Asthma in West Sweden – a translational study from epidemiology to proteomics

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Ineko AB

It always feels impossible, until it is done

Nelson Mandela

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ABSTRACT

Asthma has been increasing in prevalence and morbidity, however it is unclear if the increase continues. Asthma has long been regarded as a single disease entity, but is now recognised as a heterogenic disease with different phenotypes. The overall aim was to investigate asthma and selected phenotypes in the population with regard to prevalence, medication use and differences in mechanism.

In an epidemiologic study of 18 870 responders to a postal questionnaire, living in Gothenburg and Västra Götaland, the prevalence of physician-diagnosed asthma was 8.3%. Compared with a study conducted 18 years ago on the island of Hisingen, the prevalence of most respiratory symptoms had decreased, while there was a small increase in asthma prevalence and a significant increase in allergic rhinitis. As an epidemiological proxy to severe asthma, multi-symptom asthma (MSA) was defined from responses to the questionnaire. The prevalence of MSA was 2% in the population and 24% among asthmatics. The definition was verified in a subgroup of subjects invited to our research clinic. MSA was associated with signs of more severe disease, such as lower lung function, more airway inflammation, hyper-responsiveness and more severe health outcomes. Of subjects with MSA, 92% used asthma medication, compared with 61% of other asthmatic subjects. Inhaled corticosteroids were used by 70% of subjects with MSA, who also reported more frequent use of asthma medication. Selected participants from three phenotypes of asthma, and healthy controls were included in a proteomics study where several differences in protein expression patterns could be detected in nasal lavage fluid. In total 193 proteins was identified with a fold change of at least 1.3 as compared to healthy, these proteins represent different biological functions and pathways between phenotypes.

We conclude that the previous increase in asthma prevalence has ceased and that respiratory symptoms are decreasing. MSA is common among asthmatics and is related to signs of more severe disease, hence MSA can be used an epidemiological marker of disease severity. Medication use is high in MSA, however under-treatment occurs. Further, quantitative proteomics on nasal lavage fluid can be used to identify differences in protein expression between asthma phenotypes, and possibly to detect differences in mechanism.

Keywords: asthma, epidemiology, respiratory symptoms, medication, proteomics

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SAMMANFATTNING PÅ SVENSKA

Det är sedan tidigare känt att förekomsten av astma och symtom från luftvägarna har ökat under 1900-talet, men det är oklart om ökningen fortsätter. Tidigare har astma betraktats som en sjukdom men det blir mer och mer tydligt att astma snarare utgörs av flera undergrupper. Dessa har olika bakomliggande orsaker och olika respons på behandling. Målet med avhandlingen, som består av 4 delarbeten, var att studera astma och några av dess undergrupper med avseende på förekomst, medicinanvändning och skillnader i bakomliggande mekanismer.

Delarbete I består av en studie av befolkningen i Göteborg och Västra Götaland där 18 087 personer besvarade en postenkät. Den visade att 8,3% har läkardiagnosticerad astma och att det är något vanligare jämfört med en studie på Hisingen, Göteborg 1990. Förekomsten av luftvägssymptom har minskat medan allergisk hösnuva har ökat. Andelen rökare har nästan halverats mellan studierna, från 32% till 18%. Svår astma är problematiskt att studera i befolkningsstudier så därför definierades multisymptomatisk astma (MSA) som en markör. Förekomsten av MSA var 2 % i befolkningen och 24% bland astmatikerna.

I delarbete II och III kontrollerades definitionen av MSA i en verifieringsstudie på vår forskningsklinik bland ett urval av deltagarna. Den visade att personer med MSA har sämre lungfunktion, mer inflammation i luftvägarna och fler tecken på svår sjukdom än personer med färre astmasymptom. Av personerna med MSA använder 92% astmamedicin, jämfört med 61% av de med färre luftvägssymptom. De använder också inhalationssteroider i större utsträckning och de använder sin astmamedicin oftare och i högre doser.

I delarbete IV studerades utvalda deltagare som representerade tre undergrupper av astma samt friska kontroller i en studie där kvantitativ proteomik användes för att kartlägga proteinuttryck i nässköljvätska. Flera skillnader i proteinuttryck kunde identifieras mellan undergrupperna och friska. Totalt var 193 proteiner upp- eller nedreglerade med en faktor på minst 1,3 jämfört med friska. Dessa proteiner representerade olika biologiska funktioner mellan undergrupperna.

Från avhandlingen dras slutsatserna att den tidigare ökningen i förekomsten av astma nu har upphört och att förekomsten av luftvägssymptom minskar. MSA är vanligt bland astmatiker och är kopplat till tecken på svårare astma, alltså kan MSA användas som en markör för svårighetsgrad av astma i befolkningsstudier. Användningsgraden av astmamedicin är hög i gruppen med MSA, men undermedicinering förekommer. Vidare dras slutsatsen att kvantitativ proteomik kan användas för att hitta skillnader i proteinuttryck mellan undergrupper av astma och möjligen för att identifiera skillnader i bakomliggande mekanismer.

LIST OF PAPERS

This thesis is based on the following studies, referred to in the text by their Roman numerals:

I. Lötvall J, **Ekerljung L**, Rönmark E.P, Wennergren G, Linden A, Rönmark E, Torén K and Lundbäck B.
West Sweden Asthma Study: prevalence trends over the last 18 years argues no recent increase in asthma.
Respir Res. 2009 Oct 12;10:94.

II. **Ekerljung L**, Bossios A, Lötvall J, Olin AC, Rönmark E, Wennergren G, Torén K and Lundbäck B.
Multi-symptom asthma as an indication of disease severity in epidemiology.
Eur Respir J. 2011 Oct;38(4):825-32.

III. **Ekerljung L**, Bjerg A, Lötvall J, Bossios A, Wennergren G and Lundbäck B.
Increased use of asthma medications strengthens multi-symptom asthma as a marker of severe disease – report from the West Sweden Asthma Study.
In manuscript.

IV. **Ekerljung L,** O'Neil S.E, Sihlbom C, Bossios A, Lötvall J, Hansson S and Lundbäck B.
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ABBREVIATIONS

AF	Attributable Factor
AIA	Aspirin Induced Asthma
AR	Allergic Rhinitis
BHR	Bronchial hyper-responsiveness
BMRC	British Medical Research Council
CRS	Chronic Rhinosinusitis
ECRHS	European Community Respiratory Health Survey
EP3OS	European Position Paper on Rhinosinusitis and nasal Polyps
ESI	Electro Spray Ionization
FC	Fold Change
FeNO	Fraction of exhaled Nitric Oxide
FEV_1	Forced Expiratory Volume in 1 second
FinEsS	Studies of obstructive lung diseases in FinlandEStoniaSweden
GA^2LEN	Global Allergy and Asthma European Network
GINA	Global Initiative for Asthma
ICS	Inhaled CorticoSteroids
IPA	Ingenuity Pathway Analysis
LC	Liquid Chromatography
LTQ	Linear Trap Quadrupole
m/z	mass/charge ratio
MS	Mass Spectrometry
MS/MS	Tandem Mass Spectrometry
MSA	Multi-Symptom Asthma
NLF	Nasal Lavage Fluid
NSAID	Non Steroidal Anti Inflammatory Drugs
OLIN	Obstructive Lung disease In Northern Sweden
OR	Odds Ratio
PD20	Provocative Dose resulting in a 20% in FEV_1
TMT	Tandem Mass Tag
WSAS	West Sweden Asthma Study

1 INTRODUCTION

This thesis is based on material from the West Sweden Asthma Study (WSAS) conducted in Västra Götaland between winter of 2008 and spring of 2012. WSAS is an epidemiological study focused on respiratory diseases and aims to reach from basic to clinical epidemiology and from study of populations to studies of mechanisms. This thesis starts in an epidemiological survey of the general population and ends in a proteomics analysis of selected phenotypes of asthma thus taking advantage of the strengths in the WSAS. This thesis book will give an introduction to the research behind the parts included in the thesis, as well as short descriptions of the methods and results. Finally there is a discussion of methodology and main results.

1.1 Epidemiology of asthma

1.1.1 Reasons for conducting epidemiological studies

Epidemiological studies can be divided into two main categories, retrospective and prospective. Retrospective studies are studies of occurrences that have already happened. Prospective studies are studies of occurrences that may happen in the future. Studies can be cross-sectional (one time-point) or longitudinal (multiple time-points) and be case-control or cohort studies.

A cross-sectional study compares groups of people in terms of their current health and exposure status, and assesses their similarities. It is also common that cross-sectional studies inquire on medical and exposure history. A cross-sectional study is relatively easy to conduct as the investigator do not need to wait for the outcome to occur or try to estimate the occurrence of a risk factor several years earlier. The main disadvantage of cross-sectional studies is the inability to infer causation; however cross-sectional studies can be used to identify possible associations. An important limitation of this approach is that it does not allow for changes over time, and thus cannot accommodate diseases that take time to develop.

A cohort study follows a group of people over time to investigate what will occur in terms of what is studied. A cohort study can also have sections that are cross-sectional, where a representative group of a population is studied to determine the occurrence of disease and potential risk factors. A longitudinal

cohort study is most useful for relatively common diseases and is a desirable design because exposure precedes the health outcome, a condition necessary for determining causation. It is less subject to bias because exposure is evaluated before the health status is known. The cohort study is also expensive, time-consuming and the most logistically difficult of all the studies. The commonality of the disease and how often a risk factor occurs will determine how large the cohort needs to be for sufficient power for risk factor analyses. A high participation rate is important in longitudinal studies as not to introduce bias due to non-representative participants.

In a case-control study, one investigates the prior exposure of individuals with a particular health condition and those without it, to infer why certain subjects, the "cases," become ill and others, the "controls," do not. The case-control study is an advantageous design when rare health outcomes are studied as it is not necessary to follow very large cohorts over extended time periods. Case-control studies are generally easier, quicker and less expensive than a cohort study. A great disadvantage of case-control studies is a greater potential for bias, as cases and controls are selected after the outcome and risk has occurred the possibility to inadvertently favor certain cases. Once cases are selected based on an outcome, the subjects cannot be analyzed for other outcomes as they could in a cohort study.

1.1.2 History of asthma epidemiology

The standardization of modern respiratory questionnaires started in 1950s in the United Kingdom. The respiratory research was focused on bronchitis and as a result of different observers, diverging results were obtained regarding the prevalence of bronchitis [1]. This divergence motivated the development of standardized questionnaires about respiratory symptoms [2-4]. The British Medical Research Council (BMRC) Committee on the Aetiology of Chronic Bronchitis finalized the “Respiratory Symptoms Questionnaire” (BMRC-Q) in 1960 [5], a questionnaire that had been validated by Fletcher and co-workers [3]. Although developed 50 years ago, the BMRC-Q is still used in original or, most often, modified versions. At the same time, a strict definition of chronic bronchitis was suggested, a definition that researchers agreed upon [6]. The definition was later adopted by the World Health Organization (WHO) [7] and in most aspects by the American Thoracic Society (ATS) [8].

An important aim of the BMRC-Q was to avoid interviewer bias [2]. However, in early stages of bronchitis the standardized inquiry of symptoms still caused uncertainty, and interviewer bias could still not be completely avoided. Further, performing interviews was expensive and resource consuming. Thus, self-administrated questionnaires were developed. The first well validated self-administrated respiratory questionnaire was developed for the Tucson studies in Arizona, USA [9]. The authors concluded that the self-administrated questionnaire was a useful instrument for epidemiological studies of airway disorders although relatively large differences were found in outcomes of questions about symptoms common in asthma. Later studies has also shown that outcomes of self-administered questionnaires differ from structured interviews [10].

The first British questionnaires were focused solely on bronchitis, and questions about asthma ("Have you ever had bronchial asthma?") and attacks of shortness of breath with wheezing were included in the BMRC-questionnaire in 1966. In the 1970s, several questionnaires were developed in the USA including questions for identifying asthma. The most important included the National Heart and Lung Institute questionnaire [11], which was a modified BMRC-Q. That questionnaire was further developed by the ATS and the National Institute's Division of Lung Diseases, to a new questionnaire known as the ATS-Q with more detailed questions on asthma [12]. By that time, the new self-administered Tucson-questionnaire was already validated and used in a large scale epidemiological survey [9].

In Europe, the BMRC-Q questionnaire was translated in 1962 to French, German, Italian and Dutch and further developed by adding additional questions about asthma to form the European Community for Coal and Steel (ECCS) questionnaire [13]. Several national questionnaires for identifying asthma, chronic bronchitis and respiratory symptoms, were developed in Europe during 1970s and 1980s, most of them expanded versions from the BMRC-Q. However, there was still an urgent need for a standardized questionnaire that could be used in several languages and countries. With the focus on asthma, the International Union Against Tuberculosis and Lung Diseases (IUATLD) Bronchial Symptoms Questionnaire was developed as a result of a large international cooperation in a longer (1984) and a shorter (1986) version [14]. The widely used European Community Respiratory Health Survey (ECRHS)-Q is a short version of a modified IUATLD-Q [15]. In turn, the ECRHS-Q was edited to form the recent Global Allergy and Asthma European Network (GA^2LEN)-Q. A specific questionnaire for the study of asthma and allergies among children, the International Study of

Asthma and Allergies in Children (ISAAC) questionnaire has also been developed [16]

1.1.3 Validation of respiratory questionnaires

In the 1960s and 1970s when new questionnaires were developed the validation procedures consisted mainly of comparisons with previously used questionnaires [9, 17]. Comparisons of structured interviews and self-administered questionnaires were performed in the 1970s and the self-administered questionnaire was found to be valid [9, 18, 19]. Questions on wheezing, shortness of breath and breathlessness had a strong agreement of about 0.9, while questions on cough, phlegm, dyspnoea and physician-diagnosed diseases had a slightly weaker agreement of about 0.8.

From the different validation procedures it can be concluded that results based on self-administrated questionnaires differ from structured interviews [9, 10, 19]. Furthermore, translations create variability [14, 20], particularly the translation of "wheeze". Responses to self-administered questionnaires, before and after oral information on asthma symptoms, results in divergent results with poor agreement with kappa statistics below 0.4 [21]. The agreement may also vary depending on the subjects smoking habits and educational level [20]. The best way to validate a questionnaire on asthma seems to be a combination of clinical examinations and a clinical assessment of the symptoms [22, 23].

1.1.4 Prevalence trends of asthma and respiratory symptoms

Asthma affects approximately 300 million people all over the world and it is estimated to increase to 425 million in the next 10-15 years [24, 25]. Asthma prevalence has mainly been studied among children [26-32], with the ISAAC study as the most prominent example [16]. The studies have shown a marked increase in asthma prevalence with a possible decrease during the last 10-20 years.

The Global Initiative for Asthma (GINA) reports asthma prevalence to range from 5-18% in the world [25], with the prevalence in Sweden at about 9% [33]. There are several cross-sectional studies on asthma prevalence and symptoms common in asthma, and several attempts have been made to

determine whether asthma prevalence among adults is changing [26, 33-40]. Several studies report an increase in asthma prevalence among adults during the second half of the 20th century [26, 39]. During the last 20 years the increase seem to have plateaued, at least in Western Europe and Australia [26, 33, 38] however, increased prevalence is still observed [39, 40]. In contrast, the prevalence of asthma is still increasing in several developing countries [27]. In general, the prevalence is higher among women and among younger subjects [39, 40].

The increase in prevalence that some studies report may be a consequence of patients with a milder disease being diagnosed with asthma today compared with previously [33, 41]. The observed changes in asthma prevalence may be partially explained by systematic errors arising from changes in diagnostic practice due to a lack of standardized definitions for asthma [29]. Furthermore, the increased awareness of asthma in the public may result in increased self reporting, contributing to a further escalation in asthma prevalence.

1.1.5 Studies of asthma incidence

There are very few studies of asthma incidence. There has likely been an increase in asthma incidence during the last 35-45 years as indicated by higher incidence rates being reported in more recent studies compared to older [42, 43]. A recent comparison analysis of studies conducted over 20 years showed that the increase in asthma incidence has ceased in Sweden [41, 44, 45]. The incidence rate varies extensively between studies, ranging from 0.4-11/1000/year. Beside a true difference in incidence rate, this broad range could be due to methodological differences. In Sweden the incidence rate of physician-diagnosed asthma among adults is about 2/1000/year.

1.1.6 Risk factors associated with asthma

There are several risk factors for asthma, with the most important being heredity, allergic sensitization, socio-economic status, and environmental factors such as occupational exposures and smoking.

Type 1 allergy, atopy

The allergic reaction is caused when an allergen interacts with a specific-IgE antibody causing the immune response to react. Allergic rhinitis (AR) is the

most common immunological disorder and is estimated to affect 500 million people worldwide [46, 47]. AR and asthma have common physiological, pathological and epidemiological features [48] and AR is commonly found in subjects with asthma and is an important risk factor [49], with up to 80% of asthmatics suffering from AR [50]. In Sweden, sensitivity to pollen often results in AR, while subjects with asthma often are sensitized to furred animals [51]. The allergen(s) that causes AR and asthma varies between countries. In countries with a cold, dry climate, asthma is commonly associated with furred animals and somewhat less to grass and pollen, while mites, moulds and cockroaches commonly is associated with asthma in countries with temperate or tropical climates [52-57]. Allergic sensitization plays a minor role in the development of asthma among middle-aged and older subjects.

Family history of atopic disease

In addition to allergic sensitization, having family history of atopic disease is the strongest risk factor for the development of asthma [58-60]. The risk of a child developing asthma increased three times if one parent had asthma and was ten times higher if both parents had asthma [58].

Gender

The gender distribution for asthma is different depending on age, with the prevalence of asthma being higher in boys, while in adults, asthma is more prevalent among women [33, 58, 61]. Lower quality of life and problems with asthma control have also been associated to female gender [62]. The gender related differences might be caused by some women being hypersensitive to their sex hormones, or by other hormonal processes [63].

