UNIVERSITÀ DEGLI STUDI DI VERONA

TELETHON KIDS INSTITUTE

DEPARTMENT OF

Diagnostics and Public Health

GRADUATE SCHOOL OF

Life and Health Sciences

DOCTORAL PROGRAM IN

Applied Sciences of Life and Health

(Epidemiology and Biostatistics)

Cycle / year (1° year of attendance): 33° Cycle (2017)

Early-life predictors of lung function in the Raine Study

S.S.D. MED/01

Coordinator: Prof. Giovanni Malerba

Tutor: Prof Francesca Locatelli Dr Rachel Foong Prof Graham Hall

Doctoral Student: Dr Francesca Sanna

Early-life predictors of lung function in the Raine Study
Francesca Sanna
Tesi di Dottorato
Verona, Novembre 2020
ISBN

Abstract

The natural history of lung function is characterized by various stages of development. The first phase includes lung function increasing to a peak around young adulthood followed by a plateau phase, and finally a physiological decline with age. Early-life lung function predicts later lung function and events that happened during the lifespan might influence the lung function trajectories of an individual, resulting in poorer lung health. The fetal and the first years of life are considered vulnerable periods in which the lungs undertake rapid and dramatic changes. Investigating early life risk factors and their impact on lung function trajectories from childhood to young adulthood is critically important as a first step in preventing long term lung impairments. Indeed, there is the need to further understand which risk factors are involved in the impairment of lung function trajectories.

With this thesis, I aimed to characterize lung function trajectories and investigate early-life predictors related to low lung function trajectories identified. A total of 1512 participants with at least two spirometry measurements were investigated using data from the Raine Study. Lung function trajectories for FEV_1, FVC and FEV_1/FVC (z-scores) were identified using group-based trajectory modelling for data available at 6, 14- and 22-year follow-ups. Multivariable analysis for childhood, parental and environmental risk factors was assessed using multinomial logistic regression. We identified four lung function trajectories for FEV_1, FVC and FEV_1/FVC. Associations were found between low lung function trajectories of FVC and asthma (p=0.024), maternal smoking (p=0.015), and parental asthma (p=0.024). Childhood wheeze was associated with the very low trajectory of FEV_1 (p=0.042) and females were more likely to belong to the low trajectory of FVC compared with males (p=0.026). Early-life exposures to $PM_{2.5}$ and NO_2 were not associated with lung function trajectories.

This study provides evidence of a group of the population that followed a persistently low lung function trajectory, characterized of having asthma, wheeze, mothers smoke in pregnancy and during childhood, parental asthma and that may be partly established before six years of age.

Table of contents

List of Figures

Supplementary Figure 4.4. Spatial distribution of predicted annual concentration of $PM_{2.5Abs}$ in Perth in 1995

Supplementary Figure 4.5. Spatial distribution of predicted annual concentration of NO_2 in Perth in 2012

Supplementary Figure 4.6. Spatial distribution of predicted annual concentration of $PM_{2.5}$ in Perth in 1995

Supplementary Figure 4.7. Spatial distribution of predicted annual concentration of $PM_{2.5}$ in Perth in 2012

Supplementary Figure 4. 8. Spatial distribution of predicted annual concentration of $PM_{2.5Absorbance}$ in Perth in 1995

Supplementary Figure 4.9. Spatial distribution of predicted annual concentration of $PM_{2.5Absorbance}$ in Perth in 2012

List of Tables

Supplementary Table 4.1. Available NO_2 data obtained by Department of Environment (DER)

Supplementary Table 4.2. Available $PM_{2.5}$ data obtained by Department of Environment (DER)

Supplementary Table 4.3. A, B Descriptive summary of $PM_{2.5Absorbance}$ and NO_2 at home address for the first year of life and the lifetime average between the first year of life and 22 years of age in the Raine participants (using the last address)

Supplementary Table 5.1. Association between childhood, parental factors, air pollution exposure and FEV_1, FVC and FEV_1/FVC lung function trajectories in Raine participants with two or more spirometry measurements (unadjusted)

Supplementary Table 5.2. Association between childhood, parental factors, air pollution exposure and FEV1, FVC and FEV1/FVC lung function trajectories in Raine participants with two or more spirometry measurements

Supplementary Table 5.3. Association between childhood, parental factors, air pollution exposure and FEV_1, FVC and FEV_1/FVC lung function trajectories in Raine participants with two or more spirometry measurements

Abbreviations

AIC	Akaike information criteria
ALSPAC	Avon Longitudinal Study of Parents and Children
APP	Average posterior probabilities
AR	Allergic rhinitis
BIC	Bayesian information criteria
CRS	Tucson Children's Respiratory Study
DoHaD	Development Origins of Health and Disease theory
ECRHS	European Community Respiratory Health Survey
ERS	European Respiratory Society
ESCAPE	European Study of Cohort for Air Pollution Effects project
FEV_1	Forced Expiratory Volume in 1 sec
FVC	Forced Vital Capacity
GBTM	Group-based trajectory modelling
GEIRD	Gene-Environment Interactions in Respiratory Diseases
GERD	Gastroesophageal reflux disease
GLI	Global Lung Function Initiative
ICD-9	International Classification of Disease codes, version 9
ISAAC	International Study on Asthma and Allergies in Childhood questionnaire
LMR-LRT	Lo-Mendell-Ruben likelihood ratio test
LUR	Land use regression models
MAAS	Manchester Asthma and Allergy Study
NAR	Non-allergic rhinitis
NO_2	Nitrogen dioxide
PM	Particulate matter

$PM2_{.5Abs}$	$Pm_{2.5Absorbance}$
RRR	Relative risk ratios
TAHS	Tasmanian Longitudinal Health Study
TEPM	Tapered Element Oscillating Microbalance
WHO	World Health Organization

Acknowledgments

I wish to dedicate this thesis to my beloved grandmother.

Firstly, I would like to thank the University of Verona, specifically the Department of Diagnostics and Public Health to have given me the opportunity to start my PhD journey. I would like to express my gratitude to my supervisor Professor Francesca Locatelli who first introduced me to various research projects and guided me during the initial stages of the PhD.

I would like to acknowledge my supervisors Dr Rachel Foong and Professor Graham Hall that gave me the chance to come to Telethon Kids Institute. I am extremely grateful to Rachel that with her support and expertise mentored me through the entire period at the Institute. Her continuous advice has been invaluable. I would also like to thank Graham for his thoughtful feedback and suggestions that always helped me to lead the research project in the right direction. I am grateful for their professional and human support, especially during this challenging year.

My gratitude extends to all the Children's Lung Health team, full of precious people that welcomed me since the first day. Our weekly meeting, activities and daily chat have been an extreme add value for me.

I am also deeply grateful for all researchers that helped me at some stages during my route. In particular Dr David Blake and Professor Jane Heyworth for assisting me with the air pollution data. A special thanks to all the wonderful people who crossed my path during these years, including colleagues and my lovely friends. They all have given me inspiration, support and they have made this long journey much more enjoyable.

The biggest thanks goes to my family. To my mother and my father who have always stressed the importance of love, education, and resilience. To my sister Giorgia who

has always encouraged me and to Nicolas who was ready to cheer me up whenever I needed. Despite being far from home during these years, I could always rely on them.

Last but not least, I would like to express my wholehearted thanks to Enrico who shared the entire journey with me. His love and encouragements have been invaluable, especially during tough times.

I was fortunate to count on so many people during the time spent in Verona and in Perth. I am sure that the completion of this work would not have been possible without all of you.

Statement of candidate contribution

This thesis contains published work and/or work prepared for publication, some of which has been co-authored.

I certify that this dissertation has not been submitted previously as part of the requirements for another degree and that it is the result of my own work, unless otherwise referenced and acknowledged.

Signature

Chapter 1.

Literature review and introduction

1.1 Introduction

Lung function is a recognized marker of respiratory health. Respiratory diseases are a leading cause of mortality and disability in the world, accounting for more than 13% of the active and productive life lost due; commonly referred to as disability adjusted-life-years (DALYs) (European Respiratory Society, 2017). To prevent and control these diseases over the lifespan it is crucial to identify which risk factors have an impact on the respiratory health of the population. According to the Development Origins of Health and Disease (DOHaD) concept that was firstly explained by Barker and colleagues (1989), our health is influenced by the environment and by events that happen in sensitive developmental periods, such as *in utero* and in early childhood (Wadhwa et al., 2009).

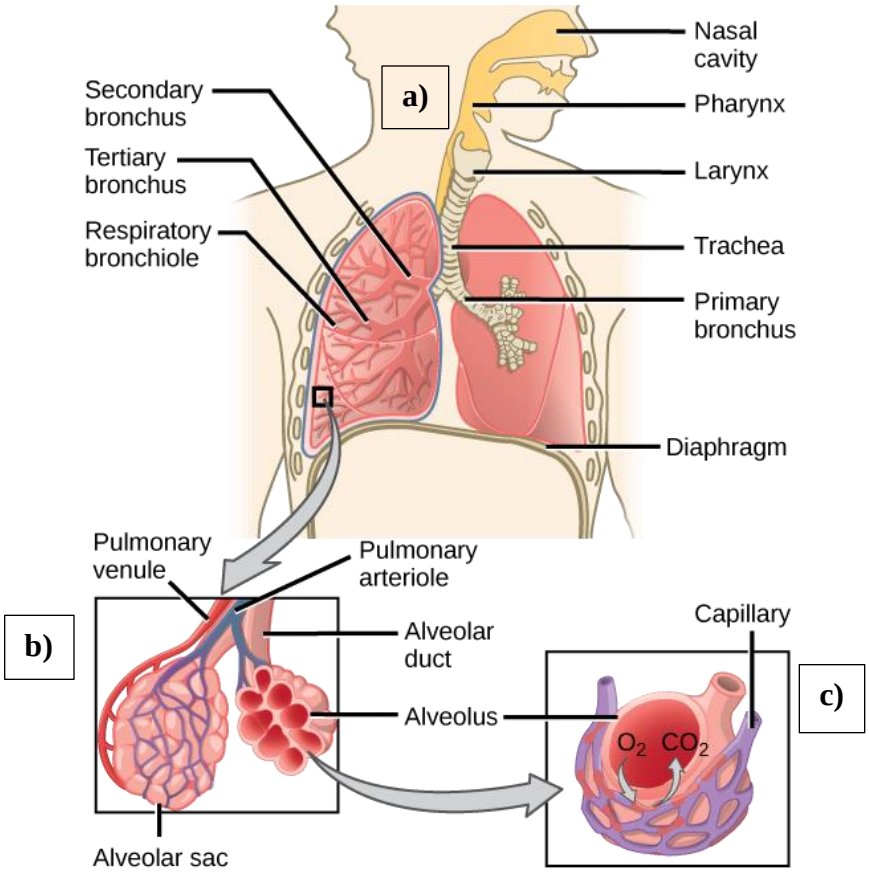
A wide range of studies have been conducted to investigate this theory, and it has been shown that determinants of chronic respiratory diseases, such as asthma and chronic respiratory obstructive diseases (COPD), have associations with insults that occur as a result of pre- and post-natal exposures (Stern et al., 2008; Maritz & Harding, 2011; Sly P., 2011). If negative events occur during these years in which the lungs are still developing, they might change the structure of the lungs, influencing lung function over the life course. Although studies have investigated the association between lung function and early-life predictors, most of them have focused on acute rather than long-term consequences. Thus, it is not clear how the early life period and which specific early-life factors are important in defining childhood and adulthood respiratory health.

This Chapter presents a review of the literature focused on the association between lung function and risk-factors in pre- and early post-natal life. To begin with, in the following sections I focus on the role of the lungs and the mechanism and development of lung function. Then, I review which risk factors have been associated with lung function impairments over the life span. The research gaps are identified, which leads to the formation of the research aims for my doctoral work.

1.2. Lung physiology and development

The respiratory system is one of the primary interfaces between the organism and the environment. Lungs are the essential organs of the respiratory system, together with the nose, oropharynx, larynx, trachea, bronchi, and bronchioles. The bronchi branch into progressively smaller airways until they terminate into the alveoli, that are the primary location of gas exchange (Figure 1.1). The oxygen enters in the lugs and it is conveyed through the alveoli into the capillaries and, ultimately, in the tissue.

