control of asthma symptoms (**Chapter 7 - 9**) and pharmacogenetics of asthma medication (**Chapter 10 - 14**). **Chapter 1** is a general introduction on childhood asthma and the different aspects of asthma medication use and efficacy that were investigated in this thesis.

In **Chapter 2** we described the formation of the PIAMA (Prevention and Incidence of Asthma and Mite Allergy) pharmacy cohort. The data of the PIAMA birth cohort study were complemented with pharmacy dispensing records. The applied method of data collection rendered a new dataset including 777 children with complete medication histories for their first eight years of life. This dataset provided the opportunity to study longitudinal medication use patterns.

In **Chapter 3** we used this new dataset to describe asthma medication use patterns among children in their first eight years of life. Asthma medication was prescribed frequently during the first years of life, particularly before age three, and only few children used medication continuously. ICS and SABA prescription occurred especially in who were more likely to develop signs of asthma at age eight.

In epidemiological studies concerning children, we often use parental reported data. Within **Chapter 4** we assessed the validity of parental reported ICS use in children using data from both the PIAMA birth cohort (n = 3963) and the PIAMA pharmacy cohort (n = 777) (described in **Chapter 2**). Parental reported ICS use (based on questionnaire data) was compared with ICS use recorded in community pharmacy records (dispensed prescriptions). At all ages overall agreement between the two methods was very high (>97%). Our finding suggested that parental report of medication use is a reliable source to asses ICS use in children. The questionnaire based medication use data collected within the PIAMA study can be used to study medication use in a large group of children, however, when more detailed information on dosing or exact time of use is needed pharmacy prescription data are essential.

Little is known about factors that determine prescribing of asthma medication in infancy. Therefore, we studied factors related to the initiation and refill of prescriptions in infancy (**Chapter 5**). We included 1202 infants who participated in the Wheezing Illnesses Study Leidsche Rijn (WHISTLER), a large population-based prospective birth cohort study including healthy newborns in a new residential area (Leidsche Rijn) near the city of Utrecht, the Netherlands. Fifteen percent of all infants in our cohort started asthma therapy during their first year of life. Respiratory symptoms were an important driver of both initiation and refill of prescriptions. For infants with physician diagnosed respiratory symptoms, day-care attendance was associated with an increased chance of therapy initiation (OR: 5.3, 95% CI: 1.8-16.2) and breastfeeding was associated with a lower chance of starting therapy (OR: 0.4, 95% CI: 0.1-1.1). Dutch children (non-immigrants) had a higher chance of refilling prescriptions during

infancy (OR: 5.3, 95% CI: 1.1-26.8). We showed that the presence of respiratory symptoms were the principal reason for physicians to prescribe asthma medication in infancy, physicians appeared not to be distracted by other (non-symptom related) factors.
Poor adherence with ICS has been reported frequently and may be associated with uncontrolled asthma. Better understanding of factors influencing adherence may help to achieve higher adherence rates for a larger part of the population, therefore, in **Chapter 6**, we investigated factors associated with therapy adherence in paediatric ICS users. Good adherence was observed in 57% of the population. Increased fractional exhaled nitric oxide (FeNO) values, which are an indication for airway inflammation, were associated with lower adherence rates (OR: 0.3, 95% CI: 0.2-0.4); this underlines the need of good adherence rates to reach sufficient disease control. Stronger parental necessity beliefs towards medication use were associated with higher adherence (OR: 2.3 95% CI: 1.6-3.4), as was Dutch ethnicity (OR: 2.1 95% CI: 1.1-4.1). Furthermore, younger age was associated with better adherence.

