Long-term exposure to outdoor air pollution and asthma

Master Thesis

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Table of Contents

List of figures

List of tables

List of Abbreviations

ALSWH: The Australian Longitudinal Study on Women's Health

CI: Confidence Interval

CO_2: Carbon dioxide

CO: Carbon monoxide

COPD: Chronic Obstructive Pulmonary Disease

DAGs: Directed Acyclic Graphs

DALYs: Disability Adjusted Life Years

ECRHS: European Community Respiratory Health Survey

ELAPSE: Effects of Low-Level Air Pollution: A study in Europe

ESCAPE: European Study of Cohorts for Air Pollution Effects

GINA: Global Initiative for Asthma

HICs: High-Income Countries

HR: Hazard Ratio

ISAAC: International study of Asthma and Allergies in Childhood

LMICs: Low-and middle-income countries

NO_2: Nitrogen dioxide

OR: Odds Ratio

PAHs : Polycyclic Aromatic Hydrocarbons

PM: Particulate Matter

RHINE: Respiratory Health in Northern Europe study

RMS: Ratio of Mean Score

RR: Relative Risk

SAPALDIA: Swiss Cohort Study on Air Pollution and Lung Diseases in Adults

SO_2: Sulphur dioxide

VOCs: Volatile Organic Compounds

WHO: World Health Organization

Abstract

Background: Asthma prevalence has increased over the last decades due to the complex interaction of genes and environmental factors. Exposure to environmental factors like low levels of air pollution greatly impacts the development of asthma throughout the lifespan and across generations. Exposure to high levels of air pollution for short periods is associated with aggravation of respiratory symptoms and increased respiratory mortality. As asthma has become a global health problem, we should have more knowledge of associations between long-term pollution exposure and asthma to enhance disease control and prevention and ensure efficient disease management.

Objective of study: To investigate if air pollution exposure 20 years ago is associated with increased risk for asthma among adults.

Method: The study design is a population-based prospective cohort study. For the study purpose, we used data from Respiratory Health in Northern Europe (RHINE) study collected from 1990 to 2010. The RHINE study was a questionnaire follow-up in 2000 and 2010 of all subjects from seven Northern European centres that participated in the ECRHS I stage 1. The participants were from seven different centres: Aarhus (DK); Gothenburg, Umea, and Uppsala (S); Reykjavik (IS); Tartu (EST); and Bergen (N). But in this study, only the participants from Gothenburg, Umea, Uppsala, and Bergen were included as the data for air pollution for the three-time points were only available for these four centres. The study population of 6,193 who have information on both asthma symptoms and exposure to air pollution and registered on sex were included in this study. The association between air pollutant with asthma severity and asthma symptoms were analysed by using logistic regression and negative binomial regression. The analyses were stratified based on sex and parental asthma.

Result: Males who were exposed to NO_2 20 years ago were associated with a small but significant increased risk of asthma severity defined as 3 or more current asthma symptoms (**OR :1.03; 95% CI: 1.00, 1.07).** However, in females, no significant association was observed between exposure to any air pollutants (NO_2, PM_{10}, and $PM_{2.5}$) in any of the three-time points with regard to asthma severity. The stratified analyses based on parental asthma history showed no significant association between exposure to air pollutants (NO_2, PM_{10}, and $PM_{2.5}$) at any of the three-time points and asthma severity. The negative binomial regression analyses stratified by sex showed that males exposed to NO_2 20 years ago were associated

with a small but significantly increased asthma symptoms risk **(OR:1.01; 95% CI:1.00, 1.03).** However, a borderline significant association was observed in females between current exposure to PM_{10} and asthma symptoms **(**O**R:**1**.06; 95% CI:0.99, 1.13)**. In the negative binominal regression stratified by parental asthma, no significant association was observed between exposure to air pollutants (NO_2, PM_{10}, and $PM_{2.5}$) at any time point and asthma symptoms.

Conclusion: The study found that exposure to NO_2 20 years ago was associated with both asthma severity and asthma symptoms only among males. But, for females**,** a borderline significant association was observed between asthma symptoms with current exposure to PM_{10}. No association was found between asthma severity and asthma symptoms with any air pollutants for three time points in the analysis stratified by parental asthma.

1. Background

Air pollution is one of the major threat to human health worldwide. According to the 2019 Global Burden of Disease study, air pollution is the third leading risk factor for mortality worldwide. In 2019, 4.51 million deaths were attributed to outdoor air pollution, while 2.31 million were attributed to indoor air pollution (1).

Many epidemiological studies have found that the exposure to air pollution is harmful to lung health. Excessive air pollution can cause the inflammation of lungs, and also changes in lung function, thereby resulting in different lung diseases including asthma, chronic obstructive pulmonary disease and bronchitis (1, 2). In the lungs immune response mechanism regulate the inflammatory response and facilitate the clearance of inhaled pathogens, such as volatile organic compounds (VOCs), metals, sulphur and nitrogen dioxides (NO_2), ozone (O_3) and particulate matter (PM) (3). However, exposure to high level of air pollution either for short-term or long-term are found to be associated with increased respiratory problem and other adverse health effects (4, 5). Many studies also demonstrates an association between short-term exposure to air pollutants and the incidence of asthma exacerbations and hospital admissions (6).

Air pollution is an important factor that enhances pulmonary disease causing greater harm in susceptible populations, such as children, the elderly, and those of low socio-economic status worldwide (2).Outdoor air pollution has been shown to adversely affect lung function during the course of life. Many cross-sectional studies and several longitudinal studies have reported lower and slower lung function growth because of exposure to air pollution in children and adolescents. This is a major global health problem in both developing and developed countries and affects mostly those who are living in urban areas (2).

1.1 Asthma

1.1.1 Definition of asthma

The Global Initiative for Asthma (GINA) describes asthma as “a heterogeneous disease, usually characterized by chronic airway inflammation. It is explained by the history of respiratory symptoms like wheeze, shortness of breath, chest tightness and cough that varies over time and in intensity, along with variable expiratory airflow limitation” (7).

Asthma is associated with airway hyperresponsiveness to specific triggers such as viruses, allergens, exercise, and smoking, that lead to repeated episodes of wheezing, breathlessness, chest tightness and/or coughing (8).

1.1.2 Risk factors for asthma

The risk factors for asthma include environmental and host factors. Genetic hereditability through a family history of asthma is a common factor, but it is neither a sufficient nor a necessary cause for asthma development (9).

Hosts are those who can get the disease. A variety of intrinsic factors that can influence an individual’s exposure, susceptibility, or response to a causative agent are all host factors. These factors can be genetic composition, nutritional and immunologic status, anatomic structure, presence of disease or medications, and psychological makeup, as well as age and sex. The external factors that affect the agent and provide the opportunity for exposure, on the other hand, are environmental factors (9).

Important host and environmental risk factors for asthma are listed below:

Host factors:

-Genetic

-Sex: Male or Female

-Obesity

Environmental factors:

-Socioeconomic factors: Low or high socio-economic status

-Occupation: Mine workers, textile industries

-Smoking

-Air Pollution

-Viral infections

1.1.3 Global burden of asthma

According to the World Health Organization (WHO), global asthma report, asthma is the 16th leading cause of years lived with disability and the 28th leading cause of burden of disease worldwide. (10). There is a huge geographic variation in prevalence, severity, and mortality of asthma. The asthma prevalence is higher in high- income countries, while asthma-related mortality is highest in low-middle income countries(10). The global asthma report of 2018 reported that 339 million people across the globe are affected by asthma. Developing countries have a mean asthma incidence of 3% to 5%, while it is more than 20% in developed countries (11). The low prevalence of asthma in developing countries can be attributed to underdiagnosis and poor data registration. However, the higher prevalence observed in developed countries may, to some degree, also be correct due to increased urbanization/westernized lifestyle and higher rates of obesity (12).

Global asthma report of 2018 also showed a 3.6% increase in age-standardized prevalence of asthma since 2006 (13). The burden of asthma is high at the age of 10-14 years and 75-79 years, while at the age of 30-34 years, the disease burden is the least. Asthma is one of the chronic non-communicable diseases with a major global burden in terms of direct and indirect costs. North America and Europe have the highest medical cost for managing asthma. The prevalence of asthma worldwide has also led to a rise in per-patient costs for asthma. Globally in 2016, asthma across all ages contributed 23.7 million disability-adjusted life years (DALYs) (14). About 1.1% of the overall global estimate of DALY per 100,000 for all causes is accounted for by asthma. It is estimated that asthma accounts for the majority of hospitalizations among children under five years old in low-and middle-income countries (LMICs) (15).

The high prevalence of asthma led to the establishment of an epidemiological study from the 1960s onwards to estimate the global asthma prevalence and incidence and to identify risk factors associated with these outcomes. The most comprehensive studies are the International Study of Asthma and Allergies in Childhood (ISAAC) and the European Community Respiratory Health Survey (ECRHS). These studies found that the prevalence of asthma in childhood and adulthood has increased in some high-income countries and levelled off in some countries, whereas an increase appears to be continued in low-and middle-income countries (10).

While many efforts have been made worldwide to address asthma, it remains a serious public health problem, particularly in low-resource settings. Globally, several epidemiological studies have shown significant contradictions in asthma prevalence, but mostly a notable rise in LMICs with high mortality rates. Some of the factors such as social inequalities, poor access to medical services and basic infrastructure, poor and weak health system and health education, modernization and ´urbanization´ of the rural environment, increasing air pollution, smoking habits, and change in food habits are associated with the increase of asthma morbidity resulting into hospitalization and mortality. Aside from affecting the quality of life, asthma negatively impacts the health of patients, their families, healthcare systems, and society. A series of asthma guidelines have been developed to raise awareness and improve asthma diagnosis and treatment. However, underdiagnosis and undertreatment continue to be problems. (16).

This shows that further research is needed on asthma risk factors to prevent further increases in asthma, especially in these vulnerable parts of the world. The research and studies conducted in countries with high incidences of asthma can contribute to more valuable knowledge for LMICs for the control and prevention of asthma.

1.2 Air pollution

"Pollution is the introduction of substances into the environment, resulting in deleterious effects of such a nature as to endanger human health, harm living resources and ecosystems." (European Environment Agency).

In other words, air pollution may be defined as the presence of substances in the air that harm human health. Although several natural causes like volcanic eruptions and wildfire cause air pollution, industrial development and various human activities such as mining, construction, and transportation made air pollution a real global problem (17).

Pollutants can be classified into gaseous and particulate matter (PM). The primary gaseous pollutants are nitrogen dioxide (NO_2), sulphur dioxide (SO_2), ozone (O_3), carbon monoxide (CO), carbon dioxide (CO_2), and heavy metals such as lead or chromium (Pb or Cr), and volatile organic compounds (VOCs) including polycyclic aromatic hydrocarbons (PAHs) (17).

Depending on the production and emission source, PM varies in number, size, shape, surface area, and chemical composition. The health effects of PM also depend on its size and

composition. PM contains sulphates, nitrates, transition metal oxides, salts, polycyclic aromatic hydrocarbons, and biological materials, such as pollen, bacteria, spores, and animal remains. Based on its diameter, PM is classified into 3 types: coarse PM_{10} (from 2.5 to 10 μm), fine $PM_{2.5}$ (from 0.1 to 2.5 μm), ultrafine $PM_{0.1}$ (UFPs) (less than 0.1 μm). As the respiratory system is generally the entry point of PM into the body, PM may increase the incidence and harshness of respiratory outcomes such as asthma attacks, exacerbating bronchitis, and other lung problems (18).

Another important pollutant concerning human health is nitrogen dioxide (NO_2). NO_2 is a traffic-related pollutant; its major emission source is automobile motor engines. As it penetrates deep into the lung, it may produce respiratory symptoms like coughing, wheezing, dyspnoea, bronchospasm, and even pulmonary oedema when inhaled at high levels. It is reported that concentrations of more than 0.2 parts per million (ppm) produce these adverse effects in humans. Moreover, long-term exposure to high levels of nitrogen dioxide is found to be associated with chronic lung disease (19).

1.2.1 Global burden of air pollution

Around 95% of the world's population has a mean annual exposure that exceeds WHO guidelines (1). In both low- and middle-income countries, air pollution is more prevalent, with Bangladesh being the world's most polluted country with 76.9 g/m^3 (only slightly lower than 77.1 g/m^3 in 2020 and 83.3 g/m^3 in 2019*)* (20). Air pollution is not only the problem of LMICs, but even the many high-income countries, for example, in Europe – the UK, Germany, and France are also exposed to a level of pollution that exceeds this threshold. Hence, it is one of the world's most significant health and environmental problems that, accounts 11.65% of death globally (1)**.**

In LMICs, air pollution contributes to 3.19 million deaths as one of the top risk factors for mortality. Air pollution is not only responsible for mortality but also one of the main contributors to the global disease burden. Health problems associated with it are one of the leading causes of death around the world. Air pollution not only takes years from people's lives but also has a significant effect on the quality of life while they are still living (1).

As air pollution increases in concentration, its negative health implications increase. Most of the research studies have found that long-term exposure to the lower level of air pollutants has adverse health effects, and that is truly a global problem. If the pollution is harmful in a

low-pollution setting like HICs, then it is more likely even more harmful in highly polluted countries. So, the studies and research on the pollution from HICs can also teach us about the health effects of LMICs.

1.2.2 Air pollution and health effects pyramid

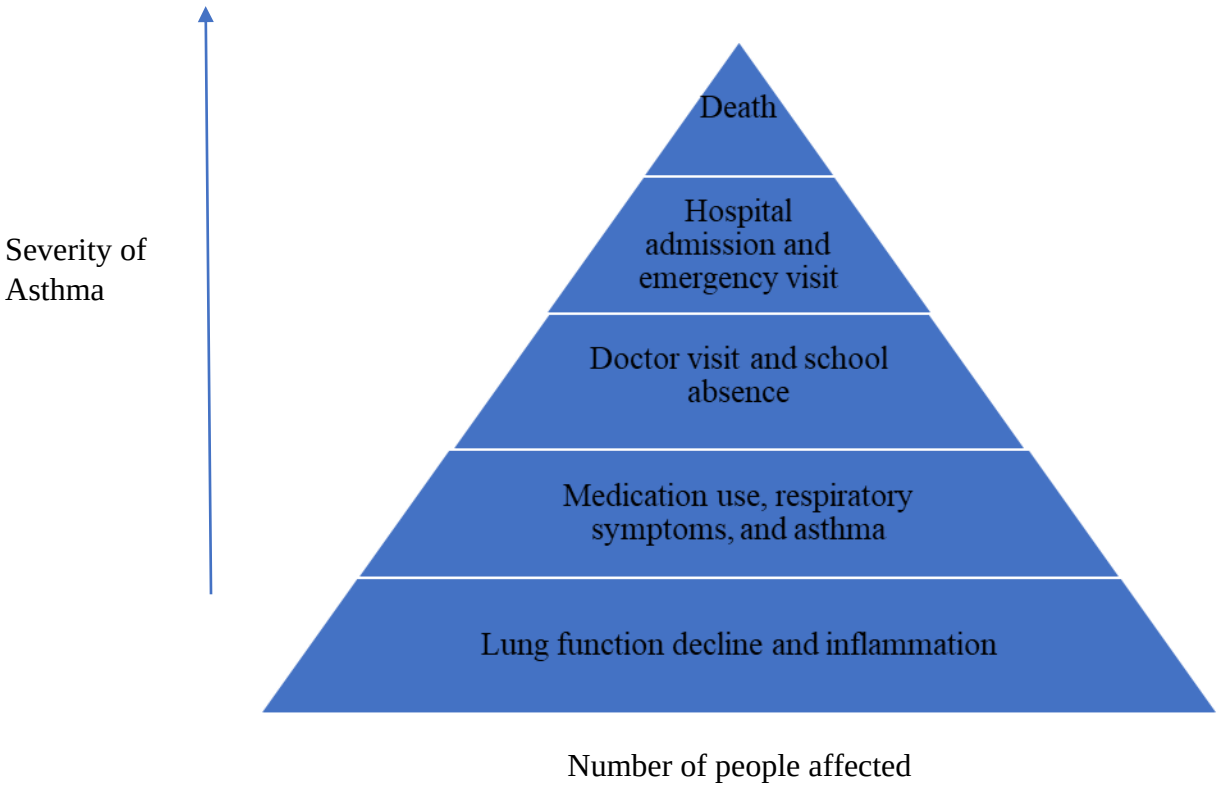

Figure 1. Pyramid of health effects caused by air pollution

Figure 1 is based on the pyramid of health effects in the ERS Report "Air Quality and Health" and illustrates how a large proportion of a population exposed to air pollution will experience milder outcomes, such as lung function decline and respiratory symptoms, while a smaller proportion of the population will experience more severe outcomes such as hospital admissions and deaths. Nonetheless, even milder outcomes, such as decreased lung function and increased respiratory symptoms, impose a heavy burden on individuals and society (21).