Figure 1.1. *Structure of the lungs.*



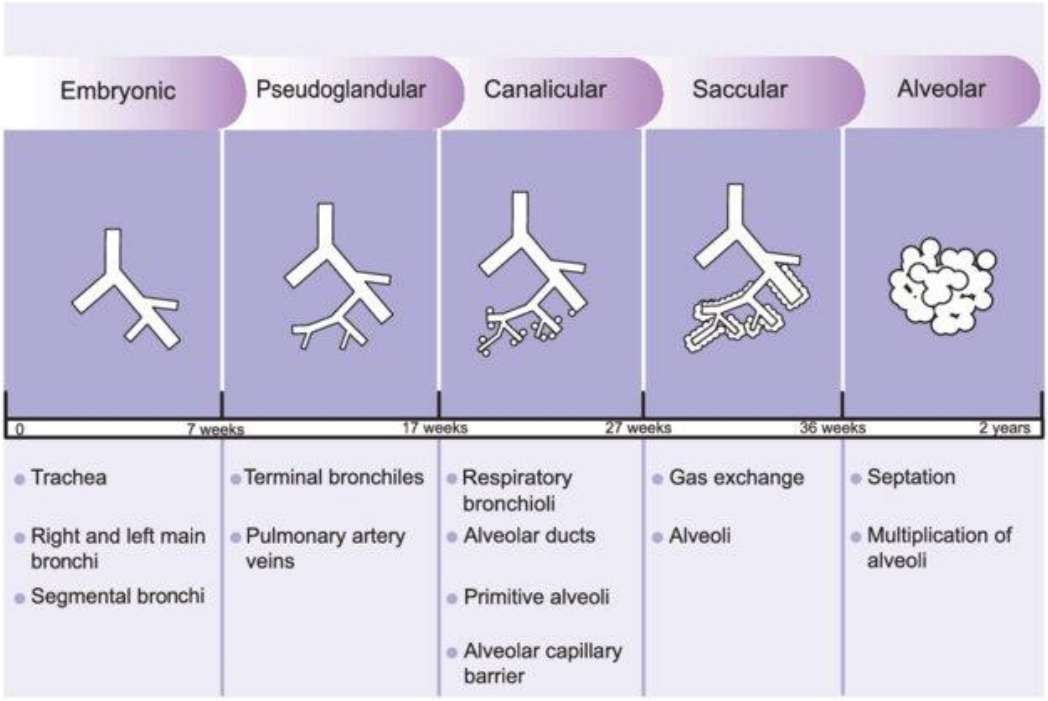
(OpenStax)

Note a) The tracheobronchial tree is the passageway from the mouth to the interior of the lungs b) gas exchange occurs in the alveoli deep into the lungs c) the lungs exchange oxygen and carbon dioxide between the air and the blood.*

Lung development starts *in utero*, as early as 3 weeks of embryonic life and continues to adolescence into early adulthood (GOLD, 2017), reaching a peak at 20-25 years of age. During early *in utero* life, the epithelial cells invade the surrounding mesenchyme to form the trachea, that branches into the main bronchi. In normal conditions, by 14 weeks, nearly 70% of the conducting airway tree present at birth has formed. Then, the acinar structures including the respiratory bronchioles, alveolar ducts and primitive alveoli are formed. During the canalicular stage, surfactant protein is detectable by 24

weeks of *in utero* life, establishing a possible platform for gas exchange. At around 28 to 36 weeks of intrauterine life, division of the airways is almost complete. The formation of double capillary walled secondary septa and multiplication of alveoli starts at around 36 weeks *in utero* and continues in the post-natal phase at least up to two- four years of age. It has been estimated that the number of alveoli at birth ranges from 20 - 50 million, and studies have reported that the number of alveoli in a fully developed adult lung reach around 300- 800 million (Joshi & Kotecha, 2007). Lung growth is completed at about 18 years in women and at about 20 years in men. The different phases of lung development are summarized in Figure 1.2.

Figure 1.2. *Principle stages of lungs development*



(Iliodromiti et al., 2013)

Note The major structures of the lung develop through the embryonic (0-7 weeks), pseudoglandular (8-17 weeks), canalicular (18-27 weeks), saccular (28-36 weeks), and alveolar (37 weeks- 2 years) stages during the gestation period.*

1.2.1 Lung function

Normal lung function development is characterized by crucial physiological phases through the life course, as a consequence of structural changes of the lung. These include a growth phase that reach the peak during early adulthood and a plateau phase followed by a steady decline due to aging (GOLD, 2017). As described in the previous section, during the lifetime and advancing with age, the lung undertakes structural and functional changes. These changes include a decrease in lung function, pulmonary remodeling, limited regeneration, and enhanced susceptibility to pulmonary diseases in people >65 years. Even in healthy individuals, lung function decreases with the age. Lung size is strongly associated with body size, dimensions of the thoracic cavity, sex, and age. Indeed, during childhood and adolescence, lung function increases 20-fold during the first 10 years of life, due to the fast growth of the lung (GOLD, 2017). One of the most common tests used to assess lung function is spirometry.

1.2.1.1. Spirometry

Spirometry is a reproducible and noninvasive lung function test (Moore, 2012). It is largely used to provide objective information for the diagnosis of lung diseases and monitoring lung health. The use of spirometry is fundamental to assess general respiratory health (Graham et al., 2019) and to check for respiratory abnormality.

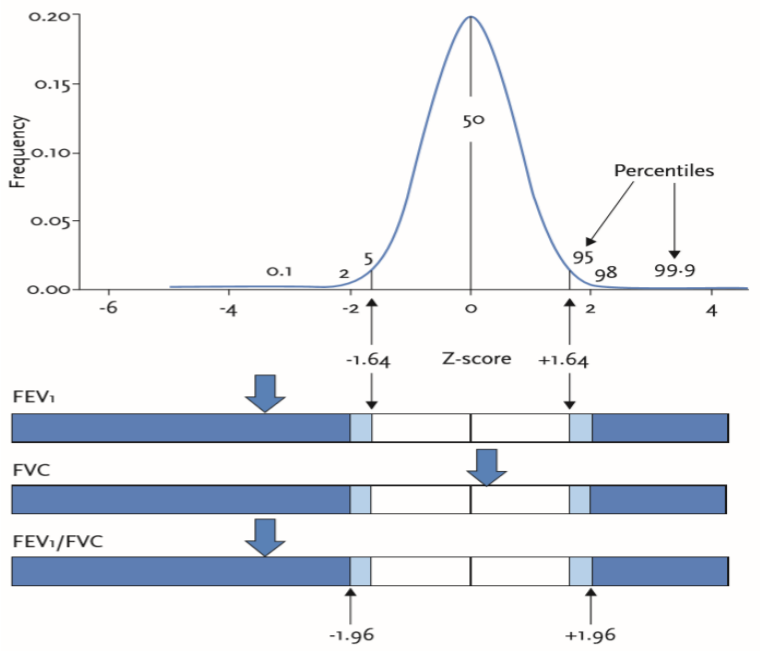
The main reported outcomes in spirometry are a) Forced expiratory volume in 1 second (FEV_1), that is the maximal volume of air exhaled in the first second of a forced expiration from a position of full inspiration. FEV can be reported at any given time, i.e. $FEV_{0.50}$ and $FEV_{0.75}$ are commonly used among young children where the lungs are less compliant; b) Forced vital capacity (FVC) that is the maximal volume of air exhaled with maximally forced effort from a maximal inspiration. c) FEV_1/FVC, that is the FEV_1 expressed as a percentage of FVC and gives a clinical index of airflow limitation; d) $FEF_{25-75\%}$, that is the average expired flow over the middle half of the FVC maneuver and is often regarded as a measure of small airway narrowing; e) Peak

expiratory flow (PEF), is the maximal expiratory flow rate achieved and occurs very early in the forced expiratory maneuver; f) Forced expiratory flow at 50% or 75% FVC ($FEF_{50\%}$ and $FEF_{75\%}$), that is the maximal expiratory flow measured at the point where 50% of the FVC has been expired ($FEF_{50\%}$) and after 75% has been expired ($FEF_{75\%}$); g) FVC_6, that is the forced expiratory volume during the first six second and is a surrogate of the FVC.

1.2.1.2. Standardization of spirometry

The major determinants of spirometric lung function are age, height, sex, and ethnicity. To facilitate the interpretation and comparison of spirometry, the results are compared to predicted values appropriate for individual being testes and standardized to the healthy population (GOLD, 2017). Recently, the Global Lung Function Initiative (GLI) addressed this issue and developed a global approach with the aim to standardize how lung function is interpreted worldwide (Cooper et al., 2017.). The main objective of the GLI was to derive "all-age" reference equations for spirometry from pre-school children to the elderly covering as many ethnic groups as possible. The GLI-2012 equations (Quanjer et al., 2012) have been approved by all major respiratory societies and have been validated in several populations, including Caucasian Australasians (Hall et al., 2012). These equations quote the various coefficients for age, height, and the residual standard deviation (RSD; standard error of the estimate) for the prediction. The range of values obtained from the healthy population is assumed to represent normal. Both the American Thoracic Society (ATS) and European Respiratory Society (ERS) recommend the use of the lower limit of normal (LLN), or upper limit, to define between health and suspected disease. By convention, the LLN is set at 5%, where 90% of the healthy population fall within the normal range. The normal range can be represented as a pictogram (Figure 1.3.), or as z-scores, in which 90% of healthy population will have a Z-scores values within ±1.64 z-scores. The z-score indicates how many standard deviations a measured value is from the predicted, removing the age-related bias.

Figure 1.3. *Normal distribution and corresponding z-scores and percentiles.*



Stanojevic et al., 2013

Note The horizontal bars demonstrated the normal range (white region), with arrows indicating how far from the normal range an observation is (z-scores). The normal range can be represented as z-scores in which 95% of healthy individuals have a z-scores within ±2 z-scores, and 90% within ±1.64 z-scores.*

1.3. Lung function trajectories

It has been shown that early lung function predicts later lung function and that different pathways may exist (Agusti & Faner, 2019). A variety of factors associated with both the environment and life- style that are present from the fetal period into adulthood may impact the natural lung function growth, resulting in abnormal lung function trajectories. Further, peak lung function reached in early adulthood has been reported to predict a diagnosis of chronic obstructive pulmonary disease (Lange et al., 2015) and to all-cause of mortality (Weinmayr et al., 2020). This may be either the consequence of an accelerated decline in lung function during adult life or the results of impaired

lung growth during childhood or *in utero (*Vasquez et al., 2017) Therefore, it is relevant to explore which factors influence lung function development, especially in childhood.

Characterizing lung function trajectories may help to recognize abnormal patterns and identify risk factors for impaired respiratory outcomes in later life. This is essential for early detection and prevention. As pointed out by Karmaus and colleagues (2019) most of the previous studies that analyzed lung function development over time used an arbitrary cut-off point of individual variability about the mean. This method does not allow us to identify unknown trajectories. Indeed, recently, more advanced statistical methods were used to identify trajectories. Most recent studies on lung function identified latent classes, but not trajectories addressing development over time. One study analyzed lung function trajectories allowing for non-linear development of lung function in individuals (Bui et al., 2018).

Studies on lung function development have been reported using different lung function outcome, where FEV_1 was the most investigated. Two studies investigated the FEV_1/FVC ratio, while other parameters explored were FVC, FEF_{25-75}. However, to the best of our best knowledge, none has examined trajectories of FEV_1, FVC and FEV_1/FVC expressed as z-score. These outcomes have been hypothesized to be the most clinical meaningful approach, especially when measured the parameters over time.

Longitudinal studies with measurements at various time points are essential to study individual lung function development over time. In the longest study, Bui et al (2018) using data from the Tasmanian Longitudinal Health Study (TAHS) analyzed FEV_1 lung function trajectories in individual followed from 5 to 53 years. Indeed, all the other studies investigated participants from adolescence to adulthood, while others investigated lung function trajectories in participants from 23 to 30 years (McGeachie et al., 2016) and from 3 to 11 years (Belgrave and colleagues, 2016). All these researchers found between two and three lung function trajectories, whereas Bui and colleagues (2018) found six trajectories, two of which had been never previously

characterized: early below average, accelerated decline and early low, accelerated growth, normal decline. These newer trajectories may be due to the longer time frame studied with the consequent ability to characterize the lung function trajectories decline. To summarize, it has been demonstrated that there are different trajectories of lung function in the general population, and that belonging to low lung function trajectories might lead to respiratory diseases later in life. Further, no studies investigated all the three respiratory outcomes from a wide period from childhood to adulthood.

1.4. Respiratory diseases

Lung diseases are mainly categorized as obstructive or restrictive diseases. In the next section a brief description of these diseases and their relationship with lung function trajectories will be described.

1.4.1. Obstructive lung diseases

Obstructive lung diseases are major global health problems, with asthma and chronic obstructive pulmonary disease (COPD) being the two main contributors. Reduced maximal attained lung function, as measured by spirometry, may identify individuals who are at risk for developing COPD (GOLD, 2017).

Asthma is a heterogeneous condition in both children and adults and the most common chronic condition among children around the world and in Australia, accounting for a consider burden of disease (National Asthma Council Australia, 2018; Global Initiative for Asthma, 2020). Most asthma starts in early childhood and around 95% of asthma patients have their first episode before preschool age (Papi et al., 2018). The disease is characterized by various respiratory symptoms, such as wheezing, shortness of breath, chest tightness, and cough, as well as variable airflow limitation. These features can be caused by numerous mechanisms that are often associated with airway inflammation and airway modelling (Stern et al., 2010).

Recently, different groups of patients with similar clinical characteristics have been defined, leading to the identification of different asthma phenotypes. Childhood-onset allergic asthma is often associated with eczema, rhinitis, food allergy, a family history of allergy and asthma, wheeze and with viral respiratory infections and tobacco exposure. Non-allergic asthma can present at any age, and in children is more likely to resolve in adolescence. Late-onset asthma is considered when asthma symptoms appear for the first-time after the completion of puberty and is common in females. However, definitions are varied and can refer to onset as early as 12 years of age or as late as over 65 years and is often underdiagnosed. This type of asthma is often non-atopic, more severe and associated with a more rapid decline in lung function. Occupational asthma is another type of asthma induced by occupational exposure to allergens or irritants and is sometimes preceded by rhinitis. Although evidence for a causative role is not exhaustive, asthma might be a risk factor for subsequent development of chronic airflow limitation and COPD (Silva, Sherrill, Guerra, & Barbee, 2004).

