From the Department of Public Health Sciences,
Division of Occupational Medicine,
Karolinska Institutet, Stockholm, Sweden

Factors associated with lung function impairment in children and adults with obstructive lung disease

Jenny Hallberg

Karolinska Institutet

Stockholm 2008

Published by Karolinska Institutet. Printed by Universitetsservice US-AB.

ISBN 978-91-7409-019-2

Premisserna var dock alltför preciseringsdiffusa
för en distinkt satisfierande konklusion.
Skalman

ABSTRACT

Obstructive lung diseases are a group of diseases in which there is a limitation in the flow of air into or out of the lungs. Two common such diseases are asthma, which is found in both children and adults, and chronic obstructive pulmonary disease (COPD), mainly found in the population over 50 years of age. Both asthma and COPD seem to have with several different subgroups, or phenotypes, which may be associated with different long term consequences in regard to morbidity and in some cases also mortality. To be able to prevent and treat these diseases in the best possible way, we need to learn more about the different phenotypes and the mechanisms behind them.

The aim of this thesis was to study factors that are associated with lung function impairment in children and adults with obstructive lung diseases. The factors that are in focus are age of onset, duration of symptoms, sex, allergy, smoking and the contribution of genes and environment.

We have, in the two first papers, measured lung function at age 4 and 8 years in a birth cohort of 4,000 children and found that asthma symptom onset in the first 4 years of life was, on a group level, associated with impaired exhaled flows. This was found irrespective of the persistence of symptoms between the age of 4 and 8. We could also show tracking of impaired flows between the age of 4 and 8. Sensitization to airway allergens was associated with lung function impairment only in children with symptom onset after the age of 4. While male sex was a risk factor for asthma symptoms, girls with asthma symptoms showed a larger negative effect on exhaled flows, at least in the four first years. In paper 3 and 4, we studied symptom data from 45,000 twins from the Swedish Twin Registry to quantify heritability for chronic bronchitis and emphysema, two of the main components seen in COPD. As smoking behaviour has genetic influences, it was necessary to study how heritability for disease was associated with heritability for smoking. The results showed that ~40% of the individuals liability to developing chronic bronchitis/emphysema can be attributed to genetic factors, and that only a small part of these factors were found to be in common with those influencing smoking habits. Women more often reported chronic bronchitis/emphysema, compared to men, and this could not be explained by different smoking habits or different genes. Two hundred of the twins took part in a clinical testing of different lung function measures. The results from this study showed that all lung function measures that were studied had a heritable component, and that it was larger for women than for men.

In conclusion, we have studied how several different factors are associated with lung function impairment in children and adults with obstructive lung disease. In summary, the first years of life are of importance for future lung function. Children that outgrow their asthma seem, on a group level, to loose their symptoms rather their lung function impairment, which might be present through life. We have furthermore shown that genes are important for the individuals' liability to disease in adult life. Sex differences exist both in children and adult disease, and there are indications of a less favourable outcome for girls/women. More work is now needed to find the individuals that belong to these susceptible groups, and to develop and apply methods to prevent and treat impaired lung function and disease.

LIST OF PUBLICATIONS

The thesis is based on the following papers, which are referred to in the text by their Roman numerals (I-IV).

I. Hallberg J, Anderson M, Wickman M, Svartengren M. Sex influences on lung function and medication in childhood asthma. *Acta Paediatr* 2006:95;1191-1196.

II. Hallberg J, Anderson M, Wickman M, Svartengren M. Development of lung function in different phenotypes of asthma among children in a birth cohort (BAMSE). Submitted.

III. Hallberg J, Dominicus A, Eriksson UK, Gerhardsson de Verdier M, Pedersen NL, Dahlbäck M, Nihlén U, Higenbottam T, Svartengren M. Interaction between smoking and genetic factors in the development of chronic bronchitis. *Am J Respir Crit Care Med* 2008;177(5):486-90.

IV. Hallberg J, Iliadou A, Anderson M, Gerhardsson de Verdier M, Nihlén U, Dahlbäck M, Pedersen NL, Higenbottam T, Svartengren M. Sex differences in heritability for lung function in twins with and without respiratory symptoms. Submitted.

CONTENTS

LIST OF ABBREVIATIONS

General abbreviations

BAMSE	Barn Allergi Miljö i Stockholm, en Epidemiologisk undersökning (Children Allergy Environment in Stockholm, an Epidemiological survey)
COPD	Chronic obstructive pulmonary disease
DZ	Dizygotic
IgE	Immunoglobulin E
MZ	Monozygotic
SALT	Screening Across the Lifespan Twin study
STR	The Swedish Twin Registry

Lung function measures

CO	Carbon monoxide
DLco	Carbon monoxide diffusion capacity
eNO	Exhaled nitric oxide
$FEV_{0.5}$	Forced expiratory volume in 0.5 seconds
FEV_1	Forced expiratory volume in 1 second
FRC	Functional residual capacity
FVC	Forced vital capacity
MEFV	Maximal expiratory flow volume
MBWO	Multiple breath wash-out
NO	Nitric oxide
PEF	Peak expiratory flow
RV	Residual volume
TLC	Total lung capacity
VC	Vital capacity

1 THESIS SUMMARY – MAIN SECTION

1.1 BACKGROUND

1.1.1 Obstructive lung diseases

Obstructive lung diseases are a group of diseases in which there is a limitation in the flow of air into or out of the lungs. Two common such diseases are **asthma**, which is found both in children and adults, and **chronic obstructive pulmonary disease (COPD),** mainly found in the population over 50 years of age.

1.1.1.1 Definition and mechanisms of asthma and COPD

The Global Initiative for Asthma (GINA)[1] states that: "The main physiological feature of asthma is episodic airway obstruction characterized by expiratory airflow limitation. The dominant pathological feature is airway inflammation, sometimes associated with airway structural changes. Asthma has significant genetic and environmental components, but since its pathogenesis is not clear, much of its definition is descriptive."

The main characteristics of asthma are reversible airflow obstruction, airway inflammation and abnormal airway responsiveness to stimuli, such as viral infections, allergens, exercise/cold air, tobacco smoke, traffic related air pollution and other bronchial irritating chemicals. As the airways react to these agents, the muscles around the airways will contract, making breathing more difficult.

According to a world-wide consensus (Global Strategy for the Diagnosis, Management, and Prevention of Chronic Obstructive Pulmonary Disease)[2], COPD is defined as the following: "Chronic Obstructive Pulmonary Disease (COPD) is a preventable and treatable disease with some significant extrapulmonary effects that may contribute to the severity in individual patients. Its pulmonary component is characterized by airflow limitation that is not fully reversible. The airflow limitation is usually progressive and associated with an abnormal inflammatory response of the lung to noxious particles or gases."

Pathological changes characteristic of COPD are found in the proximal airways, peripheral airways, lung parenchyma, and pulmonary vasculature[3]. The airway obstructing pathology of COPD is represented by obstructive bronchiolitis and emphysema. Obstructive bronchiolitis is a chronic inflammation of the airway system, resulting in narrowing of the peripheral airways, an overproduction of phlegm and destruction of the phlegm transporting system of the airways. Emphysema is the result of the destruction of the lung parenchyma. The mix of obstructive bronchiolitis and emphysema vary from person to person and probably reflects different phenotypes of the disease. In addition, COPD also has significant extrapulmonary (systemic) effects that lead to comorbid conditions[4]. COPD is closely related to smoking[5], but also indoor air pollutants (especially among women in developing countries)[6] and occupational exposures[7] have been shown to be risk factors of importance.

The characteristic symptoms of COPD are chronic and progressive dyspnoea, as well as cough and sputum production, the latter two commonly referred to as chronic bronchitis. These symptoms may precede the development of airflow limitation by many years. Conversely, significant airflow limitation may develop without chronic cough and sputum production[8].

1.1.1.2 Epidemiology of asthma and COPD

Asthma is a common affliction of the population, present throughout the ages. In epidemiologic studies, but also to a certain extent in the clinical reality, the diagnosis of asthma is a symptom based. Defining asthma from questionnaires is challenging, and although the most widespread definition used is wheeze, sometimes in combination with medication, the frequency and duration of the episodes vary between studies.

We have during the last decades seen an increase in the prevalence of asthma, particularly among children; although we now see some evidence of a plateau being reached[9-11]. Current prevalences vary greatly between geographical areas. The International Study of Asthma and Allergies in Childhood (ISAAC) estimated the prevalence of asthma to between 1.6 and 30%, with the highest prevalence found in the UK, Australia and New Zealand[12]. Studies of Swedish children report prevalences of 6-9%[13-15]. Adult asthma includes persistent disease from childhood, reactivated childhood disease and adult onset disease. The two latter phenotypes are not uncommonly related to occupational exposure[16].

In general, asthma is a disease with a good prognosis for the majority of those who are affected. However, both in children and adults, there seem to be several different subgroups, or phenotypes, of asthma, all with its own (although sometimes overlapping) implications for future disease.

COPD is a disease of the adult population, usually found in individuals over 50 years of age. The diagnosis of COPD is based on lung function parameters and the presence of symptoms. For practical reasons, lung function testing is not always available in epidemiological studies, and therefore, the reported prevalence can vary depending on the disease definition. Even though it is possible that self reported symptoms overestimate the prevalence of disease, it can be viewed as describing a population "at risk", especially in combination with smoking habits. Also, it is widely believed that the incidence, prevalence and mortality statistics grossly underestimate the burden of disease, as persons do often not seek medical attention until the disease is clinically symptomatic and quite advanced. The prevalence increases with age[17, 18] and in Sweden, a lung function based prevalence of 8-15% in the population over 45 years has been estimated[19]. According to the Global Burden of Disease Study, COPD was the sixth most important cause of death in 1990, and is expected to become the third leading cause of death worldwide by 2020[20-22]. This is likely due to the expanding epidemic of smoking in combination with more of the population living longer.

1.1.1.3 Lung function in asthma and COPD

Lung function testing is a reproducible, standardized procedure to determine lung function and is used in the process of diagnosing and monitoring respiratory disease. There are a number of indices available and the ones used are in each case chosen by the aim of the examination, and the age and cooperation ability of the patient.

Dynamic spirometry

The maximal volume of air exhaled with maximally forced effort from a full inspiration is called "forced vital capacity" (FVC). The maximal volume of air exhaled in the first second of such a forced expiration is referred to as the "forced expiratory volume in one second" (FEV_1). In some patients, a slow or unforced measure of vital capacity, referred to as VC, may provide a larger value [23].

While FVC (or VC) represents the size of the lung, FEV_1 gives the flow dimensions. In asthma and COPD, both FVC and FEV_1 can be impaired, but the latter is usually affected to a greater extent, resulting in a disproportionate reduction of maximal airflow, in proportion to the maximal volume that can be exhaled from the lung[24].