The second part of this thesis described studies related to control of symptoms. First, in **Chapter 7** we investigated determinants of uncontrolled asthma in children participating in the PIAMA study. Uncontrolled asthma at age eight was observed in 45% of the population. Low maternal education and stronger parental concerns about potential adverse consequences of medication were both associated with a higher risk of uncontrolled asthma. In addition, stronger necessity beliefs about medication use to maintain present and future health were associated with a higher risk of uncontrolled asthma. These strong necessity beliefs may reflect more severe asthma and therefore an even higher need for medication. These findings underline the need for good education of parents about asthma and its management. Health care professionals should make efforts to improve this knowledge, to positively influence parental perception towards medication use and take away unfounded concerns about medication. This could eventually lead to better asthma control in paediatric patients.
Several studies have shown that predictors of asthma treatment outcomes differ depending on the definition of the outcome chosen (lung function vs. exacerbations vs. measurements of asthma control). Therefore, to assess phenotypic and genetic predictors of asthma control, we need firm treatment outcome phenotypes. Within **Chapter 8** we investigated the association between measurements of current control (during the previous week) and long-term asthma control (during the previous season and the previous year). Overall agreement between current and long-term asthma control was limited (66% for previous season and 68% for previous year). Furthermore, we showed significant seasonal differences in asthma control. To properly define asthma control and/or treatment response in observational studies, it is important to assess asthma control over a longer period of time and to stratify or adjust for the different seasons.
In **Chapter 9** we described the previously observed seasonal patterns in asthma symptoms in more detail and sought for seasonal determinants of asthma control. We showed a decline in asthma symptoms and medication use during the summer period and a peak occurred from autumn to spring ($p < 0.0001$). Allergic rhinitis was associated with an increased risk of uncontrolled asthma during spring and summer. Eczema was associated with a higher risk of uncontrolled asthma during autumn and winter. Seasonality in asthma morbidity and health care use is most likely associated with atopic constitution and viral infections, which are common during fall, winter and spring.

The third part of this thesis focussed on pharmacogenetics of asthma medication. First, in **Chapter 10**, we described the challenges in pharmacogenetics and pharmacogenomics research and the role of using a systems biology approach. Pharmacogenomics may explain part of the inter-individual variability in drug response due to genetic variation. However, besides genetic factors, many other factors can play a role in the response to pharmacotherapy, such as disease severity, co-morbidity, environmental factors, therapy adherence and co-medication use. Therefore, a systems biology approach, which studies organisms as integrated and interacting networks of genes, proteins and biochemical reactions instead of the individual parts, may be helpful.

In **Chapter 11** we reviewed the current evidence of genetic associations and treatment outcomes with anti-inflammatory asthma drugs (corticosteroids and leukotriene antagonists). The field of asthma pharmacogenetics is still relatively new. However, there are some well-established pharmacogenetic examples described for anti-inflammatory agents, such as the *CRHR1* gene in relation to ICS response and the *ALOX5* gene in relation to response to leukotriene antagonists.

Chapter 12 described the rationale and design of the PACMAN (Pharmacogenetics of Asthma medication in Children: Medication with ANti-inflammatory effect) study. The on-going PACMAN study was initiated in April 2009 as an observational pharmacy based study, aiming at inclusion of at least 1000 children (age 4-12 years) who are regular users of asthma medication. The main goal of this study is to investigate (genetic) predictors of treatment response.

In **Chapter 13** we described our first pharmacogenetic work: we investigated the influence of the *FCER2* T2206C variant in paediatric asthmatics on ICS. The T2206C variant had previously been associated with asthma exacerbations in paediatric ICS users participating in a clinical trial. We replicated this previous finding and showed the T2206C variant to be associated with increased risk of exacerbations (OR: 1.9, 95% CI: 1.1-3.4). Furthermore, the T2206C variant was associated with a higher prevalence of asthma symptoms and asthma-related sleep disturbances during the preceding year ($p < 0.05$) and with need for increased daily steroid dose (OR: 2.5, 95% CI: 1.4-4.4).

Subsequently, we studied a larger number of SNPs with respect to ICS treatment. A total of 48 SNPs in genes that are part of the corticosteroid receptor complex (inflammatory pathway) were tested to study their influence on steroid response in **Chapter 14**. None of the tested SNPs met de Bonferroni corrected p-value of 2.08*10-4 in this preliminary study. The most significant association was found between asthma exacerbations and the rs10477211 *NR3C1* gene, coding for the glucocorticosteroid receptor gene (OR: 2.1, 95% CI: 1.3-3.3, $p = 0.002$). Currently efforts are being made to genotype the SNPs in another population (BREATHE study, UK, so that the data can be analysed together.