Chronic respiratory pulmonary disease is a common, preventable, and treatable disease characterized by persistent respiratory symptoms and airflow limitation. The disease is currently the fourth leading cause of morbidity and mortality worldwide and is projected to increase over time (GOLD, 2017). The airflow limitation or obstruction in COPD is caused by a mixture of small airway disease, parenchymal destruction and, in some cases, asthma. Chronic inflammation of the lungs leads to structural changes, restriction of the small airways and destruction of the lung parenchyma. These changes reduce the ability of the airways to remain open during expiration. Reduced maximal attained lung function, as measured by spirometry, might identify individuals with higher risk for the development of COPD. A recent study (Lange, Celli, Agustí, Jensen, et al., 2015) suggested that lung function value reached in early adulthood was associated with diagnosis of COPD in later life. The diagnostic criterion for COPD is based on spirometry confirming a reduction in the FEV_1/FVC ratio below 0.7, or below the lower normal limit. However, even in healthy individuals lung function declines

with age. Thus, the use of a fixed cut-off might result in overdiagnosis of COPD in the elderly and underdiagnosis in the youngest (Ho et al., 2019).

The Fletcher-Peto assumption on the normal lung function decline states that all individuals attained a normal lung function in young adulthood, followed by a rapid decline, where smoking was the main determinant (Fletcher & Peto, 1977). However, this hypothesis has been expanded in recent years. Specifically, the study conducted by Lange and colleagues (2015) on three large independent cohorts showed that an accelerated decline in lung function was not a characteristic feature in all participants who developed COPD. In fact, they found two main trajectories of lung function decline with age: one associated with normal initial lung function and a second with low lung function attainment in young adulthood. With this concept in mind, the pathogenesis of COPD needs to be investigated also in individuals whose low lung function trajectory starts in early life.

Few longitudinal studies attempted to investigate the childhood origins of COPD, identifying early risk factors associated with airflow obstruction later in life. In the Childhood Asthma Management cohort (McGeachie, 2016), 11% of participants with abnormal lung function trajectories at their last spirometric session (mean age, 26.0±1.8 years) met the criteria for COPD in early adulthood. Further support for this came from Bui and colleagues (2018) that used data from the Tasmanian Longitudinal Health Study and reported that 75% of COPD cases at 53 ages were attributable to the three low lung function trajectories of FEV_1 that they identified.

Many studies suggest that asthma may lead to COPD or persistent airflow obstruction or is a risk factor for COPD. Indeed, findings from 20 years of follow up using the Tucson Epidemiologic Study of Airway Obstructive disease (TESAOD) showed that adults participants with current asthma were almost 13 time more likely to fulfill COPD criteria, even after adjusting for smoking status and other potential confounders (Silva et al., 2004). Similarly, in the analyses performed using the large European Community

Respiratory Health Survey (ECRHS), Svanes and colleagues showed that children with asthma had a 10-fold increased risk of developing COPD later in life (Svanes, 2010)

All these findings provide insight into the origins of obstructive lung diseases and emphasize the importance of investigate early risk factors or adverse exposures that might lead to poorer lung function trajectories that might track into adulthood.

1.4.2. Restrictive lung diseases

Restrictive lung diseases are a rarer heterogeneous group of conditions that impair lung expansion and characterized by a reduction in lung volume and worsens with age (Burke et al., 2012). With the impaired lung expansion of restrictive lung disease, there is decreased compliance and vital capacity of the lungs, chest wall or both. This occurs in pulmonary fibrosis, pleural disease, chest wall disorders, neuromuscular disorders, pneumonectomy, pulmonary oedema and obesity. A restrictive pattern on spirometry is defined by a reduction in FVC, without bronchial obstruction, less than the LLN. If the FEV1/FVC ratio is ≥ LLN and FVC is <LLN, a restrictive pattern is suggested, and it should be confirmed evaluating the total lung capacity (Brazzale, Hall, & Swanney, 2016). To the best of our knowledge, no studies assessed the association between lung function trajectories and risk factors associated to restrictive lung diseases.

1.5 Early life risk factors of low lung function

Factors associated with both the environment and lifestyle during crucial periods, such as fetal, post-natal, and childhood can lead to permanent, physiological, structural, or epigenetic changes that persist over the life course (Chen & Zhang, 2012). While prenatal factors are more likely to impair airway development, postnatal factors are more likely to affect the airway growth, alveolarization, and microvasculature. The risk factors implicated in poor lung health include respiratory infections, environmental tobacco smoke, adverse dietary intake, premature birth, obesity, air pollution and

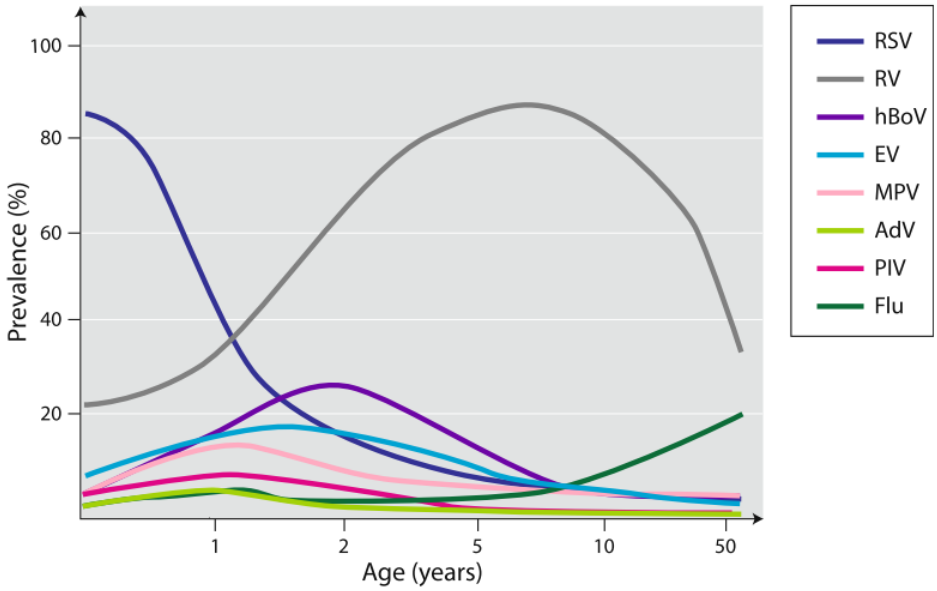
asthma (Stocks & Sonnappa, 2013) and the evidence relating to these risk factors and lung function trajectories are reviewed below.

1.5.1. Respiratory infections

Respiratory tract infections account for a large proportion of childhood illness through the world, with 156 million new episodes of pneumonia per week worldwide in children aged less than 5 years (WHO, 2019). Evidence is growing suggesting that respiratory infections in early life lead to a decline in lung function or medium and long-term development of asthma (Jackson & Lemanske, 2010). However, although respiratory infections can affect the lungs in many ways, not all individuals who contract a respiratory infection experience an asthma exacerbation.

Respiratory syncytial virus (RSV), rhinovirus (RV) and Influenza, are the most common and important causes of lower respiratory tract infections (LRTI) in young children together with others (Figure 1.4.). While most children become infected during the first years of life, the illnesses from respiratory tract infections can range from asymptomatic infections to pneumonia or bronchiolitis that may lead to hospitalizations or mortality (Lessler et al., 2009; Townsi et al., 2018).

Figure 1.4. *Virus etiology from bronchiolitis to asthma in childhood*



(Jartti, Bønnelykke, Elenius, & Feleszko, 2020)

Note RSV, respiratory syncytial virus; RV, rhinovirus; hBoV, human bocavirus; Flu, influenza virus; EV, enteroviruses; MPV, metapneumovirus; PIV, parainfluenza virus; AdV, adenovirus*

There is the chance that certain risk factors increase the susceptibility to present with wheeze after viral infections occur. In a birth-cohort population of 285 children at increased risk to develop allergies and asthma, having respiratory infections during infancy was one the main risk factors for the development of subsequent wheezing at 3 years of age (Lemanske et al., 2005). Existing evidence supported the association between respiratory infections and asthma. However, whether symptomatic low respiratory tract infections are causal in asthma development, or if it is a marker of susceptibility identifying predisposed children remains unclear.

Various studies support the hypothesis that respiratory infections, particularly those resulting in pneumonia and bronchitis, lead to diminished FEV_1. In the Tucson Cohort, Berry and colleagues (2016) identified that LRTI caused by RSV in the first three years of life represented a risk factor for having a lower lung function trajectory. Svanes and

coauthors (2010) found that severe respiratory infections before the age of 5 years were associated with a lower adult FEV_1 level, that might contribute to develop COPD. Lopez and colleagues (2013) showed that LRTI experienced between birth and 5 years of age were associated with reduced lung function measurements at age 25 for FEV_1, FVC, FEV_1/FVC, $FEF_{25\%-75\%}$, and PEF. Allinson and colleagues (2017) in an epidemiological study in a population aged between 43 and 64 years, found that participants with adverse exposure in early life, such as LRTI, had a decrease in FEV_1 in adulthood, only if they had smoked.

Although there are few epidemiological studies that agreed on the fact that childhood respiratory infections are predictors for unfavorable lung function later in life, their implications on adult respiratory health are still limited and unclear. Further, there is a disagreement as to how this relationship changes in relation to the age in which infections are experienced and if the infections are whether associated with obstructive or restrictive deficits or if it also occurs with upper respiratory tract infections (Lopez Bernal et al., 2013). Although the impact of childhood respiratory infections on lung function decline has been established, their implications in adult lung function remain uncertain.

1.5.2. Air pollution

Exposure to air pollution contributes to mortality and morbidity and it is an important cause of the global disease burden (Kim et al., 2018; Künzli et al., 2010). Further, it is known as one of the most important determinants of lung function, especially in vulnerable subgroups, such as children and in those with asthma (Losacco & Perillo, 2018). Negative health effects of air pollutants have been shown on multiple respiratory diseases, including respiratory infections, asthma, COPD, and lung cancer (Wu et al., 2018).

Although the polluting substances dispersed in the atmosphere are numerous, the major pollutants in the atmosphere include particulate matter (PM), ozone (O_3), nitrogen

oxide (NOx), sulfur dioxide (SO_2) and volatile organic compounds (VOCs) (Wu et al., 2018). Sulphur dioxide belongs to the family of sulphur oxide gases (SO_X) and is present in raw materials including crude oil, coal, and the ores of common metals, as aluminum, copper, zinc, lead, and iron. SO_X gases are formed when fuel containing sulphur is burned, when gasoline is extracted from oil and when metals are extracted from ore. NO_X is the generic term of a group of highly reactive gases, all of which contain nitrogen and oxygen in varying proportion. NO_X are emitted primarily because of high temperature combustion. Road traffic is a dominant source of NO_X. VOCs are organic chemical compounds that have sufficiently high vapor pressures under normal conditions to vaporize significantly and enter the atmosphere.

Atmospheric particulate matter (PM) is a complex mixture of primary and secondary extremely small particles and liquid droplets containing acids, organic chemicals, metals, and soil or dust particles (Losacco & Perillo, 2018). Primary PM is generated from road transport, combustion (mainly coal burning), and other industrial processes, whereas secondary PM is generated through chemical reactions among different primary particulates in the atmosphere (Xing et al., 2016). Its pathogenicity is determined by their size, composition, origin, solubility, and their ability to produce reactive oxygen. Although the bronchopulmonary tract consists of various protective mechanism, such as the mucosa cilia and air blood barrier, air pollutants can accumulate in or pass through lung tissue depending on their size and their chemical nature.

According to their diameter size, PM are classified into:$PM_{2.5}$, with a diameter $\leq$ 2.5 µm and PM_{10}, with a diameter $\leq$ 10 µm. The vapor of air pollutants tends to be absorbed by human tissues or dissolved in body fluids. Particulate matter with larger size (~10 µm) can reach the proximal airways and be mostly eliminated by mucociliary clearance. Smaller particulate matter referred to fine (PM with a diameter $\leq$ 2.5 µm) and ultrafine particles (with a diameter < 100nm, $PM_{0.1}$) can transit more deeply into the lungs (Mortimer et al., 2008). Due to their small size, they have a life-time of days

to weeks and can transport over thousands of kilometer distance. The fine particulate matters are respirable, and are small enough to travel into respiratory tract, can penetrate deeply into the lung, irritate, and corrode the alveolar wall, and consequently impair lung function. Although these ultrafine particles can be observed in many organs, their deposition patterns show that lungs are the primary site (Wu et al., 2018) and pre- and post- natal effects on lung function have been investigated. There is growing awareness of the adverse effects of both prenatal and postnatal exposure to air pollution. Evidence of air pollution exposure on lung function are reviewed below.