1.3 Rationale of the study

Although there is an increase in studies related to outdoor air pollution and the development of asthma in children, more research is needed to define and understand the role of environmental exposures in the development of asthma in adults. We need more research to identify if some groups are particularly susceptible to harmful effects of air pollution for instance, men/women and those with and without genetic predisposition through a history of parental asthma. Also, to further our understanding of the effects of long-term exposure to air pollution at low levels, we need more research. As adult-onset asthma is also common and has become a global health problem, we should know more about associations between long-term pollution exposure and asthma in this age group to enhance disease control and prevention and ensure efficient disease management. Additionally, more research in this field could aid public health authorities and governments in developing policy guidelines and taking more efficient measures to limit air pollution and improve global health. This would be particularly relevant in LMICs that have high asthma-related hospitalization rates. And mortality rate.

1.4 Literature Review

The following search terms in PubMed were used: "air pollution" AND "asthma," AND "long-term" AND "adult." Studies using the exposure metrics NO_2, $PM_{2.5}$, and PM_{10} were used in the literature review. In the search, 108 papers were found. Among them, only studies conducted on the adult population and relevant to our study were included.

The European Study of Cohorts for Air Pollution Effects (ESCAPE) (22), assessed the incidence of asthma prospectively in six European cohorts from 24 areas in eight countries with 23,704 adults over ten years. According to ESCAPE, almost all exposure metrics were related to the higher incidence of asthma, but the associations were insignificant. Positive associations with borderline significance were observed for nitrogen dioxide [adjusted odds ratio (OR) per 10 $\mu g/m^3$: 1.10; 95% CI: 0.99-1.21; $p = 0.10$] and nitrogen oxide (adjusted OR per 20 $\mu g/m^3$:1.04; 95% CI: 0.99-1.08; $p = 0.08$). Similarly no significant associations were observed for PM_{10} (adjusted OR per 10 μg/m3: 1.04; 95% CI: 0.88-1.23),$PM_{2.5}$ (adjusted OR per5 μg/m3 : 1.04; 95% CI: 0.8- 1.23), and for PM_{coarse} (adjusted OR per 5 μg/m3: 0.98; 95% CI: 0.87- 1.14) (22). Also, in this study, no significant difference was found between men and women, and no association between air pollution and incident asthma was found (OR for

NO_2: 1.06; 95% CI: 0.92-1.24) in men, (OR:1.07; 95% CI:0.97-1.19) in women, *p*-value for interaction = 0.66, (OR for PM_{10}: 1.00; 95% CI:0.63-1.59) in men, (OR:1.07; 95% CI: 0.91-1.26) in women, *p*-value for interaction = 0.80(22). In the ESCAPE study, there was no association between NO_2 and incident asthma in participants over 50 years old at baseline(22). The study showed a borderline significant association between NO_2 and incidence of asthma in ever-smokers (OR 1.13;95 % CI: 0.99-1.29) but not in never-smokers (OR :1.01; 95%CI:0.88-1.16; *p*-interact = 0.35). No significant difference was found between PM_{10} and the incidence of asthma in ever-smokers (OR:1.17; 95% CI: 0.79-1.74) and never-smokers (OR: 1.10; 95% CI:0.87-1.39) (22).

During 1991-2002, the Swiss Cohort Study on Air Pollution and Lung Disease in Adults (SAPALDIA) was designed to investigate the long-term effects of exposure to air pollution. (23). The total number of participants at baseline was 9,651. The SAPALDIA cohort study showed that out of 2,725 participants who had never smoked, 41 subjects (1.5%) developed asthma during the 11 years of follow-up, equivalent to an incidence rate of 1.39 (95% CI: 1.02 -1.88) cases per 1000 person-years. The incidence of asthma was associated with an increase in PM_{10}; a hazard ratio of 1.30 (95% CI: 1.05 - 1.61) per 1 $\mu g/m^3$ change in PM_{10}. This study suggests that traffic-related air pollution has a role in the development of adult-onset asthma among never-smokers (23).The SAPALDIA cohort study also found that among Swiss non-smokers, the association between home outdoor traffic-related PM_{10} (TPM_{10}) and asthma incidence was slightly higher for participants aged above 40 years at baseline, although no significant interaction existed (OR:1.65 significant in >40 years, OR:1.3 non-significant in ≤40 years, no exact OR available, p-value > 0.1) (23).

The Pisan longitudinal study (1991-2011) aimed to evaluate the effects of exposure to particulate matter (PM) on the incidence of respiratory diseases in 305 participants with a mean age of 47.5 years, living at the same address for ten years. The Pisan longitudinal study showed that incidences of rhinitis and chronic phlegm were associated with increasing PM2.5 OR per unit increase (p.u.i.):2.25 (95% CI: 1.07-4.98) and OR (p.u.i.): 4.17 (95% CI:1.12-18.71), respectively. Incidence of chronic obstructive pulmonary disease was associated with PM_{10}: OR: 2.96 (95% CI:1.50-7.15) per unit increase (24).

The European Community Respiratory Health Survey (ECRHS) measured asthma and allergies geographically. Adults aged between 20 and 44 years were selected randomly from population registers to participate in the survey. More than 140,000 individuals' information

was registered in the database of ECRHS. Results from the first follow-up of the European Community Respiratory Health Survey (baseline in 1990 and follow-up in 2002) showed a positive association between NO_2 and asthma incidence (OR:1.43; 95% CI:1.02 - 2.01) per 10 $\mu g/m^3$ in a fully-adjusted model. However, stratified analyses by sex showed no significant association between exposure to NO_2 and incident asthma in males (OR:1.31;95% CI:0.76-2.27) & females (OR:1.53;95%CI:0.99-2.38). Similarly, no significant association was found between NO_2 and stratified analyses by family history of asthma, no family history of asthma or atopy (OR:1.57; 95%CI 0.92-2.67) and family history of asthma and atopy (OR:1.31; 95% 0f CI: 0.84-2.04) (25). Analysis of the Swedish part of the ECRHS study showed that there was a positive association between levels of nitrogen dioxide (NO_2) and asthma onset (OR per 10 $\mu g/m^3$: 1.46, 95% CI:1.07-1.99). NO_2 was also associated with increased incident asthma, defined as the age of asthma onset between baseline age and follow-up age (OR per 10 $\mu g/m^3$:1.54; 95% CI:1.00-2.36). Males and females had no significant differences in asthma and NO_2 levels. Males had an odds ratio of 1.57; 95%CI: 0.97-2.52; and females had an odds ratio of 1.30; 95%CI: 0.96-2.04. Living near a major road was also significantly associated with asthma risk (26).

In another negative binominal regression analysis stratified by sex from the ECRHS, a significant association was found between NO_2 concentration and asthma score in men (ratio of mean asthma score (RMS)1.32;95%CI:1.12-1.56) and insignificant in women (RMS:1.14;95%CI:0.97-1.34; p-value 0.13). The significant association was found between NO_2 and family history of asthma and atopy (RMS:1.50;95%CI:1.32-1.70) (27).

A population-based cohort (CONSTANCES) study (2012-2019) among 200,000 French adults aged 18-69 years showed positive significant association between exposure NO_2 (RMS: 1.12; 95% CI :1.10 to 1.14) & $PM_{2.5}$ (.RMS: 1.12; 95% CI: 1.10 -1.14) and asthma symptoms score (28).

The Sister Cohort Study (2003-2009) in the United States was done on the sisters of women with breast cancer. In total, 6,339 participants were included in an analysis to estimate the association between ambient air pollution exposures ($PM_{2.5}$ and NO_2) and the development of asthma and incident respiratory symptoms. The Sister Cohort Study found that $PM_{2.5}$ was significantly associated with incident wheeze fully adjusted (OR:1.14;95% CI:1.04–1.26, p-value= 0.008). Similarly, NO_2 was associated with incident wheeze (OR:1.08;95% CI:1.00–1.17, p-value = 0.048). Neither $PM_{2.5}$ (0.89, 95% CI: 0.88–1.03) nor NO_2 (0.97, 95% CI:

0.93–1.07) were significantly associated with coughing (0.194 and 0.939, respectively). This study also showed an association between NO_2 and wheezed incidence among ex-/never-smokers with OR 1.14 (95% CI:1.04-1.24, p-value 0.003), but there was no association with current smokers (OR:0.89; 95% CI: 0.74–1.06, p-value 0.179) (29).

A population-based cohort study (2001-2015) in Ontario to examine the association between the incidence of COPD and adult-onset asthma with previous exposure to NO_2, $PM_{2.5}$, and O_3 was conducted on a population sample aged 35 to 85 years. Among 5.1 million adults, the investigators identified 340,733 and 218,005 incident cases of COPD and asthma, respectively. The study did not find strong evidence that the exposure to fine $PM_{2.5}$ hazard ratio HR:1.01;95%CI: 1.00-1.02) and NO_2 HR:1.00; 95%CI:0.98-1.01)was associated with adult-onset asthma. However, the positive associations of COPD with $PM_{2.5}$ was found (hazard ratio:1.07,95% CI: 1.06-1.08) (30).

The Australian Longitudinal Study on Women's Health (ALSWH) is a population-based prospective longitudinal study that started in 1996 to assess the effect of long-term exposure to ambient air pollution on the prevalence of self-reported health outcomes. This first national-scale air pollution study based on survey responses from 26,991 female participants found no association between ambient NO_2 air pollution exposure and self-reported asthma, COPD, symptoms of allergies, breathing difficulties, chest pain, or palpitations.(31).

A study that investigated the effects of both ambient air pollution and traffic noise on adult asthma prevalence, using harmonized data from three European cohort studies established in 2006–2013 (HUNT3, Lifelines, and UK Biobank) showed statistically significant associations for PM_{10} and NO_2 in relation to the prevalence of ever-had asthma. Independent of confounders, PM_{10} higher by 10 μgm^3 was associated with a 12.8% (95% CI: 9.5–16.3%) higher prevalence of ever-had asthma and 6.4% (95% CI: 1.2–11.9%) higher prevalence of current asthma. NO_2 higher by 10 μgm^3 was associated with a 1.9% (95% CI: 1.1–2.8%) higher prevalence of ever-had asthma but not current asthma. Age, smoking, and education were observed as significant effect modifiers between PM_{10} and ever-had asthma prevalence, with stronger associations for those aged $\geqslant$50 years, ever-smokers, and participants with less education. Similar findings were observed for current asthma. No significant effect modifications were seen between NO_2 and ever/current asthma prevalence by any studied variables (32).

A study in Europe (ELAPSE) examined the association between long-term exposures to particulate matter with a diameter of 2.5 m ($PM_{2.5}$), nitrogen dioxide (NO_2), and black carbon (BC) with asthma incidence in adults. The pooled data from three cohorts in Denmark and Sweden with information on asthma hospital diagnoses were studied. Of 98,326 participants, 1,965 developed asthma during 16.6 years of mean follow-up. Even at levels below the European Union limit values, asthmatic participants were more likely to be women, obese, and to have higher levels of $PM_{2.5}$ and NO_2 at their residence. The study observed associations in fully adjusted models with hazard ratios 1.22 (95% CI:1.04-1.43) per 5 $\mu g/m^3$ for $PM_{2.5}$ and 1.17 (95%CI:1.10-1.25) per 10 $\mu g/m^3$ for NO_2 (33).

A time series and case-crossover analysis (2003-2013) in Adelaide was conducted to assess the effect of air pollution on asthma hospital admissions among children aged 0-17 years (21,462) and adults aged above 18 years (14,562). The study found that there is no significant association with 10 units increase in $PM_{2.5}$ (OR: 1.14; 95% CI: 0.997-1.303) and NO_2 (OR: 1.003; 95% CI: 0.952- 1.055) with the asthma admission in hospital for any of the age groups in multi-pollutant models (34).

A survey (2007-2010) was conducted among adults over 50 years of age in six low- and middle-income countries (China, India, Ghana, Mexico, Russia, and South Africa). A total of 29,249 participants were recruited from an ongoing cohort study on global Ageing and adult health (SAGE). Among the participants, 4553 (16%) were identified with asthma. The study showed a linear relationship between exposure to $PM_{2.5}$ and asthma development. In the multivariate model, the crude prevalence ratio was 1.03 (95% CI: 1.00-1.06), and the adjusted prevalence ratio (PR) remained significant after adjusting for potential confounders (adjusted PR: 1.05; 95% CI: 1.01-1.08). The study showed that sex and smoking status were important effect modifiers, but age and alcohol consumption were not significant effect modifiers. The association between $PM_{2.5}$ and asthma was stronger among males (PR :1.09; 95% CI: 1.04-1.14) than females (PR : 1.01; 95% CI: 0.97- 1.06) and was stronger among smokers (PR : 1.07; 95% CI: 1.02, 1.13) than non-smokers (PR : 1.01; 95% CI: 0.9-, 1.05) (35).

A cross -sectional study conducted in Southern Sweden (2000) among 9,391 participants aged 18-77 years showed that living within 100 m of a road with >10 cars/minute (compared with having no heavy road within this distance) was associated with prevalence of asthma diagnosis (OR : 1.40; 95% CI: 1.04–1.89), and with asthma symptoms. Annual average NO_2 was associated with symptoms of asthma but for only >19 $\mu g/m^3$ and not with asthma

diagnosis (36). A cross-sectional study (2014-2015) entitled "Study of the health impact of oil shale sector-SOHOS" was conducted among the residents aged 18-70 years of three different counties of Estonia: Ida-Viru (2,097 participants), Lääne-Viru (403 participants) and Tartu (2,750 participants). The study showed that people living in region Ida Viru with higher levels of $PM_{2.5}$ had significantly higher odds (p-value < 0.05) of experiencing chest tightness (OR: 1.13; 95% CI 1.02–1.26), shortness of breath (OR:1.16; 95% CI: 1.03–1.31) and an asthma attack (OR:1.22; 95% CI: 1.04–1.42) (37).

It was found in a 20-year cohort study of children born between January 1984 and March 1990 in the city of Espoo, Finland, that if both parents were asthmatic, the risk of the child developing asthma was significantly higher throughout the study period. Females with maternal asthma had hazard ratios ranging from HRa0–6 2.40 to HRa0–27 1.74. Males with maternal asthma had hazard ratios greater than females: HRa0–6 3.24 to HRa0–27 2.18. Regarding paternal asthma, however, the sex differences were reversed, as females had a higher risk of developing asthma if their fathers had asthma (females, from HRa0–6 3..18 to $HR^{a}_{0–27}$ 2.63 vs. males, from $HR^{a}_{0–6}$ 2.10 to $HR^{a}_{0–27}$ 1.61) (38).

A meta-analysis that was performed to compare the effect of maternal asthma vs. paternal asthma on offspring asthma which screened the medical literature from 1966 to 2009 and included 33 studies, showed that children with asthmatic mothers are more likely to develop asthma than children of non-asthmatic mothers (OR: 3.04; 95% CI: 2.59–3.56). Children with asthmatic fathers are more likely to develop asthma than those of non-asthmatic fathers (OR: 2.44; 95% CI: 2.14–2.79). Maternal asthma has a greater risk of disease than paternal asthma (3.04 vs. 2.44, p = 0.037) (39).

A study in five area of Spain among the general population aged (20-44) yrs showed that women were at less risk of current asthma beginning after the age of 15 yrs (OR:0.46, 95% CI:0.29–0.49) while parental asthma(OR:4.53; 95%CI: 2.46–8.36) has significant association with current asthma (37).

A study in adults aged 20-65 years old in Khuzestan province, Iran, showed that having a family history of asthma (OR:2.88; CI % 95:2.23-3.71) was the potential risk factor for adult asthma (40).

A cohort study of 1,191 individuals, aged 20–44 years, who participated in baseline interviews showed, significant risk factors for the incidence of asthma were female gender (OR: 4.76; 95% CI:1.40-16.15), and maternal asthma (OR: 7.24; 95% CI: 1.66-31.55) (41).

All the selected studies in this literature overview are also presented in Table 1 and show a probable link between asthma incidence and exposure to various pollutant metrics like NO_2, $PM_{2.5}$, and PM_{10}. However, the described selection of literature shows a large variety of included exposure metrics, the definition of exposure time, methods of exposure calculations, and outcome definitions. Furthermore, the overview reveals that no studies have studied continuous air pollution exposure for as many as 20 years. To truly understand how long-term exposure to air pollution throughout adulthood affects health, there is a need to follow subjects in the same cohort for a prolonged period.

Existing studies reporting long-term effects of air pollution rarely exceed 10 years of follow-up time, and no studies have so far studied air pollution exposure for as much as 20 years in the same cohort. Although some claim to address the "lifelong impact of air pollution," the scientific evidence is made up of numerous studies covering separate time windows rather than continuous exposure throughout the lifespan. To fully understand how lifelong pollution exposure affects health, we must follow the same cohort of subjects for a prolonged period of time.