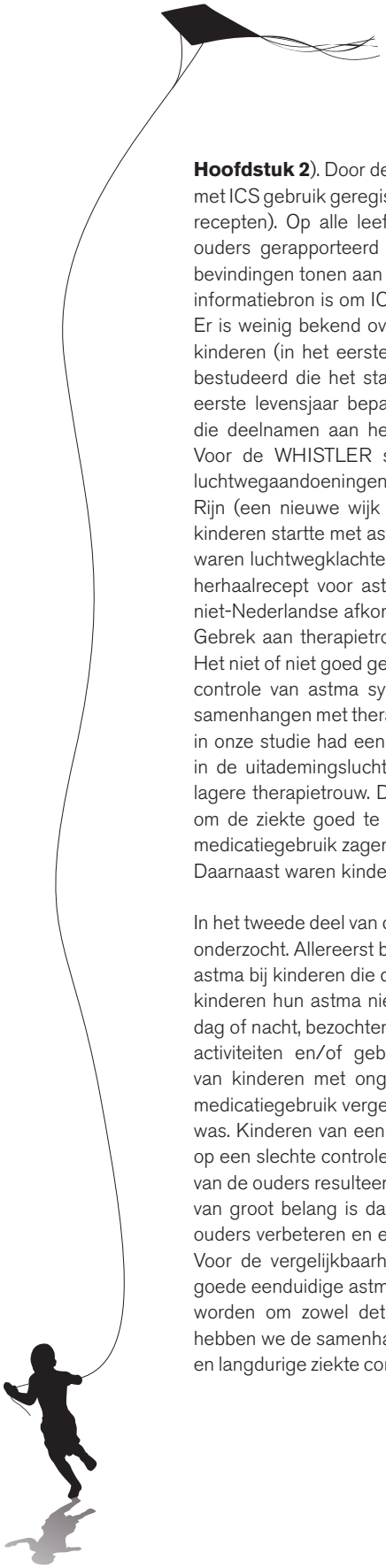
Finally, **Chapter 15** provides a general discussion of our findings in a broader perspective, including recommendations for clinical practice and future research.

SAMENVATTING

Astma is een chronische ontsteking van de luchtwegen die gekenmerkt wordt door aanvallen van hoesten, een piepende ademhaling, benauwdheid en extra slijmproductie in de longen. Het is de meest voorkomende chronische ziekte bij kinderen, ongeveer 5% van de kinderen in Nederland heeft astma. In de meeste gevallen kan de ziekte goed behandeld worden met medicijnen die ontsteking in de longen verminderen, zogenaamde ontstekingsremmers. Deze medicijnen worden meestal via de mond ingeademd (geïnhaleerd), zodat ze direct in de longen terechtkomen. Inhalatie corticosteroïden (ICS) zijn de meeste gebruikte ontstekingsremmers en zeer effectief in het onderdrukken van ontsteking in de luchtwegen. Helaas is er ook een groep patiënten waarbij behandeling met ICS onvoldoende effectief is om de ziekte onder controle te krijgen. Er zijn verschillende redenen waarom medicijnen bij de ene persoon wel werken en bij de andere persoon niet. Het niet goed gebruiken van medicatie volgens het voorschrift van de arts (niet therapietrouw), de ernst van de ziekte, het gebruik van andere geneesmiddelen (co-medicatie) en omgevingsfactoren (bijvoorbeeld contact met uitlokkende allergenen zoals huisstofmijt) kunnen daarbij een rol spelen. Ook erfelijke (genetische) verschillen, zogenaamde SNPs (Single Nucleotide Polymorphisms), dit zijn variaties in het DNA, kunnen invloed hebben op de gevoeligheid voor een ziekte of de werking van medicijnen.