1.5.2.1. Prenatal exposure to air pollution

Few studies have examined the association between exposure to ambient air pollution *in utero* and lung function over the lifespan. Mortimer and colleagues (2008) conducted a population-based study analyzing data of 232 asthmatic children from the Fresno Asthmatic Children's Environment cohort. They explored the association between exposure to air pollutants during pregnancy and lung function at 6-11 years, showing that exposure to NO_2 in the second trimester of gestational period was associated with a decrease in both FVC and FEV. First trimester exposure to PM_{10} negatively affected PEF rate, while second trimester exposure to PM_{10} had a negative effect on forced expiratory flow at 25% of FVC. Prenatal exposure to Carbon monoxide (CO) was negatively associated with FEF_{25-75}. Similarly, using advanced Bayesian statistical methods, Bose et al (2018) investigated the exposure to NO_3 during the gestational period on lung function at age 7. The authors reported that an increase in NO_3 exposure between 6 and 12 weeks of pregnancy was associated with a reduction in FEV_1 in preschoolers, with a higher effect in boys. Similar reductions were seen for FVC. In a prospective birth cohort study of 241 healthy infants, each increase of 1 $\mu g \cdot m^3$ in PM_{10} during pregnancy was associated with an increase in minute ventilation of 24.9% at five weeks of age (Latzin et al., 2009). Other studies found also that prenatal exposure to $PM_{2.5}$ was strongly associated with wheezing in the first 2 year of life (Morgenstern

et al., 2007) and that higher exposure to ambient air pollution in early life was associated with elevated risk of asthma diagnosis in preschool age children.

1.5.2.2. Early postnatal exposure to air pollution

Both cross-sectional as well as longitudinal studies have clearly shown diminished lung function in children with a short- and long-term exposure to air pollutants. However, findings are controversial. Some studies reported associations with FEV_1, whereas others showed association with FVC, or with both lung volume and expiratory flow and others found no association at all.

A review by Götschi and colleagues (2008) concluded that there is evidence for small adverse effect of outdoor air pollution on lung function in children and adolescents. A birth cohort study conducted on 1185 children of elementary-school age from the Manchester Asthma and Allergy Study (MAAS) birth cohort aimed to investigate the long-term effect of exposure to PM_{10} and to NO_2 on specific airway resistance and FEV_1 (2013). The authors found a statistically significant impairment in growth of FEV_1 with an increase in exposure to both air pollutants. Although the reduction in FEV_1 growth was small, and may had little impact on healthy individuals, the authors speculated that they could had more implications for susceptible individuals, as those with COPD or in children who will go on to smoke cigarettes. At present, there is a large difference among studies regarding the study design and exposure assessment. However, a meta-analysis conducted by Gehring et al. (2013) explored the association between individual residential exposure to air pollution and lung function, using common exposure assessment and statistical analysis protocols across five European birth cohort. They found that estimates levels of NO_2, NO_X, $PM_{2.5Absorbance}$ and $PM_{2.5}$ were associated with a small decrease in lung function in a population of school children. While most of the studies are conducted on high levels of air pollution concentrations, a population-based cross-sectional was carried out in an area in which the level of air pollutants was relatively low, using data from 2630 Australian children

aged between 7 and 11 years. The concentrations of NO_2 in the last 12 months were associated with a reduction in both FEV_1 and FVC, and an increase in FE_{NO} (Knibbs et al., 2018). Indeed, another study found similar results with regards to FEV_1, but the association was stronger in males compared to females (Gehring et al., 2013). Fuertes and colleagues (2015) assessed the association between residential exposure to air pollution and lung function in 2266 children in two German birth cohorts. They found that long-term exposure to air pollutants was not associated with lung function. However, a significant negative association for NO_2 was observed among asthmatics. Similarly, in a large population-based survey conducted in Germany, Nicolai et al (2003) found that no significant association of traffic counts on lung function. More recently research (Shao et al., 2020) investigated the effect of high-level of $PM_{2.5}$ exposure during a bushfire episode in children aged less than two years of age. Interestingly, at three-years of follow up, they had worse peripheral lung mechanics. These findings suggest that even if these children will be able to recover their respiratory over the years, the early-life exposure to a short period of air pollution might compromise their lung growth and development.

Although the association between *in utero* and post-natal exposure of air pollution and declined lung function has been established, findings are controversial and respiratory outcomes later in life might vary for different pollutants. Further, most of the studies have been conducted in areas where air pollution concentrations are high, while less is known relating to areas at low concentration levels.

1.5.3. Environmental tobacco exposure

There have been a wide range of studies showing that exposure to tobacco smoke in pregnancy or in early life is associated with impairment in lung function or increases in respiratory symptoms in childhood (Wang et al., 2020). In addition, it has been reported to result in negative pregnancy outcomes, such as low birth weight and premature birth (Rona et al., 1993).

1.5.3.1. Prenatal exposure to smoke

Maternal smoking during pregnancy is considered one of the largest risk factors for abnormal lung development (Stocks & Sonnappa, 2013). Despite the incidence of *in utero* smoking decreasing in the past decade (Zacharasiewicz, 2016), about 10% of women in Australia who gave birth still smoked in the first 20 weeks of pregnancy (Australian Government, 2019). A wide range of cohort studies investigated the association between *in utero* smoking and lung function. A study on 803 healthy infants in Norway (Lødrup et al., 1997) reported that babies of smoking mothers had impaired flow volume curves compared to babies of non-smoking mothers. Gilliland and colleagues (2003) showed that non-asthmatic children with a history of *in utero* smoke exposure, had decreased FEV_1/FVC ratio, FEF_{25-75}, and FEF_{25-75}/FVC ratio. Further, it has been shown that children exposed to *in utero* smoke and having early onset asthma reported deficits in FEV_1, and FEF_{25-75} among boys; and FEF_{25-75} among girls. The authors speculated that *in utero* exposure and early onset asthma are associated with persistent impairment on lung function, suggesting that children with early onset asthma might be a high-risk group for develop respiratory deficits over life. A longitudinal birth cohort study conducted on 2409 young adults (Hayatbakhsh et al., 2009) investigated the association between *in utero* exposure to maternal smoking and poorer lung function in early adulthood. They found that prenatal exposure to maternal smoking was associated with a reduction in the small airway flow rates in males. Stick and colleagues (1996) reported that newborns with a family history of asthma had larger deficits in lung function from *in utero* exposure compared with those without a family history of asthma.

Recent studies showed that maternal smoking have an intergenerational effect. Various studies reported also that grandmaternal smoking increased the risk of maternal asthma and, even if the mother herself does not smoke, it increased the risk of her offspring of having asthma (Accordini et al., 2018).

With regards to lung development, most research has focused on the effect of nicotine in pregnancy on the child. One of the most important effect of nicotine is its role in altering the structural development of the lung. The alveoli are bigger, and a potential mechanism for reduced lung function is a reduction in the number of alveolar-bronchiolar attachment points, with the consequence of a reduction in alveolarization. In animal models, it has been demonstrated that fetal exposure to nicotine leads to a reduction in the surface complexity of the lung parenchyma, increased collagen accumulation, upregulated surfactant protein gene expression and induction of neuro-endocrine cell hyperplasia in fetal lungs, with the consequence of lung function reduction (Maritz & Harding, 2011). Environmental tobacco smoke exposure often begins *in utero* with maternal smoking. However, the effects of postnatal tobacco smoke exposure may also lead to poorer respiratory health.

1.5.3.2. Early postnatal exposure to secondhand smoke

Early postnatal exposure to secondhand smoke is considered a risk in reducing lung function in young children. However, findings are controversial. In a large cohort of 1505 preschool children, Leung and coauthors (2013) found that children exposed to environment tobacco, quantified by urine cotinine levels, had $FEV_{0.5}$, FEV_{25-75}, and PEF lower than unexposed children. A meta-analysis of over 70 studies performed by Burke and coauthors (2012) summarized that postnatal maternal smoking was associated with significantly increased risks of onset of wheeze and asthma in children. The study found that passive smoke exposure was associated with a 20% to 85% increased risk of incidence of asthma. However, in a prospective study in 2295 children, early life exposure to secondhand smoke was not associated with lung function in infancy or at age 16 (Thacher, 2018). In a study with data from derived from the Mater University of Queensland Study of Pregnancy (MUSP), Hayatbakhsh and colleagues (2009) reported that exposure to maternal smoking between six months and 14 years was not a predictor for lower lung function at 21 years of age.

Although studies provided evidence of the association of *in utero* smoking and lung function decline in children, there is no clear evidence that postnatal exposure to maternal smoking has an independent effect on the child's lung function. In fact, there have been difficulties in separate the effect of prenatal and post-natal tobacco smoke, since women who smoke during pregnancy might continue to smoke after the birth of the child. Further, evidence is emerging in reporting that smoking in the previous generations can influence the risk of asthma in the offspring.

1.5.4. Allergens

Allergens have been consistently reported as a risk factor for bronchial hyperresponsiveness and asthma in children and adulthood (Nelson, 2000). The most common way to define atopy is by the skin prick test (SPT) or by the presence of specific IgE antibodies measured in the blood (Arasi et al., 2019). SPT and specific IgE results have been commonly dichotomized into positive or negative according to an arbitrary cut-off value. Typically, this was a 3mm wheal diameter for SPT and 0.35 kU/l for specific IgE. Atopic manifestations may persist for several years and then resolve over time. However, in atopic children, adolescents and adults, allergy manifestations may evolve following a predetermined sequence, characterized by the progression from atopic dermatitis to allergic rhinitis and asthma (Arasi et al., 2019)

Although several studies have examined the effect of allergens exposure indicating decline on various parameters of lung function in individuals with asthma, others showed that increased airway responsiveness is associated with an accelerated decline in lung function in individuals with no respiratory symptoms (Scichilone et al., 2005). Arasi and coauthors (2019) investigated the association between atopy, as determined by immediate cutaneous hypersensitivity to four common aeroallergens (house dust, ragweed, mixed trees, and mixed grasses), and the rate of decline of spirometric measurement of lung function, including FEV_1 and FEV_1/FVC ratio. They found that atopy was a significant independent factor of lung function decline among middle-aged

and older men without history of asthma. Lowe and colleagues (2004) found that children aged 3 years of age who were both sensitized and exposed to high levels of sensitizing allergen had significantly worst lung function compared with those who were either not sensitized or were sensitized but not currently exposed. These findings show that allergens might have an important role in lung function impairment, even if it is still uncertain whether they have an impact on lung function decline later in life.

1.5.5. Preterm birth

Preterm birth has been defined as any birth before 37 weeks of gestation. Rates of preterm birth have increased over the past 20 years, and 11% of all live births worldwide are estimated to be preterm (Townsi et al., 2018). As lung development is a process that is supposed to take place *in utero*, preterm born babies are at risk of later morbidity as lung development is interrupted during the canicular and saccular/ early alveolar phases.

Various studies have investigated the short and long-term outcomes on lung function in survivors of preterm infants. A cohort of preterm born participants consisting of two population-based cohort was investigated by Vollsæter and colleagues (2013). The authors found that preterm infants, especially those with bronchopulmonary dysplasia (BPD), had significantly lower FEV_1 and FEF_{25} than those born at term. Filippone and colleagues (2009) investigated lung function in preterm infants from age 5 to 15, showing lower lung function in 17 BPD survivors. Simpson and colleagues (2018) assessed longitudinal changes in lung function from early childhood to adulthood in very preterm babies (≤ 32 gestation weeks), showing that lung function trajectories in survivors of very preterm children were impaired, especially those with bronchopulmonary dysplasia and respiratory symptoms. In a cohort study assessing the lung function development of adolescence born preterm, Um-Bergström and colleagues (2017) suggested that subjects with a history of BPD preterm had a pattern of increasing airway obstruction.

Preterm birth is associated with increased respiratory symptoms, partially reversible airflow obstruction compared with those born at term (Forno et al., 2019). In a study of preterm babies, Narang and coworkers (Narang et al., 2008) reported that ex preterm infants had significantly greater respiratory symptoms compared with non-preterm infants. However, even if the prevalence of respiratory symptoms was still higher than in the control group, the prevalence was lower in adulthood than in mid-childhood for preterm babies.

It is also widely recognized that male infants have a higher rate of respiratory disease than female infants. This is shown to be true even if they are term-born (Ito et al., 2017). Stocks et al (1997) showed that male sex was associated to lower lung function in healthy preterm born infants compared with healthy preterm-born female infants. Similarly, a study on extremely preterm babies (<28 weeks of gestation) found that male sex was associated to lower lung function at birth compared to females (Bentsen et al., 2017). Doyle and colleagues (2017) examined lung function in a preterm cohort, showing that extremely preterm born infants had small airway obstruction between 8 and 18 years of age compared with controls, and that the airway obstruction increases with age. Thus, these findings suggest that preterm and very preterm babies are more susceptible to have lung function declined compared to those birth at term, and that impairment might persist into adulthood.