Brochodilator responsiveness test

Normal FVC and FEV_1 do not exclude a diagnosis of asthma, as the spirometric key feature of the disease is the *variable* obstruction. In the case obstruction is indicated, a way to tease apart whether this is due to asthma or COPD is to perform a brochodilator responsiveness test, aiming to assess the reversibility of the obstruction. After inhalation of airway dilating medication, FVC and FEV_1 are measured again. The presence of significant bronchodilation, seen as an increase of FEV_1, indicates asthma. In some cases, an additional or exclusive improvement of the FVC can be found, as an effect of decreased residual volume as small peripheral airways open up. However, not only can COPD include a reversible component, but in late asthma stages, an irreversible airway obstruction component can sometimes be found.

Peak expiratory flow

The peak expiratory flow (PEF) is the highest flow obtained during a forced expiration after maximal inhalation. If the maneuver is performed sufficiently forcefully, PEF is not effort dependent, but (in most subjects) determined by flow limitation in mainly central, but possibly also in more peripheral airways [25].

A single measurement of PEF compared to a predicted value is of little use in diagnosing disease, as variability between healthy subjects is large and it does not necessarily correlate well to FEV_1[26]. Instead, it is clinically used as a way to determine change in respiratory function over time[27]. PEF can be obtained both from a forced expiratory maneuver measured by a spirometer or with the use of a small pocket PEF meter, although the results are not always be directly comparable due to the type of scale used[28].

Static spirometry

The functional residual capacity (FRC) is the volume of gas present in the lung at end expiration during normal quiet breathing [29]. Its relation to the total lung capacity (TLC)

and residual volume (RV) is illustrated in figure 1. The FRC can be assessed by body plethysmography, gas washout or gas dilution tests, or radiography.

In severe airway obstruction, lung compartments can be incapable of complete emptying due to airway closure. This gives rise to a higher volume left in the lung at the end of expiration, i.e. a higher FRC and RV[30].

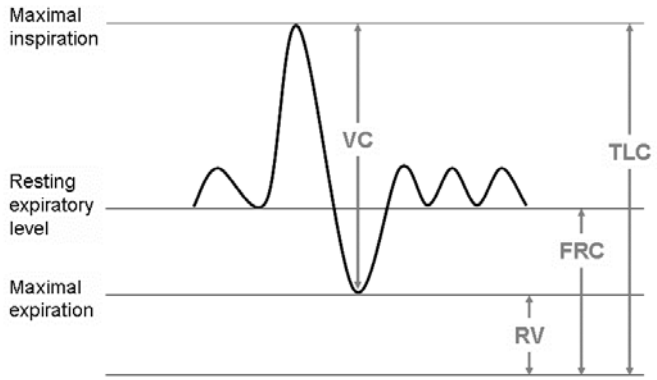

Figure 1. Lung volumes. Vital capacity (VC), total lung volume (TLC), functional residual capacity (FRC) and residual volume (RV).

Diffusion capacity
The diffusion capacity test aims to determine the efficiency of the diffusion of gas between alveolar air and the lung capillary circulation. The diffusion capacity in the lungs (DL) is most often measured using carbon monoxide (CO), hence the abbreviation DLco.

The diffusion capacity can be impaired due to small diffusion area, large diffusion way or low ability for the blood to take up CO (for example due to low hemoglobin concentration)[31]. Hence, impaired DLco is common in individuals with COPD, particularly in emphysema[32], although patients with normal DLco can have emphysema detected by computer tomography[33]. In asthma, DLco been found to be normal to increased[34].

Exhaled nitric oxide
Nitric oxide (NO) is produced from the enzymatic oxidation of arginine, and exerts a variety of biological functions throughout the body. It acts, for example, as a vasodilator, neurotransmitter, cytotoxic agent and inhibitor of platelet aggregations[35].

NO can also be measured in exhaled air, which proves to be a practical non-invasive method that can be clinically useful in diagnosing and monitoring airway diseases. After a full inhalation, immediate exhalation should be performed at a constant flow rate, until an NO plateau can be identified. The NO level at this plateau is registered in parts per billion (ppb)[36].

Elevated levels of exhaled NO (eNO) have been documented in asthma [37, 38]. Conventionally measured eNO in COPD is less useful, as the levels are usually normal or only slightly elevated, except during exacerbations[37].

1.1.1.4 Factors associated with lung function impairment in asthma and COPD

This thesis focuses on explaining how the following factors contribute to lung function impairment in children and adults with obstructive lung disease;

The timing of asthma onset and duration
It is now generally accepted that a number of distinct phenotypes of childhood asthma exist, and that some of these are more strongly associated with impaired lung function than others[39-42]. In general, early onset of symptoms, i.e. symptom onset in the first three-four years of life, has been shown to be associated with poor lung function in childhood. Further, there is evidence of tracking of lung function from birth or childhood into adolescent or adult life[43, 44], and that this might be valid both for children with transient and persistent symptoms[45].

These data indicate that, to be able to fully understand the temporal development of lung function in asthma, it is important to repeatedly assess lung function early in life, during a period where lung function tests can be very difficult to perform mainly due to the limited cooperation skills of the young child.

Asthma and sensitization to allergens
The presence of circulating IgE antibodies can be measured in serum. Individuals with increased levels of allergen specific IgE antibodies to common inhalant or food allergens are referred to as sensitized to that particular allergen. A strong association between sensitization and asthma has been shown[40, 46-49], although the association between sensitization and impact on lung function has been less extensively studied - in one study, children with allergic sensitisation were found to have impaired lung function regardless of the presence of respiratory symptoms[50], while another study showed that sensitization to indoor allergens were found to be a determinant of impaired lung function in children among current wheezers[51].

Sex differences in obstructive lung disease
Sex differences in prevalence and severity of asthma vary through life. During childhood, male sex is a risk factor for asthma. This has been attributed to differences in the structure/function relationship of the lung and airways, where girls have airways that are more proportionate to the size of their lungs, while the airways of boys are proportionally smaller, compared to lung size[52]. After puberty, especially severe asthma is more common in women[53-55], and the asthma associated sex differences in adults have been suggested to be mediated through immunological response and the effect of sexual hormones[56, 57].

Chronic bronchitis and COPD has traditionally been more common in men[58], largely due to the male predominance of smoking, although women are catching up. In 2000, the number of women dying of COPD in the United States surpassed the number of men for the first time[59]. Further, studies have shown evidence of differences between

men and women in the response to environmental agents, such as smoking and air pollutions[60-64].

COPD and smoking

The most common cause of COPD in the industrialized world is tobacco smoking, with up to 90% of all patients with COPD being smokers or ex-smokers[5]. Smoking causes a more rapid annual decline in FEV_1, compared to non-smokers[65]. Still, large individual differences in a person's susceptibility to the effects of smoking have been demonstrated[65, 66].

COPD: genes and environment

The fact that not all smokers develop clinically significant COPD[19], while some nonsmokers do[67], suggests that genetic factors modify each individuals risk. Although COPD has been shown to cluster in families[68, 69], it does not have an obvious pattern of inheritance. It is therefore likely that COPD is a complex genetic disorder, where it many genes and environmental factors interact.

The best described genetic process involved in COPD is alpha-1-antitrypsin deficiency, a where the impairment of the ability to produce a protective protein is associated with risk for premature development of emphysema, even in non-smokers [70, 71]. It does, however, account for only 1-2% of COPD cases[72], and a number of additional candidate genes have been described[73]. One way to determine if, and to what extent, a disease carries a genetic pattern is to use twin studies, in which the similarities and differences between and within monozygotic and dizygotic twin pairs are compared to calculate heritability, i.e. the relative contribution of genes to the variation of a disease. With the use of this methodology, genetic influence on chronic bronchitis has been demonstrated[69, 74-79], although the specific methods used did not allow for quantification of the relative importance of genes and environment.

Given the sex difference in disease prevalence and response to environmental exposures stated above, it will be of particular interest to study potential sex differences in the genetic and environmental contribution to lung function, especially in populations with respiratory symptoms. Furthermore, it has been shown that acquisition of "addiction" to smoking is partly genetically mediated[80-82], and thus it is of interest to examine whether smoking contributes to individual differences in lung function only as an environmental and/or as a genetic factor[83].

Childhood respiratory disorders and COPD

Even though asthma and COPD present differences in both risk factors and clinical phenotypes, it is not uncommon to observe adult asthma patients with COPD like phenotypes and vice versa. Even though they are sometimes treated as mutually exclusive entities in regard to finding mechanisms or testing the effect of treatments, it might not be optimal in order to represent the more complex reality. In addition, a number of pre- and postnatal events[84], such as prenatal insults to the developing lung, infections and the adolescents' own smoking habits[85], are important in that they may reduce maximal lung growth and might even precipitate early decline in lung function[86].

1.1.2 Aims

The aim of the thesis is to study factors related to lung function impairment in obstructive lung disease.

Specific aims are:

1. To evaluate possible sex differences in asthma diagnosis and treatment; to study whether influence on peak expiratory flow (PEF) associated to asthma severity differs between boys and girls; and to confirm results from previous studies, that children with transient asthma still show an impaired lung function.

2. To assess the relationship between lung function development over time and asthma phenotypes in a population based birth cohort (BAMSE) of 4,089 children.

3. To assess, in a population based sample of twins, (a) to what extent genetic factors contribute to the development of chronic bronchitis, including emphysema, taking sex into consideration and, (b) whether the genetic influences on chronic bronchitis, including emphysema are separate from those for smoking behaviour.

4. To assess, in a sample of twins with and without respiratory symptoms, the following: (a) to what extent genetic factors contribute to forced expiratory volume in one second (FEV_1), vital capacity (VC) and diffusion capacity (DLco), taking sex into consideration, and (b) how smoking behaviour influences these estimates.

1.2 METHODS

1.2.1 Studies of asthma in children (study I and II)

1.2.1.1 Study population

The BAMSE cohort is an ongoing prospective birth cohort study. The parents of all children born in defined areas of Stockholm between 1994 and 1996 were invited to participate in the study. In total, 7,221 children were born during the time period. After excluding 3,132 children, due to wrong address information, unwillingness to participate, parental inability to read or write Swedish, planning to move outside of Stockholm, a sibling already included in the study or serious illness of the child, the cohort consisted of 4,089 children.

When the children were 2 months of age the parents received a mailed questionnaire on parental allergy and environmental exposures. When the children were 1, 2, 4 and 8 years old, the parents received new mailed questionnaires focusing mainly of various symptoms of allergic disease.

All parents who answered to the questionnaires when the child was 4 and 8 years of age were asked to bring their child for a clinical examination, including lung function testing and blood sampling.

Table 1. Number of children followed in the BAMSE cohort and participation rate at different stages of data collection.

Age	No. of participants in questionnaires (%)	No. of participants in clinical examinations (%)
0 years	4,089(100)	
1 years	3,925(96)	
2 years	3,843(94)	
4 years	3,727(92)	2,965(73)
8 years	3,435(84)	2,630(64)

1.2.1.2 Definition of disease

Based on answers from the questionnaire, asthma at 2 years of age was defined as >3 episodes of wheezing between birth and 2 years of age, combined either with inhaled steroids or signs of hyper-reactivity (wheezing or severe coughing at exaltation and cold weather, or disturbing cough at night) without ongoing cold. At 4 and 8 years of age, asthma was defined as >3 episodes of wheezing over the last year, or 1 episode if the child had been given inhaled steroids[15, 87, 88].