In dit proefschrift hebben wij het gebruik van astmamedicatie en behandeluitkomsten bij kinderen met astma bestudeerd. We hebben geprobeerd te vinden waarom astmamedicijnen bij de ene patiënt wel werken en bij de andere niet. Daarbij hebben we een begin gemaakt met de vraag of bepaalde genen deze verschillen in reactie op medicijnen kunnen verklaren. Het proefschrift is verdeeld in drie delen: onderzoek naar het gebruik van astmamedicatie in de kinderjaren en factoren die hiermee samenhangen (**Hoofdstuk 2 t/m 6**), onderzoek naar de controle van de ziekte (uitkomst van behandeling) (**Hoofdstuk 7 t/m 9**) en onderzoek naar genetische factoren die van invloed zijn op het resultaat van behandeling (**Hoofdstuk 10 t/m 14**). **Hoofdstuk 1** is een introductie in de verschillende aspecten van astma en het gebruik van astmamedicatie op de kinderleeftijd. Hier worden de doelen en de opzet van het proefschrift beschreven.

In **Hoofdstuk 2** hebben we de opbouw van de PIAMA (Preventie en Incidentie van Astma en Mijt Allergie) apotheek dataset beschreven. De gegevens van het PIAMA onderzoek (vragenlijst gegevens van bijna 4000 kinderen) werden aangevuld met gegevens betreffende door de patiënt afgehaalde recepten uit openbare apotheken. Dit resulteerde in een dataset met de gegevens over het medicatiegebruik van 777 kinderen gedurende hun eerste acht levensjaren. Deze dataset, de PIAMA apotheek set, biedt mogelijkheden om patronen van medicatiegebruik in de loop van de tijd te bestuderen.
In **Hoofdstuk 3** hebben we deze nieuwe dataset gebruikt om gebruikspatronen van astmamedicatie voor kinderen in hun eerste acht levensjaren te beschrijven. Astmamedicatie werd veelvuldig voorgeschreven, met name vóór de leeftijd van drie jaar. Slechts weinig kinderen gebruikten continu medicatie gedurende hun eerste acht levensjaren.
In epidemiologische studies, zoals het PIAMA onderzoek, wordt vaak gebruik gemaakt van door de ouders gerapporteerde gegevens (vragenlijsten). In **Hoofdstuk 4** hebben we de betrouwbaarheid van door de ouders gerapporteerd ICS gebruik onderzocht met behulp van gegevens uit zowel de PIAMA studie als de PIAMA apotheek dataset (beschreven in



Hoofdstuk 2). Door de ouders in een vragenlijst gerapporteerd ICS gebruik werd vergeleken met ICS gebruik geregistreerd in het apotheek informatie systeem (door de patiënt afgehaalde recepten). Op alle leeftijden (nul tot acht jaar) was de overeenstemming tussen door de ouders gerapporteerd en in de apotheek geregistreerd gebruik erg hoog (>97%). Onze bevindingen tonen aan dat door de ouders gerapporteerd medicatiegebruik een betrouwbare informatiebron is om ICS gebruik in kinderen te bepalen.
Er is weinig bekend over factoren die het voorschrijven van astmamedicatie aan hele jonge kinderen (in het eerste levensjaar) bepalen. Daarom hebben we in **Hoofdstuk 5** factoren bestudeerd die het starten met astmamedicatie en het krijgen van herhaalrecepten in het eerste levensjaar bepalen. Voor deze studie gebruikten wij gegevens van 1202 kinderen die deelnamen aan het WHISTLER (Wheezing Illnesses Study Leidsche Rijn) onderzoek. Voor de WHISTLER studie, een groot geboorte-cohort gericht op het bestuderen van luchtwegaandoeningen, worden de ouders van alle gezonde pasgeborenen in Leidsche Rijn (een nieuwe wijk vlakbij Utrecht) benaderd voor deelname. Vijftien procent van deze kinderen startte met astmamedicatie in het eerste levensjaar. De belangrijkste reden hiervoor waren luchtwegklachten. Kinderen van Nederlandse afkomst (autochtoon) kregen vaker een herhaalrecept voor astmamedicatie in het eerste levensjaar vergeleken met kinderen van niet-Nederlandse afkomst.
Gebrek aan therapietrouw is bij chronische aandoeningen zoals astma vaak een probleem. Het niet of niet goed gebruiken van ICS is veelvuldig beschreven en kan leiden tot een slechte controle van astma symptomen. In **Hoofdstuk 6** hebben we onderzocht welke factoren samenhangen met therapietrouw bij kinderen die ICS gebruiken. Slechts 57% van de kinderen in onze studie had een goede therapietrouw. Kinderen met verhoogde stikstofoxide niveaus in de uitademingslucht, een indicatie voor ontsteking in de luchtwegen, hadden vaak een lagere therapietrouw. Deze bevinding benadrukt de noodzaak van een goede therapietrouw om de ziekte goed te kunnen controleren. Wanneer de ouders een hogere noodzaak van medicatiegebruik zagen, hadden de kinderen een grotere kans om beter therapietrouw te zijn. Daarnaast waren kinderen van Nederlandse afkomst beter therapietrouw.