Table.1 Overview of selected studies

First author, year, country (reference number)	Name of journal/URL	Design	Study population/ sample size	Exposure/Follow-up time	Outcome	Adjusted covariates	Results
B Jacquemin et.al 2015, Europe (22)	Environment health perspectives (https://ehp.niehs.nih.gov/doi/10.1289/ehp.1408206)	Cohort	Adult population (mean age at baseline =42), n=23,704	NO_2, PM_{10}, and $PM_{2.5}$ /10-year period	Asthma incidence	Age, sex, overweight, education, and smoking and included city/area	Positive associations of borderline significance were observed for nitrogen dioxide [adjusted odds ratio (OR) = 1.10; 95% CI: 0.99, 1.21 per 10 µg/m3; p = 0.10] and nitrogen oxides (adjusted OR = 1.04; 95% CI: 0.99, 1.08 per 20 µg/m3; p = 0.08). Nonsignificant positive associations were estimated for PM_{10} (adjusted OR = 1.04; 95% CI: 0.88, 1.23 per 10 µg/m3), PM2.5 (adjusted OR = 1.04; 95% CI: 0.88, 1.23 per 5 µg/m3), $PM_{2.5}$absorbance (adjusted OR = 1.06; 95% CI: 0.95, 1.19 per 10–5/m)
B Jacquemin et.al, 2009, Europe (25)	Epidemiology (https://journals.lww.com/epidem/Fulltext/2009/01000/Home_Outdoor_NO2_and_New_Onset_of_Self_Reported.20.aspx)	Cohort	Adults (20-44), n=4,185	Home Outdoor NO_2 /1991-2001	Onset of asthma	sex, age, socioeconomic status, atopy, family history of asthma or atopy and smoking	Positive association between NO_2 and asthma incidence OR:1.43,(95% CI:1.02 - 2.01) per 10 µg/m^3. Results were homogeneous among centers (p value = 0.59).
N Künzli , et.al 2009,Switzerland (23)	BMJ https://thorax.bmj.com/content/64/8/6	Cohort	Adults (18–60 years), n=5,734	Traffic pollutant particulate matter 10 (TPM_{10}) / 1991-	Onset /incidence asthma	Education, workplace exposure, passive smoking, parental asthma	Of 2,725 never-smokers, 41 reported asthmas onsets in 2002. Home outdoor

	64.long		participants	2002		or allergies, random area effects, lung function or co-pollutants such as regional, secondary, total PM10 or proximity to busy roads	TPM_{10} concentrations improved during the interval (mean −0.6; range −9 to +7.2; IQR 0.6 $\mu g/m^3$). The incidence of asthma was associated with a change in TPM_{10}. The hazard ratio (1.30; 95% CI: 1.05 to 1.61) per 1 $\mu g/m^3$ change in TPM_{10} (IQR) was not sensitive to further adjustments (education, workplace exposure, passive smoking, parental asthma or allergies, random area effects, lung function or co-pollutants such as regional, secondary, total PM_{10} or proximity to busy roads).
S.Fasola et.al 2020, Italy (24)	Int J Environ Res PublicHealth (https://www.mdpi.com/1660-4601/17/7/2540)	Cohort	Subjects with mean age 47.6 years at the initial interview (n = 305)	PM_{10} and $PM_{2.5}$ /1991-2011	Incidence of respiratory disease (asthma, rhinitis, chronic phlegm, COPD)	Age, smoking, occupational exposure	Incidences of rhinitis and chronic phlegm were associated with increasing PM2.5: OR = 2.25 (95% CI: 1.07, 4.98) per unit increase (p.u.i.) and OR = 4.17 (1.12, 18.71) p.u.i., respectively. Incidence of chronic obstructive pulmonary disease was associated with PM10: OR = 2.96 (1.50, 7.15) p.u.i.,low incidence of asthma restricted from providing reliable estimate(4/284=1.4%)
L.Modig,et.al 2009, Sweden (26)	European Respiratory Journal (https://erj.ersjournals.com/content/33/6/1261.long	Cohort	Adults (20-44&n= 10,800)	NO_2 /8 years	Onset and incident asthma	Body mass index (BMI), sex, age, smoking, water damage or mould in the home at any time during the last 8 years, and city, Socioeconomic index (SEI)	There was a positive association between asthma onset (odds ratio (OR) per 10 μg·m−3 1.46, 95% CI: 1.07–1.99) and incident asthma (OR per 10 μg·m−3 1.54, 95% CI:1.00–2.36) and the levels of nitrogen dioxide (NO2),

B. Jacquemin et.al 2009 (27)	The European respiratory journal (https://erj.ersjournals.com/content/erj/34/4/834.full.pdf	Cohort	Adult (20-44) n=4394	NO_2 /1991-2002	Asthma score	sex, age, social class	association between NO_2 concentration and asthma incidence was similar but slightly stronger among men OR:1.32(95 % CI: 1 1.12-1.56) than among women (OR:1.14 (95%CI :0.97-1.34; *p*-value 0.13-
MT.Young et.al, 2014, United States (29)	American Journal Respiratory Critical Care Medicine(https://www.atsjournals.org/doi/10.1164/rccm.201403-0525OC)	Cohort	Mean age 55.1yrs & n=50,884	$PM_{2.5}$ and NO_2 /2008-2012	Incident asthma	Age, BMI, race, education, occupational exposure to dust,smoking,dietary fiber, healthcare coverage	For an interquartile range (IQR) difference (3.6 µg/m3) in estimated $PM_{2.5}$ exposure, the adjusted odds ratio (adjusted OR) was 1.20 (95% CI: 0.99–1.46, p-value :0.063) for incident asthma and 1.14 (95% CI: 1.04–1.26, P-value: 0.008) for incident wheeze. For NO2, there was evidence for an association with incident wheeze (adjusted OR = 1.08, 95% CI :1.00–1.17, P = 0.048 per IQR of 5.8 ppb). Neither pollutant was significantly associated with incident cough ($PM_{2.5}$: adjusted OR:0.95, 95% CI: 0.88–1.03, p-value 0.194; NO2: adjusted OR: 1.00, 95% CI: 0.93–1.07, P value: 0.939).
S.Shin,et.al 2019, Canada (30)	American Journal Respiratory Critical Care Medicine (https://www.atsjournals.org/doi/10.1164/rccm.201909-1744OC)	Cohort	Adults aged 35-85 years, women (n= 5.1 million)	$PM_{2.5,}$ NO_2 and O_3 /2001-2015	Incident chronic obstructive pulmonary disease (COPD) and adult-onset asthma	Age, sex, province, socio economic status, education, immigration, income, unemployment, comorbidities	We found positive associations of COPD with PM2.5 per interquartile-range (IQR) increase of 3.4µg/m3 (hazard ratio, 1.07; 95% confidence interval, 1.06-1.08), NO2 per 13.9ppb (1.04; 1.02-1.05), O3 per 6.3ppb (1.04; 1.03-1.04), and Ox per 4.4ppb (1.03; 1.03-1.03). By contrast, we did not find strong evidence linking these pollutants to adult-onset asthma.

N.Lazarevic et.al,2015, Australia (31)	Occupational and environmental medicine(https://bmjopen.bmj.com/content/5/10/e008714)	Cross sectional	26 ,991 adult women	NO_2 as proxy for ambient air pollution /2006-2011	Asthma	Age group, body mass index (BMI), smoking status, alcohol intake, physical activity, fruit and vegetable consumption, degree of residential urbanisation or remoteness, annual mean temperature, marital status, educational attainment and self-assessed financial resources.	No associations were observed between any of the outcome and exposure variables considered at the 1% significance level after adjusting for known risk factors and confounders.
Y.Cai et.al, 2017, Europe (32)	European Respiratory Journal (https://erj.ersjournals.com/content/49/1/1502127)	Cross-sectional	646,731 participants aged ≥20	PM_{10} or NO_2 /2006-2013	Lifetime asthma prevalence	Age, sex, body mass index (BMI), education level (primary school or less, secondary school, or post-secondary school or above), paid employment (yes or no), smoking (current smoker, ex-smoker or never-smoker) and years at baseline	PM_{10} or NO_2 higher by 10 $\mu g \cdot m^{-3}$ was associated with 12.8% (95% CI :9.5–16.3%) and 1.9% (95% CI: 1.1–2.8%) higher lifetime asthma prevalence, respectively, independent of confounders. Effects were larger in those aged ≥50 years, ever-smokers and less educated. Noise exposure was not significantly associated with asthma prevalence.
S.Liu et.al 2020, Denmark, Sweden (33)	European Respiratory Journal (https://erj.ersjournals.com/content/early/2020/11/26/13993003.030992020)	Cohort	98,326 adult participants	$PM_{2.5}$ and NO_2	Asthma incidence	Not Available -	They observed associations in fully adjusted models with hazard ratios and 95% confidence intervals of 1.22 (1.04−1.43) per 5 $\mu g \cdot m^{-3}$ for $PM_{2.5}$, 1.17 (1.10−1.25) per 10 $\mu g \cdot m^{-3}$ for NO_2.
K.Chen et.al,2016, Australia (34)	Journal of the British Society for Allergy and Clinical Immunology(https:	Cohort	Children aged 0-17 years (21,462) and adults	$PM_{2.5}$, NO_2, PM_{10} /2003-2013	Asthma hospital admissions	Age, season	The study found that there is no significant association with 10 units increase in $PM_{2.5}$ (OR: 1.14 (95%CI: 0.997-1.303)) and NO_2 (OR: 1.003 (95% CI: 0.952-

	//onlinelibrary.wiley.com/doi/full/10.1111/cea.12795)		aged above 18 years (14,562)				1.055)) with the asthma admission in hospital for any of the age groups in multi-pollutant models.
Ai.Siqi et.al, 2019, China, India, Ghana, Mexico, Russia and South Africa (35)	Environmental research (https://doi.org/10.1016/j.envres.2018.09.028/)	Cross-sectional	29,249 adults over 50 years of age	$PM_{2.5}$ /Three-year average concentration before survey period (2007-2010)	Asthma	Sex, age, BMI, education attainment, smoking status, alcohol consumption, and occupational exposure	A total of 4553 asthma patients were identified among the 29,249 participants in this study, producing a prevalence of 15.57%. For each 10 $\mu g/m^3$ increase in $PM_{2.5}$, the adjusted prevalence ratio of asthma was 1.05 (95% CI: 1.0-1.08) Further analyses showed that males and smokers might be particularly vulnerable populations. Additionally, it was estimated that about 5.12% of the asthma cases in the study population (95% CI: 1.44%-9.23%) could be attributed to long-term $PM_{2.5}$ exposure. The association between $PM_{2.5}$ and asthma was stronger among males (PR - 1.09; 95% CI: 1.04-1.14) than females (PR : 1.01; 95% CI: 0.97- 1.06) and was stronger among smokers (PR - 1.07; 95% CI: 1.02, 1.13) than non-smokers (PR : 1.01; 95% CI: 0.9-, 1.05)
A.Lindgren et.al, 2009, Sweden (36)	International Journal of Health Geographics (https://ij-healthgeographics.biomedcentral.com/articles/10.1186/1	Cross-sectional	9,319 individuals aged 18–77	NO_x / 1 year	Asthma	Smoking habits and occupation, and exposures such as living close to a road with heavy traffic	Living within 100 m of a road with >10 cars/minute (compared with having no heavy road within this distance) was associated with prevalence of asthma diagnosis (OR : 1.40, 95% CI :1.04–1.89), and COPD

	476-072X-8-2)						diagnosis (OR : 1.64, 95% CI : 1.11–2.4), as well as asthma and chronic bronchitis symptoms.
H.Orru et.al, 2018,Estonia (37)	International journal of environmental research and public health(**https://doi.org/10.3390/ijerph15020252**)	Cross-sectional	2127 individuals aged 18-70 years	PM 2.5	Self-reported health effect in relation to air pollution	Gender, age, body mass index (BMI), environmental tobacco smoke (ETS), smoking history, and income per family member	People living in regions with higher levels of $PM_{2.5}$, had significantly higher odds ($p < 0.05$) of experiencing chest tightness (OR : 1.13, 95% CI 1.02–1.26), shortness of breath (OR:1.16, 95%of CI:1.03–1.31) or an asthma attack (OR:1.22, 95%CI:1.04–1.42) during the previous year.
EM. Paaso et.al,2013,Finland (38)	American Journal Respiratory Critical Care Medicine(https://www.atsjournals.org/doi/full/10.1164/rccm.201212-2236LE)	cohort	2,568 children	Not Available	Not Available	Not Available	Hazard rate ratios related to maternal asthma were from $HR^{a}_{0–6}$ 2.40 to $HR^{a}_{0–27}$ 1.74 for female. Among males, the adjusted hazard rate ratios related to maternal asthma were greater in magnitude: from $HR^{a}_{0–6}$ 3.24 to $HR^{a}_{0–27}$ 2.18.

1.5 Research Question

Is there association between exposure to air pollution 20 years ago and asthma? If yes, is this association still present after adjusting for current exposure?

2. Study Objectives

2.1 General objectives

To investigate if air pollution exposure 20 years ago is associated with increased risk for asthma among adults.

2.2 Specific objectives

- To investigate the association between exposure to $PM_{2.5}$, PM_{10} and NO_2 20 years ago and current asthma in men and women, and in persons with and without a family history of asthma.
- To investigate the association between exposure to $PM_{2.5}$, PM_{10} and NO_2 20 years ago and current asthma severity in men and women, and in person with and without a family history of asthma.

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3. Methods and Methodology

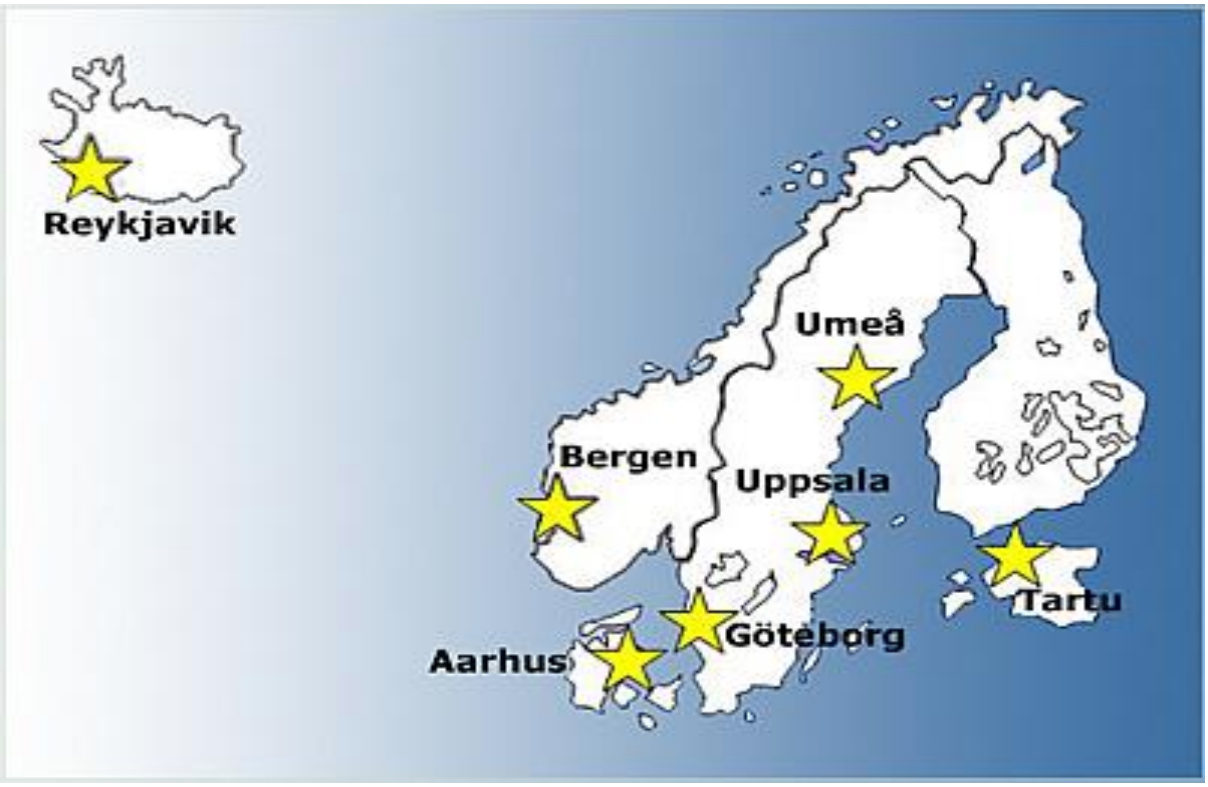
3.1 Study design

The study design is a population-based prospective cohort study. For the study purpose, we used data from Respiratory Health in Northern Europe (RHINE) study collected from 1990 to 2010. In the earlier nineties, European Community Respiratory Health Survey study (ECRHS) was established with a random population sample of young adults of 20–44-year age. ECRHS was conducted on three occasions. ECRHS I in 1990 was a postal survey (stage I) and a clinical investigation of a subsample (stage II). The clinical subsample was followed-up in ECRHSII and ECRHS III. On the other hand, the RHINE study was a questionnaire follow-up in 2000 and 2010 of all subjects from seven Northern European centers that participated in the ECRHS I stage 1. RHINE includes representative populations in Iceland, Denmark, Sweden, Norway, and Estonia. The aim of the RHINE study is to identify the incidence, prevalence, and risk factors for respiratory diseases such as asthma and chronic obstructive pulmonary disease and symptoms related to such diseases (42, 43).

3.2 Study population

The RHINE participants were 20-44 years old when they answered a questionnaire on respiratory symptoms and diseases at baseline in 1990. Further, they have responded to extensive questionnaires in two follow-ups (10 and 20 years after baseline) on respiratory symptoms, diseases, smoking habits, and other lifestyle factors such as physical activity. The participants were from seven different centres: Aarhus (DK); Gothenburg, Umea, and Uppsala (S); Reykjavik (IS); Tartu (EST); and Bergen (N). But in this study, only the participants from Gothenburg, Umea, Uppsala, and Bergen were included as the data for air pollution for the three-time points were only available for these four centres.