In study I, studying the first 4 years of life, children were divided into groups according to if they had asthma (1) in the first 2 years, but not at 4 years, (2) asthma in the first 2 years and at 4 years, or (3) asthma at 4 years, but not during the first 2 years.

In study II, studying the first 8 years of life, the definitions were altered to reflect the longer time-span of the study; transient asthma was defined as asthma in the first 4 years, but not at 8 years, persistent asthma as asthma in the first 4 years and at 8 years, and late onset asthma (representing incident cases after the age of 4) as asthma at 8 years, but not during the first 4 years.

1.2.1.3 Clinical measures

Lung function testing

Study I and II. Peak expiratory flow (PEF) was measured at both 4 and 8 years of age, using the normal range Ferraris Peak Flow Meter® (Ferraris Medical Limited, UK). Results were recorded as the largest value of several successive PEF readings, provided that the child's effort was coded as maximal by the test leader, and that the two highest readings were reproducible (within 15% of each other).

Study II. Maximum expiratory flow volume (MEFV) and multiple breath wash-out (MBWO) tests were performed at the 8 year follow up, using a spirometer (2200 Pulmonary Function Laboratory, Sensormedics, Anaheim, CA, USA). From the MEFV curves, forced vital capacity (FVC), forced expiratory volume in 1 second (FEV_1) and forced expiratory volume in 0.5 seconds ($FEV_{0.5}$) were extracted. All children performed several measurements sitting, using a nose clip. The best MEFV curve was printed, reviewed manually and used for analysis, provided that the child's effort was coded as maximal by the test leader, and that the two highest readings were reproducible (within 10% of each other).

To assess ventilation heterogeneity, the MBWO test was performed once for each child. The lung clearance index (LCI) was calculated by dividing the expired volume during the washout, by the functional residual capacity (FRC) determined from the washout. An FRC lower than 300 ml or larger than 4,000 ml was coded as technical problems. The spirometer volumes were calibrated at least daily using a 3L syringe. The nitrogen analyzer was calibrated weekly.

Exhaled nitric oxide

Study II. Measurements of exhaled nitric oxide (eNO) was performed with a NIOX chemiluminescence analyzer (Aerocrine, Solna, Sweden) in a subgroup of 482 children. To ensure that both children with symptoms and healthy controls were examined, the children performing eNO measurements was randomly sampled from those who reported symptoms of asthma, rhinitis and eczema in any, or neither, of the questionnaires. Children were encouraged to maintain a steady flow rate of 50 ml/s with the aid of a built-in incentive animation software. NO values were given as parts per billion (ppb) at the plateau of the end-expiratory reading. Tests that were accepted by the machine software and coded as maximal effort by the test-leader were included in the analysis.

Measurements of specific IgE antibodies

(Study I, II and IIII) Allergen-specific IgE-antibodies were measured against a mix of common inhalant allergens (birch pollen, timothy grass pollen, mugwort pollen, house dust mite, mould, cat, dog and horse dander) using Immuno CAP™ (Phadia AB,

Uppsala, Sweden) at a certified laboratory (Unit of Clinical Immunology and Allergy, Department of Medicine, Karolinska University Hospital). An IgE value ≥0.35 kU/l was regarded as positive. Positive tests were further analyzed for allergen-specific IgE antibodies to the allergens listed above in groups of perennial (cat, dog, horse and mite) and seasonal allergens (mould, birch, timothy and mugwort).

1.2.1.4 Statistical methods

Study I. Linear regression analysis was used to calculate differences in PEF-values between asthma groups, adjusted for sex, height and age. Mean PEF values in L/min for each group of a child in relation to mean age and height is presented when appropriate. To compare differences in PEF between asthma groups for boys and girls, an interaction term between sex and asthma group was included in the model. A p-value of the interaction term of <0.05 was considered statistically significant. Differences and disproportion of medication use and doctor's diagnosis are presented with 95% confidence intervals. Tests of differences were made with a Chi2 test, except for the "no current wheeze" group where a Fisher's exact test was used due to a limited number of subjects. Analyses were performed with STATA 7.0 software package (StataCorp LP, College Station, TX, USA).

Study II. Participating and non-participating children were compared regarding asthma, the mother's smoking habits during pregnancy, and socioeconomic index were made by chi-2-tests. The association between asthma groups and lung function variables were assessed by multiple linear regressions. All lung function variables were adjusted for age, height and sex. Socioeconomic status and maternal smoking during pregnancy (assessed as binary variables) were evaluated as possible confounders in the total sample and within each asthma group, but were not found to be significantly correlated with the outcome. To study the importance of sex or sensitization to perennial and/or seasonal inhaled allergens on lung function within each asthma group, an interaction term between sex or sensitization and asthma group was included in the model. To avoid loss of power due to exclusion of subjects, the maximal number of children available for each lung function variable was used in the analysis. eNO values did not show a Gaussian distribution, and have been described by median values and interquartile range (IQR). Mann-Whitney test was used to compare eNO values between groups. P-values of < 0.05 were considered statistically significant. Analyses were performed with the STATA 9.2 software package (StataCorp LP, College Station, TX, USA).

1.2.2 Studies of adult twins (study III and IV)

1.2.2.1 Study population and disease definition

Study III and IV are both are based on data from the Swedish Twin Registry (STR). The STR contains information on more than 80,000 twin pairs born from 1886 to 2000[89]. Between 1998 and 2002, all living twins in the STR born 1958 or earlier were contacted using a computer-assisted telephone interview in the Screening Across the Lifespan Twin (SALT) study[90]. The SALT interview included introductory items concerning zygosity and a checklist of common diseases. A series of questions related to respiratory symptoms and chronic bronchitis was also included. Special emphasis

was put on diagnostic items that could determine whether a twin was likely to have a disease, rather than simply asking the twin if they have a disease. Informed verbal consent was obtained prior to interview.

Of the 61,767 twins born 1958 or earlier who were alive in March 1998 when SALT started, 44,919 twins participated either themselves or through a proxy (if the twin was unable to take part due to cognitive impairment or hearing problems, the interviewers asked to talk to a family member or personnel at institutions), corresponding to a response rate of 73%. 42,706 individuals had complete answers to the respiratory symptoms screening questions and could be classified by zygosity. The analyses of chronic bronchitis are based on 4,178 complete monozygotic (MZ) twin pairs (with both members observed), 2,249 MZ singletons (with only one member observed), 11,670 complete dizygotic (DZ) pairs, and 8,761 DZ singletons. Information about smoking habits, measured by pack years (referring to the total smoking consumption during life), was available for 36,772 individuals, consisting of 3,430 complete MZ pairs, 2,740 MZ singletons, 9,186 complete DZ pairs, and 10,195 DZ singletons.

Study III. Individuals who responded positive to questions in SALT about recurrent cough with phlegm production, and/or had self reported chronic bronchitis were defined as having chronic bronchitis. Further, individuals who answered yes to the question on emphysema was included in the chronic bronchitis definition, using the rationale that emphysema was a unlikely to be present without prior symptoms of chronic bronchitis. Individuals with negative answers to all of the above questions were considered not to have chronic bronchitis. Individuals with self reported asthma or asthma symptoms with an onset before adulthood or that were related to allergic disease were positive for asthma.

Study IV is a clinical follow-up of study III, aiming to include 200 twin pairs. To assure that the sample would contain twins with symptoms of respiratory disease (self-reported symptoms of cough, chronic bronchitis, emphysema or asthma), disease concordant and discordant twins were prioritized over symptom free twin pairs. Due to the relatively small number of symptom concordant twins available in the population, pairs were included regardless of smoking habits, while symptom discordant and symptom free pairs were further stratified on if none, one or both of the twins in a pair had a significant smoking history, i.e. had smoked more than 10 pack years (1 pack year is equal to smoking 20 cigarettes per day for 1 year) at the time of inclusion.

1.2.2.2 Clinical measures

Study III did not include a clinical testing.

In study IV, lung function examination included measurements of dynamic and static volumes, as well as diffusion capacity. In twins with FEV_1/FVC five percent units lower than predicted or a FEV_1 below 90% of predicted, a reversibility test was performed to test for reversible obstruction.

1.2.2.3 Determination of twin- zygosity

In study III, zygosity information was obtained at the time of registry compilation on the basis of questions about childhood resemblance. Four separate validation studies using serology and/or genotyping have shown that with these questions 95-98% of twin pairs are classified correctly[90].

In study IV, zygosity of the sex-like pairs was determined by the use of a set of DNA markers from blood drawn at the clinical testing. Blood samples were not available for both members in 14 pairs, and zygosity information for these twins was instead obtained at the time of registry compilation on the basis of questions about childhood resemblance.

1.2.2.4 Statistical methods

Prevalence (study III)
The prevalence of chronic bronchitis in study III was calculated stratified on sex and smoking habits, and estimates for males and females were compared by odds ratios.

Quantitative genetic analysis (study III and IV)
Twin studies are ideal for estimating genetic and environmental effects of traits and diseases. Identical (monozygotic, MZ) twins share the same genes, whereas fraternal (dizygotic, DZ) twins share, on average, half of their segregating genes. In general, the phenotypic variance is assumed to be due to three latent factors: additive genetic factors (a2), shared environmental factors (c2), and non-shared environmental factors (e2), which also include measurement error:

$$Var(Y) = a2 + c2 + e2$$

The correlation for MZ twin pairs is assumed to be due to additive genetic and shared environmental factors (a2+c2). The within pair correlation in DZ twins is assumed to be due to the sum of half the genetic and shared environmental factors (0.5a2+c2). A genetic effect is indicated if twin similarity is greater among MZ than DZ pairs. Heritability is defined as the proportion of total phenotypic variation directly attributable to genetic effects[91]. Consequently, significant heritability reflects the importance of genetic variation for a trait or disease. The partitioning of phenotypic variance into genetic and environmental effects is usually illustrated in a path diagram. Genetic, shared, and nonshared environmental components are presented as latent variables[92]. The genetic correlation (ra) is set to 1 in MZ twins and to 0.5 in like-sex DZ twins. The shared environment correlation (rc) is set to 1 for both groups. By definition there is no correlation for the nonshared environment. Figure 2 illustrates a path diagram for an opposite-sex twin pair. The genetic, shared, and non-shared environmental variance components are noted as am, cm, em, af, cf, and ef, for males and females, respectively.