In het tweede deel van dit proefschrift wordt het onder controle hebben van astma symptomen onderzocht. Allereerst bestudeerden we in **Hoofdstuk 7** determinanten van ongecontroleerd astma bij kinderen die deelnamen aan de PIAMA studie. Op leeftijd acht jaar had 45% van de kinderen hun astma niet voldoende onder controle, zij hadden luchtwegklachten gedurende dag of nacht, bezochten een arts voor hun luchtwegklachten, waren beperkt in hun dagelijkse activiteiten en/of gebruikten extra luchtwegmedicatie in de afgelopen maand. Ouders van kinderen met ongecontroleerde astma symptomen zagen vaker de noodzaak in van medicatiegebruik vergeleken met ouders van kinderen waarbij de astma goed onder controle was. Kinderen van een moeder met een laag opleidingsniveau hadden een verhoogde kans op een slechte controle van hun astma, ook wantrouwen of zorgen omtrent medicatiegebruik van de ouders resulteerde in een slechtere astma controle. Wij concludeerden dat het daarom van groot belang is dat zorgverleners (met name de apotheker en de huisarts) kennis van ouders verbeteren en een positief beeld omtrent ziekte en medicatiegebruik stimuleren.
Voor de vergelijkbaarheid van resultaten van verschillende studies is het van belang om goede eenduidige astma controle fenotypes te beschrijven. Deze kunnen vervolgens gebruikt worden om zowel determinanten van behandelrespons te bestuderen. In **Hoofdstuk 8** hebben we de samenhang tussen de huidige ziekte controle (gedurende de afgelopen week) en langdurige ziekte controle (astma controle gedurende het afgelopen seizoen en gedurende

het afgelopen jaar) onderzocht. De overeenstemming tussen de huidige en langdurige astma controle was beperkt (66% voor het afgelopen seizoen en 68% voor het afgelopen jaar).
In **Hoofdstuk 9** hebben we seizoensgebonden patronen in astma symptomen bestudeerd en gezocht naar specifieke seizoensgebonden voorspellers van astma controle. We zagen een daling in astma symptomen en astma medicatiegebruik tijdens de zomermaanden en een piek in zowel symptomen als medicatiegebruik vanaf de herfst tot en met de lente. Kinderen met hooikoorts hadden een verhoogde kans op ongecontroleerd astma tijdens zowel de lente als de zomer. Kinderen met eczeem hadden een verhoogde kans op ongecontroleerde astma in de herfst en winter. Seizoensgebonden verschillen in astma morbiditeit hangen hoogstwaarschijnlijk samen met atopische (allergische) aanleg (zoals hooikoorts en eczeem) en het krijgen van virus infecties, die vaak voorkomen tijdens de herfst, winter en lente.