1.5.6. Breastfeeding

Breastfeeding has been associated with a variety of beneficial effect for children. Although various studies reported a protective association between breastfeeding and decline of lung function in children, this association remains uncertain (Oddy, 2017).
A study conducted on the Isle of Wight cohort, Ogbuanu and colleagues (2009) found that children who were breastfed for at least 4 months had an increased FVC, FEV_1 and

PEF (54 ml, 39.5 ml and 180.8 ml/s, respectively) compared with non-breastfed infants. A recent population-based cohort study including 4464 children have shown that a shorter duration of breastfeeding was associated with a lower FEV_1 and FVC, while nonexclusive breastfeeding with a lower FVC in school-aged children (Meel et al., 2020). However, not all studies have confirmed these associations. A prospective study including children from the ALSPAC cohort (Elliott et al., 2011) reported a modest protective effect of breastfeeding or wheezing in the first year of life. However, no effect of breastfeeding was seen on bronchial responsiveness. In a cohort of 377 healthy children, Gorlanova and coauthors (2020) found no significant association between the duration of breastfeeding and lung function outcome, or other respiratory symptoms in children. However, the authors reported that a longer duration of breastfeeding was associated with a reduced risk of atopic dermatitis in girls. The impact of breastfeeding on long term respiratory health has not largely investigated. In a study by Tennant and colleagues (2008) on 412 adults from the Newcastle Thousand Families cohort found that less than 4 weeks of breastfeeding was associated with lower adult FEV_1. Although breastfeeding is shown to have beneficial impact on health, there is not clear evidence of short- and long-term lung function outcomes.

1.6 Conclusions

There is growing evidence that life events happening from *in utero* life, childhood through adolescence have an impact in increasing the risk of impaired lung function later in life. This in line with the Developmental Origins of Health and Disease (DOHaD) theory in which early-life exposures might have a long-term impact on disease in adulthood. It has been shown that that lung function tracks throughout adulthood, and longitudinal and cross-sectional studies link various prenatal and postnatal early-life predictors such as early life infections, asthma, and outdoor air pollution to a decline in lung function during childhood and adulthood. However, although a wide range of studies have reported changes in lung function over the life

course, it is still unclear if these are associated with a certain age range or if they have an impact on lung function trajectories.

In this thesis, the main hypothesis is that early life exposure to lifestyle and environmental factors have a long-term impact on lung function trajectories. This hypothesis leads to the formulation of the general aim to investigate which early life predictors might contribute to be a risk to lung function impairment. The specific aims are described below:

Aim 1. Characterize lung function trajectories in the general population.

It has been shown that based on life experiences, individuals in the general population have their own lung function trajectories that might already be defined in early life, when the lungs are still developing. However, it has been shown that pre- and post-natal life represent susceptible periods for each individual and identify which factors influenced belonging to a particular trajectory is important to implement strategy to prevent future health respiratory deficits. In this thesis I hypothesized that different lung function trajectories exist in the Raine study population.

Aim 2. Identify early life, parental, and environmental predictors associated with low lung function trajectories identified.

Although various early life predictors have been shown to be associated with low lung function in childhood, it is uncertain whether they have a long-term impact on lung function later in life, possibly leading to respiratory health impairments in adulthood. I hypothesized that childhood factors measured before the children were six years of age, and parental risk factors were associated with low lung function trajectories throughout childhood and into adult life.

Aim 3. Investigate the association between low air pollution concentration and lung function in children.

Air pollution exposure increases the children mortality and morbidity, and it is an important cause of the global health disease. Children are considered a vulnerable group showing declined lung function when exposed to air pollution. Although the association between outdoor air pollution and lung function deficits has been largely investigated, less is known in areas at low concentrations level. I hypothesized that early-life exposure to outdoor air pollution, such as $PM_{2.5}$ and NO_2, would have a negative impact on children's lung function trajectories.

Chapter 2.
Methods

2.1. Introduction

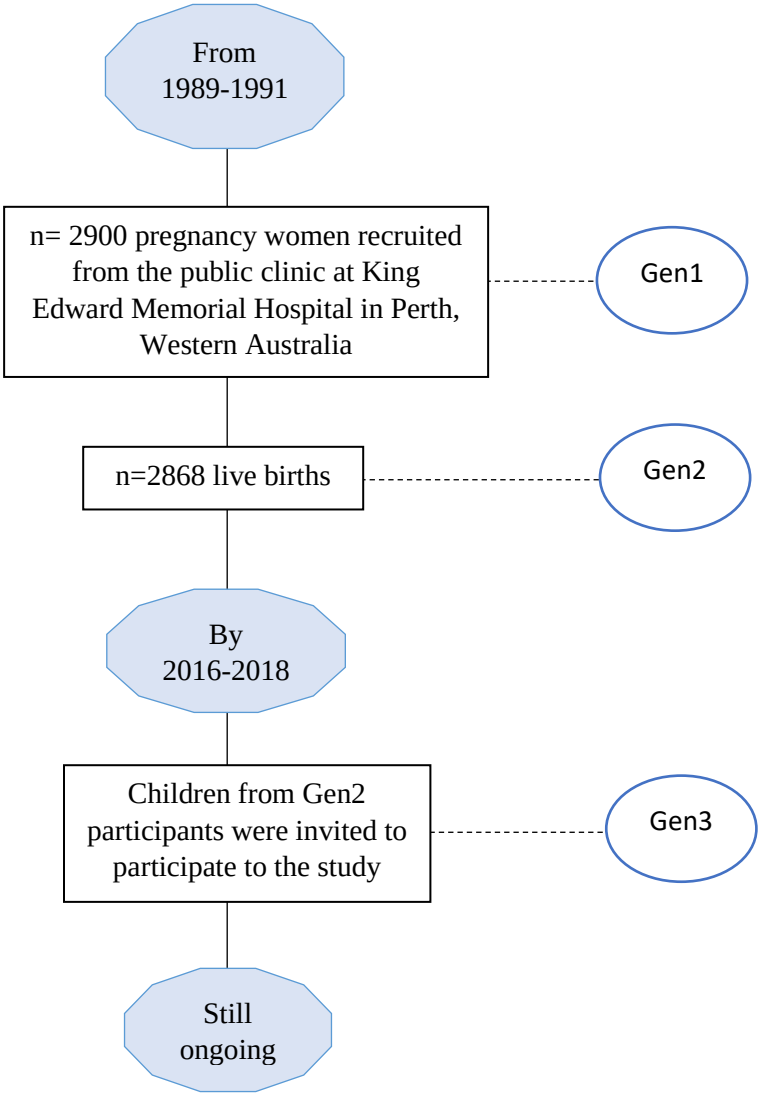
This Chapter presents the general methods used in the studies of my doctoral thesis. I have described the general methods used, including the description of the Raine Study population, the available data used, and the statistical analysis conducted. These methods are also partly covered in Chapters 3 and 4.

2.2. Population study- The Raine Study

Data used for this study are from the Raine Study. Between May 1989 and November 1991, 2900 pregnant women were recruited from the public clinic at King Edward Memorial Hospital in Perth, Western Australia (Newnham et al., 1993). Extensive data was collected during pregnancy and the infants were assessed at birth. The Raine Study established a set of core data which was then added to at each future follow up. The data included demographics and anthropometric measures on each participant, such as height, weight, and blood pressure to track them longitudinally over the life course. The families (parents) recruited for the study are known as Generation 1, while their children are known as Generation 2. As the follow-ups are still on-going, by 2016 some of the original Gen2 participants had become or were on the way to becoming parents themselves, so their children were invited to become part of the Raine Study, as Generation 3. A flow-chart of participants involved in the Raine Study is shown in Figure 2.1. From the first women enrolled in the study there were 2869 live births. Demographics, socioeconomic, and health behavior data were collected through questionnaires answered by the primary carers and children once old enough. Clinical and physical assessments were made at 18 and 34 weeks of gestation, birth and at ages 1, 2, 3, 6, 8, 10, 14, 17, 20, 22, 27 and 28 years.

For the purpose of this study, we used data from Generation2 participants retrieved by questionnaires and from clinical assessment. The variable used in this study are summarized in the next section.

Figure 2.1. *Flow chart of participants involved in the Raine Study*



2.3. Variables and clinical outcomes

2.3.1 Lung function - Spirometry

Lung function was assessed at 6, 14, and 22 years of age by spirometry, following the American Thoracic Society guidelines in place at each time point (Crapo et al., 1995; Miller et al., 2005). FEV_1 and FVC were measured from three forced expiratory curves with an acceptable start of test. The largest FEV_1 and the largest FVC were recorded after examining the data from all acceptable curves. FEV_1/FVC was calculated as the ratio of forced expiratory volume in the first one second to the forced vital capacity. Reference values were derived from the Global Lung Function Initiative (GLI) reference equations (Quanjer et al., 2012) that has been validated in a contemporary Australasian population (Hall et al., 2012). Using the GLI Tool Software developed by the GLI Task Force Group from the European Respiratory Society (ERS), we derived the z-scores for FEV_1, FVC, and FEV_1/FVC in participants that successfully completed and had acceptable and repeatable spirometry measurements.

2.3.2. Questionnaire

The respiratory questionnaire used were adapted from the International Study on Asthma and Allergies in Childhood (ISAAC) questionnaire for school children (Asher & Weiland, 1998) and applied in full at ages 6, 14 and 22. In this thesis the following respiratory outcomes were determined, in line with previous Raine study publications (Hollams et al., 2009; Collins et al., 2011; White et al., 2017).

Asthma

- Current asthma was defined as a) having current wheeze in the past 12 months and b) having taken asthma medication in the past 12 months and c) ever having a doctor-diagnosis of asthma ever.
- Asthma ever was defined as having asthma onset at or before the age of 22 years of follow-up

We also classified asthma phenotypes as early-onset, persistent, and late-onset. Asthma phenotype was defined as:

- Early-onset if asthma was present at six years of age only (i.e. not present after 6 years of age).
- Persistent if asthma was present from childhood up to adulthood
- Late-onset if asthma occurred at 14 or 24 years of age, but not earlier

Atopy

Atopy was established by measurement of specific immunoglobulins (IgE) from serum sample and was defined as positive if the participants produced IgE antibodies in response to exposure to any of the following allergens: peanuts or food mix (food allergy); rye grass pollen, house dust mite, or mould (aeroallergens).

Wheeze, Eczema and Hayfever

- Wheeze was defined as a positive response to "Has you child wheezed in the last 12 months?"
- Eczema was defined as a) a positive response to "Child has eczema?" and b) a self-reported doctor diagnosis ever
- Hay fever was defined as a) a positive response to "Child has hay fever?" and b) a self-reported doctor diagnosis

Respiratory tract infections

Lower and upper respiratory tract infections were defined based on the illnesses reported by parents through the questionnaire. We converted the diseases into International Classification of Disease (ICD-9) codes, and we determine the health outcome if it was present during the first year of life.

Maternal smoke

Maternal smoke was defined as:

- *in utero* smoking if the mother has smoked only during pregnancy at 18 or 34 weeks of gestation
- Smoking at age six if the mother was smoking at the six year of follow up, but not in pregnancy
- Smoking from pregnancy to age six if the mother has smoked during pregnancy and was still smoking at the six year follow up.

Preterm birth

Preterm birth was defined as any birth before 37 weeks of gestation.

Socio-economic status

Socio-economic status (SES) was defines based on family income per year expressed in Australian dollars ($AUD): a) less than 16.000 b) between 16.000 and 40.000 c) more than 40.000

Demographics

Demographics variables were retrieved from information provided by parents at baseline. Parental asthma, eczema, wheeze, and hay fever were defined if the mother or the father self-reported the clinical outcome at the study baseline.

2.3.3. Geocodes

Geocoding is the process of converting a description of a location, defined as coordinates, address, or a name of a place, to a location on the earth's surface. The geocodes were provided by Dr David Blake and collaborators from the Edith Cowan University (ECU), in Perth using the home addresses provided by Raine participants during the years of follow-up from pregnancy to 22 years. Data was processed using ArcGis (version 10.8)

2.3.4 Air pollution data

2.3.4.1. Air quality data

Daily air pollution data across Perth were provided by the Department of Water and Environmental Regulation in Perth from 1991 to 2012 for $PM_{2.5}$, $PM_{2.5Absorbance}$, and NO_2.

Ambient air pollution concentrations in Perth were measured routinely at ten minutes intervals, for 24 hours, over one-year period, using Tapered Element Oscillating Microbalance (TEPM) method for $PM_{2.5}$ and using Chemiluminescence method for NO_2. The ten-minute interval data for each air monitor and year were compiled into specific-annual average of daily concentrations. Quality measurements across the area of Perth started in 1990 for NO_2. However, more consistent continued NO_2 and $PM_{2.5}$ measurements started in 1994. Among the fixed air monitoring stations operating in the metropolitan area, six sites (Caversham, Duncraig, Rockingham, South Lake, Swanbourne, and Quinns Rocks) recorded NO_2 concentrations for more than 75% data over one-year period. Data for $PM_{2.5}$ in the period between 1996 and 2003 were only available from two sites (Caversham and Duncraig), while data from 2004 to 2012 were available for four sites (Caversham, Duncraig, South Lake and Quinns Rocks).

2.3.4.2 Estimation of air pollution exposure

Land use regression (LUR) models for $PM_{2.5}$, $PM_{2.5Absorbance}$, and NO_2 were developed in 2012 and validated by Dirgawati et al (2016) for the Perth Metropolitan area to estimate air pollutant concentrations across Perth. The modelling procedure was based following the procedures reported by the ESCAPE protocol (http://www.escapeproject.eu/).