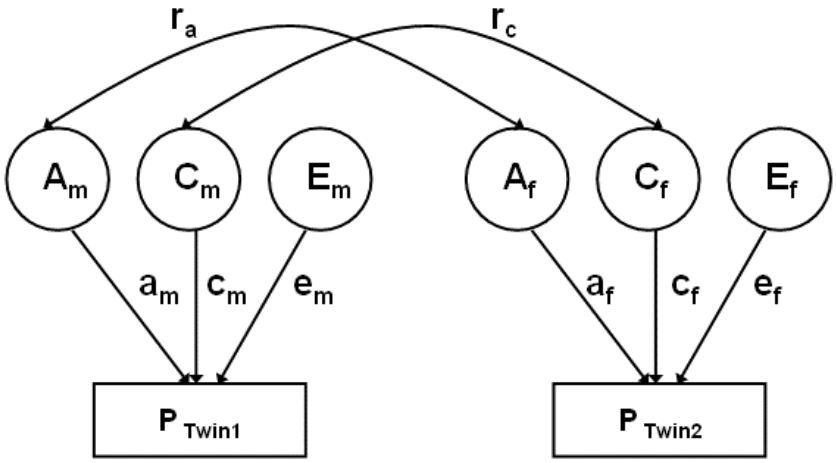

Figure 2. Basic path diagram for an opposite sexed twin pair. Am, Cm, Em, Af, Cf, Ef, ra, and rc are the genetic, shared and non-shared environmental variance components, genetic correlation, and shared environmental correlation for males and females, respectively.

Univariate structural equation modeling of dichotomous data (study III)
The relative importance of genetic and environmental influences on the liability to chronic bronchitis was evaluated using structural equation models. We assume a liability-threshold model, in which the liability to disease is normally distributed and manifested at a certain threshold as a categorical phenotype[92]. The threshold was adjusted for age and sex to account for the fact that the prevalence of chronic bronchitis depends on these factors.

A series of models, making different assumptions about the familial influence on chronic bronchitis were evaluated by raw maximum likelihood using the Mx-program[93]. By removing parameters one by one from the model and comparing its goodness-of-fit to the data compared to a larger model, the significance of the removed parameter can be assessed.

Bivariate structural equation modeling of dichotomous data (study III)
To assess to what extent the genetic influences on chronic bronchitis are associated with those for smoking behaviour, a bivariate liability-threshold model for chronic bronchitis and smoking habits was formulated. This model allows a quantification of how much of the genetic (A) and environmental (E) effect is specific to chronic bronchitis and how much is due to A and E influences on smoking. The analyses were adjusted for sex-differences and age-differences in prevalence of chronic bronchitis and smoking habits. Gene-environment interactions were not modeled.

Univariate equation modeling data with continuous data (study IV)
Three covariates (age, height and pack years) were assessed by sex stratified multiple linear regression and were found to be independently significantly correlated with each lung function measure ($p<0.05$). Therefore, in study IV, all models were tested using unadjusted measures of lung function, measures adjusted for age- and height, and

finally also measures adjusted for pack years. The unadjusted model was also tested with and without the inclusion of singleton twins.

Sex limitation models (study III and IV)

When data is available from sex-like pairs (male-male or female-female), it is possible to estimate if the variance profile is similar across the sexes or whether the magnitude of genes and environmental influence are sex dependent. The models are then fitted independently for males and females, and the results compared. To be able to estimate whether the same set of genes or shared environmental influence the trait in men and women, data from opposite-sex pairs is required. Hence, possible sex differences can be tested by comparing models with different assumptions on the influence of sex[92, 94]. A full model allows the genetic and environmental variance components to be different for women and men, and the genetic correlation is free to be estimated for opposite-sexed DZ twins. For instance, if the genetic correlation is estimated at 0, it indicates that completely different genes influence the trait in males and females. A model that tests the relative importance of genes is the same in men and women fixes the genetic and environmental variance components to be equal for males and females. In order to test whether there are different genes or environmental factors influencing phenotypic variation in the sexes the genetic and environmental variance components were allowed to be different for women and men, but the genetic correlation between the members of the opposite-sex twin pairs is constrained to 0.5. The difference in chi-squares between nested models is calculated in order to test which of the models fits better. A significant chi square difference indicates that the model with fewer parameters to be estimated fits the data worse.

All model fitting was performed with the Mx program[93].

1.3 RESULTS

1.3.1 Study I

Study sample

Of the 2,965 who participated in the clinical testing at 4 years of age, 366 children were excluded, either because they refused to do PEF measurements at all (17 children) or because of inability to perform acceptable PEF-tests (349 children). Eighty-eight percent, or 2,599 children (1,272 girls, 1,327 boys) managed to perform acceptable PEF-readings. Furthermore, 97 of these children had incomplete data on key variables in the questionnaires and could therefore not be included in the analyses. In total, 463 children were excluded from analyses (241 girls and 222 boys).

Prevalence of asthma symptoms, diagnosis and medication

This left 2,502 children with both acceptable PEF and data to be categorized in regard to asthma. Three hundred and thirty-eight (13.5%) of these fulfilled the criteria for either one of the asthma categories. More boys than girls were categorized as having past or current asthma.

A doctor's diagnosis of asthma was reported by 10.0% of the boys and 7.0% of the girls ($p=0.001$). Overall, boys were more likely to use asthma medication than girls were. Use of any asthma medication was treported by 14.0% of the boys and 10.1% of the girls ($p=0.001$), 13.2% of the boys and 9.5% of the girls reported use of inhaled beta agonist ($p=0.002$), and 8.3% of the boys and 5.1% of the girls reported inhaled corticosteroid use ($p=0.001$). When children were grouped according to if they had had current wheeze (at least one episode of wheeze in the last 12 months) or past wheeze (wheezing after the age of two, but no episodes of wheezing in the last twelve months), boys were still more likely to use asthma medication than girls were (data not shown).

Association between asthma and PEF

In general, boys had higher adjusted PEF-values than girls (Figure 3). Children with asthma symptom onset in the two first years of life presented with lower PEF-values compared to children who had never been classified as having asthma. This effect was most pronounced for both sexes among those who had persisting symptoms at the age of 4 years (boys $p<0.05$, girls $p<0.001$), and for girls whose symptoms were transient at the age of 4 ($p<0.01$). Larger differences in PEF between the never asthma and asthma groups were seen for girls than for boys. An interaction term between sex and asthma group was used to investigate if the differences in PEF between non-asthmatic and asthmatic children, i.e. the effect on PEF of asthma, were significantly larger for girls than for boys. This proved to be statistically significant for the group with asthma symptoms both at 2 and 4 years of age (Table 2). The pattern did not differ between children who fulfilled the asthma criteria at four years of age by reporting more than four episodes of wheeze and those who had less than four episodes of wheeze, but instead fulfilled the criteria by a combination of at least one symptom, and had been given inhaled steroids (data not shown).

Table 2. Difference in adjusted mean PEF in L/min at 4 years of age between no asthma and asthma groups.

	Total population[1]		Boys		Girls		P int.[2]
	ΔPEF	95 % CI	ΔPEF	95 % CI	ΔPEF	95 % CI	
Asthma at:							
2 y only	-7.5	-12.1, -3.0	-4.8	-10.7, 1.1	-11.6	-18.8, -4.3	0.157
4 y only	-5.1	-10.3, -0.0	-3.3	-9.8, 3.3	-7.9	-16.1, 0.3	0.387
2 and 4 y	-12.2	-17.6, -6.8	-8.2	-14.8, -1.6	-20.2	-29.7, -10.7	0.042

Asthma groups were compared with the no asthma group adjusted for height and age. ΔPEF is the difference in adjusted mean PEF between the no asthma and asthma group, respectively. [1)]Also controlling for sex. [2)] Interaction term between sex and asthma group.

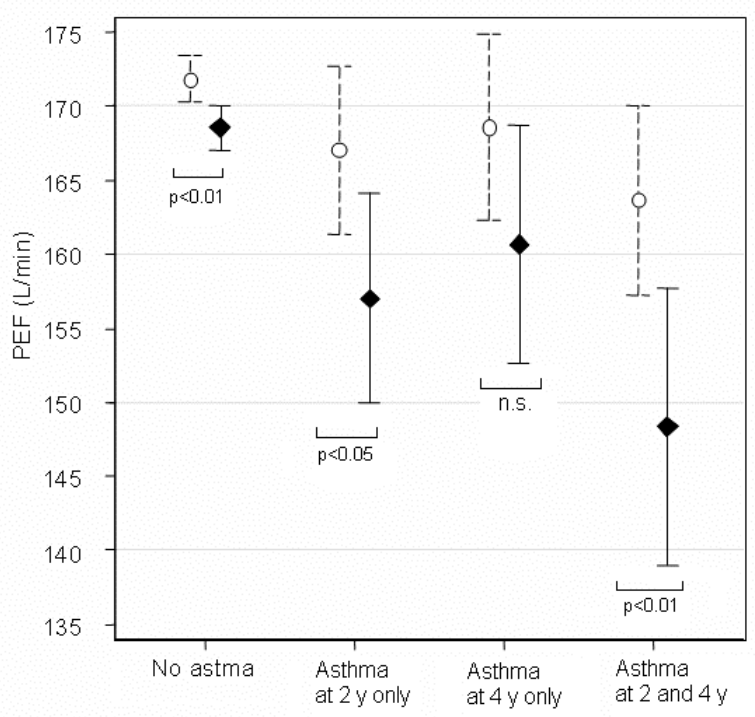

Figure 3. Estimated mean PEF values (L/min) and 95% confidence intervals for boys and girls with average age and average height in groups of asthma or no asthma. Circles denote boys and diamonds denote girls.

1.3.2 Study II

Study sample

Of the 4,089 children originally included in the BAMSE cohort, 2,498 (61%) had responded to asthma questions in all questionnaires and participated in the follow-up examination at 8 years of age. Blood samples were available for 2,329 (93%) of these children. To be able to evaluate the effect of asthma on lung function growth between 4 and 8 years of age, children had to have attended both clinical follow-ups and to have

performed acceptable PEF tests, which left 1,957 children eligible for analysis. Of the 482 children eligible for eNO testing, 459 performed technically acceptable measurements. Characteristics of participating and non-participating children are presented in Table 3, and the proportion of children with technically acceptable lung function measurements at 8 years is given in Table 4.

Table 3. Characteristics of participating and non-participating children.

	No participation in clinical examinations		**Participated at 4, not 8 years**		**Participated at 4 and 8 years**	
	%	**(total n)**	**%**	**(total n)**	**%**	**(total n)**
High socioeconomic index	78.0***	874	77.1***	585	84.6	2,359
Maternal smoking during pregnancy	13.4	880	16.0	589	12.1	2,366
Asthma 0-2 years	-	-	11.2	570	9.1	2,315
Asthma at 4 years	-	-	9.2	586	7.5	2,349

Comparing those who participated in both clinical examinations to the other groups. ***p<0.001

Children who performed technically acceptable PEF at the 4- and/or 8-year measurements were not significantly different in height, history of asthma, smoking status of their mother during pregnancy, or socioeconomic index compared to those who did not succeed; for MEFV tests, the children who succeeded were taller and more likely to have asthma after the age of 2 years, and successful MBWO tests were associated with higher mean height and high socioeconomic index (data not shown).

Table 4. Children with asthma data over 0–8 years who participated in the clinical examination at 8 years.

	Never asthma	**Transient asthma**	**Late onset asthma**	**Persistent asthma**
No. of children	2,102	220	79	107
Acceptable PEF at 8 (%)	98	97	98	99
Acceptable MEFV[§] (%)	69	72	76	81
Acceptable MBWO[§] (%)	95	95	93	97

[§]Percentage of children who performed acceptable lung function tests, excluding those who had missing data due to equipment malfunction.