3.3 Study center



Source: www.rhine.nu

Figure 2: RHINE study centre

3.4 Sample Size

The study population of 6,193 who have information on both asthma symptoms and exposure to air pollution and registered on sex were included in this study. The detail description of sample size is presented in Figure 3 below.

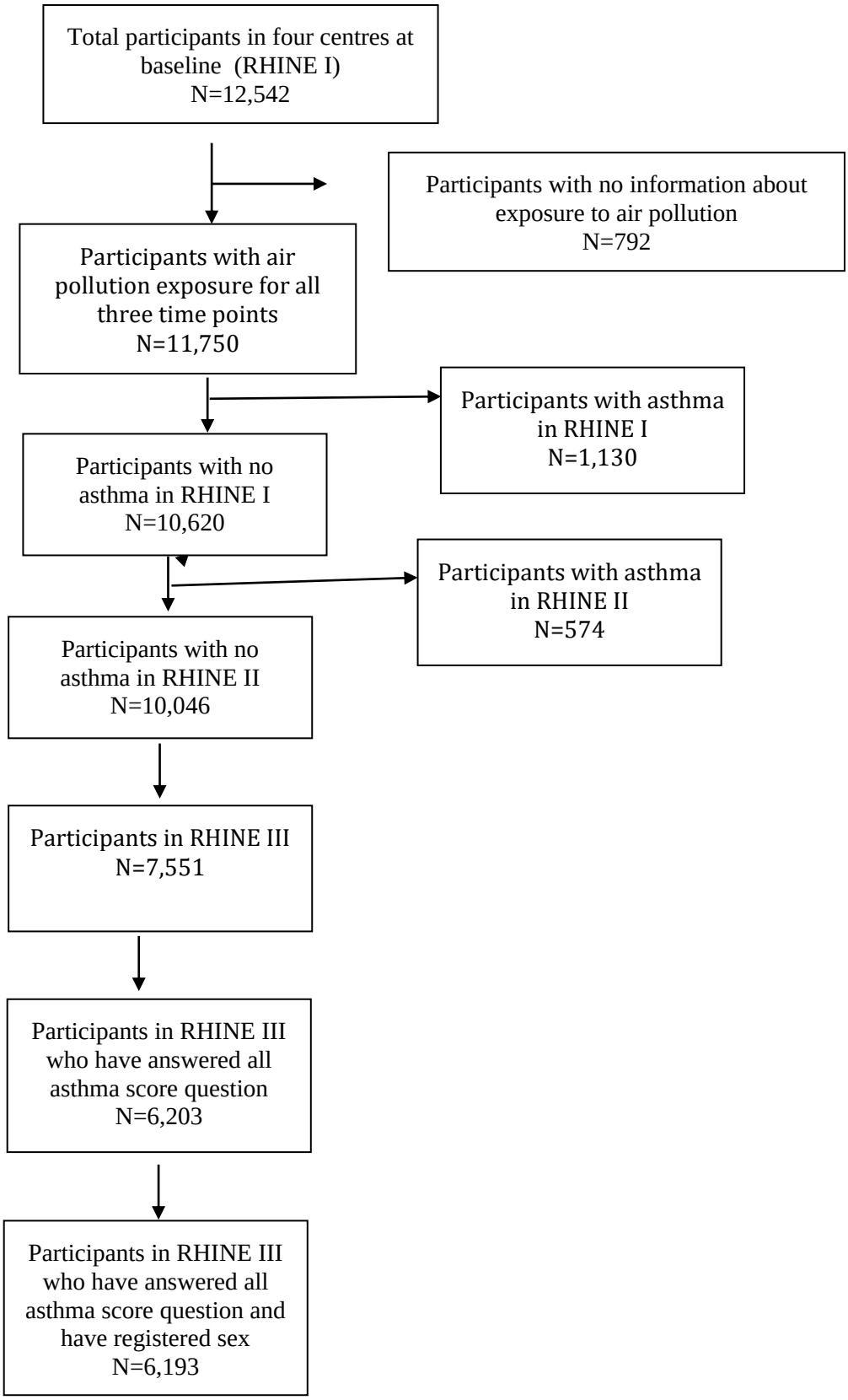


Figure 3: Flow chart presenting selected study population

3.5 Air pollution assignment

The annual mean concentrations for different pollutants (NO_2, $PM_{2.5}$, PM_{10}) in 1990, 2000 and 2010 were assigned to each participant by individual geocoded residential history. Annual mean NO_2 and PM_{10} at home addresses ($\mu g/m^3$) were assigned from the air pollution grid developed by Danielle Vienneau et al (44). Likewise, $PM_{2.5}$ at home address ($\mu g/m^3$) was assigned from the air pollution grid developed by Kees de Hoogh et al (45).Previously developed air pollution raster and Western Europe-wide hybrid land use regression models (LURs) was used for the calculation. LUR models include predictor variables i.e. land use characteristics, population density, and length of roads in zones from 0.1 km to 10 km, altitude, and distance to sea) from geographic information systems (GIS) and routine monitoring of air pollution with satellite derived and chemical transport model estimates. The routine air pollution monitoring data derives from AirBase; the European air quality database which is maintained by the European Environment Agency (EEA).

3.6 Study analysis

3.6.1 Exposure definitions

Individual residential exposures for $PM_{2.5}$, PM_{10}, and NO_2 in 1990, 2000, and 2010 were assigned based on the participants' residential addresses at each time point and were used as exposure variables.

3.6.2 Outcome definitions

Asthma symptoms and asthma severity were the primary outcome variables of the study. Asthma symptoms were defined as a positive answer to at least one of the following questions "Have you had wheezing or whistling in your chest at any time in the last 12 months? Have you woken up with a feeling of tightness in your chest at any time in the last 12 months? Have you had a wheeze with breathlessness at any time in the last 12 months? Have you been woken by an attack of coughing at any time in the last 12 months? Have you been woken by nocturnal breathlessness at any time in the last 12 months? "Based on these five questions, a new variable, "asthma symptom score", was also calculated (0-5 range score, based on the number of symptoms). The symptom score variable was further dichotomized into a severity variable, i.e., if symptoms score <= 2 then the symptoms score was considered as zero to low asthma severity, and if symptoms score>= 3 then symptoms score was considered as medium to high asthma severity. Asthma score is a good predictor of

asthma outcomes and also has a good ability to identify risk factors (46). This encourages us to use the asthma symptoms score as a measure of asthma in epidemiological studies of asthma (46). The five symptoms in the asthma symptoms score are well suited to look at the active burden of asthma because they are very common in asthmatics, and they represent a burden of disease in everyday life.

3.6.3 Confounders

A confounder is defined as having an association with both the exposure and the outcome, preceding them both in time and not being in the causal pathway between the exposure and outcome (47). All the covariates of age, sex, education, study center, parental asthma, smoking status, childhood respiratory infections, occupational exposures, body mass index, and physical activity were evaluated as potential confounders using Directed Acyclic Graphs (DAGs). In this thesis, for analysing associations between exposure to air pollution twenty years ago and asthma, only socio-economic status and study centre were identified as confounders in the DAG (Figure 4). They are associated with both the exposure and the outcome, preceding them both in time without lying in the causal pathway between them.

Even though sex and parental asthma are not confounders, we performed stratified analyses for men and women, and for participants with and without parental asthma history.

There is a difference in the prevalence and severity of asthma between males and females at different ages. A higher prevalence of asthma is found in boys during childhood, but in adulthood, the prevalence and severity are higher among women. In the case of females, differences in sex hormones during puberty, menstrual cycle, and pregnancy are also associated with asthma pathogenesis (48). Underlying mechanisms of asthma onset and progression is different for male and female, and there is reason to believe that women with their smaller lungs are more susceptible to inhalation of harmful agents than men (49). Due to these factors, we performed stratified analysis by sex.

Further, we stratified the analysis by parental asthma to understand if pollution vulnerability with regard to the development of own asthma outcome is different for those with the genetic predisposition for asthma(parental asthma) and those without a genetic predisposition for asthma (no parental asthma).A family history of asthma has long been known, and children of asthmatic parents are more likely to develop asthma. For example, the risk of having asthma in offspring with one parent having an asthma history is around 25%, and with both parents having asthma is around 50% (50). However, asthma cannot be explained only due to a single

mutation in a gene, and its transformation between the generations also does not follow the patterns of Mendelian inheritance. Asthma is polygenic and a multifactorial disorder (50). Some types of asthma are more hereditary than others with early onset or severe asthma and are more likely than individuals with mild or late-onset asthma to have a family history of asthma (51, 52).

Twin studies also showed that if genetically close relatives have asthma, one is more at risk for asthma. The risk of developing asthma is much higher in monozygotic twins than in dizygotic. A population-based study of Finnish twins and their parents showed that the heritability of asthma was approximately 79%, while 21% was due to unique environmental factors (53).

Many research had found that both maternal and paternal histories of asthma are associated with an increased risk of asthma in offspring (54). he National Health and Nutrition Examination Survey study conducted in the USA found that children with maternal history have increased asthma risk (hazard ratio of 3.71, 95% CI: 1.19–11.60) compared to those without maternal asthma history, while paternal history had a relatively more minor effect that may be only detectable in larger samples (hazard ratio of 2.17, 95% CI: 0.69–6.79) (55).

In 2010, a meta-analysis of 33 studies found that asthmatic mothers' children were more likely to develop asthma than non-asthmatic mothers' children (OR:3.04; 95% CI:59–3.56)). Likewise, children of asthmatic fathers are more likely to develop asthma than those of non-asthmatic fathers (OR: 2.44; 95% CI: 2.14–2.79) (39).

3.6.4 DAG model for air pollution and asthma

Based on knowledge from previous literature, the following asthma risk factors were discussed as potential confounders: age, sex, study center, childhood respiratory infections, socioeconomic status, occupational exposure to dust or gas, smoking, physical activity level, body mass index (BMI), birth weight, and parental asthma (47). Directed Acyclic Graphs (DAGs) was used to identify which of these are true confounders that need to be adjusted for in the multivariate model. The DAG offers a unified framework for researchers to provide a systematic representation and analysis of causal inference in epidemiology. A DAG is thus a presentation about the relationships between variables (56).When drawing the DAGs, I used the free online software on www.dagitty.net.

In this thesis, only socioeconomic status and study centres were identified as confounders in the DAG for analyzing associations between exposure to air pollution twenty years ago and asthma. They are associated with the exposure and the outcome without lying in the causal pathway between them. Socioeconomic status is a confounder because it affects where the person chooses to live or where they can afford to live, and therefore determines the exposure to air pollution. Education was used as the proxy for socioeconomic status. The study centre is a potential confounder since the level of air pollutants can be different in each study centre. The number of people with asthma in various study centers could also be different due to factors other than air pollutants, such as allergens.

In addition to the identified confounders, we want to look at associations between outcomes and smoking / physical activity/sex (in the analyses stratified by parental asthma) / parental asthma (in the analyses stratified by sex) to know whether the magnitude of these associations was like the magnitude of the primary exposures in this thesis (pollution). Therefore, all these covariates were included in univariate analyses, and if they are significant, they are also included in the multivariate analyses.

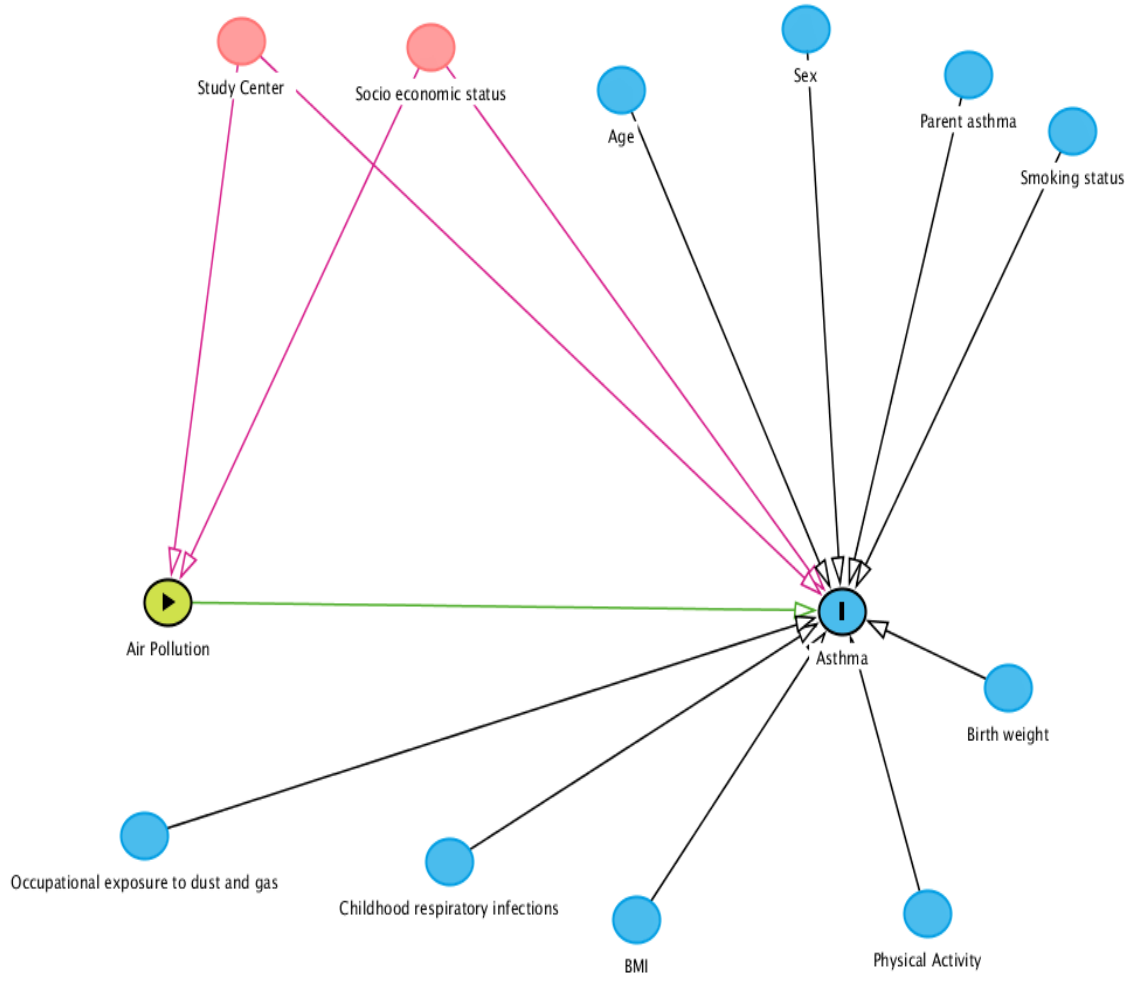


Figure 4: Direct acyclic graph linking Air pollution and Asthma

3.6.5 Statistical analysis description

3.6.5.1 Descriptive analysis

Descriptive data are summarized in percentage and mean with p-value. The percentage was used to describe categorical variables, while the mean was used to describe continuous variables. Pearson's chi-square test was used to calculate p-value for categorical variables, and the independent sample T-test was used to calculate the p-value for continuous variables.

3.6.5.2 Logistic regression

A logistic regression analyses the relationship between multiple independent variables and a categorical dependent variable by fitting data to a logistic curve. There are two logistic regression models, binary logistic regression and multinomial logistic regression. In binary logistic regression, the dependent variable is dichotomous, while the independent variables are either continuous or categorical. A multinomial logistic regression can be used when the dependent variable has more than two categories and is not dichotomous (57). This thesis used univariate and multivariate binary logistic regression models to estimate the odds ratio (OR) with 95% confidence intervals (CI) for the association between asthma symptoms and 20-year exposure to air pollutants. Univariate analyses were performed with each potential covariate identified by DAGs and outcome. Significant potential predictors from the univariate analyses were included in the multivariate logistic regression analyses.

Odds ratio is a measure of the association between exposures and outcomes. It tells about how the presence or absence of exposure (air pollutants) affects the presence or absence of outcome (asthma) in a population (58). ORs are calculated using two-by-two frequency tables: a = exposed to air pollutants with asthma, b = exposed to air pollutants without asthma, c = non-exposed to air pollutants with asthma, d = non-exposed to air pollutants without asthma, where OR = ad /bc. An OR = 1 reflects that exposure does not affect the outcome. The higher the OR, and if the 95% CI does not include 1, the greater the odds of the outcome being associated with the exposure. An OR <1 indicates that the exposed have a lower odd of the outcome. Confidence intervals represent the range of values most likely to cover the true but unknown parameter of the population. 95% CI means that we are 95 % confident that the true population parameter lies between the lower and upper limit values. The 95% CI gives an estimation of the precision of the OR. A wider CI indicates a low level of precision of the OR, whereas a narrow CI indicates a higher precision of the OR (58).