Socio-economic status

Low socio-economic status is often associated to poorer health [64]. Socio-economic status can be classified using income, occupation and educational level, or a combination of these [64-66]. Low socioeconomic status has been reported to have a variety of associations with asthma; positive, negative and no association. [64-73]. Low socioeconomic status, defined as low educational level, was related to a higher incidence and prevalence of asthma in the ECRHS [65]. An increased risk of prevalent and incident asthma and respiratory symptoms has also been reported in manual workers [64, 66, 69], especially those belonging to the socio-economic group of manual workers in service [69]. Increased asthma severity has been reported in low socio-economic classes [73]. There are many possible confounders in the observed association between socio-economic status and asthma or respiratory symptoms. The confounders include occupational exposure, smoking,

obesity, life stress, ethnicity, exposure to housing and outdoor pollution and environmental tobacco smoke [72, 74-76].

Occupational exposure

During the last decade, occupational asthma has been identified as a large problem in public health. Studies have shown that approximately 15-20% of adult asthma can be explained by occupational exposure [77]. However, the association might be an under-estimation due to the "healthy worker" effect [78]. The "healthy worker" effect is when subjects with asthma choose occupations that are free of certain exposures that might worsen their respiratory health. It might also mean that employers within certain occupations do not hire subjects with asthma. A recent study showed that those with allergic rhinitis during adolescence are less likely to choose occupations classified as having a high risk of incident asthma [79]. Common occupations that are associated to occupational asthma are bakers, hairdressers, laboratory workers, welders, cleaners, wood workers and occupations that are related to exposures to dust, gas and particles [80]. In addition to occupation related exposures, there are other factors in the work place that can worsen respiratory symptoms, these include colleagues who smoke or use perfume, cold, physical exertion and psycho-social factors [81].

Tobacco smoke

Smoking is decreasing in large parts of the Western world. In Stockholm, Sweden the prevalence of smoking decreased from 31% in 1996 to 18% in 2006 [33]. In a recent European study the prevalence of smoking was lower among subjects with asthma compared to subjects without asthma [82]. While smoking is the most important risk factor for respiratory symptoms, the association between asthma and smoking is less clear [83, 84]. Former smoking and ever smoking has been associated to asthma in several cross-sectional studies [85-88], while others have shown no association [89]. Significant association is more often found in prospective studies [43, 90-92]. A lack of association between smoking and asthma could possibly by due to the healthy smoker effect. Environmental tobacco smoke has been associated with all types of respiratory symptoms and asthma, especially in females [75, 93, 94].

1.2 Asthma

1.2.1 Bronchial hyper-responsiveness

Bronchial hyper-responsiveness (BHR) is a common feature in asthma and results in temporary bronchoconstriction and increased symptoms [95]. It is a condition in which the airways react with an exaggerated bronchoconstriction in response to stimuli [96]. In Sweden, it has been clinical praxis to use BHR to verify asthma when the anamnesis is inconclusive and cannot be verified with variable peak expiratory flow or a positive bronchodilatation test [97]. The airways of asthmatics respond with a greater constriction to a certain stimulus than the airways of healthy subjects. It is a characteristic feature of asthma and can be demonstrated in almost all patients with a clinically relevant asthma [98, 99]. The presence of BHR is often determined using direct challenge tests using methacholine or histamine. These are highly sensitive, cheap and easy to perform but are not specific to asthma [99, 100]. There is a considerable variability in the intensity of BHR between patients with asthma, and the level of reaction varies also for an individual with asthma depending on exacerbations, allergen exposure and occupational exposures. The effect of steroid use on BHR is limited among severe asthmatics, even though the underlying inflammation is reduced [99]. However, decrease in hyper-reactivity has been demonstrated when asthmatics have been free from symptoms and exacerbations during an extended time period, as a result of maintenance treatment [101]. The prevalence of BHR in different populations may vary considerably and is probably due to a wide variety of methods of measure and definitions of BHR. An epidemiological study in Finland found the prevalence of BHR to be about 20% using both methacholine and histamine [102]. Meanwhile, a Swedish study using another methacholine based method, found a prevalence of about 13% [103].

1.2.2 Defining asthma in epidemiological studies

Assessment of asthma is impaired by the lack of a clear and definite standardization of definitions of asthma and symptoms. Indeed, there is no single test or gold standard for defining or identifying asthma [22, 104]. The current definitions of asthma have four main parts: respiratory symptoms, bronchoconstriction, airway inflammation, and BHR. In the 1980s several researchers regarded BHR as a gold standard for asthma [96, 105]. A questionnaire was developed to identify asthma if possible only by symptoms

[14], the questionnaire was validated against BHR [14] and another questionnaire [106]. However, the validation showed that asthma could not be identified by solely using symptoms. A question of "Has a doctor ever told you have asthma" (physician diagnosed asthma) has a good specificity [107-109], especially when combined with symptoms and medication use in the last year. It is important in epidemiological studies of asthma to always clearly state the definition used for asthma, and preferably also the exact questions that were used [110].

1.3 Asthma – not a single disease entity

1.3.1 Phenotypes of asthma

Today there is an ongoing debate on the importance clinical phenotypes of asthma. Studies are performed using hypothesis driven phenotyping or phenotyping based on cluster analysis. When cluster analyses are performed it is important to use a study sample that is representative of the population, and the included variables have great effect on the results. Sub classification seems no less important when we consider that other important diseases such as arthritis and anemia are no longer named by their syndromes but by specific subtypes. Asthma, which affects about 10% of the population, is still referred to as the broad syndrome of asthma. A discussion about a new taxonomy for the obstructive airway diseases has been initiated by Beasley and coworkers [111].

1.3.2 Severe asthma

The global Asthma Insights and Reality (AIRE) survey reported that 18% of asthmatics have a severe asthma [112]. Severe asthma causes the most significant economical burden of asthma, despite representing a minority of those with asthma [113, 114]. It poses a great burden both on the individual and on society, as it is associated with an impaired quality of life [115], lifestyle restrictions [116], high socio-economic costs [114], increased morbidity with need of emergency care, increased risk of hospitalization and death [112, 117]. Many patients continue to have symptoms and lifestyle restrictions despite treatment and require emergency care due to asthma [112]. Clinical studies suggest that severe asthma is more common among women [112, 118] and when compared with asthma in general, a greater proportion have presence of neutrophilic inflammation [118, 119]. Severe asthmatics often have increased BHR and airway inflammation, impaired

lung function, frequent night-time awakenings and dyspnoea. Airway symptoms upon intake of aspirin is also more common among severe asthmatics [118] and asthmatics with concurrent rhinitis are at a higher risk for hospitalization and have a higher cost of asthma medication [120].

Severe asthma is difficult to define, the terminology has not been standardized and terms are still used interchangeably. Definitions are commonly based on the required need of asthma medication to achieve a controlled disease [121].. Traditionally severe asthma has been synonymous with asthma having frequent symptoms, despite the highest level of treatment [118, 122, 123]. Asthma has also been defined based on level of control rather than symptom severity [124, 125]. The level of asthma control is important, as it affects exacerbation frequency and quality of life [115, 126-128]. The GINA 2006 revision defines asthma by level of control based on symptoms, need of medication, exacerbations and lung function [124]. WHO defines severe asthma as "uncontrolled asthma" resulting in risk of frequent severe exacerbations and or adverse reactions to medications and/or chronic morbidity" [129]. According to the recent WHO definition, severe asthma includes three groups: untreated severe asthma, difficult-to-treat severe asthma and treatment-resistant severe asthma. In this definition the use of asthma medication is not included.

1.3.3 Aspirin intolerant asthma

First described in 1902 by Hirschberg [130], the mechanism causing adverse respiratory reaction to aspirin still remains to be elucidated. Hypersensitivity to aspirin can have several manifestations including asthma, rhinosinusitis, urticaria and anaphylaxis [131]. In 1968, Samter and Beers focused on the coexistence of aspirin sensitivity, nasal polyposis and chronic rhinosinusitis [132], the phenomenon is now known as Samters' triad. In aspirin induced asthma (AIA), breathing problems arise following ingestion of acetylsalicylic acid or non-steroidal anti-inflammatory drugs (NSAIDs), usually within three hours. AIA is commonly characterized by chronic rhinosinusitis with up to 26% of subjects with nasal polyps also suffering from AIA [133, 134]. The prevalence of AIA is 0.5-1.9% in the general population [131], and 4.3-11% of asthmatics [135, 136]. The pathogenesis is still unknown but involvement of the arachidonic acid metabolism, with inhibition of cyclooxygenase-1 (COX1) and an increase in inflammatory mediators such as cysteine leukotrienes (CL) is likely [137, 138]. Prostaglandin (PG) E2 has been proposed to have a crucial role as PGE2 normally act as an inhibitor on

excessive production of CLs [133]. COX is a group of enzymes that metabolize arachidonic acid to PGE, thromboxanes and prostacyclin. Deprivation of PGE2 may lead to activation of inflammatory pathways. AIA may be related to the inhibition of the COX enzyme but blockage of the CL pathway by specific inhibitors such as 5-lipooxygenase inhibitors do not completely protect against AIA [139, 140]. A decrease in inflammatory suppressors has also been suggested. To properly diagnose hypersensitivity to aspirin and NSAIDs an understanding of the underlying mechanism is necessary.

1.4 Use of asthma medication

In Western societies, prevalence of asthma reflects the prevalence of users of asthma medication [141, 142], and over the past decades an obvious increase in asthma medication has been observed [143, 144]. Issues of non-adherence are well recognized in asthma and low adherence is associated with poor asthma control, increased mortality, decreased quality of life, increased hospitalization rates and increased risk of exacerbations [112, 115, 117, 145, 146]. Still, many patients with persistent symptoms consider their disease "well controlled" [112, 147] which could explain part of their non-adherence. In the AIRE-study [112], a low usage of preventive medication, with many patients having to resort to quick-relief medication, indicative of poor asthma control, was demonstrated. There was also a poor correlation between the level of symptoms and perceived asthma control. People with severe asthma are also prone to anxiety and depression, which is also associated with non-adherence to treatment regimens [148-150], and an overestimation of adherence has been demonstrated [151]. The level of asthma control often falls short of the management goals with many patients being severely undertreated, both among mild and severe asthmatics [112, 147, 152-154]. A lower adherence in subjects with more severe disease and an overestimation of adherence has reported [112, 151, 155, 156]. Gamble et al [155] demonstrated that 35% of asthmatics had non adherence as main cause of difficult-to-treat asthma. Studies of prescription refill data have shown considerable discrepancies between self-reported adherence and prescription refills where adherence was more overestimated in severe asthmatics, implying that severe asthma is in part due to poor adherence to asthma medication [151, 156]. Studies of non-adherence according to gender have been inconclusive with some showing no gender differences [157, 158] and some showing a lower adherence among women [159].

1.5 Chronic rhinosinusitis

Chronic rhinosinusitis (CRS) is an inflammatory condition of the mucosa of the nose and paranasal sinuses that persists for at least 12 weeks [160]. The prevalence of self-reported CRS according to the European Position Paper on Rhinosinusitis and nasal Polyps (EP3OS)-criteria was 6.9-27.1% in a European comparison [161]. There are two forms of CRS; with nasal polyps (CRSwNP) and without (CRSsNP), with CRSsNP being most common [160]. The pathophysiology of the two phenotypes is largely unknown, however some evidence suggests that CRSsNP is mainly Th1-polarised, with interferon-γ more prominent than interleukin (IL)-5, while CRSwNP is mainly Th2-polarised [160]. In CRSwNP, eosinophils are the main contributor of inflammation, while myeloperoxidase and IL-8 concentrations are increased in CRSsNP indicating that in addition to eosinophils, neutrophils are also involved in the pathogenesis [162, 163]. Transforming growth factor β1 (TGF-β1) has a key role in tissue remodeling in CRS with expression reported to be significantly higher in patients with CRSsNP versus control subjects [164]. TGFβ signaling also contributes to the regulation of expression of matrix metalloproteases and their natural tissue inhibitors.

1.6 Proteomics

1.6.1 Reasons for studying the proteome

One definition of a proteome is "a set of proteins being expressed in a given type of cell or organism at a given time under defined conditions". This implies that the proteome is ever changing and proteomics of a human sample is a snapshot of that individuals' proteome at the time of sampling. It is becoming increasingly clear that while the study of the human genome has its benefits, it is not enough to understand differences and changes in complex diseases such as asthma. There has been many studies aimed at identifying asthma susceptibility genes but replication of the results has been difficult and the clinical relevance is uncertain[165]. There are many factors that influence which genes are being expressed at a given time, and how these genes are being subject to post-translational modifications. Further, the DNA sequence alone does not reveal biological function and one gene can code for several proteins by gene rearrangements and RNA splicing. The proteome varies between tissues, between different cell types, with developmental stage and depending on the environment and disease.

1.6.2 Asthma proteomics

Proteomics of human samples poses several issues. The natural variation is much higher compared with animal models or cell cultures and is further increased by external factors such as sampling, storage and processing [166]. While proteomics and quantitative proteomics has been used successfully in other fields, the area is still largely unexplored in regards to asthma [167-171], possibly due to the complexity of disease. Allergic airway inflammation has been explored in animal models of asthma, both with [172, 173] and without [174, 175] the influence of glucocorticoid treatment. Very few of these models have been confirmed in human studies [176]. Cell cultures, particularly from bronchial brushings has been used to explore differences between asthmatics and healthy, airway surface liquid with and without cytokine stimulation has been explored [177, 178] as well as fibroblast in asthmatic airways [179-181]. Biological fluids, such as bronchoalveolar lavage fluid (BLF), nasal lavage fluid (NFL) and sputum, have been used to compare asthmatics and healthy subjects [182-184], effects of airway challenge [185, 186] and effects of exposures [187, 188]. Proteomics on plasma from asthmatics has shown differential expression in T lymphocyte proteins [174].

1.6.3 Separation techniques

In this thesis, reversed phase liquid chromatography (RP-LC) was used to enhance sensitivity prior to mass spectrometry (MS) analysis. This procedure is often used as it reduces the complexity of the sample. In RP-LC samples are loaded onto columns packed with solid phase adsorbents, carrying hydrophobic groups, generally C18 is used for peptide analysis, that bind to the peptides through hydrophobic interaction, while salts and other water soluble impurities are washed away. The peptides are eluted by applying a gradient of an organic solvent (e.g. acetonitrile) and the sample containing fractions are then analyzed separately in the MS.

1.6.4 Mass spectrometry

MS is a key technique in proteomic analysis providing accurate mass measurements of small quantities of proteins, peptides and peptide fragments. Analysis of peptide fragments give information of the amino acid sequence and modifications. Three components are generally present in all mass spectrometers: an ion source, a mass analyzer and a detector. Sample

molecules are introduced into the ion source where they are converted into gas phase ions. The mass analyzer separates the ionized species according to their mass to charge (*m/z*) ratio and the detector records an ion current of the separated analytes. Results are then plotted in the mass spectra, as the ion current against *m/z*. A tandem mass spectrometer (MS/MS) has more than one analyzer or the same analyzer can be used for both MS and MS/MS. A collision cell, where selected molecules are admitted to collide with an inert gas, produces the fragments analyzed in the second MS. A peptide is selected based on information from the primary ions in the MS (MS1). Peptides of the selected *m/z* are then passed onto a collision cell where they are further fragmented to produce daughter ions which are analyzed in the second MS (MS2). Each peptide will only be fragmented once but the site of cleavage will vary between peptides with the same *m/z*. There are a number of different types of mass spectrometers employed in proteomic research.

Electrospray ionization

The ionization method used in this thesis is nano electrospray ionization (ESI) [189] since it enable production of intact gaseous ions of large biomolecules. The inventor of the ESI, John Finn, was awarded with the Nobel Prize in chemistry in 2002. With ESI the sample solution is sprayed at, atmospheric pressure, from the tip of a thin capillary and a strong electric field is applied between the capillary and a counter electrode and a fine spray of charged droplets is produced.

Quadrupole mass filters

A quadrupole mass filter separates ions according to their *m/z* by utilizing the stability of their trajectories in an oscillating electrical field. The field is created by a combination of radio frequencies and direct current voltages with only one mass permitted to pass at one time. The remaining masses collide with one of the four metal rods, not reaching the reactor. The LTQ is a linear ion trap that use a three-dimensional quadrupole field to trap and mass-analyze ions, which have a very good sensitivity and is fast, however the resolution is not so high. Furthermore the LTQ can carry out fragmentation reactions allowing high-resolution MS/MS experiments.

Orbitrap mass analyzers

The Orbitrap was invented by Alexander Makarov in the late 1990s [190]. In an Orbitrap, ions are trapped in an electrostatic field in which the ions orbit around a central electrode while at the same time oscillating along the central axis of the electrode. The oscillation ion induces an image current into the two outer halves of the Orbitrap, which are detected using a differential amplifier. The Orbitrap measure frequency (how fast the ion spin) which is

unique to the specific *m/z*. Ions of only one mass generate a sine wave signal which is converted to *m/z* spectra using a fast Fourier transform algorithm. In this thesis an LTQ Orbitrap Velos (Thermo Fisher Scientific Inc., Waltham; MA, USA) instrument was used.

1.6.5 Quantitation by mass spectrometry

There are several ways to quantify samples in mass spectrometry based proteomics. A common approach is stable isotope labeling which can be done *in vivo* or *in vitro*. *In vitro* labeling involves the incorporation of stable isotopic tags onto selective sites on the peptides, such as the amine group of the N-terminus. Depending on the selected method, up to eight samples can be quantified at the same time using isobaric tag for relative and absolute quantification (iTRAQ) or tandem mass tag (TMT) labeling techniques of the peptides.