Prevalence of asthma

Four hundred six (16%) of the participating children reported enough symptoms to fulfill the criteria of asthma at any time point during the first 8 years of life. Boys were over-represented in the asthma groups with onset before the age of 4.

Association between asthma and lung function measurements

Lung function data from the 8-year follow-up are given in Table 5. Onset of asthma between birth and the age of 4 (the transient and persistent asthma groups) was associated with impaired PEF, FEV_1, and $FEV_{0.5}$, irrespective of whether or not the

symptoms persisted beyond the age of 4, while there were no significant effects of asthma on FVC, LCI, or FRC (FRC data not shown).

Table 5. Difference in adjusted mean lung function at 8 years between groups with asthma and no asthma.

	ΔPEF (L/min)		ΔFVC (mL)	
	Coefficient	**95% CI**	**Coefficient**	**95% CI**
Never asthma	Ref.		Ref.	
Transient	-13.5	-18.7; -8.3	0.3	-34.1; 34.6
Late onset	6.5	-1.8; 14.8	16.0	-37.3; 69.4
Persistent	-12.1	-19.2; -4.8	3.8	-42.1; 49.6

	ΔFEV_1 (mL)		$\Delta FEV_{0.5}$ (mL)	
	Coefficient	**95% CI**	**Coefficient**	**95% CI**
Never asthma	Ref.		Ref.	
Transient	-43.6	-73.6; -13.6	-56.8	-84.8; -28.9
Late onset	-3.4	-50.5; 43.7	-20.4	-64.8; 23.9
Persistent	-41.8	-81.6; -2.1	-60.6	-97.2; -24.1

	ΔLCI[1)]	
	Coefficient	**95% CI**
Never asthma	Ref.	
Transient	0.08	-0.25; 0.41
Late onset	-0.10	-0.64; 0.43
Persistent	-0.03,	-0.48; 0.42

Asthma groups were compared with the no-asthma group, adjusting for height, sex and age. ΔPEF is the difference in adjusted mean PEF between the no-asthma and asthma group, etc.
[1)]Only adjusted for sex.

Children with asthma only during the first 2 years of life also presented with significantly lower PEF at both 4 and 8 years, as well as low $FEV_{0.5}$ at 8 years, compared to children who never had asthma (PEF at 4 coeff. -8.1 p=0.002; PEF at 8 coeff. -12.4 p=0.001; $FEV_{0.5}$ at 8 coeff. -45.7 p= 0.024), but did not differ significantly in LCI, FVC or FEV_1.

Transient and persistent asthma was associated with reduced PEF at both 4 and 8 years of age (Table 6). The growth of PEF between the age of 4 and 8, adjusted for change in height and age, was significantly lower only for the group of children with transient asthma. Although the children in this group no longer fulfilled the criterion of asthma, they were not necessarily completely symptom-free between the ages of 4 and 8; when comparing all the children of the transient group to those of the group that never had asthma, we found that the former group of children more often had had 1–3 episodes of wheeze (26% vs. 5%, $p < 0.001$), sleep-disturbing wheeze or cough (30% vs. 17%, $p < 0.001$), or any asthma-related treatment at the age of 8 years (18% vs. 5%, $p < 0.001$). Excluding all children with any kind of respiratory symptom or asthma treatment after the age of 4 from the transient group leaves only 139 individuals, but does not remove the negative effect seen for $FEV_{0.5}$ or PEF at 8 ($FEV_{0.5}$ -60.4, $p<0.01$; PEF at 8 -9.5, $p<0.05$). PEF growth, however, does no longer change significantly between the age of 4 and 8 ($p>0.01$).

Table 6. PEF at 4 years and PEF growth between 4 and 8 years of age, by asthma group.

	ΔPEF at 4 years (L/min)		ΔPEF growth between 4 and 8 years (L/min)	
	Coefficient	**95% CI**	**Coefficient**	**95% CI**
Never asthma	Ref.		Ref.	
Transient	-7.3	-11.4; -3.1	-5.8	-11.3; -0.3
Late onset	6.3	-0.5; 13.0	0.2	-8.8; 9.1
Persistent	-10.6	-16.3; -5.0	-2.4	-9.9; 5.1

Asthma groups were compared with the never asthma group, adjusting for sex, height and age. ΔPEF is the difference in adjusted mean PEF between the never asthma and asthma group, etc.

Association between asthma and lung function measurements: interactions with sex and sensitization to inhaled allergens

Although there was a tendency for girls to have lower $FEV_{0.5}$ than boys in the late-onset asthma group, there were no significant interactions between asthma group and sex with regard to effect on lung function at 8 years of age.

When testing for interactions between asthma group and sensitization to inhaled allergens, positive sensitization was only associated with lower FVC, $FEV_{1,}$ and $FEV_{0.5}$ in children with late-onset asthma (Table 7).

Table 7. Difference in adjusted mean lung function between never-asthma and asthma groups, stratified according to children with sensitization to airway allergens at 4 or 8 years of age, compared to never-sensitized children.

	ΔFVC (mL)						
	Neg. sensitization			Pos. sensitization			
	n	**Coef.**	**95% CI**	**n**	**Coef.**	**95% CI**	**p int.[1]**
Never asthma	958	Ref.		384	-2.1	-27.7; 23.5	
Transient	90	6.3	-40.2; 52.8	50	-38.0	-101.4; 25.4	0.268
Late onset	16	142.9	36.6; 249.2	44	-52.9	-119.9; 14.1	0.002
Persistent	32	-40.1	-115.6; 35.5	47	32.6	-32.6; 97.8	0.153

	$\Delta FEV_{0.5}$ (mL)						
	Neg. sensitization			Pos. sensitization			
	n	**Coef.**	**95% CI**	**n**	**Coef.**	**95% CI**	**p int.[1]**
Never asthma	851	Ref.		328	-4.3	-25.8; 17.3	
Transient	82	-71.4	-109.6; -33.1	50	-59.8	-110.0; -9.6	0.719
Late onset	16	37.7	-45.8; 121.2	37	-59.7	-116.9; -2.5	0.059
Persistent	30	-91.6	-152.9; -30.4	48	-60.8	-112.1; -9.6	0.449

Asthma groups were compared with the never asthma group, adjusting for sex, height and age. ΔFVC is the difference in adjusted mean FVC between the never asthma and asthma group, etc. [1)] Interaction term between sensitization and asthma group.

Association between asthma and exhaled nitric oxide measurements

Children with positive sensitization to airway allergens tended to have higher eNO levels than non sensitized children in all groups (Table 8). Compared to never asthmatic

children, elevated eNO levels were found in the groups of children with late onset and persistent asthma – in combination with positive sensitization. Again, similar results emerged from analyses on sensitization separated on the basis of perennial or seasonal allergens (data not shown).

Table 8. eNO between never-asthma and asthma groups, stratified according to children with positive and negative sensitization to airway allergens at 4 or 8 years of age.

	Neg. sensitization			Pos. sensitization			Neg. vs. pos.
	n	Median	IQR	n	Median	IQR	(p-value)
Never asthma	195	7.0	5.0–9.2	95	9.5	6.2–17.0	0.0000
Transient	28	7.9	6.4–9.8	16	12.5	8.8–22.8	0.0063
Late onset	4	6.2	4.0–9.5	12	26.0*	9.3–40.3	0.0896
Persistent	7	9.0	5.6–16.2	12	26.7**	10.3–37.9	0.0519

* p < 0.05, ** p < 0.01, *** p < 0.001 compared to never-asthma group, by Mann-Whitney test.

1.3.3 Study III

Prevalence

The crude prevalence of chronic bronchitis according to our definition in the sample of 42,706 Swedish twins was 7.1%. Out of these, 2,592 subjects reported only productive cough and/or self reported chronic bronchitis, 205 subjects reported only emphysema, and 247 reported a combination of both conditions. Prevalence estimates, stratified on smoking habits and sex are presented in Table 9. Significantly higher prevalences were seen for women than men in all groups.

Table 9. Prevalence of chronic bronchitis (CB) stratified on sex, smoking habits and asthma.

	Men		Women		Women vs. men	
	N	%CB	N	%CB	OR	(95% CI)
Total sample	16,414	6.04	20,358	8.08	1.37	1.26 - 1.48
Less than 10 PY	9,844	4.29	14,226	6.28	1.50	1.33 - 1.69
More than 10 PY	6,570	8.68	6,132	12.23	1.47	1.31 - 1.65
With asthma	1,059	18.79	1,370	28.10	1.69	1.39 - 2.05

PY = pack years.

Univariate structural equation modelling

The comparisons of different structural equation models indicated that for chronic bronchitis the same genes are of importance in men and women, and that the relative importance of genes is the same in men and women. The genetic effect was statistically significant (LRT 115.528, p-value < 0.001). In the most parsimonious model, 40% of the variance was attributed to genetic factors, and the rest (60%) to non-shared environment.

Bivariate structural equation modelling

The factors contributing to the association between smoking behaviour and chronic bronchitis were investigated using an AE bivariate liability-threshold model. We found that a genetic path mediating the association could not be excluded (LRT=58.252, p-value < 0.001). Thus, the genes involved in smoking behaviour are partly associated with the genes influencing the development of chronic bronchitis. However, they account only for a modest portion (14%) of the genetic component for chronic

bronchitis. In other words, most of the genes that are important for the development of chronic bronchitis are independent of those that are important for smoking behaviour. The bivariate model and parameter estimates are illustrated in Figure 4.

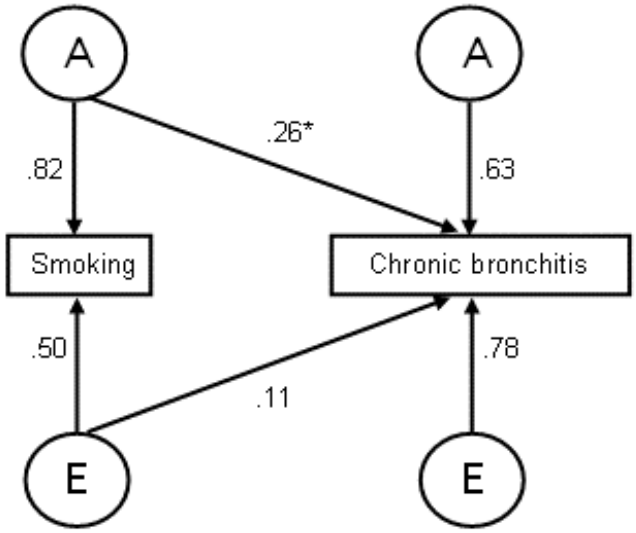

Figure 4. Bivariate path diagram describing the genetic (A) and non-shared environmental (E) effects that are common to smoking and chronic bronchitis, as well as the genetic and non-shared environmental effects that are unique to each phenotype. The observed traits (smoking and chronic bronchitis) are represented by rectangles, whereas unobserved (latent) genetic and non-shared environmental variables are represented by circles. The parameter estimates (arrows) are path coefficients, indicating the relative importance of the latent variables A and E to smoking and chronic bronchitis. The square of these estimates represents the variance of the trait accounted for by that specific latent factor. * = common genetic path.