2.4. Statistical methods

2.4.1. Air pollution exposure – Back-extrapolation of the air pollution data

To estimate concentrations of air pollutions in the Raine participants we back-extrapolated air pollution data, based on land use regression modeling developed in

2012 for the Perth Metropolitan area (Dirgawati, 2016). The air pollution trend can be estimated over a period longer than 10 years using LUR models. However, the reliability of the model depends on the characteristics of the area, whether there have been large changes in road network, emission sources and land use. Previous studies have back-extrapolated LUR models and reported good reliability on models even over a period of than 10 years (Levy et al, 2015).

We back-extrapolated air pollution data using LUR models developed and validated for the Perth metropolitan area. We first estimated air pollution concentrations for the period in which $PM_{2.5}$ and NO_2 were measured from the fixed stations (1995-2012). Then, we applied the same standardized procedure to estimate NO_2 and $PM_{2.5Absorbance}$ concentrations since 1990. Each participant's exposure to $PM_{2.5}$, $PM_{2.5Absorbance}$ and NO_2 was estimated at pregnancy, birth and at 1, 2, 3, 5, 8, 10, 13, 16, and 22 years of follow-up and from the last residential address available, using geographical information system (GIS, version). We estimated the annual $PM_{2.5}$, $PM_{2.5Absorbance}$ and NO_2 average concentrations from the LUR model. Then, each participant's exposure to $PM_{2.5Absorbance}$ and NO_2 was estimated in early life when children were aged between 3 and 4 years of age, using their exact date of birth and their year-specific address. Further details about the air pollution exposure in the Raine participants are given in Chapter 4.

2.4.2. Group-based trajectory modelling (GBTM)

To characterize lung function trajectories from 6 to 22-year follow-ups in the Gen2 participants from the Raine Study, we used a finite mixture model through group-based trajectory modelling. Group based trajectory modelling (GBTM) is a statistical methodology for analyzing individual level-development trajectories, developed by Nagin (Nagin et al., 1999).

The group-based trajectory model assumes that the population distribution of trajectories arises from a finite mixture of unknown order (Nagin et al, 2018).

Let $\mathbf{Y_1}$= $Y_{i1}, Y_{i2}, \ldots Y_{it}$ be a longitudinal sequence of measurements on individual *i* over length *T* periods and $\mathbf{P(Y_i)}$ the probability of Y_i given membership in group *j* and μ_i determines the probability of a randomly chosen population member belonging to group *j*. For individual *i* at a given age, the probability of Y_i may be written as:

$$P(Y_i|Age_i) = \sum_{j=1}^{J} \mu^j \cdot P(Y_i|Age_i, j; \beta_j)$$

The conditional distribution of Y_i given membership in *j* is indexed by the unknown parameter vector β^j which among other parameters determines the shape of the group-specific trajectory. This equation describes what is called a "finite mixture model", because it sums across a finite number of discrete groups that comprise the population. For each individual within a given trajectory group *j* we assume that the distribution of y_{it} (t=1,2,3...) is independent of the past period $y_{it-1}, y_{it-2}, y_{it-3}, \ldots$ Thus,

$$p^j(Y_i|Age_i; \beta_j) = \prod_{t=1}^{T} p^j\,(y_{it}|Age_{it}; \beta_j)$$

where $p^j(y_{it}|Age_{it}; \beta_j)$ is the probability distribution function of y_{it} given membership in group *j* and the age of individual *i* at time *T*.

The log- likelihood for the whole sample of N individuals is given by:

$$L = \prod_{i=1}^{N} P(Y_i)$$

The distribution p^j is based on the type of data available for the analysis and can accommodate count data, where p^j is assumed to follow a Poisson distribution or zero-inflated Poisson distribution, binary data, where it is assumed to follow the binary logit distribution or continuous scales, where it is assumed to follow a censored normal distribution.

The censored normal distribution is designed for the analysis of repeated data and it is the distribution used in this study. For the censored normal model, the linkage between age and the dependent variable is established by means of a latent variable y_{it}^{*j} and assumed to have an *n* degree polynomial relationship with age:

$$y_{it}^{*j} = \beta_0^j + \beta_1^j age_{it} + \beta_2^j age_{it}^2 + \cdots + \beta_n^j age_{it}^n + \varepsilon_{it},$$

where ε_{it} is an error term assumed to have a normal distribution with zero mean and a constant variance of σ^2. Where S_{min} and S_{max}, respectively, determines the minimum and maximum possible score on the measurement scale. The model assumes:

$$y_{it} = S_{min}\, if + y_{it}^{*j} < S_{min}$$

$$y_{it} = y_{it}^{*j}\, if\, S_{min} \leq y_{it}^{*j} \leq S_{max}, \text{ and}$$

$$y_{it} = S_{max}\, if + y_{it}^{*j} > S_{max}$$

Where the expected value of the latent variable y_{it}^{*j} be $\beta^j x_{it}$, a vector of length (n+1), and $x_{it}=(1, age_{it}, age_{it}^2 \ldots, age_{it}^2$. The log-likelihood equation becomes:

$$\text{LogL} = \sum_{i=1}^{N} log \left[\sum_{j=1}^{J} \pi_i \prod_{t=1}^{T} \left\{ \prod_{y_{ij}=S_{min}} \Phi\left(\frac{S_{min}-\beta^j x_{it}}{\sigma}\right) \prod_{S_{min}<y_{ij}<S_{max}} \frac{1}{\sigma}\phi\left(\frac{y_{it}-\beta^j x_{it}}{\sigma}\right) \times \prod_{y_{ij}=S_{max}} \left(1-\phi\left(\frac{S_{max}-\beta^j x_{it}}{\sigma}\right)\right)\right\}\right],$$

where Φ denotes the cumulative distribution, while ϕ denotes the density function. The estimated probability of individual *i* belonging in group *j is* given by:

$$\hat{P}(\mathrm{j}|Y_i) = \frac{\hat{P}(Y_i|j)\hat{\pi}_j}{\sum_{j=1}^{J}\hat{P}(Y_i|j)\hat{\pi}_j},$$

here $\hat{\pi}_j$ is the estimated population in group *j*, while and $\hat{P}(Y_i|\mathrm{j})$ is the estimated probability of observing *i* trajectories of Y_i given membership in *j* and is determined by using the maximum likelihood estimation.

The model selection addresses two fundamental points: 1) the determination of the optimal number of groups 2) the determination of the shape of the trajectories, using the appropriate order of polynomial function for each group. The most common criteria used to determine the model fit include the Bayesian information criteria (BIC), Akaike information criteria (AIC), Lo-Mendell-Ruben likelihood ratio test (LMR-LRT), and entropy. Because comparison between models with *k* versus *k+1* classes cannot be made via a standard likelihood comparison, BIC and AIC are commonly used to evaluate model fit by balancing model complexity. These standard procedures described above have been used in this thesis to characterize lung function trajectories from childhood to adulthood in the Raine participants. To evaluate the most appropriate model, different models with different polynomial functions and with the same number of trajectories, were compared. Then, the best model with *J* trajectories was compared with a model with *J+1* trajectories. After identifying the number of groups and the different shapes of the trajectories, model adequacy was tested using the average posterior probabilities (APP) of group membership, that Nagin (2005) recommends should exceed 0.70 for each group. An average posterior probability above 0.70 indicates that, on average, the participants are well assigned to their groups (Nagin et al., 1999).

2.5. Conclusions

Data from the Raine Study were used to characterize lung function trajectories from 6 to 22 years of age in Generation2 participants and to investigate which early-life risk factors are associated with the low lung function trajectories identified. Among other early risk factors, we estimated air pollution data as NO_2, $PM_{2,5}$ and $PM_{2,5Absorbance}$ in the participants when they were aged six. For this purpose, we back-extrapolated air pollution data from LUR models developed in 2012 for the Perth Metropolitan area. These general methods will be used for the studies in the following Chapters.

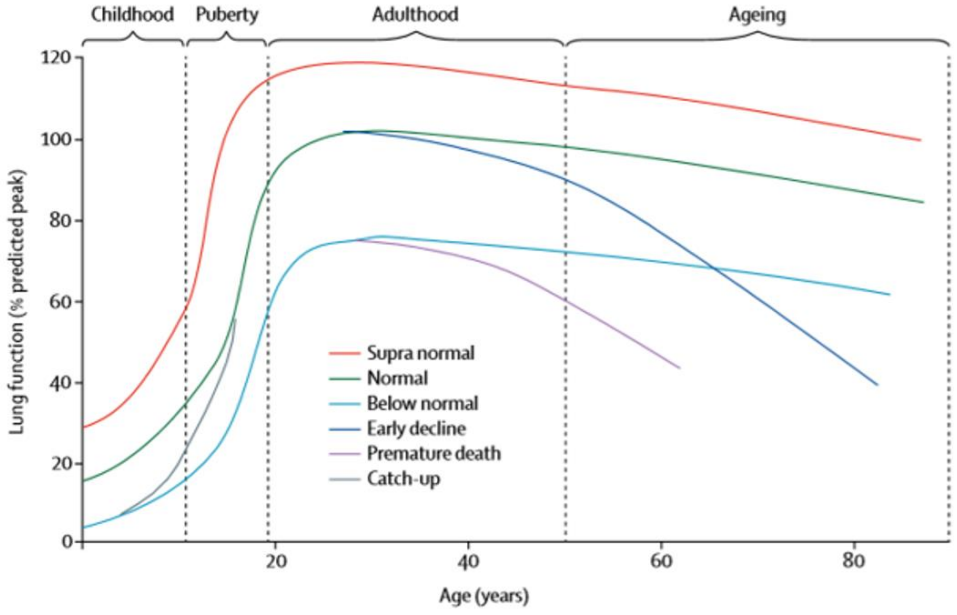
Chapter 3

Characterization of lung function trajectory through group-based trajectory modelling

3.1 Introduction

Lung function is a well-recognized marker of respiratory health, the most commonly reported outcomes being from spirometry, FEV_1, FVC and FEV_1/FVC. Lung function is characterized by three phases of development through the life course. In the first phase lung function increases as the lungs grow, reaching a peak in women at around 18 and in men at about 20 years of age (Karmaus et al., 2019). The second phase is a plateau phase enduring till early adulthood, followed by a decline due to physiological ageing (Agusti & Faner, 2019). A trajectory describes the course of a variable over age or time, as shown in Figure 3.1.

Figure 3.1. *Potential lung function trajectories in childhood, puberty into adulthood*



Agusti et al., 2019

Note The normal lung function trajectory can be divided in three phases of development from childhood to adulthood, followed by lung function decline due to ageing. Various factors might modify the path of the trajectories over life.*

Birth-cohort studies have shown that there is a distribution of lung function trajectories in the population. Bui et al (2018) identified six trajectories of lung function from 7 to 53 years of age, using the Tasmanian Longitudinal Health Study (TAHS). Another study (Belgrave et al., 2014) identified three trajectories from early school age into early adulthood using two population-based birth cohorts: the Manchester Asthma and Allergy Study (MAAS) and the Avon Longitudinal Study of Parents and Children (ALSPAC). Berry et colleagues (2016), using data from the Tucson Children's Respiratory Study (CRS) found two lung function trajectories, a low and a normal trajectory from 11 to 32 years of age.

Various statistical methods, such as hierarchical or latent curve modeling, have been used for analyzing not only developmental trajectories, but also which predictors may affect their shape. They estimate the population average trajectory and use predictors to investigate these associations. Recent advances in statistical modelling and their application to clinical research uses group-based trajectory modelling [Fan & Zhang, 2020; Jeon, Jung & Jee, 2019; Bui et al, 2018]. Unlike the other models, it assumes that the population consists of distinct groups with different trajectories (Nagin & Odgers, 2010). Group-based trajectory modelling (GBTM) was firstly reported by Nagin and Land (1993) and has been increasingly applied in clinical research (Nagin & Odgers, 2010), including respiratory health (Bui, 2018). Trajectory models have been used to understand the etiology and the development course of various diseases, such as obesity (Fan & Zhang, 2020) and diabetes (Jeon, Jung & Jee, 2019).

The aim of the group-based trajectory model is to identify complex patterns in longitudinal analysis, as well as identify clusters of individuals with similar trajectories. This approach identifies individuals that follow similar development of a variable over time and estimates the effect of predictors on different trajectories identified. This method may be preferable over other approaches such as hierarchical and latent curve modeling when handling trajectories that do not vary regularly in the population. It has been shown that based on early- life exposures, individuals in the general population have their own lung function trajectories that might be already defined in early life, when the lungs are still developing. Pre- and post-natal life represents susceptible periods for each individual and identify which factors influenced the belonging to a particular trajectory is important to implement strategy to prevent future health respiratory deficits.

In this thesis it is hypothesized that different lung function trajectories exist in the Raine Study population.

In the Chapter I have provided the procedure of the group-based statistical modelling used for characterizing lung function trajectories, as we hypothesized that insults in early life are associated with low lung function trajectories. Specifically, I have described the procedure I have applied to identify lung function trajectories from 6 to 22 year of follow ups in the Raine Study participants. The results from this study are reported in Chapter 5, in which I will investigate which early-life factors are associated with the identified lung function trajectories.