A mass tag for isobaric labeling has three parts; firstly, a group that will react with the peptide that is to be labeled, secondly, a part that act as a mass balance so that all tags have the same mass in the first MS, and finally, a mass reporter that has a different mass between the labels (Figure 1). The TMT isobaric tags have identical structures that covalently attach to the amino-group of lysine and the N-terminal of the peptides. During MS, the labeled peptides cannot be distinguished from each other and thus are progressed to the second MS as one *m/z*. In the MS/MS, each tag produces a unique reporter ion signature, enabling quantification by comparing intensities of the reporter ions. If several sets are being analyzed within the same experiment, one of the tags is used to label a pool consisting of small amount from each sample, making comparisons between sets possible.

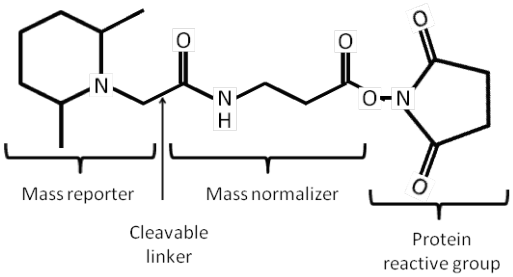

Figure 1. Schematic of a TMT mass tag

1.6.6 Proteins identification

Depending on the used MS technique, different methods for the identification of proteins is used. When MS/MS has been used amino acid sequence analysis is usually applied and identification of proteins is done by matching mass spectrometric data to databases containing known protein sequences.

Amino acid sequence analysis using tandem mass spectrometry

In MS/MS, a spectrum is created that is more or less unique for the investigated peptide [191]. This is achieved by isolating a single peptide precursor and inducing fragmentation along the peptide bonds. The identified masses are then compared with theoretical fragmentation patterns of proteins in a database search.

In the LTQ Orbitrap Velos fragmentation can be done using collision induced dissociation (CID) or higher-energy C-trap dissociation (HCD). The CID process includes multiple low-energy collisions of the peptide precursor ion with an inert gas, usually argon, which finally leads to dissociation of the precursor ion [192]. The HCD process involves fragmentation of ions in a collision cell and then the ions are transferred back to the C-trap for analysis in the Orbitrap [193]. Which protein database that is used will affect the outcome, in this thesis UniProtKB Swiss-Prot was used. UniProtKB Swiss-Prot is non-redundant, manually curated and cross-referenced.

1.6.7 Interpreting the results

Ingenuity Pathway Analysis

Ingenuity Pathway Analysis (IPA) is a web based licensed bioinformatics tool which takes information from the Ingenuity® Knowledge Base (Ingenuity® Systems, www.ingenuity.com). The Ingenuity® Knowledge Base is a repository of biological interactions and functional annotations created from millions of individually modeled relationships between proteins, genes, complexes, cells, tissues, metabolites, drugs and diseases. It is manually reviewed and updated regularly. It allows the user to explore biological functions, networks and pathways associated to the analyzed dataset.

Protein analysis using gene ontology

The Protein Analysis THrough Evolutionary Relationships (PANTHER) Classification System [194] is a web based resource that classifies genes by their function. The classification is performed by expert biologist who uses

scientific evidence and evolutionary relationships to describe the gene products in terms of biological process, cellular components and molecular functions. PANTHER is part of the Gene Ontology (GO) Reference Genome Project [195].

As for PANTHER, GO Term Finder [196] is part of the GO consortium in which gene products may be annotated to one or more GO nodes. It can be used to draw conclusions from microarray and other biological data, calculating the statistical significance of each annotation and thus, can identify the GO terms significantly enriched in a submitted list of genes.

2 AIM

The aims of this thesis were:

1. To estimate the current prevalence of asthma and respiratory symptoms in West Sweden.

2. To evaluate if the prevalence of asthma and respiratory symptoms are still increasing.

3. To investigate if markers of severe asthma can be identified by a postal survey.

4. To investigate patient reported use of asthma medication in West Sweden.

5. To investigate if differences in protein expression between asthma phenotypes can be identified in a non-invasive samples.

3 METHODS AND MATERIALS

3.1 Study area

The region of Västra Götaland reaches from the northern part of Sweden's west coast to the lakes of Vänern and Vättern in the central part of Southern Sweden (Figure 2) and is referred to as West Sweden in the papers included in this thesis. The region is very diverse with rural areas, small and medium sized towns and a big city. In the beginning of 2008, when this study was initiated 1.6 million people were living in the county, representing 1/6th of Sweden's population. Gothenburg is situated on the west coast and is the second largest city in Sweden with 700 000 living in the city or in the surrounding urbanized area. The population in the area is representative of Sweden in regards to age and gender distribution. The climate is oceanic according to the Köppen climate classification with warm summers, mild winters and high humidity. The average temperature is between 15 to 16°C in July and between -1 to -4 degrees in January. The average precipitation 500-1000 mm/year [197].

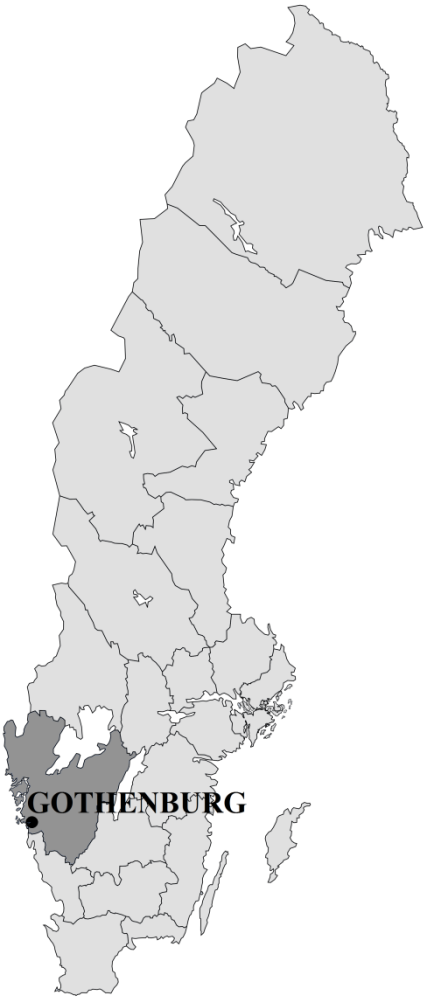

Figure 2. Sweden with the study area of Västra Götaland in darker grey. Modified from a image by Lokal_Profil under license agreement CC-BY-SA-2.5

3.2 Study design and study population

The first part of the WSAS took place in 2008 when a postal questionnaire was mailed to 15 000 randomly selected subjects living in the metropolitan area of Gothenburg and 15 000 randomly selected subjects living in remaining study area. The population was stratified by 10-year age groups and gender to best represent the population in Västra Götaland. Of the randomly selected subjects, 782 could not be traced, were deceases or were unable to participate. **Paper I** is based on the 18 087 subjects who responded to the questionnaire.

From responders to the questionnaire, a random sample of 2000 was selected for clinical examinations. In addition, all subjects who reported physician-diagnosed asthma or reported every having asthma and either use of asthma medication, wheeze or attacks of shortness of breath during the last year were also included in the clinical cohort. In total, 3536 subjects were included, of which 1736 had reported asthma in the questionnaire. **Paper II** includes data from the 18 087 responders to the questionnaire and clinical data from the 843 subjects who had participated in the clinical examination between February 2009 and December 2010. **Paper III** includes data from the 1755 subjects who had participated in clinical examinations between February 2009 and December 2011. **Paper IV** included 36 well characterized subjects who had participated in the clinical examinations and had successfully given NLF, thus enabling separation of three phenotypes of asthma and a healthy control group. A schematic of the study design is shown in Figure 3.

The clinical study was completed in May 2012 and included 2002 subjects, 1172 from the random sample and 830 from the asthmatic group. Of participating subjects from the random sample, 132 were considered asthmatic based on their responses in the questionnaire survey which meant that a total of 962 asthmatic subjects had attended the clinical examinations.

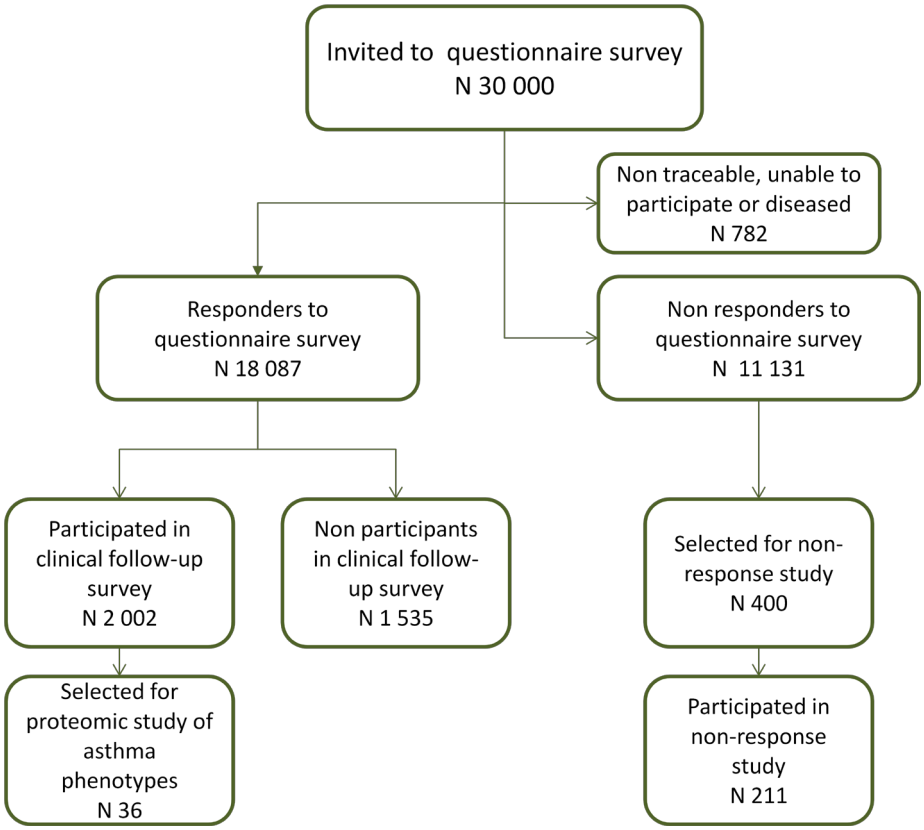

Figure 3. Study design of the West Sweden Asthma Study.

3.3 Postal questionnaire

The questionnaire consisted of two parts that were included in a folder mailed to the selected subjects, together with a pre paid response envelope. The participants could also choose to respond by using a web based questionnaire with unique user names and passwords.

The first part of the questionnaire was the Obstructive Lung disease In Northern Sweden (OLIN)-questionnaire [198], which has been extensively used in Sweden and within the FinEsS-studies (epidemiological studies of obstructive lung diseases and allergy in Finland, Estonia and Sweden) [199] and has also been used in Vietnam [200]. Additional questions were added to the OLIN-questionnaire, focusing on occupation, airborne occupational and environmental exposures, health status and socio-economic status. The second part consisted of the Swedish version of the GA^{2}LEN-questionnaire

[201, 202]. The two questionnaires complement each other as the OLIN-questionnaire more thoroughly covers bronchitis and chronic obstructive pulmonary disease (COPD), while the GA^{2}LEN-questionniare has detailed questions on rhinitis, chronic rhinosinusitis and eczema. The questionnaire is included in Swedish as Appendix I. The questions on asthma were similar or identical [110]. From the questionnaire the definition of multi-symptom asthma (MSA) was created. This definition includes subjects who report p*hysician-diagnosed asthma* and *asthma medication* and *attacks of shortness of breath* and *recurrent wheeze* and at least one additional respiratory symptom.

3.3.1 Study of prevalence trend

In **paper I**, the prevalence of asthma and respiratory symptom was studied. The questions included in the questionnaire allowed for comparisons with two studies performed within the study area in the 1990s. The comparisons were performed to determine if there has been a change in prevalence of asthma and respiratory symptoms in Gothenburg from the early 1990s to 2008. Only questions that were similar between the questionnaires were used. In the first comparison, the Gothenburg part of the ECRHS survey performed on the island of Hisingen in 1990 [34] was used. In this comparison, only subjects from the current study matching the study population from 1990 were used so the comparison only included subjects 20-44 years of age and living on the island of Hisingen. The second comparison was performed against a study conducted in the county of Södra Älvsborg in 1994 [43]. In this comparison, only subjects aged 16-50 years of age and living in Södra Älvsborg were included. Södra Älvsborg is included in the county of Västra Götaland since 1998.

3.3.2 Study of non-response

A non-response study should always be performed as a part of epidemiological surveys and hence, a non-response study was performed during the summer of 2008 [203]. The non-response study is not part of this thesis but is vital for the validity of the results and therefore, described briefly. From the non-responders that could be identified, a random sample of 400 was selected. Phone numbers were acquired from two publicly available phone records, with phone numbers for 72.2% of the subjects being identified. However, 13.8% did not respond to any of the five phone calls that were conducted before a subject was considered unreachable. The study was a structured interview conducted by a single interviewer containing selected

questions from the original postal questionnaire and additional questions on reasons for non-response. Of the contacted 234 subjects, 90.2% agreed to participate.

3.4 Clinical examinations

3.4.1 Structured interview

The extensive structured interviews were conducted by trained nurses and contained questions on airway symptoms and diseases, rhinitis and allergies, detailed questions on many potential risk factors, utilization of health care due to respiratory symptoms and questions on comorbid diseases. The Swedish version of the questionnaire is included as Appendix II. In addition, separate questionnaires were included for subjects with asthma or COPD. Of particular interest for this thesis was the questionnaire on the use of asthma medication. This questionnaire contained questions about which type of medication the asthmatic had used during the previous year, including short (SABA) and long (LABA) acting beta antagonists, inhaled (ICS) and oral glucocorticosteroids, combination therapies, anti-cholinergic medication etc., with examples of brand names given. The subjects were also asked how often they used the medication (never, occasionally, most days) and in the case of steroids, how much medication they used per day. Doses were converted to beclomethasone dipropionate (BPD) equipotent doses. The Swedish version of the questionnaire is included as Appendix III.

3.4.2 Skin prick test

Atopy to airborne allergens were tested using a standardized panel of 11 allergens *Dermatophagoides pteronyssinus, Dermatophagoides farinae, Alternaria alternate, Cladosporium herbarium, Blatella germanica,* dog, cat, horse, timothy, mugwort and birch (ALK, Hørsholm, Denmark). Histamine (10 mg/ml) was used as a positive control, while glycerol was used as a negative control. The allergens were applied using a lancet on the forearm using standardized methods, however, the allergens was only applied to one arm [204]. A mean wheal diameter equal or larger than 3 mm, measured after 15 minutes, was considered positive. Subjects were asked to refrain from anti-histamines for at least 72 hours prior to the visit.

3.4.3 Lung function, reversibility and methacholine test

Lung function tests were performed using a Masterscope Spirometer (Jaeger, Höcjberg, Germany). The tests were performed with the subject seated and using a nose clip. FEV_1% predicted was calculated using the ECCS reference equation [205]. Subjects were asked to refrain from long-acting bronchodilators for 24 hours and short-acting bronchodilators for eight hours prior to the visit.

Reactivity to methacholine was determined using the Spira equipment (Spira Respiratory Care Center Ltd, Hämeenlinna, Finland) following a shortened protocol. The highest cumulative dose was 1.96 mg. The cumulative dose where a 20% decrease in FEV_1 was reached was calculated using the following formula: PD20 = A+((20-B)*(C-A))/(D-B), where A = administered dose methacholine prior to 20% decrease in FEV_1, B = % decrease in FEV_1 after A, C = administered dose methacholine causing a minimum of 20% decrease in FEV_1 and D = % decrease in FEV_1 after C.

Reversibility was tested at the same visit to the clinic as the methacholine challenge, meaning that some subjects performed the reversibility test without a prior methacholine challenge and some performed it after a methacholine challenge. As a consequence, not all subjects have been reversibility tested in an optimal way. In cases where the subject first underwent a methacholine challenge, the subjects were given 4x0.1 mg of salbutamol (Ventoline®) followed by two capsules of 4 µg ipratropium bromide (Atrovent®) with the reversibility spirometry measured 30 minutes after. A spacer was used for both drugs. In cases where no methacholine was given, the subject was administered 4x0.1 mg of Ventoline and spirometry was performed after 15 minutes.

3.4.4 Exhaled nitric oxide

Fraction of exhaled nitric oxide (FeNO) was measured using a NIOX (Aerocrine AB, Solna, Sweden) at three flow rates; 50, 100, and 270 ml/s. This was performed to identify inflammation in different parts of the lung. In this thesis, only the value from the 50 ml/s measurement is used. The subject performed two exhalations per flow and the average was recorded.

3.4.5 Nasal lavage fluid

NLF was collected from all patients who gave their consent. With the head tilted back 30 degrees and the pharynx closed, 5 ml of 10% saline was instilled into the left nostril using a plastic syringe. Immediately after insertion the head was tilted forward and the fluid passively collected. The NLF samples were centrifuged at 300 x g for 10 minutes at 4°C to remove cells and stored in -80°C.

3.5 Proteomics

3.5.1 Study population

Subjects in **paper IV** were selected based on characteristics identified in the structured interview and clinical examinations. They were stratified into four groups; multi-symptom asthma (MSA), multi-symptom asthma with chronic rhinosinusitis (CRS-MSA), aspirin induced asthma (AIA) and healthy controls.

Subjects considered to have MSA (please, see *Definitions* below) in the proteomics study had to fulfill the criteria from the postal questionnaire, as well as those in the structured interview. They also had to be methacholine reactive, or if no methacholine test was performed, have a reversibility of more than 12%. If neither was performed, the FEV_1% predicted had to be less than 90%.