1.3.4 Study IV

Descriptive statistics

Participating twins were, compared to the total available sample, younger (55.3 vs. 59.4 years, $p<0.001$) and more likely to be female (63 vs. 53%, $p<0.001$). Due to the selection, participating twins were also more likely to have smoked at least 10 pack years (45% vs. 33%, $p < 0.001$), and had more often self reported respiratory symptoms (asthma, recurrent cough, chronic bronchitis or emphysema) (43 vs. 13%, $p<0.001$), compared to all twins in the Swedish Twin Registry database (according to data from telephone screening). All evaluated covariates (age, height and pack years) were found to be significantly related to lung function for both men and women ($p<0.05$).

Model fitting

The results from structural equation modeling indicated that the same genes are of importance in men and women, although the relative importance of genes and environment differ between men and women.

Variance components

Table 10 shows the partitioning of the total variance into variance attributable to genetic and environmental factors, while Figure 5 shows the absolute variance values.

Variance components unadjusted measures
For unadjusted FEV_1 and DLco, the total variance was primarily attributable to genetic factors for women, and only to environmental variance in men. Unadjusted VC demonstrated genetic and environmental influence for both men and women. The inclusion of singleton twins did not change the estimates (data not shown).

Variance components adjusted for age and height
Adjustment for age and height resulted in a decrease of the total variance of FEV_1, VC and DLco, as the variance that is common to the covariates and lung function measures is removed from total variance (Figure 5). In both men and women, this decrease in total variance was largely attributable to reduced shared environmental variance. In fact, in women, height and age explained all or nearly all of the shared environment seen in the unadjusted measures of lung function. Although genetic variances were stable or only slightly decreased, the fact that heritability is the ratio of the genetic variance to the total variance will cause the heritability estimates to increase as the total variance decreases. The non-shared environment remained similar before and after adjustment for age and height for all three lung function measures.

Variance components further adjusted for pack years
As seen in figure 5, genetic variance became more apparent for men (while shared environment decreased) for all lung function measures when pack years were added to the equation. In women, pack years caused genetic variance to decrease, with a redistribution to shared environmental variance. Hence, adjustment for pack years resulted in higher heritability estimates in men, and lower (or unchanged) heritability estimates in women.

Excess variance in men
The total variances for all lung function measures were higher in men even after the introduction of age, height and pack years. Dividing the total variance by the mean values did not make the variance equal between the sexes (data not shown).

Table 10. Parameter estimates (with 95% confidence intervals) for the sex limitation model for unadjusted and adjusted FEV_1, VC and DLco.

	Men			Women		
	a^2 (95%CI)	**c^2 (95%CI)**	**e^2 (95%CI)**	**a^2 (95%CI)**	**c^2 (95%CI)**	**e^2 (95%CI)**
FEV_1						
Unadjusted	0.00 (0.00-0.44)	0.56 (0.00-0.72)	0.44 (0.26-0.67)	0.57 (0.17-0.85)	0.23 (0.00-0.61)	0.20 (0.14-0.30)
Adj age, height	0.02 (0.00-0.53)	0.31 (0.00-0.56)	0.67 (0.39-0.98)	0.66 (0.13-0.78)	0.02 (0.00-0.51)	0.32 (0.22-0.46)
Adj age, height, PY	0.25 (0.00-0.60)	0.08 (0.00-0.51)	0.67 (0.38-1.00)	0.46 (0.00-0.77)	0.22 (0.00-0.67)	0.33 (0.23-0.47)
VC						
Unadjusted	0.32 (0.00-0.73)	0.40 (0.03-0.78)	0.28 (0.16-0.50)	0.39 (0.05-0.75)	0.43 (0.07-0.74)	0.18 (0.12-0.27)
Adj age, height	0.41 (0.00-0.74)	0.15 (0.00-0.64)	0.44 (0.25-0.75)	0.69 (0.13-0.79)	0.01 (0.00-0.53)	0.30 (0.21-0.44)
Adj age, height, PY	0.51 (0.00-0.73)	0.00 (0.00-0.58)	0.49 (0.27-0.87)	0.50 (0.00-0.78)	0.20 (0.00-0.67)	0.31 (0.21-0.45)
DLCO						
Unadjusted	0.01 (0.00-0.51)	0.70 (0.20-0.82)	0.29 (0.18-0.47)	0.44 (0.07-0.70)	0.19 (0.00-0.52)	0.37 (0.25-0.53)
Adj age, height	0.01 (0.00-0.68)	0.61 (0.00-0.76)	0.38 (0.23-0.60)	0.40 (0.00-0.61)	0.06 (0.00-0.42)	0.54 (0.38-0.75)
Adj age, height, PY	0.25 (0.00-0.69)	0.21 (0.00-0.63)	0.54 (0.30-0.90)	0.40 (0.00-0.57)	0.00 (0.00-0.46)	0.60 (0.43-0.81)

a^2, c^2 and e^2, genetic, shared and non-shared environment in proportion of the total variation, respectively. PY= pack years

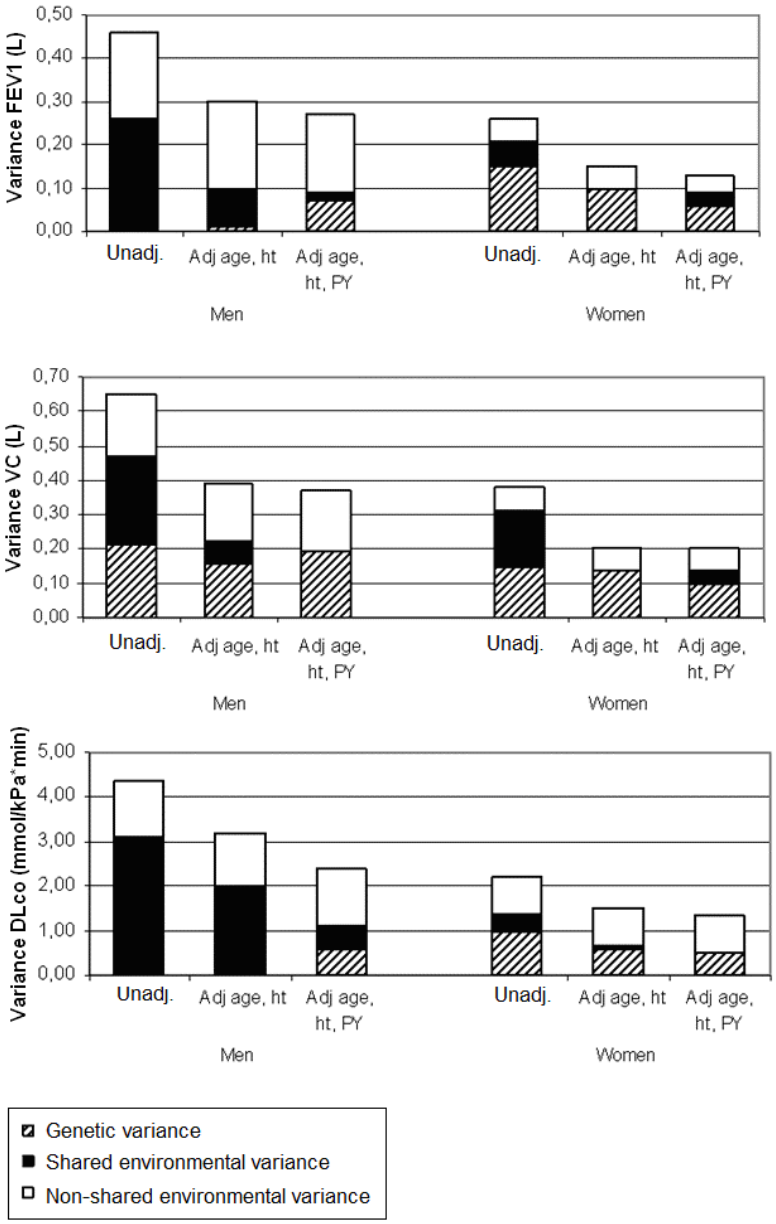

Figure 5. Unadjusted and adjusted genetic, shared and non-shared environmental variance components for FEV_1, VC and DLco in men and women.

1.4 DISCUSSION AND CONCLUSIONS

1.4.1 Asthma: Timing of symptom onset and duration

We have shown that not only onset of symptoms of asthma such as wheeze up to the age of 4 years, but also asthma alone during the first 2 years of life, is associated with impaired air flows at 8 years of age, compared to children without such symptoms. For PEF, which was the only lung function measured followed longitudinally, this association could be seen already at 4 years of age, while no further impairment was seen between the age of 4 and 8 (given that no symptoms were allowed after the age of 4 in the transient asthma group). Although it is possible that children with well-treated persistent asthma will show few or no symptoms after 2 to 4 years and hence be assigned to the transient asthma group, these results indicate that even though symptoms are transient, the effect on lung function is not.

The present findings are in agreement with earlier international studies showing a relationship between early onset symptoms and impaired flows at school age [40, 95], and further adds to this knowledge by reporting data from a large number of children 4 years of age. The tracking of lung function here reported from 4 to 8 years, has by other overlapping studies been reported to last from early childhood to school age and into adult life[42-44, 95-97]. An important point that remains to be answered is whether these early onset lung function impairments were present already at birth, or if they developed during the first years of life. Recent results from studies examining lung function very early in life has presented different conclusions[40, 98]; however, it is not unlikely that both variants exist.

1.4.2 Asthma: Does allergy matter?

When children with and without sensitization were compared in respect to level of lung function, sensitization was related to lower FVC and $FEV_{0.5}$, but only for the group who started having symptoms after 4 years of age, the late onset group. Similar results were recently published, but reduced lung function at 7–13 years of age in children with wheeze at school age (5–7 years) was found only in association with early sensitization to perennial allergens[99]. However, the latter study did not take age of onset into account, which possibly can contribute to the partly diverging results between the two studies. It is interesting to note that the pattern of FVC and $FEV_{0.5}$ is different between the sensitized and non sensitized late onset group. Non sensitized children with late onset asthma actually performed higher FVC and $FEV_{0.5}$ than healthy children, although an obstructive pattern is seen as the two volumes are compared, while the sensitized children have similar reductions of both volumes. We cannot say whether the nature, here referring to chronic vs. reversible, of the lung function impairment associated with early/late onset asthma, with or without sensitization, etc, is the same in all different groups, as reversibility was not tested for. Furthermore, as sensitization did not have an effect on PEF at either four or eight years, and as more extensive flow measurements were measured only at 8 years of age, we cannot say anything about the temporal development of these changes.

Elevated eNO levels were associated with positive sensitization status, particularly in children with current asthma symptoms. High ENO levels have been connected to

increased blood eosinophil count, which indicates some ongoing process in the airways, possibly of an inflammatory origin[100]. This further supports the presence of several different asthma phenotypes. It should be noted that as eNO was tested in a subgroup of children, some of the groups were small and one should be cautious about drawing any firm conclusions. Due to insufficient number of children, we could not assess the importance of inhaled corticosteroids and exposure to allergens, both factors that could contribute to the level of eNO.