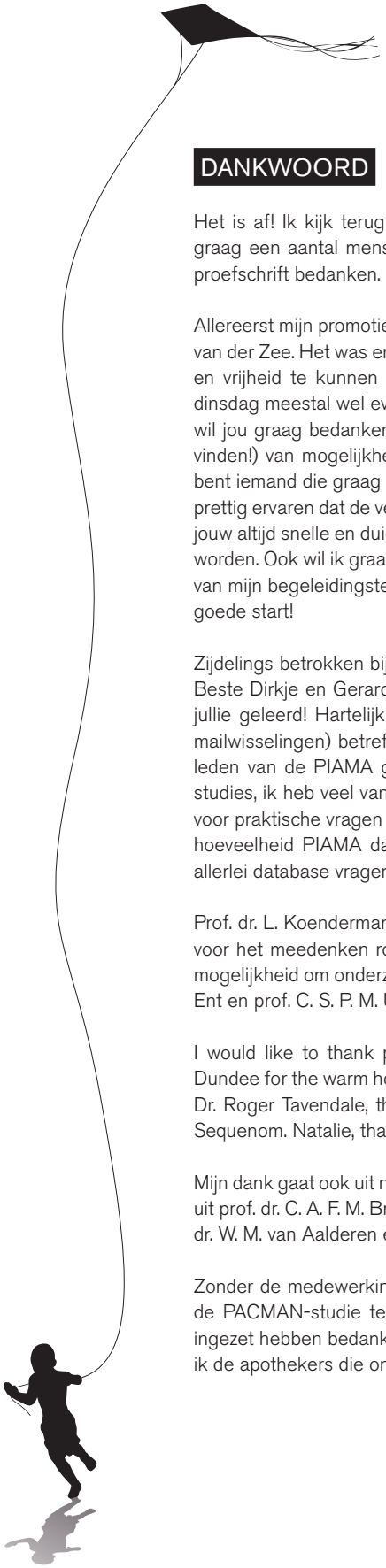
Het derde deel van dit proefschrift bevat allereerst twee hoofdstukken die de concepten van de farmacogenetica (in astma) introduceren en daarnaast beschrijft dit derde deel studies naar de farmacogenetica van ICS. Het onderzoeksgebied van de farmacogenetica heeft als doel meer inzicht te geven in de bijdrage van erfelijke verschillen (genetische variatie) in de reactie op geneesmiddelen. In **Hoofdstuk 10** hebben we de uitdagingen in farmacogenetisch onderzoek en de rol van een “systeembiologie” aanpak beschreven. Systeembiologie is het onderzoeksgebied dat biologische systemen bestudeert als een geheel, in plaats van de losse componenten (zoals genen, eiwitten en cellen) afzonderlijk. Farmacogenetica probeert de interindividuele verschillen in respons op geneesmiddelen te verklaren aan de hand van genetische variatie. Maar, naast genetische verschillen, kunnen ook andere factoren een rol spelen in de effectiviteit van behandeling met medicatie, zoals de ernst van de ziekte, omgevingsfactoren, therapietrouw en gebruik van andere medicatie. Daarom zou een zogenaamde systeembiologie aanpak, die een organisme als een netwerk van geïntegreerde en samenwerkende genen, eiwitten en biochemische reacties ziet in plaats van de individuele losse onderdelen te bestuderen, hierbij kunnen helpen.
In **Hoofdstuk 11** geven we een overzicht van wat er al bekend is over de genetische variatie en verschillen in behandelrespons op het gebied van ontstekingsremmende astmamedicatie. Farmacogenetica van astmamedicatie is een relatief nieuw onderzoeksveld. Toch zijn er wel een aantal farmacogenetische voorbeelden te noemen, zoals het *CRHR1* gen en respons op ICS en het *ALOX5* gen en respons op leukotriene receptor antagonisten.
Hoofdstuk 12 beschrijft de achtergrond en opzet van de PACMAN (Pharmacogenetics of Asthma medication in Children: Medication with ANti-inflammatory effect)-cohort studie. Deze nog lopende studie is in april 2009 van start gegaan in diverse openbare apotheken in Nederland om informatie te verzamelen van minimaal 1000 kinderen (leeftijd 4-12 jaar) die regelmatig astmamedicatie gebruiken. Inmiddels hebben al ruim 700 kinderen deelgenomen. Het doel van de PACMAN studie is om (genetische) voorspellers van behandelrespons te vinden.
In een eerder uitgevoerde klinische trial naar de effectiviteit van ICS vonden de onderzoekers dat dragers van *FCER2* variant genotype T2206C vaker ernstige astma exacerbaties (plotselinge verergering van de ziekte) hadden, dit zou kunnen komen doordat bij deze kinderen ICS minder goed werken. In **Hoofdstuk 13** hebben we de invloed van de T2206C variant van het *FCER2* gen op de effectiviteit van ICS onderzocht. Uit onze studie bleek ook dat dragers van twee variant allelen van de *FCER2* T2206C SNP een verhoogd risico hadden op het doormaken van een astma exacerbatie. Daarnaast hadden dragers van variant allelen vaker astma symptomen in het afgelopen jaar en kregen dragers van de T2206C variant een hogere dagelijkse ICS dosering.