The selection of CRS subjects was based on answers from the postal questionnaire. Subjects classified as CRS-MSA had to fulfill the EP3OS-criteria, where chronic rhinosinusitis is characterized by two or more symptoms (please, see *Definitions* below) [206].

Subjects with AIA reported *physician-diagnosed* asthma and breathing difficulties within three hours of using aspirin or a NSAID. Most subjects in the AIA group also fulfilled the criteria for MSA. The report of breathing problems after aspirin intake was confirmed via a phone call by a physician, where the subjects were asked to describe the event(s) and the severity of the reaction.

Healthy controls reported no respiratory symptoms, *physician-diagnosed asthma, use of asthma medication* or *allergic rhinitis* and they had a negative

skin prick test and methacholine test. Furthermore, they were chosen to match the other groups regarding age and gender. In all groups, subjects were excluded if they reported ever smoking, chronic obstructive pulmonary disease, chronic bronchitis, emphysema, heart disease, claudicates intermittens, stroke, transient ischemic attack, elevated blood fats, diabetes mellitus, rheumatic disease or other diseases with a systemic inflammation, or had a FEV_1% predicted of less than 50%.

3.5.2 Study design

The experiment was run in nine different sets, with one sample per group in each set, the samples were randomly assigned to a set. To enable quantitative comparisons between sets, samples were diluted so that all samples had the same protein concentration and a pool containing small amount from each sample was created and included in all sets. This pool was always ran with the TMT-127 Da tag. As each TMT set can be run using six labels and one was always allotted to the pool, five labels were available for samples. The samples were randomly assigned one of the five labels, and in each set one label was not used. After the samples and pool had been labeled, the samples within each set were pooled and fractionation by chromatography was performed. Based on protein content, fractions that were to be subjected to MS were chosen, Due to low protein content some fractions were pooled two and two. On overview of the study design is shown in Figure 4. After MS analysis, all sample intensities in a set was compared to the intensity for the corresponding peptide in the pool and a ratio was created. In this fashion all peptides were normalized, thus enabling comparison between sets.

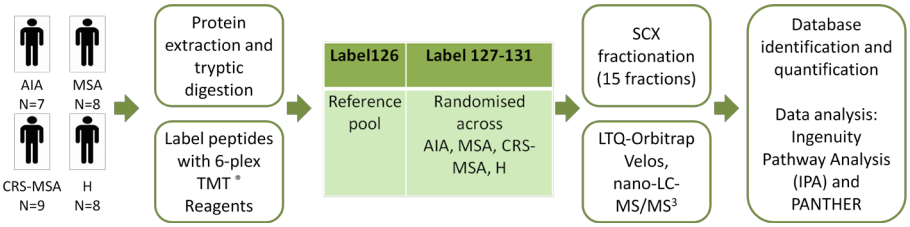

Figure 4. Study design of the proteomics experiment

3.6 Definitions

3.6.1 Outcomes

Ever asthma: "Have you ever had asthma".

Physician-diagnosed asthma: "Have you been diagnosed as having asthma by a doctor".

Active (or current) asthma: Ever asthma or *physician-diagnosed asthma* and at least one of *use of asthma medication*, *attacks of shortness of breath*, *any wheeze* and *recurrent wheeze*.

Multi-symptom asthma: Physician-diagnosed asthma and *asthma medication* and *attacks of shortness of breath* and *recurrent wheeze* and at least one symptom out of *dyspnoea, breathlessness (exercise), breathlessness (cold)* and *breathlessness (exercise in cold)*.

Asthmatic (in the clinical follow-up): Physician-diagnosed asthma or *ever asthma* and, in the latter case, either *asthma medication*, *any wheeze* or *attacks of shortness of breath* during the last year.

Asthma medication: "Do you currently use asthma medicine (permanently or as needed)".

Attacks of shortness of breath: "Do you presently have, or have you had in the last 10 years, asthma symptoms (intermittent breathlessness or attacks of shortness of breath; the symptoms may exist simultaneously with or without cough or wheezing)" and "Have you had these symptoms within the last year". Only the latter, i.e. symptoms within the last year, has been included in the analyses.

Recurrent wheeze: "Do you usually have wheezing or whistling in your chest when breathing".

Any wheeze: "Have you had whistling or wheezing in the chest at any occasion during the last 12 months".

Wheeze with breathlessness: "Have you had whistling or wheezing in the chest at any occasion during the last 12 months" and "Have you been at all breathless when you had wheezing or whistling in the chest".

Wheeze apart from cold: "Have you had whistling or wheezing in the chest at any occasion during the last 12 months" and "Have you had this wheezing or whistling in your chest when you have not had a cold".

Wheezing with breathlessness apart from cold: Any wheeze and *Wheeze with breathlessness and Wheeze apart from cold.*

Dyspnoea: "Do you get breathless when you walk on level ground with people of your own age".

Breathlessness – exercise: "Do you usually have breathlessness, wheeze or severe cough when you exercise".

Breathlessness – cold: "Do you usually have breathlessness, wheeze or severe cough in cold weather".

Breathlessness – exercise in cold: "Do you usually have breathlessness, wheeze or severe cough when you exercise in cold weather".

Breathlessness – dust: "Do you usually have breathlessness, wheeze or severe cough in dusty environments".

Breathlessness – strong smell: "Do you usually have breathlessness, wheeze or severe cough from strong smells".

Difficult breathing after use of pain-killer: "Have you ever had any difficulty with your breathing within 3 hours after taking a pain killer".

Chronic bronchitis ever: "Have you ever had chronic bronchitis, COPD or emphysema".

Longstanding cough: "Have you had longstanding cough during the last year".

Sputum production: "Do you usually have phlegm when coughing or do you have phlegm in the chest which is difficult to bring up".

Chronic productive cough: Sputum production for at least 3 months during two subsequent years.

Chronic rhinitis: Yes to "Do you have a blocked nose more or less constantly" and/or "Do you have a runny nose more or less constantly".

Allergic rhinitis: "Have you now, or have you ever had, allergic rhinitis (hay-fever) or allergic eye catarrh".

Chronic rhinosinusitis: Two or more nasal symptoms, one of which should be either *nasal blockage* or *nasal discharge*, being present for more than 12 weeks during the last 12 months. The other two symptoms are *facial pain/pressure* and *reduction or loss of smell.*

3.6.2 Risk factors

Occupational exposure to gas, dust or fumes: "Have you been heavily exposed to gas, dust or fumes at work".

Smokers reported smoking during the year preceding the survey.

Ex-smokers reported having quit smoking at least 12 months preceding the survey.

Ever smoker were either smokers or ex-smokers.

Non-smokers reported neither smoking nor ex-smoking.

Age at onset: response to "How old were you when you developed asthma".

Area of residence was categorized according to population density and location. Metropolitan Gothenburg with more than 500 000 inhabitants and towns/villages in West Gothia according to size: towns with more than 10 000 inhabitants, towns with 2 000 - 10 000 inhabitants, villages with 500 – 2 000 inhabitants and villages and rural areas with less than 500 inhabitants.

*Family history of asthma: "*Do any of your parents or sibling have, or have had, asthma?"

*Family history of allergy: "*Do any of your parents or sibling have, or have had, allergic rhinitis or allergic eye catarrh"

*Rural childhood: "*Did you live in country-side (i.e. not town or suburb) during your first five years of life"

Farm childhood: "Did your family live on a farm during your first five years of life"

3.7 Analyses and statistical methods

3.7.1 Epidemiology

For all epidemiological original papers in this thesis, the author managed the database and conducted all analyses. Quality control of data computerization was done by entering 10% of the data from the postal survey twice; errors amounted to 0.1-0.2%. Data obtained during the structured interview the data was entered directly into the computers. The clinical data has been checked for logical errors. Missing data from the postal questionnaire was handled as follows: For calculations of prevalence of symptoms and diseases a missing answer was considered as a negative answer. For independent variables included in risk analyses, all missing answers was coded as missing and included in the analyses. All statistical analyses were performed using SPSS version 16.0-18.0 (IBM, Somers, NY, USA). In comparisons of prevalence, the two-sided χ^2-test were used, reported p-values are two-sided Fisher's exact test where a p-value less than 0.05 was considered as statistically significant. An unpaired, two-tailed Student's t-test was used to compare means. A Mantel-Haenszel test-for-trend was used when appropriate. Univariate and multivariate risk analyses were performed using logistic regression, with results presented as odds ratios (OR) with 95% confidence intervals. In **paper II**, the attributable fraction (AF), i.e. the proportion of cases caused by a specific factor, was calculated according to the formula ((RR-1)/RR)*p, where RR is the Risk Ratio and p is the proportion of exposed cases [77]. RR was calculated according to the formula (a/(a+b))/(c/(c+d)) where a is the number of subjects with MSA exposed to the factor, b is the number of subjects without asthma exposed to the factor, c is the number of subjects with MSA not exposed to the factor and d is the number of subjects without asthma not exposed to the factor.

3.7.2 Proteomics

To be considered for further analyses, a protein had to have been quantified in at least three samples in each group. As the expression of most proteins followed a normal distribution, the mean ratio of each quantified protein in the sample against the same protein quantified in the pool was calculated. The fold change (FC) was calculated for each of the asthma groups compared to the healthy group. The Student's t-test was used to determine differences in protein expression between the groups. A p-value of <0.05 was considered statistically significant. In IPA, molecules that met a 1.3 FC were considered and a Benjamini-Hochberg multiple testing correction was applied.

4 RESULTS

4.1 Part 1: Questionnaire survey (Paper I)

4.1.1 Participation and demographics

The response rate was higher among women than among men (67% vs. 56%, $p<0.001$) and among subjects living outside the metropolitan area of Gothenburg compared to those living in the city (64% vs. 60% respectively, $p<0.001$). Participation increased significantly by age ($p<0.001$), from 51% among those aged 16-25 years to 77% among the oldest aged 66-75 years.

4.1.2 Prevalence of respiratory symptoms and asthma

The prevalence of asthma varied depending on the definition examined. Most common was *ever asthma,* which was reported by 9.7% of subjects. The prevalence of *physician-diagnosed* asthma was 8.3%. Not everyone who reported *physician-diagnosed asthma* reported *ever asthma*, so when the two were combined, the prevalence was 10.2%. The prevalence of subjects with *physician-diagnosed asthma* currently having symptoms was 6.9%. The prevalence of *ever asthma* was highest in the 26-36 year age group at 10.2% and then decreased with age.

Use of asthma medication was reported by 8.6% of the population. Of those reporting *physician-diagnosed asthma*, 70% reported using asthma medication, which increased to 84% among *physician diagnosed asthmatics* reporting symptoms. Of subjects with *physician-diagnosed asthma* and reporting at least three of the symptoms that were included in the definition of *multi-symptom asthma* (MSA), 88% used medication. This shows a clear relationship between the degree of symptoms and the use of asthma medication.

Respiratory symptoms were common in the study population, with more than one-third of the population reporting at least one respiratory symptom. The most common respiratory symptom was *any wheeze* (16.6%), followed by *sputum production* (13.3%), *longstanding cough* (11.4%) and *attacks of shortness of breath* (9.5%). Forty-six percent of the 5.9% reporting *wheezing with breathlessness apart from cold* had not reported they had *ever asthma* or

physician-diagnosed asthma, a result corresponding to 2.7% of the participating study sample.

Gender aspects

In general, the prevalence of asthma was higher among women than among men: *ever asthma* 10.5% and 8.7% respectively ($p<0.001$) and *physician-diagnosed asthma* 9.1% and 7.4% ($p<0.001$). In addition, most symptoms common in asthma were significantly more prevalent among women, while bronchitic symptoms were equally common in men and women. The use of *asthma medicines* was reported by 6.8% of men and 10.1% of women ($p<0.001$).

Prevalence trends

Compared with studies performed on the island of Hisingen, Gothenburg, in 1990 [34] and a study performed in the county of Södra Älvsborg in 1994 [43], a decrease in the prevalence of most respiratory symptoms was observed, while there were increases in the prevalence of asthma and use of asthma medication. Notably, there was a large decrease in smoking during the period from 32% in 1990 to 18% in 2008, when subjects in the same ages are compared.

4.1.3 Risk factors

Current smoking was a strong risk factor for all investigated symptoms, with ORs ranging from 1.81-3.88. Additionally, *ex-smoking* was a risk factor for respiratory symptoms and for *physician-diagnosed asthma*, but the effect was not as strong. *Allergic rhinitis* and a *family history of asthma* were strong risk factors, especially for *physician-diagnosed asthma* and *attacks of shortness of breath*. Neither *gender* nor *regions of domicile* were strongly associated with any of the respiratory symptoms or with asthma. *Occupational exposure to gas, dust or fumes* was significantly associated with all investigated symptoms and with asthma. The strongest associations were found for *recurrent wheeze* (OR 2.2) and *attacks of shortness of breath* (OR 1.8) (Figure 5).

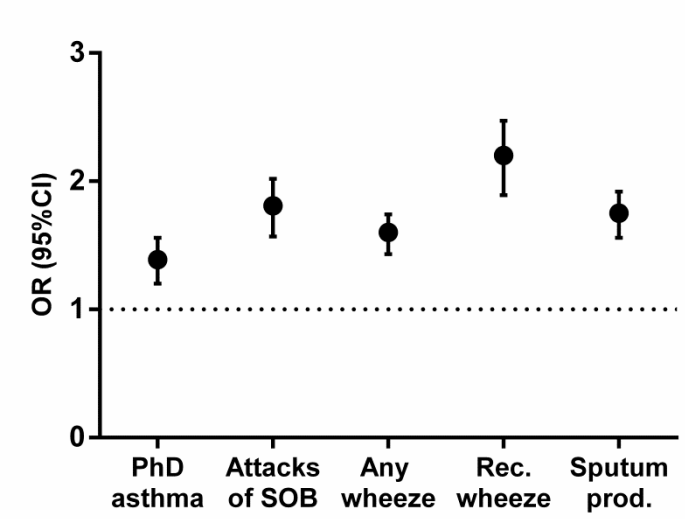

Figure 5. Occupational exposure to gas, dust or fumes as a risk factor for physician-diagnosed asthma and respiratory symptoms. Abbreviations used in the figure: PhD-physician diagnosed, SOB-shortness of breath, Rec. – recurrent, prod. - production

4.2 Part 2: Questionnaire survey and clinical examination (Paper II and III)

4.2.1 Prevalence of multi-symptom asthma

Multi-symptom asthma was detected in 2.0% of the population; comprising 24% of the subjects with *physician-diagnosed asthma* and more common among women. Unlike asthma as a whole, no significant differences were found by age. The prevalence among other asthma for the symptoms that were required to be included in the MSA group was 59% (*asthma medication*), 51% (*attacks of shortness of breath*) and 15% (*recurrent wheeze*). All investigated respiratory symptoms were more common among MSA compared to other asthma except *allergic rhinitis* which was equally common at 65% (Figure 6).

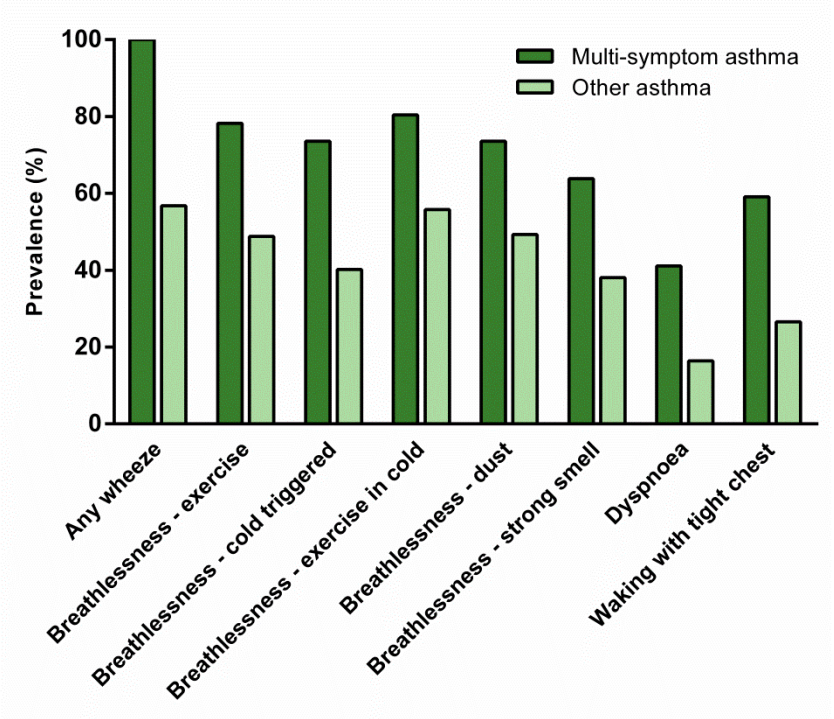

Figure 6. Prevalence of symptoms among subjects with multi-symptom asthma and other asthma.

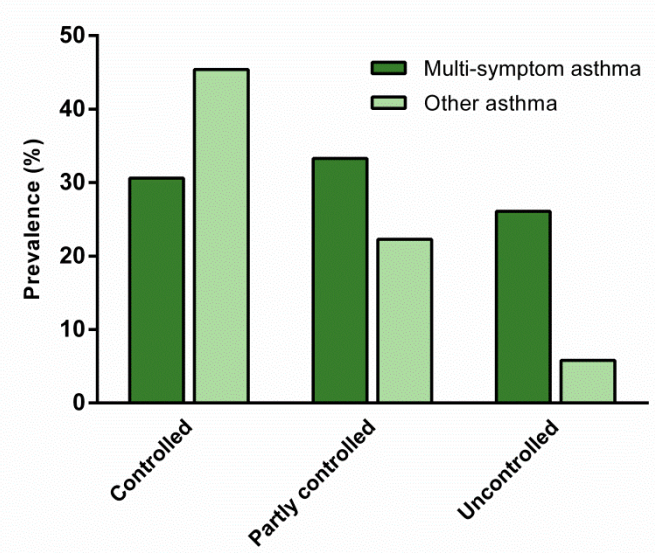

Figure 7. Asthma control according to GINA in multi-symptom asthma and other asthma.