It is clear that allergy does matter for the afflicted individual. However, here, its impact on lung function seems to be limited to children with a late symptom start. To better understand whether this finding is due to mechanistical differences between the groups, or can be attributed to differences in symptom reporting, a more detailed analysis and a longer follow up span will be required.

1.4.3 Sex differences in prevalence and lung function in asthma and COPD

We found significant sex differences in the prevalence of both childhood asthma and adult COPD. In agreement with other studies [53-55], childhood asthma was more common in boys, and chronic bronchitis more common in women. However, the results from the 4 year follow up of the BAMSE study (study I) showed that the impact on PEF of asthma with onset before the age of 2 was significantly larger in girls than in boys. At the 8 year follow up (study II), the asthma groups were defined differently, and although there was no longer a significant difference between boys and girls, there was a tendency for girls to have lower values also at 8 years.

Even though asthmatic girls were always compared to healthy girls, and boys to boys, it is possible that there are anatomical and/or physiological differences between the airways of boys and girls that make them prone to react in different ways to the same situation. It has also been discussed that boys may have more symptoms because they are exposed to different environmental risk factors than girls, such as pets and sports[101], although, in this case, this reasoning only holds if the factors that provoke symptoms have nothing to do with measurable effects on lung function.

Instead, it is possible that these differences between boys and girls might be caused by bias in how symptoms are apprehended and interpreted between the sexes, with girls having to express more severe disease to be noticed, which can then be associated with a larger relative lung function impairment. Another possible explanation could be that boys and girls express different symptoms, or patterns of symptoms, of asthma[102] and that the use of a single common base criterion such as wheeze will cause only a fraction of the girls with asthma will be detected, in this case the ones with more severe disease. It was also more common for boys to report use of asthma medication at 4 years of age, but it should be noted that the group showing the greatest relative sex specific differences in the use of medication, the no current wheeze group, was very small. Underestimation of asthma symptoms by girls themselves, parents or doctors has previously been reported in international studiews[103, 104], and we find that this might also be true for Swedish children.

It has been suggested that the reason that particularly chronic bronchitis is more common in adult women, compared to men, is because more women smoke, or because women would be more aware of symptoms than men and therefore report more chronic bronchitis[18]. On the other hand, it has been shown that women are less likely to get a diagnosis of COPD when presenting with the same symptoms as men[105]. In study III, we could show that even though more women reported symptoms of chronic bronchitis, more men than women had smoked at least 10 pack years. Disease was also more common in females in opposite-sexed twin pairs, which by definition have the same age, indicating that it is not only a reflection of the higher age in women. As discussed above in regard to asthma, it is possible that anatomical and/or physiological differences between the sexes cause the same exposure to have different effects. For example, the effect of smoking classified in number of cigarettes or pack years might not be directly comparable due to the relatively smaller area available for exposure in women. Another possibility is that genetic factors can be involved in this difference of susceptibility.

1.4.4 The importance of genes and environment

Both asthma and COPD are likely to be complex genetic disorders were both a number of genes and environments contribute to the development of disease. Study III and IV of this thesis focused on the interplay between genes and environment in COPD and chronic bronchitis.

It has since long been suggested that COPD and chronic bronchitis are diseases that run in the family[68, 69, 74-79]. However, familial is not necessarily the same as genetic, since many families share predisposing environments as well as genes. Therefore, a reasonable point to start was to try and determine the magnitude of the genetic effects in relation to the influence of the environment. Since lung function data was not available from all twins in the Swedish twin registry, we chose to begin with studying chronic bronchitis, one of the main symptom groups associated with COPD. By comparing similarity of disease presence in monozygotic and dizygotic twin pairs in study III, we were able to show that genetic factors do play a role in the individuals' liability to develop chronic bronchitis.

Others have reported that different aspects of smoking behavior are, like other addictions, strongly heritable. This was also the case for the variable used to assess smoking in study III. Since the association between smoking and chronic bronchitis is strong, genetic factors that influence smoking behaviour will seem to be associated with chronic bronchitis. Hence, it was necessary to investigate if the genetic factors seen for chronic bronchitis were independent for those of smoking habits. From the results of this extended analysis, it was concluded that the genetic factors influencing chronic bronchitis are largely different from those influencing smoking behavior, although a small overlap was found. This confirms and extends the studies of familial aggregation of chronic bronchitis[69, 74-79]. Our results should, however, not be interpreted as if smoking has no effect in certain individuals. Considering that the prevalence of chronic bronchitis in smokers was twice as high as in non-smokers, the conclusion should be that smoking and genes share culpability for chronic bronchitis. Having said that, it should be noted that there are many other environmental factors of importance, known and unknown, that we did not considered in this analysis.

The next step in the process of understanding how genes and environment interplay in the development of COPD was to assess the magnitude of heritability of different lung function parameters. This was done in a subset of twin pairs with and without respiratory symptoms from study III. All lung functions measures studied expressed a heritable pattern, although to different extents. Even though the influence of smoking could not be studied as extensively as in the previous study, attempts were made to describe its effect on the different estimates.

The presence of genetic variance for lung function found in this disease enriched population is in agreement with the results of family studies, where it was shown that relatives of probands with COPD had a higher risk of airflow obstruction, compared to controls [68, 69, 106]. In another subpopulation of the Swedish twin registry, the SATSA twins[107], heritability estimates adjusted for age, height and smoking was higher for FEV_1 (67% compared to 25-46%), but similar for VC(48% vs. 50-51%), compared to the current study. Since heritability represents the portion of the total phenotypic variance that is attributable to genetic variance, the heritability of the same trait will differ between populations that differ in the distribution of environmental risk factors for that trait, and even though both populations were of the same nationality and age range, the prevalence of smoking habits and symptoms would have been lower in the unselected SATSA material. Further, as stated above, studying lung function in healthy individuals may not be the same thing as studying diseased individuals, particularly for measures of lung function that are known to be susceptible to these factors, such as FEV_1. The genetic influences on lung function in adults can stand for a number of different processes. They can be genes associated with lung function decline or the impact of the adverse effects of smoking, but also protective factors and factors related to lung function growth in childhood and adolescence. To be able to assess factors specifically related to lung function decline, lung function needs to be measured at more than one time point.

In the long run, these studies can contribute to the identification of which genes are involved in susceptibility and severity of chronic bronchitis and COPD. The understanding of what these genes do and what substances they produce can eventually contribute to the development of drugs to treat or even prevent disease.

1.4.5 COPD: sex differences in heritability

In common for both chronic bronchitis and lung function measures were that the same genes are of importance in men and women. However, for lung function, but not for chronic bronchitis, the relative importance of genes and environment differs between men and women, with genetic factors playing a proportionally larger role in women. The discrepancy between the two phenotypes in regard to sex is perhaps not surprising, as chronic bronchitis is not necessarily related to lung function impairment, and vice versa. The interpretation on how this sex difference in heritability is associated to the prevalence of disease is not straightforward, but might help explain sex differences in COPD and chronic bronchitis. One study have specifically stated the lack of sex differences in heritability of lung function measures[107], while another found similar results of higher heritability estimates in females compared to males, despite large differences in the study populations (theirs was a young healthy population)[108]. What

we can say is that sex is an important factor to take into account, at least for some phenotypes of disease, and that it needs to be further explored. The results of the here presented studies present interesting possibilities to further explore the importance of sex, genes and environment, possibly in the form of sex by gene by environment interactions.

1.4.6 Methodological aspects

Generalizability of data (study I and II)
The BAMSE study is a population based observational study free of interventions. The included families were living in three different areas in and around Stockholm that were chosen to be representative of the general population[109]. Children who participated in both clinical testing sessions were more likely to come from families with higher socioeconomic index than those who did not participate, or only participated at 4 years. There were tendencies, although not statistically significant such, of higher proportions of smoking mothers (during pregnancy) and asthma during the first 2 years and between 2 and 4 years among the groups of non participants. Mean PEF values were not different in the group of children who only participated at 4 years compared to those who participated in both sessions. If these biases have any effect on out findings it would be to attenuate the differences observed.

The first questionnaire assessed the environment of the child and lifestyle factors of the family. The following questionnaires asked about symptoms, particularly during the last 12 months, hence, exposure was collected before outcome, and any misclassification should be non differential. There was no specific feedback on the lung function measurements from the 4 year follow up given to the parents, so it is unlikely that the examination in itself could have influenced the treatment of the child between the age of 4 and 8.

Generalizability (study III and IV)
The criterion used to define chronic bronchitis in study III was wider than the classical criteria of "cough with phlegm production for at least 3 months during at least 2 consecutive years"[110]. The broader the definition, the more genes are likely to be involved, each contributing with a small effect. This is more likely to reflect the population, but makes it more difficult to find *the* genes. It might also falsely classify healthy subjects as diseased. However, as the data were used for heritability estimates, the misclassification will only be a problem if it is differential between MZ and DZ twins. In the same study, smoking was dichotomized, using ten pack-years as a cut-off, which means that light smokers are classified as non-smokers. The motivation for using this measure was to make sure that those classified as smokers had smoked enough to damage the lungs. Again, misclassification of smoking habits will only be a problem if the misclassification is differential between MZ and DZ twins. It is conceivable that part of the genetic influence attributed to chronic bronchitis could partly be due to heritability for atopy. However, the exclusion of all subjects with asthma symptoms did not influence the heritability estimates for chronic bronchitis substantially.

Twin data might not be generalizable to singletons. Factors that are importance from the respiratory point of view are for example mode of delivery, birth weight, and neonatal respiratory morbidity. However, frequencies and prevalences of chronic

bronchitis and smoking habits were similar to results obtained from singleton populations and results are likely to apply also to the general population.

The twin pairs studied in study IV was a selected sample from a population. As the sample contains a larger proportion of smoking and diseased individuals, these results are not directly generalizable to the general population, but to populations with similar background. Further, the number of twin pairs included in the study was insufficient to evaluate possible gene environment interaction. A solution to both these issues would be to examine a larger number of twins, or to pool this dataset with data from other studies.

Spirometry in children

The BAMSE studies (study I and II) offer a unique opportunity to examine how different lung function indices can be used in response to describing asthma related impairment in the epidemiological setting.

As mentioned in the introduction, the choice of lung function variable is strongly dependent on aim of the investigation and the cooperative abilities of the patient or subject, which in children is largely determined by the age of the child. Therefore, even though the diagnostic abilities of PEF can be criticized, it was in study I and II only used to compare the effect of asthma on airflow between groups of children. Although a complete dynamic spirometry examination could (and probably should) be attempted in individual children at the age of four, the striving to gain a high number of technically acceptable examinations was prioritized before extensiveness in the first follow up.

As the more extensive examination including flow volume curves was performed at the 8 years follow up, we found that many of the children had nearly reached, or reached, their FVC within one second. Even in those children who had a portion of lung volume left to exhale after one second, the FEV_1 measure in itself might not represent the same thing as in adults. An alternative is therefore to use a shorter time span, here 0.5 seconds, denoted as $FEV_{0.5}$. The interpretation of PEF results was similar to those of $FEV_{0.5}$, indicating that PEF, although not necessarily informative for the individual, is a viable option for lung function measurements in small children in larger studies.