Vervolgens hebben we een groter aantal SNPs die de effectiviteit van ICS zouden kunnen beïnvloeden bestudeerd. In **Hoofdstuk 14** hebben we in totaal 48 SNPs in verschillende genen die allemaal deel uitmaken van het corticosteroid-receptor complex, de plek waar het medicijn in het lichaam bindt zodat het zijn werking kan uitvoeren, bestudeerd in relatie tot ICS behandelrespons. Geen van de door ons bestudeerde SNPs in deze genen voldeed aan de statistische grens voor significantie. De meest significante associatie vonden we tussen een variant in het *NR3C1* gen, coderend voor de corticosteroid receptor (rs10477211) en een verhoogde kans op exacerbaties (OR: 2.1, 95% BI: 1.3-3.3, p = 0.002).

Tenslotte omvat **Hoofdstuk 15** een algemene discussie van onze bevindingen en plaatsen we de resultaten van de verschillende studies in een breder perspectief. Ook doen we op basis van de studies beschreven in dit proefschrift enkele aanbevelingen voor de kliniek en vervolgonderzoek.

CHAPTER 17

DANKWOORD
LIST OF PUBLICATIONS
ABOUT THE AUTHOR



DANKWOORD

Het is af! Ik kijk terug op een leuke en leerzame periode en wil op deze laatste pagina's graag een aantal mensen die op verschillende wijze een bijdrage hebben geleverd aan dit proefschrift bedanken.

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Zijdelings betrokken bij mijn onderzoek waren prof. dr. D. S. Postma en dr. G. H. Koppelman. Beste Dirkje en Gerard, ik heb onze samenwerking als erg prettig ervaren en heb veel van jullie geleerd! Hartelijk dank voor jullie bijdragen tijdens de diverse brainstormsessies (en mailwisselingen) betreffende zowel PIAMA als PACMAN in Utrecht dan wel Groningen. De leden van de PIAMA groep wil ik bedanken voor de kritische input bij de diverse PIAMA studies, ik heb veel van deze soms zeer uitgebreide discussies geleerd. Dr. A. H. Wijga, Alet, voor praktische vragen rondom PIAMA kon ik altijd bij jou terecht. Ada, jij beheert de enorme hoeveelheid PIAMA data, bedankt voor jouw snelle reacties en acties met betrekking tot allerlei database vragen.

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I would like to thank prof. dr. Colin Palmer and all of his colleagues at the University of Dundee for the warm hospitality. In a short period of time, I learned so much about genotyping. Dr. Roger Tavendale, thanks for all the help and patience in teaching me to work with the Sequenom. Natalie, thanks for letting me stay at your house, I really enjoyed the company.

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Met plezier heb ik vier jaar gewerkt op de afdeling Farmacoepidemiologie & Klinische Farmacologie. De gezamenlijke borrels, congresbezoeken, lunches en afdelingsuitjes hebben zeker bijgedragen aan het voltooien van dit proefschrift. Alle F&F'ers bedankt daarvoor! De dames van het secretariaat (Ineke, Suzanne, Anja (en daarvoor Addy)) wil ik bedanken voor de ondersteuning. Patrick, fijn dat ik met SPSS en andere database gerelateerde vragen bij jou kon aan kloppen. Svetlana, bedankt voor jouw ondersteuning bij het Markov stuk. Ook mijn kamergenoten, eerst Maarten en Helga en daarna Michelle en Marloes, wil ik bedanken voor de gezelligheid en vele theerondjes in de soms tropische hitte van N807. Simone, tegelijk van start en bijna tegelijk klaar. In de nieuwbouw deelden we daarom het "peeskamertje" op de 5e. Bedankt voor de gezelligheid en fijn dat we in deze laatste periode de regeldingen rondom het boekje en de promotie konden delen! Susanne, het was fijn om een "sparring partner" te hebben en samen over PACMAN te kunnen nadenken. Ons tripje naar Istanbul was een geslaagde afsluiter! Heel veel succes nog!