On the other hand, associations between asthma symptoms score and exposure variables were analyzed by negative binominal regression with a relative risk (RR). In cases of over-dispersed count data, where the conditional variance exceeds the conditional mean, negative binomial regression can be used (59). As Figure 5 shows, asthma score in our study population was over-dispersed with excess zeros. The relative risk is defined as the probability of an event occurring in the exposed group compared to the non-exposed group. Relative Risk = 1 means no risk difference between the two groups. Relative risk >1 means

there's a higher risk of the disease or event occurring in the exposed group than in the non-exposed group, and a relative risk < 1 means the risk is lower. (60).

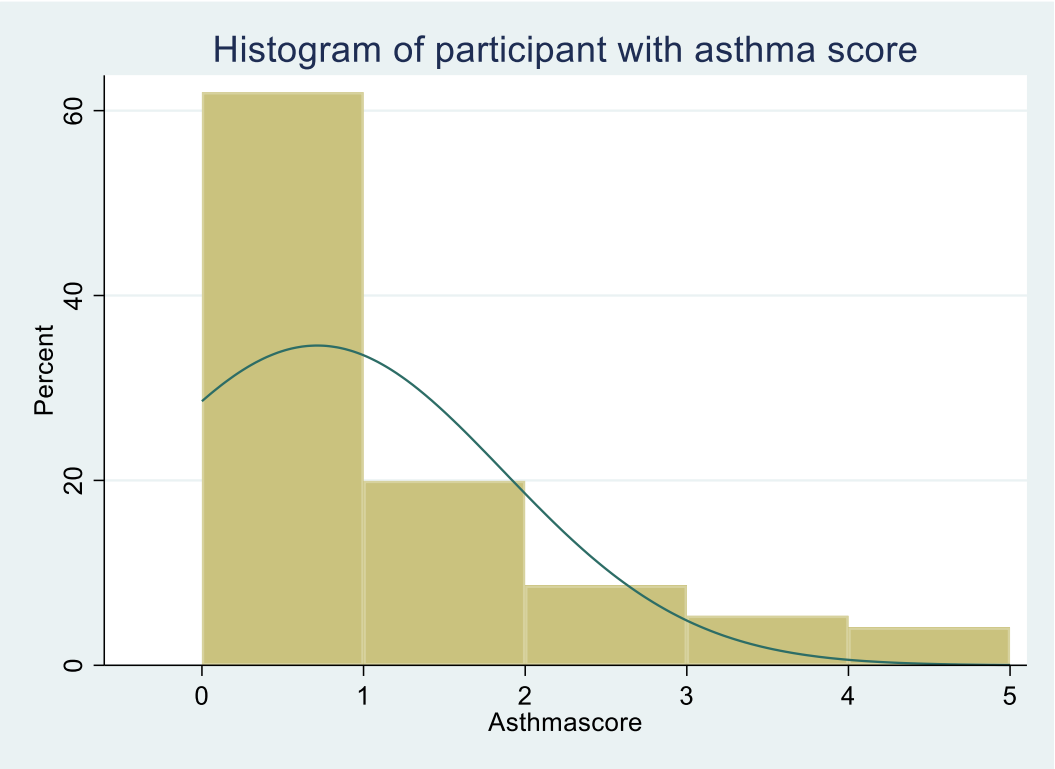


Figure 5 : Participants with asthma symptoms score

In this study, 20-year exposure is the main predictor, but 10-year exposure and current exposure were also included in multivariate logistic regression and negative binomial regression models to identify which of these is the most important predictor of asthma. For each exposure, we ran separate logistic regression models, i.e., one for NO_2, one for PM_{10}, and one for $PM_{2.5}$. All statistical analyses were conducted using STATA version 16.1.

4. Results

Table 2: Characteristic of 6,193 participants from the Respiratory Health in Northern Europe (RHINE) study, means (standard deviation) for continuous variables, and counts (%) for categorical variables.

	Male n= 3,015 n=(%)	**Female n= 3,178 n=(%)**	**p-value[1]**	**Total (n=6,193 n=(%)**
Age mean (std.dev)	52.2(7.15)	51.9(7.24)	**0.04[2]**	52.0 (7.19)
Prevalence of Asthma symptoms score[3]			**<0.001**	
0	2,121(70.4)	2,081(65.5)		4,202(67.9)
1	537(17.8)	710(22.3)		1,247(20.1)
2	220(7.3)	202(6.4)		422(6.8)
3	80(2.7)	121(3.8)		201(3.2)
4	34(1.1)	37(1.2)		71(1.2)
5	23(0.7)	27 (0.8)		50(0.8)
Asthma severity[4]			**0.02**	
Low	2,878(95.5)	2,993(94.2)		5,871(94.8)
High	137(4.5)	185(5.8)		322(5.2)
Smoke Status[5]			**0.06**	
Never-smoker	1,593(54.0)	1,595(51.5)		3,188(52.7)
Ex-smoker	935(31.6)	1,064(34.3)		1,999(33.0)
Current smoker	426(14.4)	439(14.2)		865(14.3)
Parental Asthma			**0.01**	
No	2,674(88.7)	2,756(86.7)		5,430(87.7)
Yes	341(11.3)	422(13.3)		763 (12.3)
Education level[6]			**<0.001**	
Primary school	347 (11.5)	330 (10.4)		677(11.0)
Lower secondary	1,350(44.9)	1,244(39.3)		2,594(42.0)
College & university	1,310(43.6)	1,591(50.3)		2,901(47.0)
Physical Activity[7]			**<0.001**	
Never or once a week	1,332(44.4)	1,055(33.4)		2,387(38.7)
2-3 times in a week	1,127 (37.6)	1,285(40.7)		2,412 (39.2)
Everyday	539(18.0)	819(25.9)		1,358(22.1)

[1] Pearson's Chi-squared test
[2] P-value from Independent sample T test
[3] Asthma symptoms score(0-5) is computed by combining five main symptoms of asthma i.e. wheezing, chest tightness, wheezing with breathlessness, nocturnal breathlessness and nocturnal cough.
[4] Asthma severity variable is generated first by creating asthma symptoms score i.e. by combining wheezing, chest tightness, wheezing with breathlessness, nocturnal breathlessness and nocturnal cough, and then recoding asthma symptoms score <=2 as 0 (low severity) and asthma score>=3 as 1 (high severity).
[5] Missing value :Smoking status=141
[6] Missing value :,education =21
[7] Missing value : Physical activity=36

The above table presents the general characteristics of participants with sex differences. The results show significant differences between men and women in all characteristics, except for smoking status, where the difference was borderline significant.

The mean age of participants was 52 years. Only 4.5% of males had high asthma severity, while for females, it was 5.8%. The result showed that 2.5% more male participants were never smokers compared to females, while the percentage of current smokers was almost similar for both sex. Female participants have 2% more parent asthma history as compared to male participants. More male participants than female participants have lower education levels. At the same time, more female participants with college and university education than males. More female participants were found to involve in physical activity than males.

Table 3 : Univariate logistic regression analysis of asthma severity expressed with odd ratios with 95% CI in relation to air pollutants(NO_2, PM_{10} & $PM_{2.5}$)[8], study centre, smoking, parental asthma, educational level, and physical activity, stratified by sex in N=6, 193 participants from the RHINE study.

Asthma severity[9]	**Male**		**Female**	
	OR (95% CI)	**p**	**OR (95% CI)**	**p**
Centre				
Bergen	1			
Gothenburg	0.90 (0.58, 1.39)	0.64	1.14 (0.76, 1.71)	0.51
Umea	0.71 (0.45, 1.10)	0.13	0.95(0.63, 1.44)	0.84
Uppsala	**0.33(0.19, 0.59)**	**<0.001**	0.75 (0.49, 1.15)	0.19
Smoke status				
Never-smoker[10]	1		1	
Ex-smoker	**1.51(1.00, 2.27)**	**0.04**	1.05 (0.73, 1.50)	0.77
Current smoker	**3.05(1.99, 4.68)**	**<0.001**	**2.36 (1.61, 3.45)**	**<0.001**
Parental Asthma				
No[11]	1			
Yes	**2.49.(1.65, 3.76)**	**<0.001**	**1.80 (1.25,2.61)**	**0.002**
Education level				
College and university[12]	1		1	
Lower secondary	1.31(0.91, 1.90)	0.14	**1.57(1.14, 2.16)**	**0.005**
Primary school	1.34(0.77, 2.33)	0.28	1.30 (0.78, 2.16)	0.30
Physical Activity				
Everyday[13]	1		1	
2-3 times in a week	0.68(0.41, 1.12)	0.13	0.86 (0.58, 1.28)	0.17
Never or once in a week	1.05(0.66, 1.66)	0.81	1.30 (0.89, 1.89)	0.47
NO_2 in 1990	**1.02(1.00, 1.04)**	**0.04**	1.00(0.98, 1.02)	0.54
NO_2 in 2000	1.01(0.98, 1.03)	0.34	1.00(0.98, 1.02)	0.37
NO_2 in 2010	0.99(0.97, 1.02)	0.88	1.00(0.98, 1.02)	0.58
PM_{10} in 1990	1.02(0.95, 1.08)	0.54	1.02(0.96, 1.08)	0.48
PM_{10} in 2000	1.02(0.95, 1.09)	0.47	1.01(0.95, 1.07)	0.66
PM_{10} in 2010	1.00(094, 1.08)	0.78	1.02(0.96, 1.08)	0.48
$PM_{2.5}$ in 1990	1.00(0.94, 1.08)	0.79	1.01(0.95, 1.07)	0.71
$PM_{2.5}$ in 2000	0.98(0.92, 1.05)	0.74	1.00(0.94, 1.06)	0.79
$PM_{2.5}$ in 2010	0.97(0.91, 1.04)	0.49	0.99(0.94, 1.05)	0.98

[8] Micrograms per cubic meters ($\mu g/m^3$) measurement unit is used for air pollutant variables

[9] Asthma severity variable is generated first by creating asthma symptoms score i.e. by combining wheezing, chest tightness, wheezing with breathlessness, nocturnal breathlessness and nocturnal cough, and then recoding asthma symptoms score <=2 as 0 (low asthma severity) and asthma symptoms score>=3 as 1 (high asthma severity).

Reference categories:

[10] Never smoker,

[11] No parental asthma

[12] College and university education

[13] Everyday

This table presents the univariate model analysis between asthma severity and other variables like air pollutants, parent asthma, study centre, education, smoking status, and physical activity stratified by sex. The results showed that risk factors for asthma severity for men were being a current smoker, ex-smoker, and having parents with asthma. Also, there were significant positive associations with higher NO_2 exposure in 1990. The risk for asthma severity was less for the male participants from Uppsala than for Bergen.

Males who were current smokers had more risk of having asthma severity as compared to never smokers **(OR:3.05; 95%CI:1.99, 4.68)**. Similarly, males who were ex-smoker also had more risk of having asthma severity as compared to never smokers (**OR:1.51; 95%CI:1.00, 2.27)** . Male participants having parents with asthma history had 2.49 times higher odds of having asthma severity than males with no parental asthma (**95%CI: 1.65, 3.76**). Males who had only secondary and primary levels of education had a tendency for increased risk of developing high asthma severity as compared to those who had college and university level education. Increased exposure to NO_2, 20 years ago increased the risk of asthma severity in males with positive significance (**OR**:**1.02, 95%CI: 1.00, 1.04)**.

The risk factors for asthma severity for women were being current smokers with lower secondary level education and having parents with an asthma history. There was a significant positive association between asthma severity and female participants who were current smokers **(OR:2.36; 95% CI:1.61, 3.45)** having parents with asthma **(OR:1.80; 95%CI: 1.25, 2.61).** Females who had only lower secondary were at more risk of developing asthma severity as compared to those who have college and university-level education **(OR**:**1.57; 95%CI:1.14, 2.16).**

Table 4: Multivariate logistic regression analysis of association between study centre, parent asthma, educational level, smoking status, air pollutants (No_2 ,Pm_{10}& $Pm_{2.5}$)[14]and asthma severity[15] expressed with odd ratios with 95%confidence interval and stratified by sex in N=6,193 participants from the RHINE study

Asthma severity	Male		Female	
	OR (95% CI)	**p**	**OR (95% CI)**	**p**
Centre				
Bergen	1			
Gothenburg	0.89(0.52, 1.51)	0.67	1.32(0.82, 2.12)	0.24
Umea	1.00(0.57, 1.78)	0.97	1.03(0.62, 1.71)	0.90
Uppsala	**0.39(0.21, 0.70)**	**0.002**	0.86(0.54, 1.36)	0.52
Parental Asthma				
No	1			
Yes	**2.44(1.60, 3.74)**	**<0.001**	**1.95(1.33, 2.85)**	**0.001**
Education level				
College and university	1		1	
Lower secondary	1.09(0.58 ,1.89)	0.86	**1.31(0.93, 1.83)**	**0.11**
Primary school	1.05(0.73, 1.62)	0.65	0.92(0.52, 1.61)	0.77
Smoke Status				
Never-smoker	1		1	
Ex-smoker	1.39(0.91,2.11)	0.12	1.00(0.70, 1.45)	0.96
Current smoker	**2.70(1.71, 4.27)**	**<0.001**	**2.29(1.54, 3.40)**	**<0.001**
NO_2 in 1990	**1.04(1.00, 1.07)**	**0.04**	0.98(0.94,1.01)	0.27
NO_2 in 2000	0.99(0.96, 1.02)	0.95	1.00(0.97, 1.04)	0.67
NO_2 in 2010	0.97(0.95, 1.01)	0.18	1.00(0.97, 1.03)	0.83
PM_{10} in 1990	1.04(0.90, 1.20)	0.57	0.98(0.86, 1.11)	0.77
PM_{10} in 2000	1.05(0.91, 1.22)	0.45	0.96(0.84, 1.10)	0.37
PM_{10} in 2010	0.94(0.81, 1.18)	0.40	1.06(0.94, 1.19)	0.60
$PM_{2.5}$ in 1990	1.13(0.96, 1.33)	0.11	0.97(0.84, 1.11)	0.71
$PM_{2.5}$ in 2000	0.94(0.81, 1.09)	0.46	1.02(0.89, 1.16)	0.77
$PM_{2.5}$ in 2010	0.95(0.84, 1.08)	0.51	0.98(0.87, 1.10)	0.76

This table shows the multivariate analysis that is adjusted for covariates that were significant in the univariate analyses: study centre, parental asthma, education, smoking status, and air pollutants (NO_2, PM_{10}, and $PM_{2.5}$) and stratified by sex. Even after adjusting for each other,

[14] All three time points are included in the same model, but each pollutant has its own model (three PM_{10} in one model, three NO_2 in one model, three $PM_{2.5}$ in one model). Measurement unit for the air pollutants is micrograms per cubic meters (µg/m3)

[15] Asthma severity variable is generated first by creating asthma symptoms score i.e. by combining wheezing, chest tightness, wheezing with breathlessness, nocturnal breathlessness and nocturnal cough, and then recoding asthma symptoms score <=2 as 0 (low asthma severity) and asthma symptoms score>=3 as 1 (high asthma severity).

current smokers, having parental asthma, and increasing exposure to NO_2 20 years ago remained significant risk factors for asthma severity among male participants. The male participants who live in Uppsala had less risk of asthma severity than Bergen. For males, even after the adjustment with the other variables in the model ,significant association was found between asthma severity and current smoker **(OR:2.70; 95% CI: 1.71,4.27),** having parental asthma (**OR:2.44; 95%CI: 1.60, 3.74**) and exposure to NO_2 (**OR: 1.04; 95% CI:1.00, 1.07) 20 years ago.** For females, the results showed that being a current smoker and having a parental asthma history were still risk factors for asthma severity, even after adjusting for the other variables in the model. There was a significant positive association between asthma severity and female who were current smokers (**OR:2.29;95%CI:1.54, 3.40)** and have parental asthma **(OR: 1.95; 95% CI:1.33, 2.85)**. The significant association between asthma severity and female with lower secondary education disappeared after adjustment. No significant associations were observed between the air pollutants and asthma severity in females.