3.2. Methods

3.2.1. Study population

Raine participants attending at least two visit that included the spirometry measurement within the 6, 14 or 22 years of follow-up were included in this study. The FEV_1, FVC, and FEV_1/FVC (z-scores) individual measures of spirometry were derived from the Global Lung Initiative reference equations (Quanjer et al., 2012), validated in the Australian population (Hall et al., 2012).

3.2.2. Statistical analysis

3.2.2.1. Group based trajectory modelling

Group based trajectory modelling was used to identify trajectories of lung function from 6 to 22 years in the Raine participants. Modelling was performed using the STATA (version 16.0; StataCorp, College Station, TX) "traj" plugin (Jones & Nagin, 2013). A censored normal model was fitted to the data because the FEV_1 and FVC values (z-scores) were continuous. The shape of the trajectories was defined by a polynomial function of age, that can be modeled with up to a fifth-order polynomial function. We determined the optimal number and shape of lung function trajectories based on the smallest absolute Bayesian Information Criterion (BIC) value and average posterior probabilities. We considered an average posterior probability of >0.7 as an indicator of good model fit, as indicated in the Nagin recommendations (Nagin & Odgers, 2010). We first started with a model that consisted of one group with the highest polynomial order (cubic), and

then we increased the groups until the number of groups that best fitted the data was identified (Peristera et al., 2020). Once the number of trajectories was identified, we reduced the polynomial orders until the highest order polynomial for each group was significant at the confidence level alpha (x)= 0.05. After selecting the best fitting model, participants were automatically assigned to one of the groups based on their highest estimated group membership probabilities, that is the probabilities of each participant's belonging to each of the trajectory groups. This results in a distribution over classes. We modelled trajectories for FEV_1, FVC and FEV_1/FVC separately. Sensitivity analysis were conducted to test the reliability of trajectories identified. We performed the analysis with participants having two spirometry measurements during the years of follow-up.

3.3. Results

To characterize the lung function trajectories, we included data from 1512 children in the cohort (768 males and 744 females, mean age at baseline 5.9 SD± 0.2 years). All included participants had at least two valid spirometry measurements of FEV_1 and FVC with spirometry performed between 6 and 22 years (Figure 3.2). Characteristics of children are shown in Table 3.1. Boys had a higher proportion of childhood asthma ($p=0.03$) and allergies in childhood ($p<0.001$), while wheezing and eczema in young adulthood were more prevalent in females ($p=0.03$ and $p=0.001$, respectively). Late-onset asthma was prevalent in females ($p<0.001$). Compared with the 1512 included participants, excluded participants had lower SES, less respiratory tract infections, childhood and parental hay-fever, asthma, atopy, and more of them were born preterm or had mother smoking (Supplementary Table 1.1).

Figure 3.2. *Flow chart of participants in this study from the Raine Study*

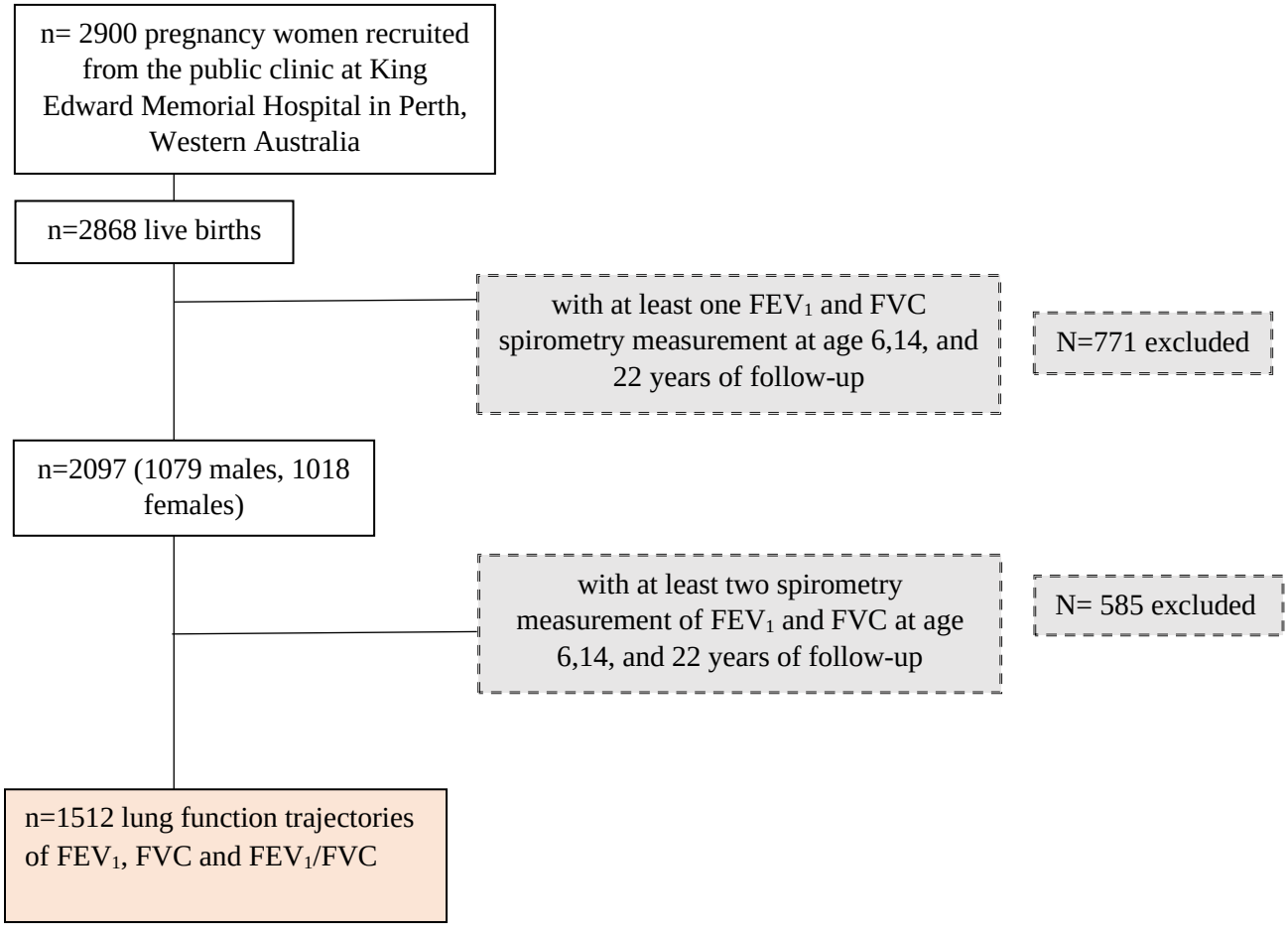


Table 3.1. *Childhood and parenthood characteristics in participants with two or more spirometry measurements according to sex.*

Participant demographics (n=1512)			
Variable	**Male**	**Female**	**P-value**
Gender, %	768 (50.79)	744 (49.21)	
Socioeconomic status			**0.713**
≤16.000	67 (9.45)	70 (10.14)	
16000-40000	316 (44.57)	293 (42.46)	
≥40000	326 (45.98)	327 (47.39)	
Respiratory infections, %			0.879

URTI only	245 (35.61)	244 (36.31)	
LRTI or both	182 (26.45)	182 (27.08)	
Preterm (<37 gestational age),	45 (6.09)	56 (7.94)	0.167
Asthma phenotypes			**0.007**
Early-onset asthma	75 (10.37)	57 (8.01)	
Persistent	13 (1.80)	10 (1.40)	
Late-onset	38 (5.26)	69 (9.69)	
Current asthma at age 6, %	133 (17.32)	99 (13.31)	**0.030**
Current asthma at age 14, %	78 (10.16)	69 (9.27)	0.563
Current asthma at age 22, %	40 (5.21)	63 (8.47)	**0.012**
Current wheeze at age 6, %	172 (23.40)	142 (20.03)	0.120
Current wheeze at age 14, %	98 (13.78)	94 (13.49)	0.871
Current wheeze at age 22, %	84 (17.54)	118 (22.96)	**0.034**
Current eczema at age 6, %	166 (22.90)	174 (24.96)	0.361
Current eczema at age 14, %	127 (17.81)	147 (21.06)	0.123
Current eczema at age 22, %	45 (9.53)	82 (16.43)	**0.001**
Current hay fever at age 6, %	51 (6.64)	53 (7.12)	0.711
Current hay fever at age 14, %	156 (21.88)	135 (19.37)	0.244
Current hay fever at age 22, %	73 (15.37)	104 (20.47)	0.037
Atopy, %			**<0.001**
Food Allergy only	44 (5.73)	52 (6.99)	
Aeroallergens only	65 (8.46)	41 (5.51)	
Both	273 (35.55)	184 (24.73)	
Maternal smoking			0.287
Only in pregnancy	31 (4.41)	35 (5.21)	
Only at age 6	52 (7.41)	54 (8.04)	
Both in pregnancy and at age 6	130 (18.49)	147 (21.88)	
Parental Asthma, %			0.268
Mother only	111 (16.82)	104 (16.48)	
Father only	53 (8.03)	71 (11.25)	
Both	18 (2.73)	18 (2.85)	
Parental Eczema, %			0.445
Mother only	117 (17.67)	106 (16.80)	

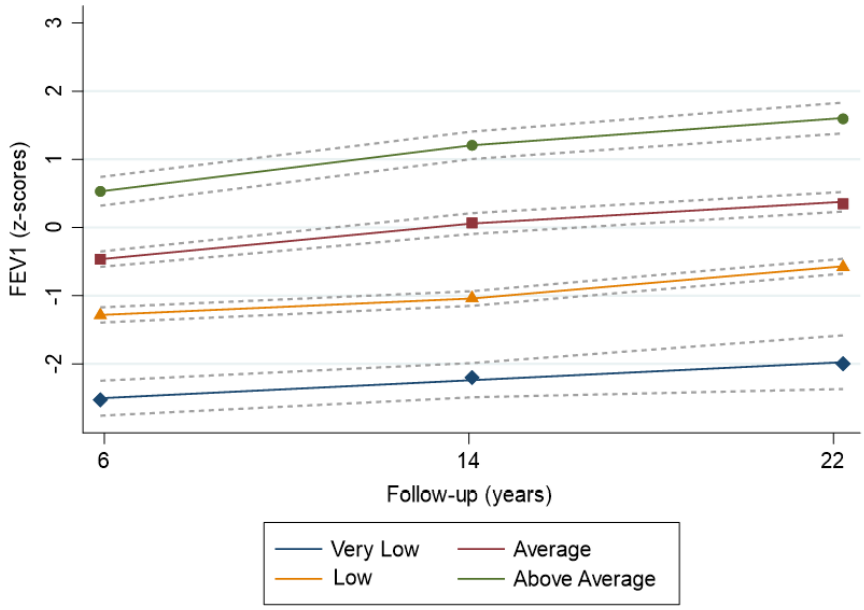
Father only	42 (6.34)	55 (8.72)	
Both	21 (3.17)	19 (3.01)	
Parental Hay fever, %			0.481
Mother only	231 (30.08)	210 (28.23)	
Father only	123 (16.02)	116 (15.59)	
Both	97 (12.63)	114 (15.32)	
Parental Wheeze, %	313 (43.05)	313 (44.78)	0.512

**SES=socio economic status was defined by the family incomes, expressed in Australian dollars ($AUD), LRTI= low respiratory tract infections in the first year of life, URTI= upper respiratory tract infections in the first year of life. **Full details of the variable definitions are reported in the Chapter 2.*

3.3.1. Lung function trajectories

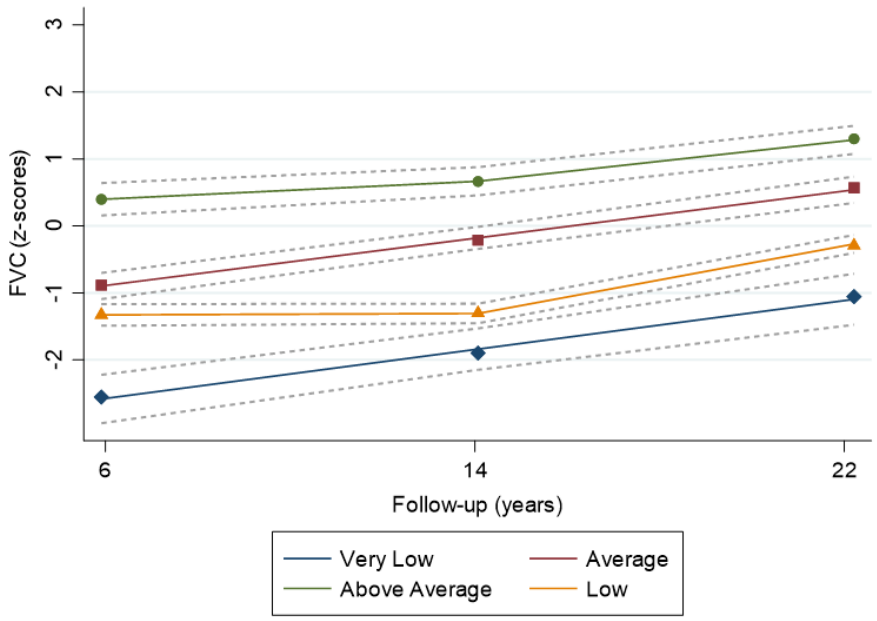
We fitted individual models to analyze the lung function trajectories of FEV_1, FVC, and FEV_1/FVC (z-scores). Longitudinal modeling identified four lung function trajectories for FEV_1 (Figure 3.3), FVC (Figure 3.4) and FEV_1/FVC (Figure 3.5), that best fitted our data with a membership probability of >0.7 for each group. The best fitted models are reported in Supplementary Tables 3.2 A, B and C, while the average posterior probabilities for each group within the lung function parameters are reported in the Supplementary Table 3.3. FEV_1 and FVC were characterized by four trajectories that we have called respectively: "very low", "low", "average" and "above average". FEV_1/FVC was also characterized by four trajectories based on their shapes, these are: "very low", "low-high", "high-low" and "average" trajectories. The distribution of FEV_1, FVC and FEV_1/FVC in the lung function trajectories of the Raine participants with two or more spirometry measurements are described in Table 3.2, showing that most of participants were in the low and average of FEV_1 and FVC trajectories, while in the average and high-low trajectories for FEV_1/FVC. We also assessed the overlap of participants both in FEV_1 and FVC lung function trajectories, as shown in Table 3.3.