Different asthma groups, possibly representing different phenotypes of asthma, showed different patterns of lung function impairment. While some groups showed impaired volumes compared to never asthmatic children, others had disproportionally increased volumes. We therefore support the need for taking both $FEV_{0.5}$ (or FEV_1) and FVC into account, rather than only the $FEV_{0.5}$/FVC measure.

It seems that children with asthma performed technically better lung function tests than children without asthma. It is feasible that the former group may have had the possibility of practicing their technical skills at lung function testing. Greater lung function values in asthmatic children would, however, result in smaller differences between them and the group who were never asthmatic, suggesting that differences between the asthmatic and the never-asthmatic groups are in fact even larger than shown here.

The children of the early persistent group were shorter than their healthy peers. Assuming that the lower height is associated with asthma, adjusting lung function values for height might cause an overestimation of values for this group, which would, also in this case, result in a dilution of the differences seen between the asthmatic and the never-asthmatic groups.

Spirometry in adults

All lung function tests in study IV were carried out in a specialized clinic with highly experienced staff. Therefore, it is not likely that the sex differences seen in the current study originate from a methodological error, such as poor performance at lung function testing. Men and women did not differ in disease level (measured as FEV_1/VC, data not shown) and it seems unlikely that the sex differences in the genetic and environmental estimates are due to studying differently diseased populations for men and women.

1.4.7 Conclusions

Specific conclusions that can be drawn from the studies included in this thesis are:

- Asthma symptom onset before the age of 4 is associated with impaired expiratory flows at the age of 8, irrespective if the symptoms persist or not.
- For measured variables, these changes were present already at 4 years of age and the differences did not change after that.
- Sensitization is of importance for lung function in the group of children who onset of asthma symptoms between the age of 4 and 8.
- Female sex was associated with reduced air flow at 4 years, and a tendency remained at 8 years of age.
- Chronic bronchitis was more common in women, and the difference could not be explained by smoking habits.
- Smoking behavior is determined both by environmental and genetic factors.
- A persons liability to develop chronic bronchitis is determined both by environmental and genetic factors. The genetic factors are largely separate from those influencing smoking behavior. The genes are the same for men and women.
- Men have larger variability in lung function even after adjustment for height, age and smoking habits.
- There are sex differences in the relative impact of genetic and environmental factors on FEV_1, VC and DLco in a symptom-enriched sample of twins, but the genes are the same for men and women.

1.5 CLINICAL IMPLEMENTATION AND FUTURE PERSPECTIVES

The ultimate aim of these studies is contribute to strategies for the prevention and treatment of obstructive lung diseases. In order to do that, we need to understand the genetic, environmental and developmental mechanisms behind the diseases studied in this thesis. We have here focused on how various factors relate to lung function and lung function development in childhood asthma, and COPD, found in the other end of the life span.

We want to stress the need of careful phenotyping, and perhaps even genotyping, in future studies aiming to understand the mechanism behind asthma and COPD. At the same time, there are many similarities not only within, but also between the diseases and we and others support the importance of a life long perspective when dealing with obstructive lung diseases. It might therefore not be optimal to view them as completely exclusive entities in this type of studies, or clinical work, depending on the situation. Furthermore, all four studies included in this thesis highlight the presence of sex differences in obstructive lung disease, albeit in different aspects. Although there are obvious differences in the anatomy of the respiratory systems of males and females, there are implications that girls and women with obstructive lung diseases might experience less favorable outcomes - this is another field that deserves further investigation.

1.6 POPULÄRVETENSKAPLIG SAMMANFATTNING

Astma och kroniskt obstruktiv lungsjukdom (KOL) är två av de vanligaste obstruktiva lungsjukdomarna. Obstruktiv betyder hindrad eller tilltäppt, och i detta fall är det passagen av luft in och/eller ut ur lungorna som är hindrad. Graden av obstruktivitet kan mätas med hjälp av lungfunktionstestning. Astma förekommer både hos barn och vuxna, medan KOL återfinns framförallt i populationen över 50 år.

Både vid astma och vid KOL tycks det finnas flera olika undergrupper av sjukdom som också förknippas med olika långtidsutsikter vad gäller sjuklighet och i vissa fall även dödlighet. För att kunna förebygga och behandla dessa sjukdomar på bästa sätt krävs att vi lär oss mer om de olika undergrupperna och deras bakomliggande mekanismer.

Syftet med denna avhandling var att belysa faktorer som är kopplade till lungfunktionssänkning hos barn och vuxna med obstruktiva lungsjukdomar. De faktorer som vi har valt att speciellt undersöka är debutålder, symtomduration, kön, allergi, rökning och genetik.

I de två första studierna mätte vi lungfunktionen på c:a 2500 barn vid 4 och 8 års ålder och fann att de som insjuknade med astmasymtom som pipande och/eller väsande andning under de fyra första livsåren hade (på gruppnivå) lägre utandningsflöden är barn som aldrig hade hast astmasymtom. Detta gällde oavsett om symtomen fanns kvar mellan 4 och 8 års ålder eller inte. Vi kunde vidare visa att, oberoende av symtomförekomst, fanns de sänkningar av lungfunktion som sågs vid 4 år kvar vid 8 års ålder, utan att förvärrats ytterligare däremellan. Astmasymtom var vanligare förekommande hos pojkar än flickor. Trots detta tycktes flickor med astma ha större påverkan på sina utandningsflöden, åtminstone under de första fyra åren, jämfört med barn som aldrig hade haft astma.

De barn som var sensibiliserade mot luftvägsallergen, det vill säga de som hade förhöjda nivåer av antikroppar i blodet mot pollen, pälsdjur, mögel eller kvalster, hade generellt sett inte sämre lungfunktion än barn utan sensibilisering, med ett undantag. I gruppen med barn som insjuknat med symtom för första gången efter fyra års ålder hade de sensibiliserade barnen sämre utandningsflöden och andetagsvolymer än de som aldrig haft astmasymtom.

I de två följande studierna studerades hur 45.000 tvillingar hade svarat på frågor om symtom på kronisk bronkit och emfysem, två av huvudkomponenterna i KOL. Genom att jämföra hur mycket mer lika enäggstvillingpar (som i princip är genetiskt identiska) var jämfört med tvåäggstvillingpar (som delar i genomsnitt 50% av sina gener) kunde vi kvantifiera betydelsen av gener och miljö i en persons individuella känslighet för att utveckla sjukdom. Då rökning, i likhet med många andra beroenden, är till viss del genetiskt betingat, ville vi också visa att den genetiska betydelse vi kunde påvisa för sjukdom inte endast var en spegling av rökningens genetik. Resultaten visade att c:a 40% av den individuella känsligheten för att utveckla kronisk bronkit och/eller emfysem kunde härledas till genetiska faktorer, att denna till en mycket liten del var kopplad till genetiken till rökning, och att den inte skilde för kvinnor och män. Två

hundra tvillingpar deltog dessutom i en lungfunktionsundersökning, från vilken vi kunde visa att samtliga mått på lungfunktion som studerades hade en tydlig ärftlig komponent, men att den inte nödvändigtvis var lika stor för kvinnor och män.

Sammanfattningsvis har vi påvisat en mängd olika faktorers betydelse för lungfunktionsnivå vid obstruktiv lungsjukdom hos vuxna och barn. Resultaten visar att de första livsåren är av betydande vikt för den framtida lungfunktionsnivån. Barn som växer ifrån sin astma tycks, åtminstone på gruppnivå, växa ifrån sina symtom snarare än sin lungfunktionssänkning, vilken möjligtvis kan bestå livet ut. Vi har dessutom visat att genetiken är viktig för vem som kommer att utveckla obstruktiv lungsjukdom i vuxenlivet. Alla fyra studierna har påvisat könsskillnader både när det gäller astma och KOL. Ytterligare arbete krävs nu för att identifiera individer som tillhör särskilt känsliga undergrupper, samt att utveckla metoder för att förhindra och förbättra uppkomsten av lungfunktionssänkning och sjukdom.

2 ACKNOWLEDGEMENTS

I want to express my gratitude to everyone who has helped and supported me and thereby made this thesis possible. I would especially like to thank **all children and families participating in the BAMSE study and all twins in the Swedish Twin registry**. Without you, these studies would not have been possible to perform.

A large number of friends and colleges have contributed to this thesis and I wish to thank you all, and in particular:

My main supervisor Magnus Svartengren, for introducing me to the world of research and sharing your immense scientific knowledge.

My co-supervisor Martin Anderson, for patiently teaching me how lungs really function and how to climb mental and actual mountains.

My co-supervisor Magnus Wickman, for sharing your knowledge on paediatric allergy research with me, your incredible enthusiasm, and for always letting me know when I have done something right.

Centre for Allergy Research at KI - Bengt Björkstén, Lars Gustafsson, Sven-Erik Dahlén, Gunilla Hedlin, Marianne Heibert-Arnlind, Gunilla Jacobsson Ekman and Anne Renström, for making it possible for me to do my PhD studies within the Cfa network, and my fellow Cfa PhD students for inspiring and entertaining discussions about allergy research.

The research group, in particular: Britt-Marie Larsson, inspiring mentor and friend, Anne-Marie Windahl for friendship and solving all administrative issues, Ingemar Rödin for entertaining discussions on everything, Gun Nise, Anna Klepczynska Nyström and Maria Lindström for your enthusiasm and support.

Pernilla Wiebert, ultrafine friend and college, for always being there, one step ahead, leading the way.

Bamsegänget - Eva Hallner, André Lauber, Inger Kull, and many more, for sharing your knowledge and making it so entertaining to work with the BAMSE study. Stina Gustavsson för att du alltid är villig att hjälpa till, lära ut och heja på.

My co-authors at the Swedish Twin Registry, Nancy Pedersen, Ulrika Ericsson, Annica Dominicus and Anastasia Iliadou, for excellent advice and for guiding me through the field of genetic epidemiology.

Co-authors and collaborators at AstraZeneca, in particular Maria Gerhardsson de Verdier, Magnus Dahlbäck, Ulf Nihlén and Tim Higenbottam, for stimulating collaboration and inspiring discussions.

Helena Svensson and Marie Mulder for helping me with the statistics.

All collaborators at Södersjukhuset for the immense work you have put into the clinical part of twin study, in particular Kerstin, Anna, Isabella, Kristina, Linda and Astrid at the Department of Clinical Physiology.

Ylva Rodvall, for chocolate, support, and cheering me on at all times.

All PhD students and ex-Phd students at the Department of Occupational Medicine, you have been a great support. Everyone at Occupational and Environmental Medicine, Stockholm County Council for making me feel so welcome.

All my friends, and in particular Hanna, Lina and Marie with families, for encouraging me to keep going and reminding me of what is important in life.

My mother Christina, father Roland, brothers Johan and Joakim and hela släkten – for providing excellent genes and environment.

Alejandro ♥

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