Table 5:Univariate logistic regression analysis between between air pollutants (NO_2,PM_{10}&$PM_{2.5}$)[16], smoking, sex, educational level, physical activity and asthma severity[17] expressed with odd ratios with 95% confidence interval and stratified by parental asthma in N=6,193 participants from the RHINE study

Asthma Severity	**Yes parent asthma**		**No parent asthma**	
	OR (95% CI)	**p**	**OR (95% CI)**	**p**
Centre				
Bergen	1		1	
Gothenburg	0.95(0.48 ,1.86)	0.89	1.07(0.76, 1.49)	0.68
Umea	0.67(0.34, 1.32)	0.25	0.89(0.64, 1.25)	0.53
Uppsala	0.70(0.36, 1.37)	0.30	**0.50(0.34, 0.75)**	**0.001**
Sex				
Female	1		1	
Male	1.010.62, 1.66)	0.94	**0.73(0.57 ,0.95)**	**0.01**
Smoke Status				
Never-smoker	1		1	
Ex-smoker	1.52(0.88, 2.65)	0.13	1.15(0.84, 1.56)	0.37
Current smoker	1.80(0.91, 3.55)	0.08	**2.85(2.08, 3.90)**	**<0.001**
Education level				
College & university	1		1	
Lower secondary	1.26(0.75, 2.12)	0.37	**1.48(1.13, 1.94)**	**0.004**
Primary school	0.95(0.40, 2.24)	0.90	1.40(0.92, 2.12**)**	0.92
Physical Activity				
Everyday	1		1	
2-3 times in a week	0.68(0.41, 1.12)	0.13	0.86(0.58, 1.28)	0.47
Never or once a week	1.05(0.66,1.66)	0.81	1.30(0.89, 1,89)	0.17
NO_2 in 1990	1.00(0.97, 1.04)	0.73	**1.01(0.99 1.03)**	**0.07**
NO_2 in 2000	1.01(0.98,1.04)	0.54	1.00(0.99, 1.02)	0.26
NO_2 in 2010	1.00(0.97, 1.03)	0.82	1.00(0.98, 1.01)	0.77
PM_{10} in 1990	1.02(0.92, 1.12)	0.65	1.02(0.97, 1.06)	0.41
PM_{10} in 2000	1.02(0.93, 1.13)	0.57	1.01(0.96, 1,06)	0.50
PM_{10} in 2010	1.04(0.94, 1.15)	0.36	1.01(0.96, 1.06)	0.68
$PM_{2.5}$ in 1990	1.00(0.90, 1.10)	0.95	1.01(0.96, 1.06)	0.60
$PM_{2.5}$ in 2000	1.02 (0.92, 1.12)	0.68	0.99(0.94, 1.04)	**0**.83
$PM_{2.5}$ in 2010	1.01(0.92, 1.11)	0.75	0.98 (0.93, 1.03)	0.52

[16] All three time points are included in the same model, but each pollutant has its own model (three PM_{10} in one model, three NO_2 in one model, three $PM_{2.5}$ in one model). Measurement unit for the air pollutants is micrograms per cubic meters (µg/m3).

[17] Asthma severity variable is generated first by creating asthma symptoms score i.e. by combining wheezing, chest tightness, wheezing with breathlessness, nocturnal breathlessness and nocturnal cough, and then recoding asthma symptoms score <=2 as 0 (low asthma severity) and asthma symptoms score>=3 as 1 (high asthma severity).

This table presents the univariate analyses of associations between asthma severity and air pollutants, sex, study centre, education, smoking status, and physical activity, stratified by parental asthma. For participants with no parental asthma, the association between asthma severity and males living in Uppsala, who were current smokers and had lower secondary education was significant. There was borderline significance between asthma severity and exposure to NO_2 20 years ago for participants without parental asthma. No significant association was observed between asthma severity and air pollutants for participants with parental asthma history.

On the other hand, for participants with no parental asthma history, significant association was found between lower secondary education level and asthma severity (**OR: 1.48;95%CI: 1.13, 1.94).** This group also had a significant positive association between asthma severity and current smoking **(OR: 2.85; 95% CI: 2.08, 3.90)**. In addition, living in Uppsala with no parental asthma history seemed to be associated with less asthma severity **(OR: 0.50;95% CI:0.34, 0.75).**

Table 6: Multivariate logistic regression analysis of association between study centre, sex, educational level, smoking status, air pollutants (No_2, Pm_{10}, & $Pm_{2.5}$)[18] and asthma severity[19] expressed in odd ratios with 95% confidence intervals and stratified by parental asthma in N=6 ,193 participants from the RHINE study

Asthma severity	**Yes Parent asthma**		**No Parent asthma**	
	OR (95% CI)	**p**	**OR (95% CI)**	**p**
Centre				
Bergen	1			
Gothenburg	1.10(0.50, 2.40)	0.81	1.08(0.73, 1.59)	0.69
Umea	0.70(0.30, 1.62)	0.41	1.09(0.71, 1.67)	0.67
Uppsala	0.78(0.38, 1.59)	0.50	**0.57(0.37, 0.87)**	**0.009**
Sex				
Female	1			
Male	0.98(0.59, 1.62)	0.94	**0.75(0.57, 0.98)**	**0.03**
Education level				
College and university	1		1	
Lower secondary	1.18(0.67, 2.05)	0.55	1.22(0.91, 1.62)	0.17
Primary school	0.84(0.34, 2.06)	0.70	1.02(0.65, 1.60)	0.92
Smoke Status				
Never-smoker	1		1	
Ex-smoker	1.44(0.82, 2.54)	0.19	1.08(0.79, 1.49)	0.59
Current smoker	1.67(0.83, 3.37)	0.14	**2.63(1.89, 3.66)**	**<0.001**
NO_2in 1990	0.98(0.93, 1.04)	0.62	1.01(0.98, 1.04)	**0**.39
NO_2 in 2000	1.01(0.95, 1.07)	0.66	1.00(0.97, 1.03)	0.88
NO_2 in 2010	0.99(0.93, 1.04)	0.72	0.99(0.96, 1.01)	0.50
PM_{10} in 1990	0.93(0.76, 1.14)	0.52	1.02(0.92, 1.14)	0.63
PM_{10} in 2000	0.96(0.77, 1.20)	0.74	1.01(0.90, 1.12)	0.84
PM_{10} in 2010	1.09(0.89, 1.34)	0.38	0.99(0.89, 1.10)	0.92
$PM_{2.5}$ in 1990	0.91(0.74,1.12)	0.41	1.08(0.96, 1.22)	0.19
$PM_{2.5}$ in 2000	1.05(0.84, 1.30)	0.66	0.96(0.86, 1.08)	0.55
$PM_{2.5}$ in 2010	0.99(0.81, 1.21)	0.96	0.96(0.87, 1.06)	0.48

This table shows the multivariate analysis that is adjusted for study centre, sex, education, smoking status, and air pollutants (NO_2, PM_{10} & $PM_{2.5}$) and stratified by parent asthma. For

[18] All three time points are included in the same model, but each pollutant has its own model (three PM10 in one model, three no2 in one model, three PM 25 in one model. Measurement unit for the air pollutants is micrograms per cubic meters (µg/m3).

[19] Asthma severity variable is generated first by creating asthma symptoms score i.e. by combining wheezing, chest tightness, wheezing with breathlessness, nocturnal breathlessness and nocturnal cough, and then recoding asthma symptoms score <=2 as 0 (low asthma severity) and asthma symptoms score>=3 as 1 (high asthma severity).

those with parental asthma history, none of the variables had a significant association with asthma severity, even after adjusting for the other variables in the model.

Those participants with no parental asthma history and living in Uppsala were at less risk of having asthma severity than those living in Bergen. Similarly, male participants with no parental asthma history were at less risk of asthma severity as compared to females. For those with no parental asthma history, significant association was found between being a current smoker and asthma severity. The association remained positively significant between asthma severity and current smoker **(OR:2.63;95%CI:1.89,3.66)** after adjusted for the other variables**.** At the same time, the significant association between asthma severity and lower secondary education for participants with no parental asthma disappeared after adjustment for other variables in the model. No significant associations were observed between the air pollutants and asthma severity in participants with or without parental asthma history.

Table 7:Univariate negative binomial regression between asthma symptom score and study centre, parental asthma, education level, smoking status and air pollutants(NO_2, PM_{10}&$PM_{2.5}$)[20]expressed in relative risks with 95%confidence interval and stratified by sex in N=6,193 participants from the RHINE study

Asthma symptoms score[21]	Male		Female	
	RR (95% CI)	**p**	**RR (95% CI)**	**p**
Centre				
Bergen	1			
Gothenburg	1.06 (0.83, 1.36)	0.59	1.09 (0.86, 1.39)	0.43
Umea	0.84 (0.66, 1.07)	0.17	1.15(0.91, 1.45)	0.22
Uppsala	**0.75(0.58, 0.96)**	**0.02**	0.95 (0.75, 1.21)	0.71
Smoke status				
Never-smoker[22]	1		1	
Ex-smoker	**1.26(1.03, 1.54)**	**0.02**	1.10 (0.91, 1.33)	0.31
Current smoker	**2.53(2.00, 3.21)**	**<0.001**	**1.71 (1.35, 2.18)**	**<0.001**
Parental Asthma				
No[23]	1			
Yes	**1.84(1.42, 2.39)**	**<0.001**	**1.69 (1.34 ,2.14)**	**<0.001**
Education level				
College and university[24]	1		1	
Lower secondary	**1.28(1.06, 1.55)**	**0.01**	**1.24(1.03, 1.48)**	**0.01**
Primary school	**1.57 (1.19, 2.08)**	**0.001**	1.06 (0.80, 1.42)	**0**.64
Physical Activity				
Everyday[25]	1		1	
2-3 times in a week	0.89(0.68, 1.15)	0.38	0.92(0.74, 1.14)	0.46
Never or once in a week	1.24(0.97, 1.59)	0.08	**1.25 (1.01, 1.55**)	**0.03**
NO_2 in 1990	**1.01(1.00, 1.02)**	**0.01**	0.99(0.98, 1.01)	0.92
NO_2 in 2000	1.00(0.99, 1.01)	0.27	1.00(0.97, 1.04)	0.26
NO_2 in 2010	0.99(0.98, 1.00)	0.51	1.00(0.99, 1.01)	0.38
PM_{10} in 1990	**1.03(1.00, 1.07)**	**0.04**	0.99(0.95, 1.02)	0.57
PM_{10} in 2000	1.03(0.99, 1.06)	0.07	1.00(0.94, 1.04)	0.62
PM_{10} in 2010	1.01(0.97, 1.05)	0.43	1.01(0.98, 1.05)	0.33
$PM_{2.5}$ in 1990	1.02(0.99, 1.06)	0.15	0.99(0.96, 1.03)	0.89
$PM_{2.5}$ in 2000	1.01(0.98, 1.05)	0.32	1.00(0.97, 1.04)	0.62
$PM_{2.5}$ in 2010	0.99(0.96, 1.03)	0.80	1.00(0.97, 1.07)	0.71

[20] All three time points are included in the same model, but each pollutant has its own model (three PM_{10} in one model, three NO_2 in one model, three PM $_{2.5}$ in one model). Measurement unit for all air pollutants are micrograms per cubic meters (µg/m3).

[21] Asthma symptoms score(0-5) is computed by combining five main symptoms of asthma i.e. wheezing, chest tightness, wheezing with breathlessness, nocturnal breathlessness and nocturnal cough.
Reference categories
[22] Never smoker
[23] No Parental asthma
[24] College and University education
[25] Everyday

This table presents the univariate negative binomial regression analyses of associations between asthma symptoms score and air pollutants, study centre, education, smoking status, and physical activity, stratified by sex. For male participants, being ex-smoker and current smokers with a parent having asthma and with a lower level of education and exposure to NO_2 and PM_{10} 20 years ago and PM_{10} ten years ago were risk factors for increased asthma symptoms. The association between these variables and asthma symptoms score were positively significant, except for exposure to PM_{10} 10 years ago which had a borderline significant association with asthma symptom (p-value 0.07). But those men who live in Uppsala were less likely to have higher asthma symptoms score than those who live in Bergen. With one change in smoking status, the asthma symptoms score increased by 1.26 times and 2.53 times in males who were ex-smoker and current smokers. Compared with males having no parental asthma, asthma symptoms increased by 1.84 times in males with parental asthma. Compared with university education, asthma symptom scores increased by 1.28 times in males with secondary-level education and by 1.57 times with primary education. With one unit increase in NO_2 and PM_{10} among males who were exposed to NO_2 and PM_{10} 20 years ago, the asthma symptoms score increased by 1.01 times and 1.03 times, respectively.

For female participants, being a current smoker, having lower secondary level education and having a parental asthma history, and who never or once a week did physical activity were risk factors for increased asthma symptoms. There were no observed associations between air pollutants and asthma symptom scores for females. However, with one unit change in smoking status in females, asthma symptoms score increased by 1.71 times among current smokers. Compared to females having no parental asthma, the asthma symptoms score increased by 1.64 times in females with a parent asthma history. Asthma symptoms score increased by 1.25 times in females who never or once a week did physical activity compared to a female who did physical activity every day.

Table 8: Multivariate negative binomial regression between asthma symptom score and study center, parent asthma, education level, smoking status, physical activity and air pollutants(NO_2 , PM_{10}&$PM_{2.5}$)[26] expressed in Relative risk with 95%confidence interval and stratified by sex in N=6,193 participants from the RHINE study

Asthma symptoms score[27]	**Male**		**Female**	
	RR (95% CI)	**p**	**RR (95% CI)**	**p**
Centre				
Bergen	1		1	
Gothenburg	1.13(0.85,1.49)	0.38	1.06(0.80, 1.39)	0.66
Umea	1.07(0.79,1.45)	0.63	1.34(1.01, 1.77)	0.04
Uppsala	0.93(0.72,1.20)	0.63	1.07(0.83, 1.37)	0.57
Parental Asthma				
No	1		1	
Yes	**1.80(1.39, 2.33)**	**<0.001**	**1.65(1.30, 2.10)**	**<0.001**
Education level				
College and university	1		1	
Lower secondary	1.13(0.93,1.38)	0.20	1.11(0.92,1.35)	0.24
Primary school	1.09(0.80,1.47)	0.56	0.98(0.65, 1.21)	0.46
Smoke Status				
Never-smoker	1		1	
Ex-smoker	**1.21(0.99, 1.49)**	**0.05**	1.10(0.91, 1.34)	0.29
Current smoker	**2.21(1.71, 2.85)**	**<0.001**	**1.68(1.31, 2.16)**	**<0.001**
Physical Activity				
Everyday	1		1	
2-3 times in a week	0.91(0.70, 1.19)	0.51	0.96(0.77, 1.20)	0.74
Never or once in a week	1.08(0.84, 1.39)	0.50	**1.26(1.01, 1.57**)	**0.03**
NO_2 in 1990	**1.01(1.00, 1.03)**	**0.03**	0.99(0.97, 1.01)	0.52
NO_2 in 2000	1.00(0.98, 1.02)	0.81	1.01(0.99, 1.03)	0.29
NO_2 in 2010	**0.98(0.96, 0.99)**	**0.03**	1.00(0.98, 1.02)	0.59
PM_{10} in 1990	1.06(0.99, 1.14)	0.07	0.96(0.89, 1.03)	0.27
PM_{10} in 2000	1.02(0.95, 1.09)	0.54	1.00(0.94, 1.08)	0.81
PM_{10} in 2010	0.96(0.89, 1.02)	0.23	1.06(0.99, 1.13)	0.05
$PM_{2.5}$ in 1990	1.05(0.97, 1.13)	0.19	0.99(0.91, 1.07)	0.86
$PM_{2.5}$ in 2000	1.02(0.95, 1.10)	0.50	1.03(0.95, 1.11)	0.45
$PM_{2.5}$ in 2010	0.93(0.87 ,1.00)	0.06	1.00(0.94, 1.08)	0.78

[26] All three time points are included in the same model, but each pollutant has its own model (three PM_{10} in one model, three NO_2 in one model, three PM $_{2.5}$ in one model)

[27] Asthma symptoms score(0-5) is computed by combining five main symptoms of asthma i.e. wheezing, chest tightness, wheezing with breathlessness, nocturnal breathlessness and nocturnal cough.

This table shows the multivariate negative binomial analysis that is adjusted for study centre, parent asthma, education, smoking status, physical activity, and air pollutants (NO_2, PM_{10} & $PM_{2.5}$) and stratified by sex.

For males being current and ex-smoker, having a parental asthma history and exposure to NO_2& PM_{10} 20 years ago were risk factors for increased asthma symptoms after adjustment for the variables. With one unit change in smoking status, the asthma symptoms score increased by 1.21 and 2.21 times in males who were ex-smoker and current smokers, respectively, keeping other variables constant. Compared to males without parental asthma, the asthma symptoms score increased by 1.80 times in males having parental asthma holding other variables constant. With one unit increase in NO_2 among males who were exposed to NO_2 20 years ago, the asthma symptoms score increased by 1.01 times and decreased by 0.98 times for current exposure to NO_2. However, for exposure to PM_{10} 20 years ago in males, asthma symptoms score increased by 1.06 times and had a borderline significant relationship with asthma symptoms with p-value 0.07. For current exposure to $PM_{2.5}$ in males, asthma symptom scores decreased by 0.93 times and had a borderline significant relationship with asthma symptom scores.

Female participants being a current smokers, having a parental asthma history, who never or once a week did physical activity, and current exposure to PM_{10} were risk factors for increased asthma symptoms after adjusting for other variables. However, with one unit change in smoking status in females, asthma symptoms score increased by 1.68 times among current smokers keeping other variables constant. Compared to females with no parental asthma, asthma symptoms score increased by 1.65 times in females with parental asthma history keeping other variables constant. The asthma symptoms score increased by 1.26 times in females who never or once a week did physical activity compared to those females who did physical activity every day, keeping other variables constant. With a change in PM_{10}, asthma symptom scores increased by 1.06 times for females and had a borderline significant relationship.