4.2.2 Validating the questionnaire definition using clinical variables

Lung function, expressed as FEV_1% of predicted, was lower among MSA than in other asthma (89% vs. 99%) and MSA displayed more hyper-reactivity to methacholine, with 83% having a PD20 less than 1.96 mg compared to 59% among other asthma. The methacholine challenge had a sensitivity of 82.5 and a specificity of 60%. The airway inflammation, as measured by exhaled NO, was higher in the MSA group, and morbidity variables, particularly emergency unit visits, asthma exacerbations and night-time awakening, were considerably more common among MSA compared to other asthma. According to the GINA 2006 classification, 59.4% of subjects with MSA had uncontrolled or poorly controlled asthma compared with 28.2% among subjects with other asthma (Figure 7).

4.2.3 Differences in use of asthma medication

Of the whole asthmatic population, 65% were using asthma medication at the time of clinical examinations. The prevalence of medication use for asthma was 92% among MSA and 58% among other asthma. Almost twice as many subjects with MSA used ICS compared with other asthma (71% vs. 40%, $p<0.001$). About half of the subjects using ICS also used LABA, with a majority using a combination inhaler. Among subjects not using steroids, the prevalence of SABA use was 70% in the MSA group and 28% in the OA group ($p<0.001$). With increasing age, all groups reported an increased prevalence in the use of ICS and combination treatment. In general, the prevalence of medication use was higher among women.

One-third of the subjects with asthma used ICS daily, or on most days. More than half of the subjects with MSA used ICS most days, but only one-fourth of the subjects with other asthma used ICS to the same extent. Thirty seven percent of subjects with multi-symptom asthma used a combination treatment most days, compared with 15% of the subjects with other asthma. Oral steroids and bronchodilators through a nebulizer were predominantly used periodically or occasionally.

Most asthmatic subjects were using a medium dose of ICS, with only 3% of asthmatics using a high dose of more than 1000 µg BPD, or equipotent dose of other ICS, daily. Using a high dose of ICS was more common in the multi-symptom asthma group (8%), compared to the other asthma group, where only 2% used a high dose.

4.2.4 Risk factors

Having a *family history of both asthma and allergy* was the strongest risk factor for MSA compared with non-asthma, with an OR of 7.3, while the OR for having a *family history of either asthma or allergy* were approximately 2.7. Other significant risk factors included *occupational exposure to gas, dust or fumes* (OR 2.0), *female gender* (OR 1.6), age 61-75 (OR 1.5) and *current smoking* (OR 1.3).

When other asthma was used as a reference, older age became a stronger risk factor, while having a *family history of asthma or allergy* had decreased importance. Older age was a "protective" factor for other asthma, in contrast to MSA, where older age was a risk factor.

The attributable fraction of several independent factors on MSA was calculated. Having a family history of allergy explained 37%, a family history of asthma explained 33% and occupational exposure to gas, dust or fumes explained 17% of the MSA in the study population. In a stratified calculation, occupational exposure to gas, dust or fumes was found to be a much stronger attributable factor for MSA among men (AF 35%) than for women (AF 13%).

Mainly as a result of the definition of MSA, having MSA was a considerably stronger risk factor for asthma medication use than having other asthma in adjusted logistic regression models. There were few other significant risk factors for medication use, with increasing age and living in a town of 2 000-10 000 inhabitants reaching significance.

4.3 Part 3: Proteomics of asthma phenotypes

4.3.1 Demographics

There was more airway inflammation, measured as FeNO, in the CRS-MSA group compared to AIA, MSA and healthy. No differences in FEV_1/FVC ratio, blood neutrophils, blood eosinophils, or in differential cell count of cytospins of the NLF, between the groups were found. There were slightly more women in the group with AIA.

4.3.2 Protein analysis

In total, 790 proteins were identified, with 721 also quantified. Of the 721 proteins, 474 proteins had been quantified in at least three subjects per group and were included in the functional analysis. In IPA, a FC of 1.3 was applied as a cut-off and 193 proteins had a FC of more than 1.3 compared to healthy. Of these 193 proteins, 50 were unique to AIA, 39 to CRS and 31 to MSA. The remaining 73 proteins were common to two or all asthma groups.

GO Term Finder identified the 73 proteins that were common between groups to be enriched in immune defense processes such as defense response, response to stress, complement activation, innate immune response and response to stimulus. Decreased expression of specific proteins suggests impaired protective defenses, increased defense response and response to stress.

In proteins uniquely altered in AIA, the enriched biological processes included regulation of catalytic activity, specifically endopeptidase activity, while proteins unique to CRS were enriched in response to stress and epithelial cell differentiation. Proteins unique to MSA were enriched in biological processes of negative regulation of molecular function, immune response and defense response.

The 73 proteins common to all three groups of asthma had a more even distribution (percent hits against total number of proteins in the dataset) over the PANTHER protein classes, than the proteins that were unique to the different asthma groups. In general, all groups had protein classes involved in active metabolic and immune system processes, response to stimulus and few protein classes involved in apoptosis. The unique proteins of subjects with CRS-MSA had an overrepresentation of processes involved in structural changes, in contrast with AIA and MSA,

which had very little structural activity. The molecular function of transporter activity was common in AIA, but was rare in MSA and CRS-MSA.

IPA identified the biological category of "Respiratory Disease" to be associated with all groups. The top categories in all groups were "Dermatological Conditions and Diseases", "Immunological Disease" and "Inflammatory Disease. "Asthma" was among the top five associated biological functions for MSA. "Hypersensitive Reaction" was strongly associated to AIA and MSA. For all three groups, the main associated biological functions were involved with skin diseases such as dermatitis, psoriasis and atopic dermatitis. IPA identified several significantly associated networks for each of the asthma phenotypes. Proteins previously found to be involved in asthma, CRS and the COX pathway were present in the top networks.

5 DISCUSSION

5.1 Discussion of methodology

5.1.1 Validity

The validity of a study is its ability to measure what it is suppose to measure. The internal validity is how well results from the study population represent the population the sample was drawn from. The external validity is how well results from a study can be applied to other populations.

The representativeness of the study population is crucial for the validity of a study. This study was a cross-sectional study performed on a representative sample of the population in Västra Götaland, selected in a stratified manner. Different response rates in the different age groups and between genders might influence the results; therefore a non-response study was performed to investigate the internal validity of the questionnaire survey. This non-response study indicated no differences in regards to the prevalence of respiratory symptoms between responders and non-responders. However, the non-responders were more often male, young, smokers and living in Gothenburg. This implies that the prevalences reported here can be extrapolated to the population of Västra Götaland. However, the importance of young age and smoking as risk factors might have been underestimated, although the study gave no such implications [203]. Some caution must be taken when the results from the non-response study are analyzed as it was performed among a randomly selected sample were 53% participated.

Participation in the clinical survey was 59% in the random sample and 55 % among asthmatics. All of these subjects are not included in the papers included in this thesis and it is difficult to evaluate the representativeness of the subjects. The participants in the clinical examinations are more likely to be females and of older age, much like the responders to the postal questionnaire. It is also clear that asthmatics are slightly overrepresented among the participants in the clinical survey, as 9.6% were identified as asthmatics in the postal questionnaire, but made up 11.3% of the participants from the random sample in the clinical examinations. A comparison of responses to the postal questionnaire for participants and non-participants in the clinical survey has not been performed yet. However, the data from the clinical follow-up has not been used to estimate prevalence in the entire population, but only within a group and hence the somewhat skewed participation should not play a major influence on the results.

The large sample size and the use of well validated questionnaires contribute to the high internal validity of the results. The questionnaires used in the study have been used in various studies, and the questions on physician diagnosed asthma and respiratory symptoms have been evaluated [107, 207]. The use of the two questionnaires also allows for more precise comparisons with other studies, as questions from the OLIN-questionnaire can be used when comparisons are made with studies in Northern Europe that have used the OLIN-questionnaire, and the GA^{2}LEN-questionnaire can be used in comparisons to other centers within the GA^{2}LEN network and ECRHS. The estimates of prevalence reported here are similar to recent reports from other parts of Sweden and Northern Europe [33, 39, 199] suggesting a high external validity of the questionnaires used in this study.

5.1.2 Bias

Bias is a systematical error that might occur in a dataset, there are many different forms of bias, some of which will be discussed here.

Selection bias

Once a study question has been formulated and study subjects identified, it is important that these subjects be recruited uniformly and that data about the health and exposure be collected consistently. If certain subjects are not enrolled in the study, or if information is collected differently for different subjects, the resulting bias could invalidate the study. Selection bias can occur when not everyone eligible to be in a study can be selected as a subject, and when those selected are different from those excluded, in a systematic way. To avoid selection bias, comparison populations must be selected that are similar, except for the specific factors under study, and that is often difficult to achieve. This study was a random population study but certain parts of the population may be hard to reach, or choose not to answer. As the non-response study shows, it is unlikely that this has impacted on the outcomes in a major way.

Recall bias

Sometimes the subjects' ability to recall and report past experiences may be affected by their preconceived ideas about a possible health hazard, such as exposures in the work environment. This is hard to control for in cross-sectional studies, but by asking for symptoms and exposures during the last year recall bias can be reduced. The variables used in the definition of MSA have slightly different time spans as part of their definitions, this might cause symptoms being anterior to asthma medication, which might influence recall.

5.1.3 Confounding

Confounding is when an association is found for the incorrect reason and is a common and important factor to take into consideration. It is associated with both the risk factor and the disease being studied, but does not need to be a risk factor for the disease under study. The confounding variable can either inflate or deflate the true association. To investigate the true association one has to investigate all factors thought to cause confounding at the same time. Several variables may be confounders in any study, the effects may be small or large but failure to account of the most important confounders may lead to the validity being questioned. It is not possible to account for all potential confounders as there are so many of them.

5.1.4 Misclassification

Misclassification is a technical term for mislabeling or mischaracterizing a study subject, and may occur with diseases or exposures. For example, older subjects, particularly those who smoke, might prefer to have asthma to having COPD. Subjects who only smoke occasionally, or a couple of times a year might not regard themselves as smokers. When we defined MSA, we did not intend to define severe asthma, but to find an epidemiological marker of severe disease. Our definition is not able to distinguish between, for instance, subjects with a persistent severe asthma and subjects with brittle asthma and lack of adherence to treatment. It may be that subjects identified using the current method could be more sensitive to symptoms, rather than having a more severe disease. It might even be argued that some subjects with MSA may have some features of COPD, however, this is contradicted by the mean age of onset of asthma at 25 years and the presence of only nine smokers above the age of 50 having a FEV_1/FVC ratio <0.7. There might also be a misclassification in regards to medication use. The main weakness in this regard, is the lack of objective measurements of medication use, such as blood levels of pharmaceutical metabolites. In the proteomics study, two subjects included in the AIA groups had CRS, so these subjects were excluded to reduce complexity and keep the groups as pure as possible. A limitation in the inclusion of subjects in the proteomics study was the absence of questions in the structured interview regarding CRS, and as a consequence, the inclusion was based on responses to the questionnaire survey.

5.1.5 Statistical Variation

Statistical variation is really chance fluctuations and the risk of the results being influenced by statistical variation decreases with increasing sample size. The statistical variation is an important factor in power calculations. Epidemiological studies are made on samples of a larger population, and because of statistical variation, the results will be at least a little different than if the entire population was tested. There are several ways statisticians estimate the effect of statistical variation and hence the uncertainty of the findings: 95% confidence intervals and p-values being two of the most common. In the proteomics study, the individual variation at protein level is known to be high, and the method itself causes further variation. However, multiplexing using the TMT-technique reduces experimental variance and to reduce the impact of the individual variation, a minimum of three values per group were required with a FC of at least 1.3. To have a power of 0.8, with a variation of 40% and a 1.5 FC would require eight samples per group.

5.1.6 Determinants of disease

Data of exposures and other determinants of disease were collected using the postal questionnaires and no objective measures were used. Missing answers for questions on exposures were treated as missing, while missing answers were treated as "no" for symptoms and diseases, in line with the standards of interpretation of data after validation of the questionnaire [197]. In this way, no over estimation of prevalences or risk is done. The cross-sectional design has the weakness of not being able to conclude anything about causality for possible determinants which makes discussion of cause or consequence, as well as detected associations, more difficult.

5.1.7 Definitions

We have used different definitions of asthma to enable comparisons with different studies. The definitions for papers I-III was based on questionnaire data while clinical data was used in paper II and III. For paper IV, the definition of asthma was based on the structured interview and on clinical data.

Defining multi-symptom asthma

In the late 1980s and early 1990s it was found that recurrent wheeze and attacks of shortness of breath, two of the core symptoms of MSA, correlated with impaired lung function [208] and hyper-reactivity [209], particularly in asthmatics. This was verified in an Estonian study in 1996 [210]. In 2007-2008 a large population based interview study of subjects with physician-

diagnosed asthma in Stockholm, Sweden (unpublished data) identified certain morbidity variables as considerably more common in those reporting the above symptoms. The triggers exercise and cold were selected, because they are less biased compared with symptoms caused by irritants such as tobacco smoke and dust, which also strongly correlate with sensoric hypersensitivity without bronchial obstruction. Further, the large European Network For Understanding Mechanisms of Severe Asthma study (EMFUMOSA) has shown that triggers connected to allergy, i.e. pollen and furry animals, are inappropriate to use in identifying severe asthma.

5.1.8 Choice of proteomics method

Isoelectric focusing (IEF) and gel electrophoresis (GE), while not used in this thesis, are important tools in proteomic research and are briefly described here. IEF relies on the fact that the net charge of a protein varies with the surrounding pH proteins are separated according to their isoelectric point (pI). In two dimension gel electrophoresis (2-DGE), proteins are separated by both pI and mass enhance separation and then detected. At this stage, the identity of the proteins are unknown additional analysis, for example using MS, are required. In theory, 2-DGE can reveal virtually all proteins in a cell or tissue at any given time. 2-DGE has the advantage of identifying all isoforms of a protein, a feature that is hard to achieve in MS.

As, 2-DGE MS also has advantages. MS allows for a large proportion of the proteins in a sample to be identified and quantified. MS however has the limitation that certain proteins are more difficult to ionize, resulting in failure to identify to protein. The reason for this feature still remains to be elucidated. When quantitative studies are performed some peptides are more likely to be labeled, which might cause some bias. However, the TMT-technique is designed to minimize this problem.

In ESI, the solvent is evaporated, reducing the size of the droplets until the droplet disintegrates and solvent-free gas-phase ions are produced. When the amount of sample is limited a low-flow electrospray, nano-ESI [211], is used. In nano-ESI the spray needle is very thin and positioned close to the mass analyzer. These adjustments result in very small droplets and consequently a reduction in the amount of sample needed. ESI allows for direct coupling with separation techniques such as nano LC. The Orbitrap have a high resolution, a high mass accuracy in the low ppm range, a high mass-to-charge range and a dynamic range of more than three orders of magnitude [212,

213]. When the Orbitrap is combined with a LTQ, an instrument is created that benefit both from the high resolution and mass accuracy of the Orbitrap and the speed and the sensitivity of the LTQ. The LTQ Orbitrap Velos MS have a rapid quantitation of low level isobaric labeled peptides and is fast which is important when complex samples are being analyzed [214]. Connecting a nano LC to the mass spectrometer increases the resolution up to 100 times. The separation and concentration of components results in higher sensitivity and efficiency and at the same time salts and other impurities are removed.

Which database that is used for protein identification depends on data source and in which species the search is performed. In addition to the database several factors will affect the search results. In this thesis MASCOT, a software search engine, was used for MS/MS spectra searches where experimental MS/MS spectra were compared against theoretical spectra in the UniProt Swiss-Prot database. Peptides were identified and fit into proteins sequences for protein identification. To reduce false positives, the results are also search against a decoy database where the proteins sequences are reversed or random, a false discovery rate (FDR) of <1% is usually required. Proteome Discoverer (Thermo Fisher Scientific Inc., Waltham; MA, USA) is a for database searches to identify proteins which can incorporate several database search algorithms and validate proteins with FDR determination. The 1.3 FC threshold was chosen based on the variability of human samples, which often ranges up to 20%. As most proteins, but not all, were found to be normally distributed, Student's t-tests was used identify the significantly different proteins. No correction for multiple comparisons was used, as there is currently no consensus on how to perform these corrections in proteomic samples. It is important to use as many individuals as possible in clinical proteomics as the variation is high and peptides are not always quantified which reduces the power.

MS studies without quantification usually results in a higher number of identified proteins, compared with studies where quantification is performed, due to the lower complexity of the sample. In our study, the aim was not to describe the protein content of different asthmatic groups, but to compare them with regards to expression levels and hence a quantitative approach was chosen. There are several available quantification techniques. In a pilot-study performed in our lab, iTRAQ and TMT labeling techniques were compared for NLF. iTRAQ resulted in more quantified proteins but only allows for three samples and one pool per set while TMT allows for five samples and one pool per set. TMT was chosen as the reduced variability with a reduced

number of sets was considered as more important than having more quantified proteins.

A problem with using a database such as the Ingenuity® Knowledge Base is that the results are influenced by what has been included in the database. The Ingenuity® Knowledge Base, despite being very comprehensive and manually reviewed, has an overrepresentation of research on cancer and cancerous cell lines, and a protein, although involved in many diseases, may have been associated with many types of cancer, resulting in a bias.