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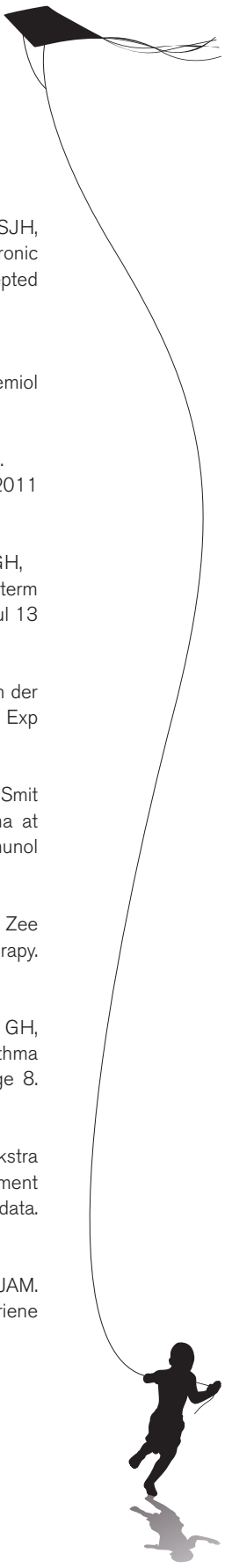

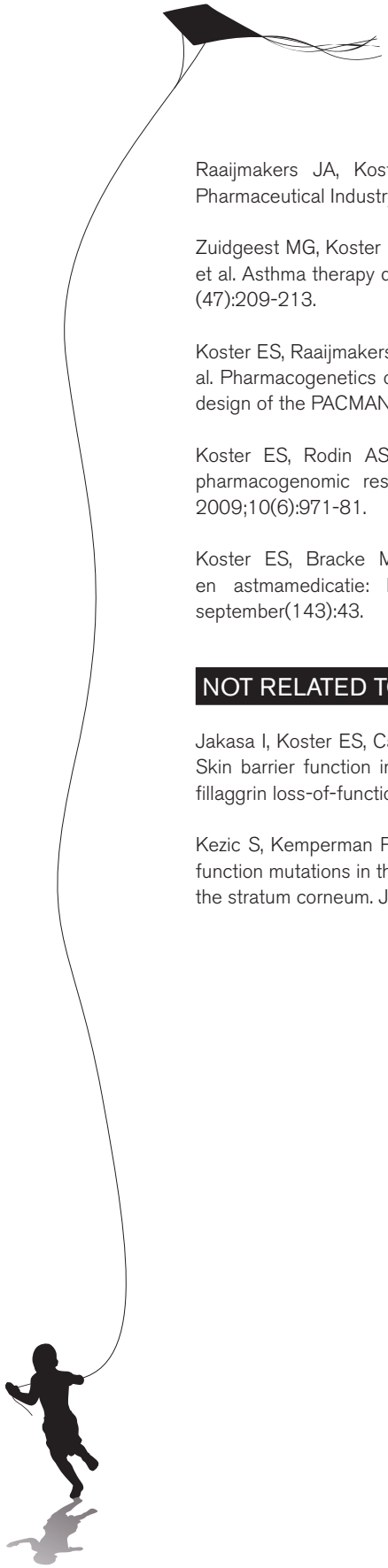

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Ellen Koster was born in Hengelo (ov) on December 8th, 1983. In 2002, she completed secondary school (Atheneum) at SG Twickel in Hengelo. In the same year she started her study Biomedical Sciences (majors Epidemiology and Toxicology) at the Radboud University Nijmegen where she obtained her Master's degree (MSc) in 2007. During her studies she completed research traineeships at the Coronel Institute for Occupational Health in Amsterdam and TNO Quality of Life in Zeist. In October 2007, she started her PhD on "Pharmacogenetics of asthma medication in children" at the division of Pharmacoepidemiology and Clinical Pharmacology, Utrecht University. During the last phase of her PhD-project, she received a grant for a short-term fellowship from the Dutch Asthma Foundation (Astma Fonds) for a study visit to the Population Pharmacogenetics Group at the University of Dundee, United Kingdom. Since September 2011 she holds a position as a post-doctoral researcher on a "Patient Safety" at the Nivel institute (Netherlands institute for health services research).