Table 9:Univariate negative binomial regression between asthma symptom score and study Centre, sex, education level, smoking status, physical activity and air pollutants (NO_2, PM_{10}, &$PM_{2.5}$)[28] expressed in relative risk with 95% confidence interval and stratified by parental asthma in N=6,193 participants from the RHINE study

Asthma symptoms score	**Yes Parental asthma**		**No parental asthma**	
	RR (95% CI)	**p**	**RR (95% CI)**	**p**
Centre				
Bergen	1			
Gothenburg	1.18 (0.77, 1.81)	0.44	1.07 (0.89, 1.28)	0.46
Umea	0.87 (0.57, 1.34)	0.54	1.02(0.85, 1.22)	0.80
Uppsala	0.82(0.53, 1.27)	0.37	0.86 (0.72, 1.04)	0.13
Smoke status				
Never-smoker	1		1	
Ex-smoker	1.28(0.90, 1.82)	0.15	**1.15(0.99, 1.34)**	**0.05**
Current smoker	**2.19(1.44, 3.34)**	**<0.001**	**2.05 (1.71, 2.46)**	**<0.001**
Sex				
Female	1		1	
Male	0.99(0.72, 1.35)	0.96	0.91 (0.79 ,1.03)	0.16
Education level				
College and university	1		1	
Lower secondary	1.16(0.82, 1.63)	0.38	**1.25(1.09, 1.44)**	**0.001**
Primary school	1.26 (0.79, 2.03)	0.32	**1.26 (1.02, 1.57)**	**0.03**
Physical Activity				
Everyday	1		1	
2-3 times in a week	0.70(0.45, 1.09)	0.11	0.93(0.78, 1.12)	0.49
Never or once in a week	1.31(0.87, 1.97)	0.19	**1.18 (0.99, 1.41**)	**0.05**
NO_2 in 1990	1.00(0.98, 1.03)	0.38	1.00(0.99, 1.01)	0.16
NO_2 in 2000	1.01(0.99, 1.03)	0.16	1.00(0.99, 1.01)	0.29
NO_2 in 2010	1.00(0.98, 1.02)	0.63	1.00(0.99, 1.00)	0.93
PM_{10} in 1990	1.03(0.97, 1.10)	0.23	1.00(0.98, 1.03)	0.52
PM_{10} in 2000	1.05(0.99, 1.12)	0.07	1.01(0.98, 1.03)	0.33
PM_{10} in 2010	1.05(0.98, 1.11)	0.13	1.00(0.98, 1.03)	0.47
$PM_{2.5}$ in 1990	1.01(0.95, 1.07)	0.68	1.01(0.98, 1.03)	0.39
$PM_{2.5}$ in 2000	1.03(0.97, 1.09)	0.30	1.00(0.98, 1.03)	0.54
$PM_{2.5}$ in 2010	1.01(0.95, 1.07)	0.59	0.99(0.97, 1.02)	0.83

[28] All three time points are included in the same model, but each pollutant has its own model (three PM_{10} in one model, three NO_2 in one model, three $PM_{2.5}$ in one model). Measurement unit for all pollutants is micrograms per cubic meters ($\mu g/m^3$).

This table presents the univariate negative binominal analyses of associations between asthma symptoms score and air pollutants, sex study centre, education, smoking status, and physical activity, stratified by parental asthma. The result shows that for the participant with parental asthma history, only exposure to PM_{10} 10 years ago had a borderline significant association with asthma symptoms score. For those participants with the history of parental asthma, current smoker was only found as the risk factor for increased asthma symptoms.

For the participants with parental asthma, with one unit change in smoking status, the asthma symptoms score increased by 2.19 times among the current smoker.

For participants with no parental asthma history, being current and ex-smoker, having lower secondary and primary level education, and never or once a week involved in physical activity were risk factors for increased asthma symptoms score.

For participants with no parental asthma, with one unit change in smoking status, for ex-smoker and current smokers, the asthma symptoms score increased by 1.15 times and 2.05 times, respectively. Compared to university and college level education, the asthma symptoms score increased by 1.25 times for lower secondary education and 1.26 times for primary level participants with no parental asthma. For participants with no history of parental asthma, asthma symptoms score increased by 1.18 times for those who never or once a week did physical activity compared to those who did the daily physical activity.

Table 10 : Multivariate negative binominal regression between asthma symptoms score and study center, sex, education level, smoking status, physical activity and air pollutants(NO_2, PM_{10} &$PM_{2.5}$)[29] expressed in Relative risk with 95% confidence interval and stratified by parent asthma in N=6,193 participants from the RHINE study

Asthma symptoms score[30]	**Yes Parent asthma**		**No Parent asthma**	
	RR (95% CI)	**p**	**RR (95% CI)**	**p**
Centre				
Bergen	1			
Gothenburg	1.26(0.78, 2.06)	0.33	1.06(0.86, 1.31)	0.56
Umea	1.07(0.64, 1.79)	0.78	1.23(0.98, 1.54)	0.07
Uppsala	0.97(0.62, 1.53)	0.90	1.00(0.82, 1.21)	0.97
Sex				
Female	1			
Male	0.95(0.69, 1.31)	0.78	**0.86(0.75, 0.98)**	**0.03**
Education level				
College &university	1		1	
Lower secondary	1.03(0.71, 1.47)	0.87	1.12(0.97, 1.30)	0.11
Primary school	1.05(0.64, 1.72)	0.84	0.98(0.77, 1.24)	0.77
Smoke Status				
Never-smoker	1		1	
Ex-smoker	1.25(0.87, 1.78)	0.22	1.14(0.98, 1.33)	0.06
Current smoker	**2.02(1.30, 3.13)**	**0.002**	**1.95(1.60, 2.37)**	**<0.001**
Physical Activity				
Everyday	1		1	
2-3 times in a week	0.69(0.44,1.08)	0.11	0.99(0.82, 1.19)	0.93
Never or once in a week	1.06(0.69, 1.64)	0.76	**1.19(0.99,1.42)**	**0.05**
NO_2 in 1990	.99(0.96, 1.03)	0.79	1.01(0.99 1.02)	0.16
NO_2 in 2000	1.02(0.98, 1.06)	0.18	1.00(0.99, 1.01)	0.50
NO_2 in 2010	0.98(0.95, 1.01)	0.41	0.99(0.97, 1.00)	0.23
PM_{10} in 1990	0.99(0.87, 1.13)	0.94	1.01(0.96, 1.07)	0.52
PM_{10} in 2000	1.06(0.93, 1.21)	0.32	1.00(0.95, 1.06)	0.83
PM_{10} in 2010	1.01(0.90, 1.15)	0.76	1.00(0.95, 1.06)	0.73
$PM_{2.5}$ in 1990	0.93(0.82, 1.06)	0.29	1.04(0.98, 1.11)	0.12
$PM_{2.5}$ in 2000	1.08(0.94, 1.24)	0.23	1.01(0.96, 1.07)	0.61
$PM_{2.5}$ in 2010	0.95(0.84, 1.08)	0.46	0.97(0.92, 1.02)	0.29

This table presents the multivariate negative binominal analyses of associations between asthma symptoms score and air pollutants, sex, study centre, education, smoking status, and

[29] All three time points are included in the same model, but each pollutant has its own model (three PM_{10} in one model, three NO_2 in one model, three $PM_{2.5}$ in one model)

[30]Asthma symptoms score(0-5) is computed by combining five main symptoms of asthma i.e. wheezing, chest tightness, wheezing with breathlessness, nocturnal breathlessness and nocturnal cough

physical activity, stratified by parental asthma. No association was observed between asthma symptoms and air pollutants for participants with and without parental asthma history, even after adjusting for other variables.

For participants with parental asthma, only a current smoker was a risk factor for increased asthma symptom scores, even after adjusted for other variables. With one unit change in smoking status, the asthma symptoms score increased by 2.02 times for current smokers with parental asthma history holding other variables constant.

Participants with no parental asthma, being male, current smokers, and who never or once a week did physical activity are risk factors for increased asthma symptoms. The relationship between asthma symptom scores and current smokers was positively significant after adjusted for other variables. However, there was the borderline significance with never or once a week involvement in physical exercise. The association between lower secondary and primary level education with asthma symptom scores disappeared after adjustment. With one unit change in smoking status, the asthma symptom scores increased by 1.95 times in participants with no parental asthma keeping other variables constant. Compared to females with no parental asthma history, males with no parental asthma keeping other variables constant in the model have 0.86 times fewer asthma symptoms than females.

5. Discussion

5.1 Summary of the main findings

In this prospective study, we found that males exposed to NO_2 for 20 years were associated with a small but significant increased risk of asthma severity, defined as three or more current asthma symptoms. However, in females, no significant association was observed between exposure to any air pollutants (NO_2, PM_{10}, and $PM_{2.5}$) in any of the three-time points with regard to asthma severity. Further, being a current smoker and having a parental asthma history were associated with increased risk of asthma severity in both sex. The stratified analyses based on parental asthma history showed no significant association between exposure to air pollutants (NO_2, PM_{10}, and $PM_{2.5}$) at any time point and asthma severity. Males participants with no parental asthma history were at less risk of asthma severity than females. For those with no parental asthma history, being a current smoker is also a risk factor for the development of asthma severity. On the other hand, none of the investigated covariates were associated with asthma severity for participants with parental asthma history. The negative binomial regression analyses stratified by sex showed that males exposed to NO_2 20 years ago were associated with a small but significant risk of increased asthma symptoms. In contrast, current NO_2 exposure was associated with less risk of having asthma symptoms. In this stratified analysis, being a current and ex-smoker and having a parental asthma history are also the risk factors for increased asthma symptoms. However, a borderline significant association was observed between current exposure to PM_{10} and asthma symptoms in females. In the negative binominal regression stratified by parental asthma, no significant association was observed between exposure to air pollutants (NO_2, PM_{10}, and $PM_{2.5}$) in any of the time points and asthma symptoms. However, for participants with parental asthma, only a current smoker is a risk factor for increased asthma symptoms. For participants with no parental asthma, being a male, being a current smoker, and never or once a week physical activity are risk factors for increased asthma symptoms.

5.2 Comparison with previous findings

Our study findings cannot be easily comparable with other studies due to methodological heterogeneity in the definition of asthma, follow-up time, and variation in types of analyses. Nevertheless, our estimates for outdoor air pollution are in accordance with other studies for NO_2 in (22, 24, 26, 31-35, 37, 38) ,PM_{10} (22) and $PM_{2.5}$ (22, 31, 35)

Our findings on stratified analysis for females are consistent with results from the ESCAPE study, where they found no significant association between exposure to NO_2 and PM_{10} and asthma incidence in 10 years among older females. However, our male findings contradicted that in ESCAPE, as they found no significant association between NO_2 and asthma incidence. But for PM_{10}, it was similar to our findings that show insignificant association (22).

Compared to the ECHRS study, our finding for stratified analyses by sex is consistent for females that showed no association between asthma incidents and NO_2 exposure. However, our finding contradicts its finding for males, which found no association between asthma incidence and NO_2. However, their finding based on stratification by family history of asthma is in line with our findings, which found no association between NO_2 and asthma incidence (26).Likewise, the finding of a population-based cohort study (2001-2015) in Ontario is in line with our finding, which showed no association between exposure to $PM_{2.5}$ (HR:1.01;95%CI: 1.00-1.02) and onset asthma (30).

However, some of our findings contradict the findings of other studies for pollutants, including NO_2 (31, 35, 37), PM_{10} (25), and $PM_{2.5}$ (34, 36). For instance, a study in Adelaide showed no association between NO_2 (OR:1.003;95% CI: 0.95-1.05) and asthma admission in hospitals for any of the age groups in multi-pollutant models (34). The ELAPSE study observed associations in fully adjusted models with hazard ratios 1.22 (95%CI:1.04-1.43) per 5 μg/m3 for $PM_{2.5}$, which is not in line with our findings (33).

For asthma symptoms score, the finding of our studies was in line with two other studies (27, 28) that showed a significant association between NO_2 and asthma symptoms score. For instance, an ECHRS study with negative binomial regression showed a significant association in men, ratio of mean score (RMS:1.32;95%CI:1.12-1.56) and insignificant in women (RMS:1.14; 95%CI:0.97-1.34) (27).

5.3 Biological plausibility for exposure to air pollution and asthma

The exact biological mechanisms of how exposure to outdoor air pollution contributes to the development of asthma in adults are not yet clear. However, it has been proposed that NO_2 and other pollutants can act as an airway irritant and can deposit in the respiratory tract and lung alveoli, causing oxidative stress. Even at a low concentration, this can cause infection in the respiratory -tract by interacting with the immune system and leading to inflammation in the upper and lower respiratory tract, which is considered a key physiological change in the

development of asthma. In addition, the interaction of air pollutants with genetic and epigenetic determinants has also been suggested. It has been proposed that exposure to air pollutants causes changes in DNA methylation levels, which in turn affect gene expression, and might aid in asthma development. (61, 62).

Further, asthma triggers also include allergic (e.g. house dust, mites, cockroach, residue animal dander, mold, and pollens) and non-allergic (e.g. exposure to tobacco smoke, viral infections, cold air, occupational agent like chlorine, ammonia, and exercise) stimuli. Together with ambient air pollution, all such triggers may produce a series of events leading to chronic airway inflammation, which can cause asthma or exacerbate the asthma symptom (10, 63).

5.4 Strengths and limitations of the study

The strengths of our study include the large population-based study sample over the 20-year follow-up period. This made it possible to examine the long-term association between exposure to air pollution and the occurrence of asthma in adults. As a result of the large sample size, we were able to stratify the analysis by sex and parental asthma history, thereby increasing our analysis's statistical power. This multi-centre study includes different geographic locations, and the comparison between the centres increases the study's generalizability. Generalizability can be described as the degree to which the findings and conclusions from a study conducted on a sample population are applicable to the population at large. In other words, generalization is interpreting a study's results once they are determined to be internally valid in a large population (64). Hence, with the inclusion of four study centers from Norway and Sweden, we can at least generalize our results to Nordic countries. Further, the prospective data collection of both exposure and outcome over 20 years ensures temporality (the exposure precedes the outcome), and the results are not susceptible to reverse causation, which refers to the situation in which the outcome precedes and causes the exposure rather than the other way around (47).

In our study, we did the individual assessment of exposure to long-term air pollution based on complete individual geocoded residential moving history retrieved from population registers. This forms a definitive source of unbiased exposure data in Northern Europe, including an impressive long duration. Another strength is our assessment of the outcome based on the positive answer to asthma symptoms questions. Our severity outcome (high versus low) with

a cut-off of 3 respiratory symptoms, all of which were active during the last 12 months, ensured an asthma outcome with relevant and active disease burden for the participants. Furthermore, using the asthma symptoms score as a continuous variable improved the analyses' power and gave additional information to the dichotomous definitions of asthma. For these reasons, epidemiological studies recommend that asthma symptoms be analysed as a continuous asthma score (65).

To achieve valid and precise estimates, we have to minimize the measurement error that can occur during all stages of the study, from the study design through the data collection and in the analyses and interpretation of the study results. Bias is a systematic error in the design, conduct, or analysis of a study that leads to a mistake in estimating an exposure's effect on the risk of the outcomes (47).

A number of limitations should be considered when interpreting the findings of this study, in particular concerning the three main biases in epidemiology: Confounding, selection bias, and information bias. These three forms of bias are a concern in epidemiologic studies, which can threaten the internal validity of a study. A study's internal validity is determined by the extent to which the results from the study represent the truth in the study population and is not influenced by methodological errors (47).

Confounding is an effect of a third variable that distorts the association between the dependent and the independent variable. Confounders are variables that are associated with both exposure and outcome, which precedes them in time. It can cause an over or underestimation of the true effect between exposure and outcome (47). In order to minimize the bias due to confounding, we included the possible confounding variables as identified in DAGITTY as covariates in the logistic and negative binomial regression model. Further, we stratified the analysis based on the parental history of asthma which can be used as a proxy for genetic factors and therefore be an important confounder. However, we cannot rule out that there are other measures for genetic and other potential confounders that might lead to residual confounding in our study.

Smoking status as far back in time as before pollution exposure could be considered to be such a potential confounder. Many previous studies have stratified analyses of air pollution and asthma outcomes by smoking status (22, 23). In our study, we only adjusted for smoking status instead of stratifying by it. Since our main exposure was pollution exposure 20 years

ago, we would also need to define smokers and non-smokers more than 20 years ago, and we did not have that information available in our study. We could have used current smoking status as a proxy, but quite many would probably have quit during the 20-year-period, and some would have started smoking during the 20-year-period. It would blur the non-smoker/smoker stratification groups, with smokers in the non-smoking group and non-smokers in the smoking group.

Selection bias is a systematic error due to the method used to select the study population or if participants are selectively lost to follow-up. It occurs when the study population is not truly representative of the target population. It can cause an overestimate or underestimate of association (47)The participants response rate of RHINE I after ten years (i.e., RHINE II) was 77% and after 20 years (i.e., RHINE III) was 53%. Since the loss-to-follow-up is more than 20%, we cannot rule out the chance of attrition bias in this study (66, 67). The study's internal validity is compromised if too many subjects are lost to follow-up. It is generally recommended that the number of participants lost to follow-up should not exceed 20%. Attrition bias is a selection bias due to systematic differences in the quantitative and qualitative characteristics due to the loss of follow of the study population during the study progress. Loss may be due to withdrawal, dropout, change in protocol, and migration (68). Loss to follow-up may also be due to a healthy survivor effect which is most commonly observed in association with occupation, in that persons who remain employed tend to be healthier than those who leave employment. However, it can also be relevant for our type of study with long follow-up time: it is more likely that persons who continue to be in a study are healthier than persons who leave the study. Several studies have suggested that loss to follow might alter the prevalence estimates but not estimates for the association between exposure and outcome. This has also been shown in the RHINE study (42). Since the objective of our study was to assess the association between asthma and air pollutant exposures and not to assess the prevalence of asthma, we assume that selection bias will probably not threaten the internal validity.