5.1.9 Choice of sample for asthma proteomics

Studies of respiratory proteomics have identified several biomarkers but there has been difficulties verifying the results. This could partly be due to patient selection where the syndrome of asthma, rather than specific phenotypes, has been studied. Accessing disease relevant samples is a problem when proteomic studies of respiratory diseases are being performed, therefore cell cultures and animal models have frequently used [215]. Studies on human samples have been performed in sputum [216], BLF [183, 186, 217], biopsies [218] and exhaled breath condensate [219]. While BLF and bronchoalveolar biopsies are taken from the site of disease they are very invasive. Serum is easily accessible but is not from site of disease and there are issues with abundant serum proteins obscuring expression of less common proteins. Sputum is usually easy to induce in symptomatic symptoms but this can be difficult in healthy control subjects. It is also unknown how the instilled hypertonic saline affects the sample. NLF suffers from problems with dilution, as does BLF, but is non-invasive and can be obtained from most subjects. While it is not site of disease the upper airways may reflect the milieu in the lower airways in line with the united airway concept [220]. Studies previously performed on NLF have commonly used 2-DE, followed by protein identification of selected spots by MALDI-TOF [183, 187, 221]. As these studies only identify selected protein spots, and not all visible on the 2-DE gel, these studies have identified far less proteins than the current study; however Lindhal *et al* detected approximately 1000 proteins in the 2DE gel using image analysis [187]. Benson *et al* identified 197 proteins using LC-MS/MS on extensively fractionated pools of NLF containing up to 2 mg of protein, far less than in the current study [222]. Wang *et al* identified 451 proteins using LC-MS/MS, comparable with the current s [171]. Differences might be due to differences in the method for collecting NLF, different search criteria or different versions of the database being searched.

5.2 Discussion of main results

5.2.1 Paper I - III

Prevalence of asthma and respiratory symptoms

The prevalence of physician-diagnosed asthma in this study was estimated to be 8.3%, similar to what has been found in other parts of Sweden [93, 199, 210, 223]. Compared with the results from the ECRHS study in Gothenburg in 1990 [34] and the Södra Älvsborg study preformed in 1994 [43], a very small increase in asthma prevalence could be observed. It is however unclear if the increase is true or a result of changed diagnostic criteria. The small increase is in line with studies of asthma incidence that have shown a stable incidence of about 2/1000/year since the mid 1980s (Figure 8). Increasing asthma prevalence in older age groups has been reported previously, however, this was not observed in the current study. Use of asthma medication was reported by 8.6%, a prevalence that is in accordance with the ECRHS study [141, 224] and with recent studies from the Nordic countries [33, 142, 143, 201, 225]. Asthmatics reported less respiratory symptoms and only 6.9% had active asthma. Of these subjects, only half reported both use of asthma medication and one or two symptoms. The finding suggests that a greater proportion of mildly symptomatic asthmatics are being diagnosed as having asthma, as those who were diagnosed with asthma in the 1980s and 1990s had more symptoms of asthma than found in the current study and other recent studies (Figure 9) [90, 198].

A clear decrease in respiratory symptoms compared with the 1990s was observed. This was true for both genders and all age groups. Symptoms that may be related to smoking, such as any wheeze, sputum production and longstanding cough, as well as symptoms closely related to asthma, such as wheezing with breathlessness, had decreased. The observed increase of allergic rhinitis might reflect an increase in allergic sensitization in the area, possibly leading to a future increase of allergic asthma. The reduction in symptoms related to smoking is not surprising, as the prevalence of smoking had reduced by 44% during the time between the studies.

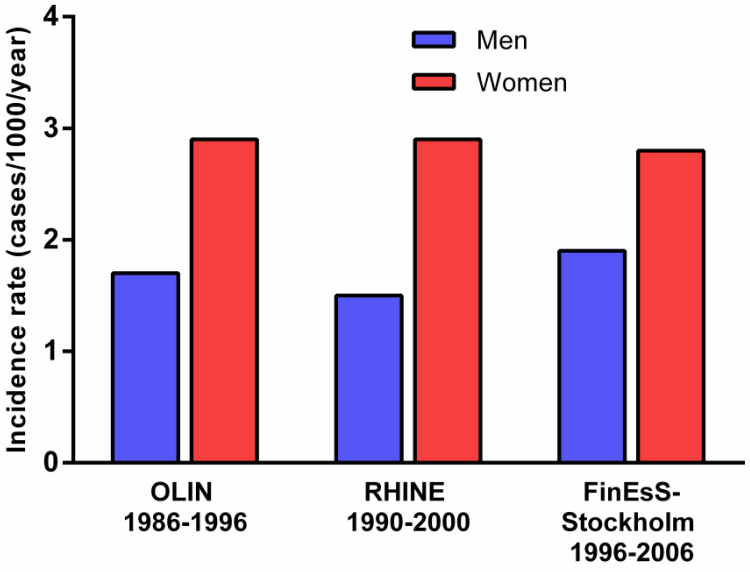

Figure 8. Trend in asthma incidence. Reprinted from Ekerljung et al, Resp Med 2008, 102:1730 with permission from Elsevier.

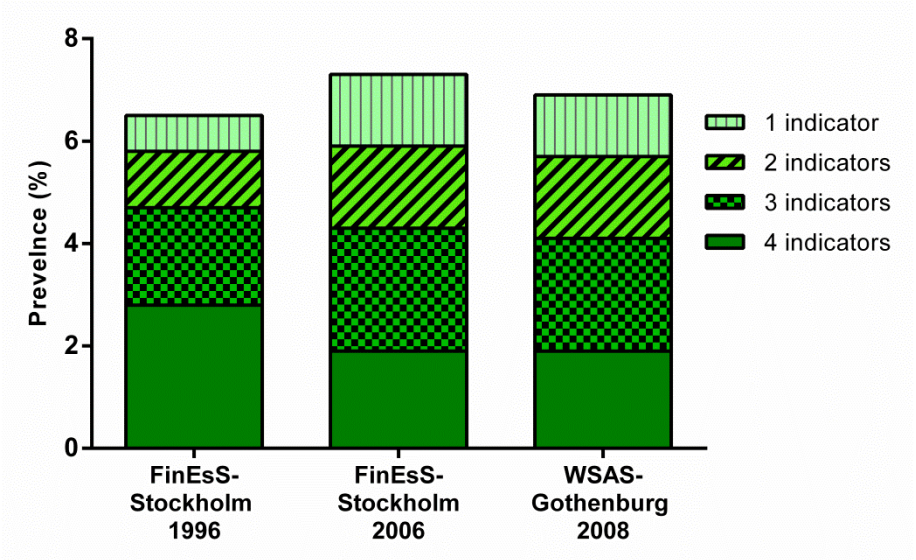

Figure 9. Prevalence of active asthma in Stockholm and Gothenburg with numbers of indicators (asthma symptoms or use of asthma medication). Modified from Ekerljung et al Int J Tuberc Lung Dis 14(6):764–771 and Lötvall et al Respir Res. 2009 12;10:94.

Multi-symptom asthma as a marker of severe disease

MSA was present in 2% of population and in one of four asthmatics. Subjects who report multiple symptoms, despite use of asthma medication, were more likely to suffer from more severe asthma as they have significantly lower lung function, express signs of increased airway inflammation, are more hyper-responsive, have more night-time awakenings and report more symptoms due to environmental triggers, compared to with those with less symptoms of asthma. Almost all subjects with MSA still used asthma medication one to four years after the postal survey, indicating stability in the definition. Despite the use of asthma medication, a large majority of the multi-symptom group also had signs of uncontrolled or poorly controlled asthma as defined by GINA 2006 [127]. The current study reflects a population with signs of more severe disease with a similar gender distribution to what was reported in the population based ECHRS study [226] and to the patient based EMFUMOSA study [118, 148], with women being more frequently affected. The definition is not sufficient to encompass all aspects of severe asthma, but many similarities are present.

Medication use

Of those having asthma according to the postal survey, 8.6% used asthma medicines regularly or as needed [142]. The random sample in the clinical cohort is not completely representative of the responders to the postal questionnaire. In the random sample, 11.4% reported physician-diagnosed asthma compared with 8.3% in the postal questionnaire. The prevalence of use of asthma medication in the interview was 9.8% when adjusted for the higher proportion of asthmatics.

One to four years after the postal survey, almost all asthmatics with multiple symptoms still reported use of asthma medication, which was one of the inclusion criteria of MSA in 2008, compared with 63% among asthmatics with fewer symptoms. Subjects with MSA used their medication more frequently and in higher doses than those classified as having other asthma. Further, maintenance treatment was considerably more common among those with MSA. In our study, 70% of MSA and 40% of other asthma used steroids, comparable with a study by Liou *et al* [154] and higher than a study by Cerveri *et al* [227]. Suboptimal dosing has been found to be similar between severe and mild asthma [154].

Our estimation that up to 30% of MSA can be due to low adherence or under-treatment, is comparable to a study by Gamble et al [155] where 35% of asthmatics had non-adherence as the main cause of difficult-to-treat asthma.

Risk factors

As the study design was cross-sectional, the results only verify an association and conclusions on cause or consequence are difficult to make. However, risk factors that are stabile over long time periods, such as smoking, family history of asthma and, not least, gender, can often mean causality. Female sex and ex-smoking were closely associated with asthma, with risk factor patterns that were similar to previous studies, [90, 198, 199, 223]. The reduced importance of a family history of asthma on the risk of asthma could possibly be explained by the inclusion of patients with milder disease in this category than previously [90, 198]. This is supported by family history of asthma and allergy being the strongest risk factors for MSA. In our study, the prevalence of asthma was highest in the age group of 26-35 years, while it was lower in the age group of 16-25 years, arguing against a further increase in prevalence of asthma in the lower age group. In studies performed in the 1990s, asthma was most common in adolescents and young adults [198]. Respiratory symptoms were less age-dependent when compared to previous Swedish studies [198, 228, 229]. However, chronic respiratory symptoms increased with increasing age. Particularly symptom of bronchitis have been strongly age and smoking dependant, this trend cannot be seen in the current study, probably due to the considerable decrease in smoking and a decrease in outdoor air pollution. The lack of difference between the metropolitan area of Gothenburg and the non-metropolitan area may also reflect an improvement in the outdoor air pollution in the metropolitan areas in Sweden [230]

Smoking and occupational exposure to gas, dust or fumes had a greater impact on MSA compared with other asthma. Occupational exposures and air pollutants are known risk factors for asthma [77] and with similar exposures at work, severe asthmatics more frequently reported that work affected their breathing [231, 232]. When investigating subjects with multiple risk factors, we found that the hereditary factors are the most prominent factors related to increased risk of MSA. In the absence of a hereditary factor, an increased risk at the population level was observed only if both smoking and occupational exposure of gas dust or fumes were present. If a hereditary factor was present, the concurrent presence of smoking and/or occupational exposure to gas, dust or fumes resulted in a ten-fold increased risk of having MSA compared with subjects who have none of the risk factors.

5.2.2 Paper IV

The current proteomics study is considered a screening, hypothesis generating, survey for future studies. Interesting results must be verified in a larger patient sample using complementary methods like ELISA or Western blot. It is difficult to draw finite conclusions in regards to potential biomarkers but it is clear that a quantitative MS/MS approach on NLF can be used to identify differences between the phenotypes in regards to mechanistic differences, with different biological functions and pathways associated with the phenotypes. Proteins that were common between the phenotypes (compared to healthy) include molecules involved in defense response, response to stress, innate immune response and coagulation related processes. The samples in the CRS-MSA group were more different than the AIA and MRS group. This could possibly be due to the site of sampling which, in the case of CRS, is also the site of disease. In hindsight it would have been useful to have a group with CRS but without asthma to identify features unique to CRS. The absence of AIA relevant proteins previously identified could be partly explained by those studies using samples obtained during aspirin provocation. There are also proteins that are significantly different between a phenotype and the healthy individuals. The question we have to ask is whether the differences identified between the phenotypes are relevant; this needs to be studied in further experiments.

The advantage of using NLF is mainly easy access and relatively non-invasive sampling procedure. However, the site of sampling is distant from the main site of bronchial inflammation, which poses a limitation in detecting lower airway disease relevant proteome information. However, both proteins common in asthma, and proteins previously associated to the investigated phenotypes are present in the samples [174, 184, 186, 233, 234]. Additionally, the 2-DE profiles of samples from NLF and BLF have been found to be similar [183]. When our data is compared to data from other NLF proteomic studies [171, 222, 235, 236], 295 proteins had not previously identified. The high number of quantified proteins suggests that the method used in this study was quite thorough. The combination of the fractionation, with the sensitivity of the LTQ-Orbitrap Velos instrument, resulted in a high yield of proteins.

6 CONCLUSION

1. The prevalence of asthma was 8.3%.

2. The previous increase prevalence of asthma has leveled off in West Sweden.

3. The prevalence of most respiratory symptoms decreased.

4. The prevalence of multi-symptom asthma was 2% in the general population and 24% among asthmatics.

5. The definition of multi-symptom asthma does not define severe asthma, however can be used as an epidemiological marker of more severe disease.

6. The prevalence of asthma medication use was 8.6%, of subject with a physician-diagnosed asthma, 70% used asthma medication.

7. Subjects with multi-symptom asthma used more asthma medication and in higher doses compared with subjects with other asthma.

8. Multi-symptom asthma is only partly due to non-use of medication.

9. Differences in functions, pathways and protein expression of phenotypes of asthma compared to healthy can be detected using MS/MS in NLF.

10. Proteins detected in NLF reflect, at least in part, the milieu in the lower airways.

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APPENDIX

Appendix I – Postal questionnaire in Swedish.

Appendix II – Interview questionnaire in Swedish.

Appendix III – Questionnaire on use of asthma medication in Swedish.

Frågeformulär

Astma, allergi och KOL i Västra Götaland

Vi är tacksamma om Du besvarar alla frågor i bägge frågeformulären, trots att de i vissa fall kan verka lika.

GÖTEBORGS UNIVERSITET

FRÅGEFORMULÄR
Astma, allergi och KOL i Västra Götaland 2008

Besvara frågorna genom att kryssa i lämplig ruta.
Om Du är osäker om svaret, välj ”Nej/vet ej”

		NEJ/ VET EJ	JA
1.	Är det någon av Dina föräldrar eller syskon som har eller har haft		
	a) astma	☐	☐
	b) allergiska ögon- eller näsbesvär (hösnuva)	☐	☐
	c) kronisk luftrörskatarr (bronkit), KOL eller emfysem	☐	☐
2.	Har Du nu eller har Du haft någon av följande sjukdomar:		
	a) astma	☐	☐
	b) allergiska ögon- eller näsbesvär (hösnuva)	☐	☐
	c) kronisk luftrörskatarr (bronkit), KOL eller emfysem	☐	☐
	d) annan lung- eller luftvägssjukdom	☐	☐
	Om “ja”: Vilken eller vilka? ……..…….……		
3.	Har Du av läkare fått diagnosen astma?	☐	☐
	Om ”ja”: Hur gammal var Du när Du fick astma? ……år		
4.	Har Du av läkare fått diagnosen kronisk luftrörskatarr (bronkit), KOL eller emfysem?	☐	☐
5.	Använder Du astmamediciner (ständigt eller vid behov)?	☐	☐
6.	Har Du nu eller har Du under de senaste 10 åren haft astmabesvär? (Dvs. periodvisa eller anfallsvisa andningsbesvär/andfåddhet, besvären kan uppträda med eller utan hosta och med eller utan pip i bröstet)	☐	☐
	Om ”ja”: Har Du haft sådana besvär under det senaste året (de senaste 12 månaderna)?	☐	☐
7.	Har Du haft långvarig hosta under det senaste året?	☐	☐
8.	Brukar Du hosta upp slem eller har Du slem i bröstet som Du har svårt att få upp?	☐	☐
	Om ”ja”: a) Har Du fått upp slem de flesta dagar under perioder som varat minst 3 månader?	☐	☐
	b) Har Du haft sådana perioder minst 2 år i rad?	☐	☐
9.	Brukar Du ha pip, skrål eller väser det i bröstet då Du andas?	☐	☐

	NEJ/ VET EJ	JA
10. Har Du haft pip eller väsningar i bröstet vid **något tillfälle** under de senaste 12 månaderna?	☐	☐
Om ”ja”:	☐	☐
a) Har Du överhuvudtaget varit **det minsta andfådd** när Du haft pip eller väsningar i bröstet?		
b) Har Du haft detta pip eller väsande i bröstet när Du **inte** samtidigt varit förkyld?	☐	☐
11. Har Du vaknat med trånghetskänsla i bröstet vid något tillfälle under de senaste 12 månaderna?	☐	☐
12. Måste Du gå långsammare än jämnåriga på slät mark på grund av andfåddhet?	☐	☐
13. Brukar Du bli andfådd, få pip i bröstet eller hostattacker:		
a) vid ansträngning	☐	☐
b) i kyla	☐	☐
c) vid ansträngning utomhus i kallt väder	☐	☐
d) i dammiga miljöer	☐	☐
e) av cigarett- eller tobaksrök	☐	☐
f) av bilavgaser	☐	☐
g) av starka dofter (parfym, kryddoft, trycksvärta, rengöringsmedel, blommor etc)	☐	☐
h) av pollen från växter som gräs och/eller träd	☐	☐
i) vid kontakt med pälsdjur (katt, hund, häst eller andra pälsdjur)	☐	☐
14. Har Du någon gång reagerat med andningssvårigheter inom 3 timmar efter att ha tagit en värktablett?	☐	☐
Om ”ja”:		
Kommer Du ihåg namnet på medicinen? …….……		
15. Har Du nästäppa mer eller mindre ständigt?	☐	☐
Oavsett ”ja” eller ”nej”:		
Har Du snuva mer eller mindre ständigt?	☐	☐
16. Är Du rökare? (Som rökare räknas även de som röker enstaka cigaretter eller pipstopp per vecka och de som slutat röka under det senaste 12 månaderna.)	☐	☐
Om ”ja”:		
Hur många cigaretter röker Du per dag?		
Mindre än 5 ☐ 5 – 14 ☐ 15 – 24 ☐ 25 eller mer ☐		
Om ”nej”:		
Har Du tidigare varit rökare och slutat röka för mer än ett år sedan?	☐	☐
Om Du är eller har varit rökare:		
Hur gammal var Du när Du började röka? ……år		

	NEJ/ VET EJ	JA
17. Är Du yrkesverksam**?**	☐	☐
Om ”ja”:		
Arbetar Du heltid?	☐	☐

18. Vilket har varit Ditt **huvudsakliga** yrke eller sysselsättning?

…….....…….....