Information bias is a systematic error that occurs when there is an error in the information collected from a study population or if there is a misclassification of the exposure or outcome in a study (47)The misclassification can be further divided into a differential or non-differential misclassification.

Differential misclassification occurs if misclassification in the exposure is different for those with and without the outcome or if misclassification in the outcome is different for those with and without the exposure. A common kind of differential misclassification is recall bias, which can lead to both an over- and underestimation of the exposure-outcome association (47)Potential recall bias for exposure could be if participants with asthma (outcome) overreported higher exposure levels of outdoor ambient air pollution compared with participants without asthma. However, our study is free from such bias as we did not rely upon self-reported exposures but collected objective data on outdoor ambient air pollution exposures based on each participant's residential address history. There is, of course, always a risk for recall bias also in the reporting of outcomes – which in our study was self-reported for asthma, but it is unlikely that misclassification of outcomes would be differential, i.e., dependent on the residential addresses.

Misclassification in exposure and outcome is non-differential if it is independent of the other if it affects equally exposed and non-exposed, or those with and without a disease. In our study, the definition of asthma was based on self-report that might be prone to recall bias (47)The adults might have failed to recall the past event of asthma symptoms, and we might have misclassified them as unexposed. Such information errors could have caused bias towards the null, i.e., we get an estimate which shows no effect rather than the actual effect. However, in our study, the respiratory symptoms questions are about the last 12 months, and most remember a year back in time. If we had defined asthma as a self-reported diagnosis ever, on the other hand, the study would be more vulnerable to recall bias because a person who got a diagnosis 30 years ago may not remember that he had a diagnosis.

5.5 Implication for clinical, public health practice and future research

This prospective study was conducted in Norway, and Sweden which has relatively lower level of outdoor air pollution (69, 70). However, our estimates mainly for NO_2 showed that even the adults living in Nordic countries with low level of air pollution can develop asthma when exposed to such pollutants for over a longer period of time.

So, considering the dose-response relationship plus the multi-factorial causation of asthma, we can assume that the association between NO_2 and asthma which we see in this study from Norway and Sweden would probably be extremely much larger in a low-and middle income countries, which has higher level of air pollution. Multifactorial causation of disease theory describes how disease is not only caused by germ theory but other multiple factors and that

both environmental factors and genetic factors are responsible (71). Asthma is also a multifactorial disease since it is not only the result of one factor but a combination of both genetic and environmental factors including exposure to air pollutants, occupational exposures to gas or fumes, dust, mites, smoking , poor nutrition and poor living condition.

Hence, our findings for long-term exposure to NO_2 in adults and the development of asthma have public health implications for both developed and low and middle-income countries. A better knowledge of the negative impact of air pollution on asthma outcomes could encourage clinicians to ask a specific questions on possible recent exposure to air pollution that might lead to asthma in adult patients. It will aid in educating patients about ways to minimize exposure to outdoor air pollution and manage their asthma.

Further, our findings are important to stimulate public health authorities and governments particularly of low-and middle-income countries to take more efficient measures to limit the exposure to air pollutants while conducting asthma screening programme.

Although our study was a long-term multicentre study with large sample size, it was confined to Nordic centres. Therefore, future multicentre studies need to include larger geographical areas. Also, future research should use multiple exposure levels of air pollution (indoor, outdoor residential, and occupational) and adjust for genetic and other potential confounding factors.

6. Conclusion

The study found that exposures to NO_2 20 years ago was associated with both asthma severity and asthma symptoms among adult males even after adjustment for current exposure to outdoor air pollution and other variables. For females, a borderline significant association was observed between asthma symptoms and current exposure to PM_{10}. When analysing men and women together but stratifying them according to parental asthma history, no association was found for asthma severity and asthma symptoms with any of the air pollutants.

7.Ethical Consideration

This master thesis involves the use of data collected over more than 20 years. The RHINE study was approved according to national legislations in each study centre by regional committees of medical research ethics. All data collection has complied with the principles of the Declaration of Helsinki, and all participants have provided written informed consent prior to participation. Appropriate Data Protection measures have been taken to ensure safe storage of information.

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9. Annex

Kompetansesenter for klinisk forskning
HAUKELAND SYKEHUS

Institutt for indremedisin
Seksjon for lungemedisin

Ernst Omenaas

Airways symptoms

1. Have you had wheezing or whistling in your chest at any time **in the last 12 months?** ☐ No ☐ Yes

   If NO go to question 2, if YES:

   1.1 Have you been at all breathless when the wheezing noise was present? ☐ No ☐ Yes

   1.2 Have you had this wheezing or whistling when you did not have a cold? ☐ No ☐ Yes

2. Have you woken up with a feeling of tightness in your chest at any time **in the last 12 months?** ☐ No ☐ Yes

3. Have you been woken by an attack of shortness of breath at any time **in the last 12 months?** ☐ No ☐ Yes

4. Have you been woken by an attack of coughing at any time **in the last 12 months?** ☐ No ☐ Yes

5. Have you had an attack of asthma **in the last 12 months?** ☐ No ☐ Yes

6. Are you currently taking any medicine *(including inhalers, aerosols or tablets)* for asthma? ☐ No ☐ Yes

7. Do you have any nasal allergies including hay fever? ☐ No ☐ Yes

8. Do you have any nasal allergies including hay fever? ...../...../.....

9. What is today's date? ...../...../.....

10. Are you male or female ☐ Male ☐ Female

11. How tall are you? ..... cm

12. How much do you weigh? ..... kg

13. In recent years, have you been troubled by a protracted cough? ☐ No ☐ Yes

14. Do you usually bring up phlegm or do you have phlegm in your lungs which you have difficulty bringing up? ☐ No ☐ Yes

    If NO go to question 18, if YES:

15. Do you bring up phlegm in this way almost every day for **at least three months every year?** ☐ No ☐ Yes

    If NO go to question 18, if YES:

16. Have you had periods of this kind for at least two years in a row? NO YES

    If NO go to question 18, if YES:

17. How old were you when these problems began? ..... years

Smoking habits

18. Are you a smoker *(this applies even if you only smoke the odd cigarette/cigar or pipe every week)?* ☐ No ☐ Yes

19. Are you an ex-smoker?

If NO to question 18 and 19 go to question 20, if YES:

19.1 Smoke/smoked .....cigarettes/day

.....cigars/week

.....pkts pipe tobacco/week

How old were you when you started smoking? .....(age)

Smoked for .....years *(applies to both smokers and ex-smokers)*

Stopped smoking in.....(year)

Upper and lower airways

20. Do you have or have you ever had asthma? ☐ No ☐ Yes

If NO go to question 24, if YES:

21. Have you ever had asthma diagnosed by a doctor? ☐ No ☐ Yes

22. How old were you when you first experienced asthma symptoms? ..... years

23. In which year did you last experience asthma symptoms? 19...../ 20.....

24. Has a doctor ever told that you have COPD (BOLD) ☐ No ☐ Yes

25. Have you ever had wheezing or whistling in your chest? ☐ No ☐ Yes

25.1 If "Yes", how old were you when you first noticed wheezing or whistling in your chest? ..... years

25.2. If "Yes", when was the last year you noticed wheezing and whistling in your chest? 19...../ 20.....

26. Have you ever experienced nasal symptoms such as nasal congestion, rhinorrhoea (runny nose) and/or sneezing attacks without having a cold? ☐ No ☐ Yes

If NO go to question 25, if YES:

26.1 How old were you when you experienced them for the first time? ..... years

26.2 Have you had these kind of nasal symptoms in the last 12 months? ☐ No ☐ Yes

26.3 At which time of the year are your nasal symptoms worst?

Spring	Summer	Autumn	Winter	Always	Don't know
☐	☐	☐	☐	☐	☐

27. Has your nose been blocked **for more than 12 weeks during the last 12 months?** ☐ No ☐ Yes

28. Have you had pain or pressure around the forehead, nose or eyes **for more than 12 weeks during the last 12 months?** ☐ No ☐ Yes

29. Have you had discoloured nasal discharge (snot) or discoloured mucus in the throat **for more than 12 weeks during the last 12 months?** ☐ No ☐ Yes

30. Has your sense of smell been reduced or absent **for more than 12 weeks during the last 12 months?** ☐ No ☐ Yes

In-door and out-door environment

31. In which type of accommodation do you live?

Detached house	Semidetached or terraced house	Apartment	Other
☐	☐	☐	☐

32 When did you move to your current home? 19 .....

33. How many hours per day do you spend in your home most days? Approx. ..... hours/day

34. Does tobacco smoking take place in your present home?

Yes every day	Yes, frequently 1-4 times/week	Yes, sometimes 1-3 times/month	No never
☐	☐	☐	☐

35. Have any of the following been identified in your home during **the past 12 months:**

35.1 *Water leakage or water damage indoors in walls, floor or ceilings ☐ No ☐ Yes

35.2 *Bubbles or yellow discoloration on plastic floor covering, or black discoloration of parquet floor ☐ No ☐ Yes

35.3 *Visible mould growth indoors on walls, floor or ceilings. ☐ No ☐ Yes

36. Have you seen any signs of damp, water leakage or mould in your home at any time during the past X years? ☐ No ☐ Yes

37. Have you seen any signs of damp, water leakage or mould in your workplace at any time during the past X years? ☐ No ☐ Yes

38. Is your bedroom window towards a nearby street (<20 m)?

☐ No
☐ Yes a street with little traffic
☐ Yes a street with moderate traffic
☐ Yes a street with much traffic

39. Can you in your bedroom hear traffic noise?

- ☐ Not at all
- ☐ A little
- ☐ Much
- ☐ Very much

40. How much time do you usually spend walking or travelling along streets with busy traffic a typical weekday? Approx ..... minutes/day

Marital status

41. What is your marital status? *(more than one alternative may be true)*

- ☐ 1.Single
- ☐ 2 Currently married
- ☐ 3 Cohabitating
- ☐ 4 Separated or divorced
- ☐ 5 Widowed
- ☐ 6 Do not wish to answer

Marital status

42. Please mark the educational level which best describes your level:

- ☐ 1) Primary school
- ☐ 2) Lower or upper secondary school, or technical school
- ☐ 3) College or university

Occupation and work

43. Are you currently working? ☐ No ☐ Yes

44.. Which is your current or most recent work or occupation?

……

How many years have you worked or did you work in this occupation? .....years

45. We assume that your work ability, when it was as best, was 100 percent. How would you rate your current work ability, expressed in percent? ..... %

46. Have you ever changed job because the job affected your breathing? ☐ No ☐ Yes

46.1 If "Yes", in which years? .....

46.2 If "Yes", from which occupation/job did you change? *(could be several)* .....

47. Have you ever changed job because of hayfever or nasal symptom ☐ No ☐ Yes

47.1 If Yes, in which years? .....

47.2 If "Yes", from which occupation/job did you change? *(could be several)* .....

48 Have you ever changed job because of other health problems/diseases? ☐ No ☐ Yes

48.1 If Yes, in which years? .....

48.2 If "Yes", which occupation/job did you change from? *(could be several)* .....

49. Have you ever worked as a painter? ☐ No ☐ Yes

If "Yes", between which years? .....

50. Have you ever worked as a cleaner? ☐ No ☐ Yes

If "Yes", between which years? .....

51. Have you been reporting any days of sick leave during the last 12 months? ☐ No ☐ Yes

51.1 If yes, how many days have you been on sick leave?

☐ 1 – 7 days ☐ 8-30 days ☐ 31 days – 90 days ☐ More than three months

52. Have you been reporting any days of sick leave because of breathing problems during the last 12 months? ☐ No ☐ Yes

52.1 If yes, how many days have you been on sick leave for breathing problems?

1 – 7 days	8-30 days	31 days – 90 days	More than three months
☐	☐	☐	☐

Childhood and family

53. What term best describes the place you lived most of the time when you were under the age of five years?

☐ Farm with livestock
☐ farm without livestock
☐ village in rural area
☐ small town
☐ suburb of city
☐ inner city

54. When you were a child, which of the following were regularly used for heating?

Open wood	Coke or coal fire	Paraffin	Electricity	Gas or oil fired boiler
☐	☐	☐	☐	☐

55. Did you have a serious respiratory infection before the age of five years? ☐ Yes ☐ No ☐ Don't know

56.1. Did your father ever smoke regularly during your childhood? ☐ Yes ☐ No ☐ Don't know

56.2 Did your mother ever smoke regularly during your childhood? ☐ Yes ☐ No ☐ Don't know

56.3 Did other people (other than parents) smoke regularly at home during your childhood? ☐ Yes ☐ No ☐ Don't know

57. When you were a child, how often did you eat fresh fruits?

Never	Rarely	Every week	Almost daily	Almost daily in the autumn season
☐	☐	☐	☐	☐

58. Did your biological parents ever suffer from any of the following:

	Mother (yes)	Father (yes)
Asthma	☐	☐
Chronich bronchitis, emphysema and/or COPD	☐	☐
Heart disease	☐	☐
Hypertension	☐	☐
Stroke	☐	☐
Diabetes	☐	☐
Cancer	☐	☐

59. Do you have children (including grown-up children)? ☐ No ☐ Yes

If yes, how many? ..... children

Please write the years when your children were born, and tick "yes" if they have had any of the following:

Child	Birth year of child (year)	Asthma before 10 year (yes)	Asthma after 10 years (yes)	Hayfever/ rhinitis (yes)	Atopic eczema/Skin allergies (yes)
1					
2					
3					
4					
5					
6					
7					

Sleep and daytime symptoms

The numbers mean
1: Never or almost never
2: Less than once a week
3: once or twice a week
4: 3- 5 nights/days a week
5: Almost every day or night

How often has it occurred in the last months:

60. that You snore loudly and disturbingly? 1 2 3 4 5

61. that You have heartburn or belching when you have gone to bed? 1 2 3 4 5

62. that You have difficulty in getting to sleep at night? 1 2 3 4 5

63. that You wake up repeatedly during the night? 1 2 3 4 5

64. that You perspire heavily during the night? 1 2 3 4 5

65. that You feel drowsy in the daytime? 1 2 3 4 5

66. that You wake up too early and have difficulty in getting to sleep again? 1 2 3 4 5

67. Have you ever had sleep apnoea diagnosed by a doctor? ☐ No ☐ Yes

If "No" go to question 69, if "Yes":

67.1 What year did you get the diagnosis of sleep apnoea? Year .....

67.2 If you are currently treated for sleep apnoea, what treatment do you have?

- ☐ CPAP
- ☐ Oral appliance (bite splint)
- ☐ Previous surgery in the throat or nose
- ☐ Others

68 How long time do you usually sleep per night?

I usually sleep .....hours and .....minutes.

Other diseases

69. Have ever had hypertension (high blood pressure) diagnosed by a doctor? ☐ No ☐ Yes

If yes:

69.1 When did you get the diagnosis hypertension (high blood pressure)? Year .....

69.2 Are you currently taking any medication for hypertension (high blood pressure)? ☐ No ☐ Yes

70. Have you ever had stroke? ☐ No ☐ Yes

70.1 If you have had stroke, in which year was it? Year .....

71. Have you ever been treated in hospital because of heart infarction or angina pectoris? ☐ No ☐ Yes

If yes:

71.1 When were you treated (for the first time) at a hospital because of heart infarction or angina pectoris? Year .....

72. Have you ever had diabetes diagnosed by a doctor? ☐ No ☐ Yes

If yes:

72.1 What year did you get the diagnosis diabetes? Year: .....

72.2 What treatment are you currently using for diabetes?
- ☐ Insulin
- ☐ Tablets
- ☐ Both insulin and tablets
- ☐ Only diet

73. Do you have or have you ever had ulcerative collitis? ☐ No ☐ Yes

73.1 If yes: how old were you when the disease started? ..... years

74. Do you have or have you ever had Crohn's disease? ☐ No ☐ Yes

74.1 If, yes, how old were you when the disease started? .....years

General health

75 Does your gum bleed when you brush your teeth?
- ☐ Always
- ☐ Often
- ☐ Sometimes
- ☐ Rarely
- ☐ Never

76 How often do you usually brush your teeth?
- ☐ 2 times/day or more
- ☐ Once daily
- ☐ Less than daily

77. How frequently do you exercise? (Give an average)

Never	Less than once a week	Once a week	2-3 times a week	Almost every day
☐	☐	☐	☐	☐

77.1. If you do such exercise as frequently as once or more times a week: How hard do you push yourself? *(Give an average)*
- ☐ I take it easy without breaking into a sweat or losing my breath /
- ☐ I push myself so hard that I lose my breath and break into a sweat /
- ☐ I push myself to near-exhaustion

77.2. How long does each session last? (Give an average)

Less than 15 minutes	6-30 minutes	30 minutes to 1 hour	More than 1 hour
☐	☐	☐	☐

78. Body silhouettes

Information and contact conscent

In case we need to get in touch with you again please write your telephone number below

Telephone number: Daytime .....

Evening .....

THANK YOU FOR YOUR HELP