Hur många år sammanlagt har Du arbetat i detta yrke? .....…år

19. Har Du **nu** något annat yrke eller sysselsättning (är studerande, arbetssökande, hemmafru, förtidspensionerad, har ålderspension osv.)? ☐ ☐

a) Vilket? ……..……..……

b) Sedan hur många år? ……..år

20. När arbetet blir *kroppsligt besvärande*, har Du då möjlighet att dra ner på takten eller arbeta annorlunda, så att besvären minskar?

☐ Ja, ofta ☐ Ja, ibland ☐ Nej, sällan ☐ Nej, aldrig/nästan aldrig

21. När arbetet *blir psykiskt påfrestande*, har Du då möjlighet att påverka det Du gör, så att påfrestningen minskar?

☐ Ja, ofta ☐ Ja, ibland ☐ Nej, sällan ☐ Nej, aldrig/nästan aldrig

22. Känner Du Dig utvilad och återhämtad när Du börjar arbetet?

☐ Ja, ofta ☐ Ja, ibland ☐ Nej, sällan ☐ Nej, aldrig/nästan aldrig

23. Hur stor är Din arbetsförmåga i Ditt nuvarande arbete (förutsatt heltidsarbete och uttryckt i procent)

……%

Frågan syftar att ta reda på Din totala arbetsförmåga oavsett Din arbetstid.
Om Du arbetar 30 timmar i veckan men skulle orka arbeta 40 timmar, ange då 100% som svar.
Om Du arbetar 40 timmar i veckan, men orkar egentligen bara 30 timmar i veckan, ange då 75% som svar.

24. Har Du varit sjukskriven vid något eller några tillfällen under de senaste 12 månaderna? ☐ ☐

Om Du är sjukskriven just nu, räkna inte med den nuvarande sjukskrivningsperioden.

Om ”ja”:

☐ 1 – 7 dagar ☐ 8 – 30 dagar ☐ 2 – 3 månader ☐ 4 – 12 månader

	NEJ/ VET EJ	JA
25. Har Du varit sjukskriven p g a andningsbesvär under de senaste 12 månaderna?	☐	☐
26. Har Du någon gång ändrat arbetsuppgifter p g a astma eller andra andningsbesvär?	☐	☐

Om "ja":
Vilket år? ……..
Vilket yrke hade Du då?…….

27. Har Du någon gång ändrat arbetsuppgifter p g a andra hälsoskäl?	☐	☐

Om "ja":
Vilket år? ……..…
Vilket yrke hade Du då?……

28. Hur störd är Du när Du befinner Dig **hemma** av luftföroreningar utomhus (från trafik, industrier etc) om **Du har Dina fönster öppna**? *(Om Du inte alls känner Dig störd välj 0, om Du känner Dig oerhört störd välj 10 och om Du känner Dig någonstans däremellan välj en siffra mellan 0 och 10)*

0 1 2 3 4 5 6 7 8 9 10

Inte alls störd — Outhärdlig störning

29. Har Du under de senaste 10 åren någon gång haft en vattenskada i Din bostad?	☐	☐

Om "ja":
Vilket år? ……..

30. Har Du under de senaste 10 åren någon gång haft en synlig mögelskada i Din bostad?	☐	☐

Om "ja":
Vilket år? ……..

31. Har Du varit mycket utsatt för damm, gaser eller rök i arbetet?	☐	☐

32. Hur många gånger per vecka (i genomsnitt) äter Du fisk? ……..……

33. Hur många gånger per vecka tränar eller sportar Du så mycket att Du blir svettig eller andfådd, eller går på långpromenad, skidåkning eller motsvarande? ……...

34. Bodde Du på landsbygden (dvs inte stad eller tätort) under Dina fem första levnadsår?	☐	☐
Hade Din familj jordbruk under Dina fem första levnadsår?	☐	☐

Besvara frågorna genom att kryssa i rätt alternativ.

	NEJ	JA
		X

Om Du är osäker vid ”nej-ja-frågor”, välj ”nej”-rutan.

1. Har Du haft pip eller har det väst i bröstet vid något tillfälle under **de senaste 12 månaderna?** NEJ ☐ JA ☐

OM SVARET ÄR ”NEJ” GÅ TILL FRÅGA 2 ***OM ”JA” GÅ TILL FRÅGA 1.1***

1.1 Har Du överhuvudtaget varit det minsta andfådd när Du haft detta pipande ljud? NEJ ☐ JA ☐

1.2 Har Du haft detta pip eller väsande i bröstet när Du **inte** samtidigt varit förkyld? NEJ ☐ JA ☐

2. Har Du vaknat med en trånghetskänsla i bröstet vid något tillfälle under **de senaste 12 månaderna**? NEJ ☐ JA ☐

3. Har Du vaknat av andnödsattack vid något tillfälle **de senaste 12 månaderna**? NEJ ☐ JA ☐

4. Har Du vaknat av hostattack vid något tillfälle **de senaste 12 månaderna?** NEJ ☐ JA ☐

5. Brukar Du under vintern få upp **slem från bröstet** nästan varje dag under **åtminstone tre månader varje år**? NEJ ☐ JA ☐

6. Har Du **någonsin** haft astma? NEJ ☐ JA ☐

OM ”NEJ” GÅ TILL FRÅGA 7 ***OM ”JA” GÅ TILL FRÅGA 6.1***

6.1 Hur gammal var Du när Du hade Ditt första astmaanfall? ÅLDER ☐☐
(Om osäker, ange Din bästa gissning!)

6.2 Har Du **någonsin** varit inlagd på sjukhus på grund av astma? NEJ ☐ JA ☐

NEJ JA

6.3 Har Du haft något astmaanfall under **de senaste 12 månaderna**?

6.4 Tar Du för närvarande någon **astma**medicin, inklusive inhalatorer, sprejer eller tabletter**?**

7. Har Du hösnuva eller någon annan allergisk snuva?

OM "NEJ" GÅ TILL FRÅGA 8 ***OM "JA" GÅ TILL FRÅGA 7.1***

7.1 Har Du haft problem med allergisk snuva **under de senaste 12 månaderna?**

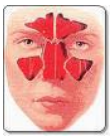
7.2 Har Du någonsin haft problem med allergisk snuva under **mer än 4 dagar under en enskild vecka**?

7.3 Om "ja", hände detta under **mer än 4 veckor i sträck?**

7.4 Har Du **klåda eller irritation i ögonen** samtidigt med Dina näsbesvär?

8. Har Du varit täppt i näsan i **mer än 12 veckor under de senaste 12 månaderna?**

9. Har Du haft värk eller tryck runt pannan, näsan eller ögonen i **mer än 12 veckor under de senaste 12 månaderna?**



10. Har Du haft missfärgat nässekret (snor) eller missfärgat slem i halsen **i mer än 12 veckor under de senaste 12 månaderna?**

11. Har Ditt luktsinne varit nedsatt eller borta i **mer än 12 veckor under de senaste 12 månaderna?**

12. Har en läkare **någon gång** sagt att Du har **kronisk** bihåleinflammation?

NEJ JA

13. Har Du någonsin under **minst 6 månader** haft besvär av återkommande kliande utslag? ☐ ☐

OM "NEJ" GÅ TILL FRÅGA 14 ***OM "JA" GÅ TILL FRÅGA 13.1***

NEJ JA

13.1 Har Du haft det kliande utslaget **under de senaste 12 månaderna**? ☐ ☐

13.2 Drabbar detta **endast** Dina händer?

NEJ JA ☐ ☐

14. Har Du någonsin haft eksem eller någon form av hudallergi? NEJ JA ☐ ☐

15. Har Du någon gång haft svårt att andas inom 3 timmar efter att Du intagit smärtstillande läkemedel? NEJ JA ☐ ☐

OM "NEJ" GÅ TILL FRÅGA 16 ***OM "JA" GÅ TILL FRÅGA 15.1***

15.1 Var vänlig skriv ner läkemedlets namn……

NEJ JA ☐ ☐

16. Har Du någonsin rökt under minst ett års tid?

["JA" betyder *minst en cigarett om dagen eller en cigarr i veckan under minst ett år*]

OM "NEJ" GÅ TILL FRÅGA 17 ***OM "JA" GÅ TILL FRÅGA 16.1***

16.1 Hur gammal var Du när Du började röka? ÅLDER ☐☐

NEJ JA

16.2 Har Du rökt alls under **sista månaden**? ☐ ☐

OM "JA" GÅ TILL FRÅGA 16.3 ***OM "NEJ" GÅ TILL 16.2.1***

16.2.1 Hur gammal var Du när Du slutade röka? ÅLDER ☐☐

16.3 I genomsnitt, hur mycket röker (rökte) Du?

Cigaretter per dygn ☐☐

17. Är Du **för närvarande**: ***Kryssa bara i en ruta!***

anställd	1.	☐
egen-företagare	2.	☐
arbetslös	3.	☐
sjukskriven, sjukbidrag	4.	☐
hemarbetande full tid	5.	☐
studerande, full tid	6.	☐
pensionerad	7.	☐
övrigt	8.	☐

18. Arbetar Du **för närvarande**:

	NEJ	JA
a. inom sjukvården (t.ex. som sjuksköterska, undersköterska, medicintekniker, läkare, ambulanssjukvårdare eller liknande)?	☐	☐
b. i ett jobb som huvudsakligen innefattar någon typ av arbete med rengörning eller städning	☐	☐

19. Hur **lång** är Du? ☐☐☐ cm

20. Hur mycket **väger** Du?  ☐☐☐ kg

21. Ange Ditt **födelsedatum** DAG ☐☐ MÅNAD ☐☐ **19** ☐☐ ÅR

22. Ange **dagens datum** DAG ☐☐ MÅNAD ☐☐ **20** ☐☐ ÅR

23. Är Du **man** eller **kvinna**? MAN ☐ KVINNA ☐

24. Vad har Du för **postnummer**? ☐☐☐☐☐

25. Hur många år har du bott på **nuvarande adress**? ☐☐ ÅR

26. Hur **lång tid per dygn** vistas Du vanligtvis i bostaden? ☐☐ TIMMAR

27. Hur ofta brukar Du uppleva luften i **Ditt bostadsområde** som **irriterande**? ***Kryssa bara i en ruta!***

Dagligen/nästan dagligen	1.	☐
Ibland/periodvis	2.	☐
Sällan/aldrig	3.	☐

28. Hur **besvärande** är avgaserna från trafiken i **Ditt bostadsområde**?

Kryssa bara i en ruta!

Inget/lite	1.	☐
Något	2.	☐
Mycket	3.	☐

29. Hur lång tid reser/går Du **omgiven av stadstrafik** en vanlig vardag? MINUTER ☐☐

30. Har något av följande konstaterats i Din bostad **de senaste 12 månaderna?**

	NEJ	JA
a Vattenskador/fuktskador inomhus på väggar, golv, eller tak?	☐	☐
b ”Buckliga” plastmattor, gulnade plastmattor eller svartnad parkett?	☐	☐
c Synlig mögelväxt på väggar, golv eller i taket?	☐	☐

31. Vilken är den **högsta utbildning** Du har? ***Kryssa bara i en ruta!***

Gått i skola mindre än 5 år	1.	☐
Folkskola eller grundskola	2.	☐
Realskola eller flickskola	3.	☐
2-årigt gymnasium eller yrkesskola	4.	☐
3-4-årigt gymnasium	5.	☐
Universitet eller högskola, 2,5 år eller kortare (mindre än 120 p)	6.	☐
Universitet eller högskola, 3 år eller längre (mer än 120 p)	7.	☐

32. Har Du någon gång haft ett arbete där Du utsatts för **gas, rök eller damm**? NEJ ☐ JA ☐

33. Har en läkare någon gång sagt att Du har **kroniskt obstruktiv lungsjukdom (KOL)**? NEJ ☐ JA ☐

34. Frågor om sömn och sömnkvalité:

Siffrorna betyder

1: *aldrig eller sällan*
2: *mindre än en gång i veckan*
3: *1 till 2 ggr i veckan*
4: *3 till 5 ggr i veckan*
5: *nästan varje dag eller natt*

Hur ofta har det hänt under de senaste månaderna:

Ringa in rätt svar

a. att Du snarkar högt och störande?	1	2	3	4	5
b. att Du har svårt att somna på kvällen?	1	2	3	4	5
c. att Du vaknar flera gånger under natten?	1	2	3	4	5
d. att Du känner Dig sömnig under dagen	1	2	3	4	5
e. att Du vaknar för tidigt och kan inte somna om?	1	2	3	4	5

35. Tar Du för närvarande **medicin** för

		NEJ	JA
högt blodtryck	1	☐	☐
KOL	2	☐	☐
sockersjuka/diabetes	3	☐	☐
sömnbesvär	4	☐	☐

36. Har Du någonsin snusat dagligen **under minst 6 månader**? NEJ ☐ JA ☐

36.1 Om ”ja” snusar Du **fortfarande**? NEJ ☐ JA ☐

37. Använder Du **tuggtobak**, **nikotinplåster** eller **nikotintuggummi**? NEJ ☐ JA ☐

38. **Hur ofta** brukar Du **motionera** så mycket att Du blir andfådd eller börjar svettas?

Kryssa bara i en ruta!

varje dag	1.	☐
4-6 gånger per vecka	2.	☐
2-3 gånger per vecka	3.	☐
en gång i veckan	4.	☐
en gång i månaden	5.	☐
mindre än en gång i månaden	6.	☐
aldrig	7.	☐

39. Hur **många timmar per vecka** brukar Du **motionera** så mycket att Du blir andfådd eller börjar svettas?

Kryssa bara i en ruta!

aldrig	1.	☐
ungefär ½ timma	2.	☐
ungefär 1 timma	3.	☐
ungefär 2-3 timmar	4.	☐
ungefär 4-6 timmar	5.	☐
7 timmar eller mer	6.	☐

40. Får vi ta kontakt med Dig igen för ytterligare hjälp med projektet eller för att be om ytterligare information? NEJ ☐ JA ☐

40.1 Om ”ja”, på vilket **telefonnummer** kan vi lättast nå Dig?

☐☐☐☐☐☐☐☐☐☐☐☐

Tack för hjälpen!

Vuxna intervjuformulär vid klinisk us 2008

Undersökningsdatum _____ Intervjuare _____

Löpnummer _____ Födelsedatum _____

3. Etnisk tillhörighet ○ Kaukasier ○ Afrikan ○ Asiat/oiental ○ Annan

4. Kön ○ Man ○ Kvinna 5. Tätortsgradient _____

Hosta och expektorat

6. Har Du haft långvarig hosta under det senaste året (12 månaderna)? ○ Nej ○ Ja

7. Brukar Du hosta eller harkla Dig på morgonen? ○ Nej ○ Ja

8. Brukar Du hosta eller harkla Dig under andra tider på dygnet? ○ Nej ○ Ja

9. Brukar Du hosta eller harkla upp slem från bröstet, eller känner Du att det sitter slem i bröstet som Du har svårt att hosta eller harkla upp? ○ Nej ○ Ja

10. Hostar eller harklar Du upp slem (eller har slem som det är svårt att få upp trots hosta) de flesta dagar i perioder om minst 3 månader per år? ○ Nej ○ Ja

11. Om ja, sedan hur många år? ____ år

Pip i bröstet eller väsande andning

12. Brukar Du ha pip eller väser det i bröstet då Du andas? ○ Nej ○ Ja

13. Har Du någonsin, nu eller tidigare, vid något tillfälle haft pip eller väsningar i bröstet då Du andas? ○ Nej ○ Ja

14. Har Du haft pip eller har det väst i bröstet vid något tillfälle under de senaste 12 månaderna? ○ Nej ○ Ja

Om ja på 14 besvara fråga 15-17

15. Har Du varit det minsta andfådd när Du haft pip eller väsningar i bröstet? ○ Nej ○ Ja

16. Har Du haft detta pip eller väsande i bröstet utan att samtidigt vara förkyld? ○ Nej ○ Ja

17. Har Du pip i bröstet eller väsande andning de flesta dagarna i veckan? ○ Nej ○ Ja, periodvis ○ Ja

Andnöd

18. Är Du rörelsehindrad (av andra skäl än ev. hjärt- eller lungbesvär)? ○ Nej/el rel ○ Ja

19. Om ja, av vilka skäl? Kryssa för ett eller flera alternativ

Cerebrovaskulär sjukdom	❑
Muskelsjukdom	❑
Rörelseinskränkning i extremitet	❑
Rullstolsburen	❑

Övrigt: _____

20. Har du någonsin besvär med din andning? ○ Nej ○ Ja

21. Om ja, har du dessa besvär

○ För jämnan så att andningen aldrig är riktigt bra
○ Återkommande men avlöst av besvärsfria perioder
○ Endast vid enstaka tillfällen
○ Numera inga besvär

Anfåddhet vid ansträngning - MRC dyspne skala

22. Vilket påstående stämmer bäst överens med dig? [ ]

0: Jag blir bara andfådd när jag anstränger mig rejält, inte när jag tar en snabb promenad eller går i uppförsbacke

1: Jag blir andfådd när jag tar en snabb promenad eller går i uppförsbacke.

2: Jag blir andfådd när jag går på slät mark i samma takt som andra personer i min egen ålder. (och/eller) Jag blir andfådd vid gång på slät mark så jag måste stanna upp trots att jag går i min egen takt.

3: Jag måste stanna på grund av andfåddhet efter cirka 100 m gång på slät mark.

4: Jag blir andfådd när jag tvättar mig eller klär på mig.

99: Frågan ej tillämplig pga nedsatt rörelseförmåga av annan anledning.

23. Har Du någon gång haft hastigt påkommande andnöd eller andfåddhet? O Nej O Ja

Om Ja, besvara fråga 24

24. Om ja, har Du någon gång under de senaste 12 månaderna haft hastigt påkommande andnöd eller andfåddhet? O Nej O Ja

25. Har Du någonsin haft hastigt påkommande andnöd med pip eller väsningar i bröstet? O Nej O Ja

Om Ja, besvara fråga 26-27

26. Har Du haft hastigt påkommande andnöd med pip eller väsningar i bröstet under de senaste 12 månaderna? O Nej O Ja

27. Har Du någonsin haft anfall av andnöd med pip eller väsningar i bröstet eller astmasymtom på Din arbetsplats? O Nej O Ja O N/A

Astma, kronisk bronkit, emfysem och KOL

28. Har Du eller har Du haft astma? O Nej O Ja O Vet ej

29. Har Du av läkare fått diagnosen astma? O Nej O Ja O Vet ej

30. Hade Du pip eller väsningar i bröstet i tidig barndom eller astma under barndomen? O Nej O Ja O Vet ej

Om Ja på någon av frågorna 28-30, annars gå till fråga 32

31a. Hur gammal var Du när Du första gången hade pip i bröstet eller hade besvär av andnöd eller märkte av astma? ____ år

31b. Om du inte minns tydligt, var det:

- O Före skolåldern
- O Under skolåldern men före 20 års ålc
- O Mellan 20 och 30 års åldern
- O Mellan 30 och 40 års åldern
- O Mellan 40 och 50 års åldern
- O Efter 50 års åldern
- O Minns inte alls

31c. Hur gammal var du när du senast hade astmabesvär? _____ år

32. Har du använt astmamediciner regelbundet eller vid behov under de senaste 12 månaderna?
- O Nej
- O Ja

Om NEJ, besvara fråga 33.

33. Har Du tidigare använt astmamedicin?
- O Nej
- O Ja

34. Har Du av läkare fått diagnosen kronisk luftrörskatarr eller kronisk bronkit?
- O Nej
- O Ja
- O Vet ej

35. Har Du av läkare fått diagnosen KOL?
- O Nej
- O Ja
- O Vet ej

36. Har Du av läkare fått diagnosen emfysem?
- O Nej
- O Ja
- O Vet ej

37. Har du använt mediciner regelbundet eller vid behov mot kronisk luftrörskatarr, KOL eller emfysem under de senaste 12 månaderna?
- O Nej
- O Ja

Om NEJ, besvara fråga 38

38. Har Du tidigare använt medicin mot kronisk luftrörskatarr, KOL eller emfysem.
- O Nej
- O Ja

39. Reagerar Du på någon av följande exponeringar?

	Inga besvär	Ögon-besvär	Näs-besvär	Klåda i mun och svalg	Andnings-besvär	Kliande utslag / eksem	Diarré eller ont i magen
Pollenexponering (gräs, björk, gråbo, mm)	☐	☐	☐	☐	☐	☐	☐
Pälsdjursexponering (katt, hund, häst, kanin, marsvin, mm)	☐	☐	☐	☐	☐	☐	☐
Födoämnen (fisk, skaldjur)	☐	☐	☐	☐	☐	☐	☐
Födoämnen (nötter, kärnförande frukter)	☐	☐	☐	☐	☐	☐	☐
Mjölk (Laktosintolerens)	☐	☐	☐	☐	☐	☐	☐
Mjöl (Glutenintolerens)	☐	☐	☐	☐	☐	☐	☐
Kvalster/damm	☐	☐	☐	☐	☐	☐	☐
Annan 1	☐	☐	☐	☐	☐	☐	☐
Annan 2	☐	☐	☐	☐	☐	☐	☐
Annan 3	☐	☐	☐	☐	☐	☐	☐

Specificera eventuella andra ämnen:

Annat 1: _____ Annat 2: _____ Annat 3: _____

40. Har Du, eller har Du haft, någon annan lung/luftvägssjukdom än astma, kronisk luftrörskatarr, KOL eller emfysem?

O Nej
O Ja

41. Om ja, vilken/vilka? _____

42. Har Du haft tbc?

O Nej
O Ja, lungtbc
O Ja, annan tbc

Sjukvårdsbehov pga lung- eller luftvägsbesvär eller sjukdom

43. Har du någonsin sökt läkare eller sjukvård pga andfåddhet, andnöd eller pip i bröstet, hosta, slemhosta eller andra luftvägsbesvär inklusive förkylning? O Nej O Ja

44. Om ja, har du under senaste 12 månaderna sökt för detta beskrivet ovan? O Nej O Ja

45. Har Du någon gång behövt uppsöka akutmottagning pga andningsbesvär? O Nej O Ja

46. Om ja, har Du sökt akut under de senaste 12 månaderna? O Nej O Ja

47. Har Du någon gång varit inlagd på sjukhus för andningsbesvär? O Nej O Ja

48. Om ja, har Du varit inlagd under de senaste 12 månaderna? O Nej O Ja

Frågor om allergiska näs-ögonbesvär

49. Har Du, eller har Du haft, allergiska näsbesvär eller hösnuva? O Nej O Ja

49 a. Om JA, hur gammal var Du när Du första gången hade hösnuva eller allergiska näsbesvär? ____ år

50. Genomgår du, eller har du genomgått allergivaccinering (hyposensibilisering)? O Nej O Ja

51. Har Du någon gång haft besvär med nysningar, rinnande näsa eller nästäppa utan att Du varit förkyld? O Nej O Ja

Om JA,besvara fråga 52-53

52. Har Du haft besvär med nysningar, rinnande näsa eller nästäppa utan att Du varit förkyld under senaste 12 månaderna? O Nej O Ja

53. Har dessa näsproblem uppträtt samtidigt med kliande eller rinnande ögon? O Nej O Ja

Om JA på fråga 52

54. Vilka faktorer kan utlösa dessa problem?	JA	NEJ
a) Pollen från träd som björk, rönn, al m.m.	❑	❑
b) Pollen från gräs	❑	❑
c) Pälsdjur som katt, hund, häst, kanin etc	❑	❑
d) Mögel	❑	❑
e) Parfymer, lukter eller rök	❑	❑
f) Temperaturförändringar	❑	❑
g) Trycksvärta	❑	❑
h) Kvalster/damm	❑	❑

55. Har du under de senaste 5 åren använt mediciner mot hösnuva eller andra näsbesvär som rinnsnuva eller nästäppa utan att du samtidigt varit förkyld?
O Nej
O Ja

56. Har du under de senaste 12 månanderna använt mediciner mot hösnuva eller andra näsbesvär som rinnsnuva eller nästäppa utan att du samtidigt varit förkyld?
O Nej
O Ja

Om JA på fråga 56, besvara nedan om mediciner

57. Antihistaminer i tablettform?
(Aerius, Cetirizin, Clarityn, Kestine, Mizollen, Periactin, Polaramin prolongatum, Semprex, Tavegyl, Teldanex, Telfast, Zyrlex, Versal)
O Nej
O Ja

58. Nasala steroider?
(Becotide nasal, Flutide nasal, Nasacort, Nasonex, Rhinocort)
O Nej
O Ja

59. Övrigt för näsa eller ögon? (Inkl Antasten privin, Atrovent nasal, Emadine, Nasin; Nezerli, Lastin, Livostin, Lomudal, Pollyf Tilavist, Zaditen, Zincfrin)
O Nej
O Ja

60. Kortisonspruta? (Depo-Medrol el dyl)
O Nej
O Ja

Övriga sjukdomar

61. Har du eller har du haft hjärtproblem eller hjärtsjukdom? Flera alternativ är möjliga

Nej	❑
Kärlkramp	❑
Hjärtinfarkt	❑
Hjärtsvikt	❑
Rytmrubbning	❑
Annan hjärtsjukdom	❑
Kranskärlsopererad/ (Kärlkrampsopererad)	❑

62. Använder du hjärtmediciner (inklusive proppförebyggande)?

O Nej O 1 medicin O 2 mediciner O 3 mediciner O 4 mediciner eller fler

63. Har du eller har du haft högt blodtryck? O Nej O Ja

64. Använder du mediciner mot högt blodtryck? O Nej O Ja

65. Har du eller har du haft fönstertittarsjukan (klaudikatio, kärlkramp i benen)? O Nej O Ja

66. Har du eller har du haft blodpropp eller blödning i hjärnan (TIA, stroke)? O Nej O Ja

67. Har du eller har du haft förhöjda blodfetter? O Nej O Ja

68. Tar Du medicin mot förhöjda blodfetter? O Nej O Ja

69. Har du eller har du haft diabetes? O Nej O Ja

70. Har du eller har du haft reumatisk sjukdom? O Nej O Ja

71. Använder Du hormontabletter, plåster, spiral, p-stav eller p-piller? (endast kvinnor) O Nej O Ja

72. Har eller har du haft halsbränna eller sura uppstötningar (reflux)? ⊙ Nej ⊙ Ja

72b. Inträffar detta efter måltider? ⊙ Nej ⊙ Ja ⊙ Båda

73. Har du eller har du haft några andra sjukdomar än vad som nämnts ovan?

73b. Tar du några övriga mediciner? ⊙ Nej ⊙ Ja

Uppväxttid

74. Rökte någon av dina föräldrar eller andra anhöriga i Din hemmiljö under Din uppväxttid innan Du började i skolan?

Nej	❑
Mor	❑
Far	❑
Annan	❑

75. Rökte Din mor då hon var gravid och väntade Dig? ⊙ Nej ⊙ Ja ⊙ Vet ej

76a. Hade ni pälsdjur eller burfåglar i hemmiljön eller i den nära omgivningen under Din uppväxttid innan Du började i skolan? ⊙ Nej ⊙ Ja

76b. Om Ja, vilka?	JA	NEJ
Katt	❑	❑
Hund	❑	❑
Marsvin/smågnagare	❑	❑
Häst	❑	❑
Kor	❑	❑
Burfåglar	❑	❑

77a. Finns det pälsdjur, djur eller burfåglar i hemmet nu? ○ Nej ○ Ja

77. Om Ja, vilka?	JA	NEJ
Katt	❑	❑
Hund	❑	❑
Marsvin/smågnagare	❑	❑
Häst	❑	❑
Kor	❑	❑
Burfåglar	❑	❑

78. Hade Du någon allvarlig luftrörs- eller lunginfektion före skolåldern, t. ex. kikhosta eller krupp? ○ Nej ○ Ja ○ Vet ej

79. Brukade Du dela sovrum med andra barn före skolåldern? ○ Nej ○ Ja ○ Vet ej

80. Hur många syskon som du vuxit upp tillsammans med, har Du eller har Du haft? Antal ____

81. Hur många äldre syskon, som du vuxit upp tillsammans med, har Du eller har Du haft? Antal ____

82. Vistades Du över ett år på daghem, lekskola eller barnhem tillsammans med andra barn före skolåldern? ○ Nej ○ Ja ○ Vet ej

83. Hur bodde Du mestadels före skolstart? ○ Villa/radhus ○ Lägenhet

84. Var bodde Du mestadels före skolstart? ○ Landsbygd ○ Förort ○ Stad/tätort

85. Bodde Du i Sverige mesta delen av tiden före skolstart? ○ Nej ○ Ja

86. Om nej, vilket land bodde Du mestadels i? _____

87. Vilken var din födelsevikt?

○ < 2500 g ○ 2500-3000 g ○ 3000-4000 g ○ > 4000 g ○ Vet ej

88a. Vilket är ditt nuvarande yrke eller senaste yrke?

_____

88b. Antal år: _____

89. Har Du arbetat mer än 5 år i något annat yrke? ○ Nej ○ Ja

Om JA på fråga 89 - specifiera vilket/vilka yrken:

90a. Yrke 1: _____

90b Antal år: _____

90c.Yrke 2: _____

90d. Antal år: _____

90e. Yrke 3: _____

90f. Antal år: _____

Rökning och nikotinanvändning

91. Är du... ○ Icke rökare
○ Före detta rökare
○ Rökare

92. Har du någonsin rökt minst ett år? ○ Nej
(minst en cigarett/dag - minst en cigarr/vecka eller minst 30 gram tobak/månad - under minst ett års tid) ○ Ja

Om ja,

93. Hur gammal var Du när Du började röka? ____ år

Fråga 94-95 besvaras av icke-rökare och före detta rökare

94. Händer det att du röker ibland? (mindre än varje vecka) ○ Nej
○ Ja

Om ja,

95. Hur många cigaretter per månad? Antal ____

Fråga 96 besvaras av före detta rökare

96. Hur gammal var Du när Du slutade röka? ____ år

Frågorna 97-99 besvaras av rökare

97a. Om Du röker cigaretter, hur många röker Du i genomsnitt per dag? Antal ____

97b. Om Du röker cigaretter, hur många röker Du i genomsnitt per dag?

○ Röker inte cigaretter ○ 1-4 ○ 5-14 ○ 15-24 ○ >24

98. Om Du röker cigarrer/cigariller, hur många röker Du i genomsnitt per dag?

○ Röker inte cigarr ○ 0-1 ○ 2-4 ○ > 5

99. Om Du är piprökare, hur mycket förbrukar Du i genomsnitt per dag?

○ Röker inte pipa ○ < 50 g ○ 50-100 g ○ > 100 g

Frågorna 100-102 besvaras av rökare och ex-rökar

100. Hur mycket har Du rökt?

Ålder	Rökår	Cigaretter/dag	Cigarrer/dag	Piptobak(g/vecka)
0-20	_____ år	0-20 _____ st	0-20 _____ st	0-20 _____ g/v
21-40	_____ år	21-40 _____ st	21-40 _____ st	21-40 _____ g/v
41-60	_____ år	41-60 _____ st	41-60 _____ st	41-60 _____ g/v
60 +	_____ år	60 + _____ st	60 + _____ st	60 + _____ g/v

101. Hur många år har Du rökt? _____ år

102. Uppskattat antal pack-years: _____ år

103. Är Du eller har Du varit utsatt för rökning i din hemmiljö?
- ⊙ Nej
- ⊙ Ja, tidigare ej nu
- ⊙ Ja, nu

104. Är Du eller har Du varit utsatt för rökning på arbetsplatsen?
- ⊙ Nej
- ⊙ Ja, tidigare ej nu
- ⊙ Ja, nu

105. Hur mycket passiv rökning har Du utsatts för under Ditt liv?

I hemmet		*Arbete/skola/fritid*	
Rökår	Timmar/dygn	Rökår	Timmar/dygn
Ålder	Ålder	Ålder	Ålder
0-7 _____ år	0-7 _____ h/dygn	0-7 _____ år	0-7 _____ h/dygn
8-15 _____ år	8-15 _____ h/dygn	8-15 _____ år	8-15 _____ h/dygn
16-25 _____ år	16-25 _____ h/dygn	16-25 _____ år	16-25 _____ h/dygn
26-40 _____ år	26-40 _____ h/dygn	26-40 _____ år	26-40 _____ h/dygn
41-60 _____ år	41-60 _____ h/dygn	41-60 _____ år	41-60 _____ h/dygn
60 + _____ år	60 + _____ h/dygn	60 + _____ år	60 + _____ h/dygn

Mediciner för luftvägarna (Astma, KOL, kronisk bronkit, emfysem

M1. Kortverkande beta-2 stimulerare i inhalationsform? (Airomir, Buventol, Salbutamol, Ventoline, Salbuvent, Inspiryl, Bricanyl, Berotec)

- O Nej
- O Ja

M1b. Hur ofta använder du något av ovanstående?

- O Aldrig
- O Någon gång per månad
- O Minst 3 ggr per vecka
- O Enstaka gånger per år
- O Högst två gånger per vecka
- O Dagligen eller nästan dagligen

M2. Inhalationssteroider? (Pulmicort, Becotide, Flutide, Beclomet, Asmanex)

- O Nej
- O Då och då
- O De flesta dagar i veckan

Sort _____ Dygnsdos _____

M3. Kombinationspreparat? (Seretide Symbikort)

- O Nej
- O Ja

Sort _____ Dygnsdos _____

M3d. Hur ofta använder du ovanstående

- O Aldrig
- O Periodvis
- O Minde än 1 ggr/vec
- O Några ggr/vecka
- O Dagligen eller näst

M4. Antikolinergika i inhalationsform? (Atrovent, Spiriva)

- O Nej
- O Då och då
- O De flesta dagar i veckan

M5. Långverkande Beta-2 stimulerare i inhalationsform? (Serevent, Foradil, Oxis)	○ Nej ○ Då och då ○ De flesta dagar i veckan
M6. Beta-2 stimulerande medel i tablettform? (Ventoline, Bricanyl, Bambec)	○ Nej ○ Då och då ○ De flesta dagar i veckan
M7. Teofyllinpreparat? (Theo-Dur, Teovent, Euphylong)	○ Nej ○ Då och då ○ De flesta dagar i veckan
M8. Luftrörsvidgande medel i inhalationsform genom inhalationsapparat? (Ventoline, Inspiryl, Bricanyl, Atrovent, Combivent)	○ Nej ○ Då och då ○ De flesta dagar i veckan
M9. Perorala steroider mot luftvägssjukdom? (kortisontabletter; Betapred, Decadron, Dexacortal Medrol, Prednisolon, Deltison, Cortal)	○ Nej ○ Då och då ○ De flesta dagar i veckan ○ Endast vid exacerbation ○ Ej tagit i år men tidigare
M10. Leukotrienreceptorantagonist (Singulair)	○ Nej ○ Då och då ○ De flesta dagar i veckan