Figure 3.3. *Lung function trajectories (FEV_1) from 6 to 22 years of follow up in the Raine participants with two or more spirometry measurements*



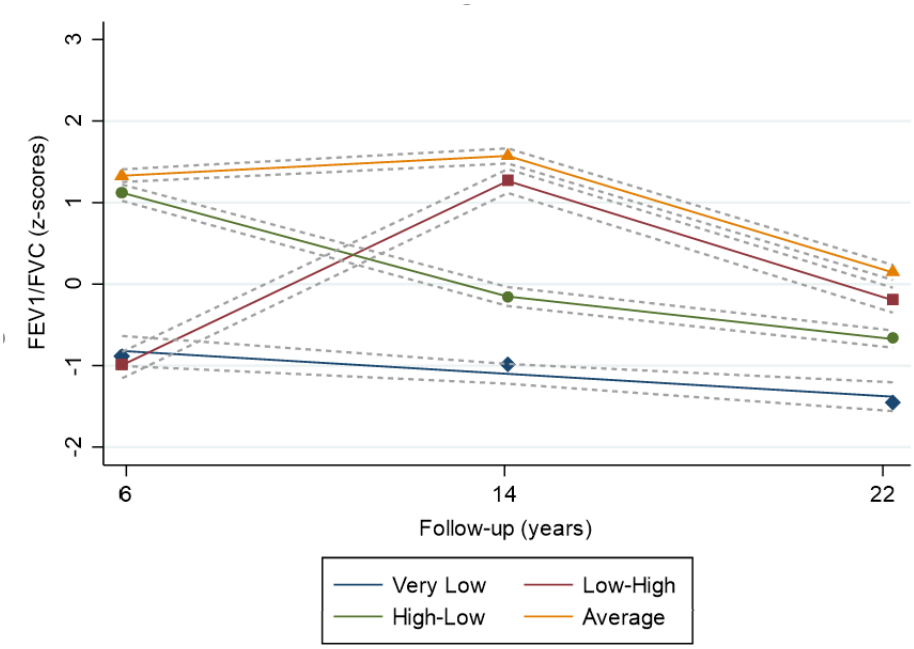
Note The 95% confidence intervals are represented by dashed lines*

Figure 3.4. *Lung function trajectories (FVC) from 6 to 22 years of follow up in the Raine participants two one or more spirometry measurements*



Note The 95% confidence intervals are represented by dashed lines*

Figure 3.5. *Lung function trajectories (FEV_1/FVC) from 6 to 22 years of follow up in the Raine participants two or more spirometry measurements*



Note The 95% confidence intervals are represented by dashed lines*

As the trajectories of FEV_1/FVC were not what we expected, showing either decline or growth between 6 and 14 years of age, we investigated its reliability as shown in Supplementary Figure 1 and Supplementary Table 3.4. We investigated whether the membership to trajectories for participants with two or more spirometry measurements at any age was different to those with spirometry at both 14 and 22 years. Trajectory membership was unchanged, with most of the participants (95%, n= 820) being in the same trajectory of FEV_1/FVC (Supplementary Table 3.5)

Table 3.2 *Distribution of forced expiratory flow in one second (FEV_1), forced vital capacity (FVC) and their ratio (FEV_1/FVC) in the lung function trajectories in the Raine Study participants with two or more spirometry measurements.*

	FEV_1 n (%)				**FVC n (%)**				**FEV_1/FVC n (%)**			
Trajectory	**Very Low**	**Low**	**Average**	**Above Average**	**Very Low**	**Low**	**Average**	**Above Average**	**Very Low**	**Low-High**	**Average**	**High-Low**
	51 (3.37)	746 (49.34)	611 (40.41)	104 (6.88)	61 (4.03)	855 (56.55)	457 (30.22)	139 (9.19)	131 (8.66)	198 (13.10)	654 (43.25)	529 (34.99)
Total	1512				1512				1512			

*Note. *All values are given as frequencies with percentages in parentheses.*

Table 3.3 *Individual distribution of FEV_1 and FVC lung function trajectories in the Raine Study participants with two or more spirometry measurements.*

	FVC				
FEV_1	**Very low n (%)**	**Low n (%)**	**Average n (%)**	**Above Average n (%)**	**Total**
Very low	27 (44.26)	23 (2.69)	1 (0.22)	1 (0.72)	52 (3.44)
Low	0 (0.00)	206 (24.07)	342 (75.00)	62 (44.60)	610 (40.34)
Average	34 (55.74)	627 (73.25)	82 (17.98)	3 (2.16)	746 (49.34)
Above average	0 (0.00)	0 (0.00)	31 (6.80)	73 (52.52)	104 (6.88)
Total	61	456	139	856	1,512

*Note. *All values are given as frequencies with percentages in parentheses.*

3.4. Conclusions

In this study we characterized lung function trajectories using spirometry measurements at age six, 14 and 22 years from the Raine Study cohort. By using a finite mixture model through group-based trajectory modelling, we identified four trajectories for FEV_1, FVC and FEV_1/FVC. Lung function trajectories for FEV_1 and FVC were similar in shapes, being characterized by a very low, low, average, and above average trajectories, where the low trajectory was the most populated trajectory. FEV_1/FVC was characterized by a very-low, low-high, average, and high-low trajectories, based on their shape, where nearly half of participants were in the average trajectory. To date, few studies have investigated lung function trajectories over time. The Tucson's Children Respiratory Study (CRS) identified lung function trajectories in 599 individuals aged between 11 and 32 years, identifying a persistently low trajectory and a normal trajectory for FEV_1/FVC (Berry et al., 2016). Karmaus and colleagues (2019) studied lung function trajectories in 1158 participants from 10 to 26 years in males and females, separately. They identified two trajectories for FEV_1, FVC and FEV_1/FVC, and three trajectories for FEF_{25-75}. In the CAMP Study, 684 individuals with asthma were followed from 23 to 30 years of age and four trajectories of FEV_1 were identified within the group (McGeachie et al., 2016). In the Manchester Asthma and Allergy Study, Belgrave and colleagues (2014) identified three trajectory of lung function in 1,051 individuals for male and female, separately, using specific airway resistance measurements from 3 to 11 years of age. The study with the largest age range was conducted by Bui et al (2018) using the Tasmanian Longitudinal Health Study (TAHS) and they analyzed lung function trajectories in individuals followed from 5 to 53 years of age. They identified six paths, of which two (the early below average, accelerate decline and early low, accelerate growth, normal decline) were new compared with previous studies. Even if lung function trajectories have been subject of few studies, to the best of our best knowledge, none has examined trajectories of FEV_1, FVC and FEV_1/FVC from childhood to adulthood in a large study population and expressed these as z-scores, hypothesized to be the most clinical meaningful approach (Vaz Fragoso et al., 2016). Differently from our study, Bui et al found six trajectories of FEV_1. This can be due to the fact that the participants involved in the TAHS were older compared with those in our study and thus we could not detect lung function decline with age.

One strength of our study is the large sample of children and a long period of follow-up which covers developmental periods from childhood to adolescence and the adulthood, with available measurements over the years. This allowed us to established lung function trajectories over sixteen years from early life to young adulthood. This study has some limitations. Although spirometry is considered to be reliable and acceptable in pre-school children, one of the limits encountered when performing spirometry is that patients might be unable to exhale completely. Indeed, a limitation of the study is that in our population sample, the forced vital capacity measured at six years may be underestimated, and therefore potentially resulting in an overestimation of lung function trajectories of FEV_1/FVC ratio. However, in a sensitivity analysis, when we included participants with spirometry measurement at 14 and 22 years of age (might have also 5), the ratio FEV_1/FVC still resulted in four trajectories with the same shapes, suggesting that misrepresentation of FEV or FVC at five to six years of age was unlikely to have contributed to inappropriate trajectories in FEV_1/FVC.

Thus, group-based trajectory modelling has been found to be a reliable statistical model that can be applied in the respiratory context, and we identified groups of individuals in the population that follow similar lung function trajectories over time. This finding suggested that lung function trajectories are established before six years of age. The lung function trajectories identified would serve as a basis to identify which are the early-life factors associated with low lung function trajectories, as described in Chapter 5.

3.5. Supplementary material

Supplementary Table 3.1. *Childhood and parenthood characteristics in included and excluded participants*

Participant demographics			
Variable	**Included (n=1512)**	**Excluded (n=1356)**	**P-value**
Female Gender, %	744 (49.21)	670 (49.41)	0.913
Socioeconomic status			**<0.001**
≤16.000	137 (9.79)	95 (13.46)	
16000-40000	609 (43.53)	359 (50.85)	
≥40000	653 (46.68)	252 (35.69)	
Respiratory infections, %			**<0.001**
URTI only	503 (33.27)	346 (25.52)	
LRTI or both	333 (33.27)	227 (16.74)	
Preterm (<37 gestational age), %	101 (6.99)	117 (9.07)	**0.046**
Asthma phenotypes			**<0.001**
Early-onset asthma	135 (9.41)	90 (6.75)	
Persistent	22 (1.53)	1 (0.08)	
Late-onset	107 (7.46)	41 (3.08)	
Current asthma at age 6, %	235 (15.54)	114 (8.41)	**<0.001**
Current asthma at age 14, %	147 (9.72)	49 (3.61)	**<0.001**
Current asthma at age 22, %	103 (6.81)	18 (1.33)	**<0.001**
Current wheeze at age 6, %	314 (21.75)	168 (22.70)	0.610
Current wheeze at age 14, %	192 (13.64)	61 (15.72)	0.296
Current wheeze at age 22, %	202 (20.34)	28 (18.42)	0.582
Current eczema at age 6, %	340 (23.91)	155 (21.03)	0.131
Current eczema at age 14, %	274 (19.42)	64 (16.49)	0.192
Current eczema at age 22, %	127 (13.08)	24 (16.11)	0.314
Current hay fever at age 6, %	104 (6.88)	50 (3.69)	**<0.001**
Current hay fever at age 14, %	291 (20.64)	66 (17.01)	0.113
Current hay fever at age 22, %	177 (18.01)	26 (17.22)	0.814
Atopy, %			**<0.001**
Food Allergy only	96 (6.35)	28 (2.06)	

Aeroallergens only	106 (7.01)	29 (2.14)	
Both	457 (30.22)	124 (9.14)	
Current FEV_1 at age 6, (z-scores)	-0.85 (0.98)	-0.89 (0.99)	0.261
Current FEV_1 at age 14, (z-scores)	-0.46 (1.04)	-0.63 (1.07)	**0.024**
Current FEV_1 at age 22, (z-scores)	-0.11 (0.99)	-0.29 (1.07)	0.071
Current FVC at age 6, (z-scores)	-1.08 (1.05)	-1.04 (1.31)	0.753
Current FVC at age 14, (z-scores)	-0.79 (1.06)	-0.87 (1.01)	0.181
Current FVC at age 22, (z-scores)	0.08 (0.93)	-0.02 (0.82)	0.189
Current FEV_1/FVC at age 6, (z-scores)	0.71 (1.18)	0.66 (1.26)	0.263
Current FEV_1/FVC at age 14, (z-scores)	0.71 (1.19)	0.52 (1.32)	**0.030**
Current FEV_1/FVC at age 22, (z-scores)	-0.34 (0.92)	-0.50 (1.03)	0.078
Maternal smoking			**0.003**
Only in pregnancy	66 (4.80)	45 (6.34)	
Only at age 6	106 (7.71)	62 (8.73)	
Both in pregnancy and at age 6	277 (20.15)	183 (25.77)	
Parental Asthma, %			0.291
Mother only	215 (16.65)	88 (13.97)	
Father only	124 (9.60)	55 (8.73)	
Both	36 (2.79)	23 (3.65)	
Parental Eczema, %			0.701
Mother only	223 (17.25)	118 (18.47)	
Father only	97 (7.50)	44 (6.89)	
Both	40 (3.09)	15 (2.35)	
Parental Hay fever, %			**<0.001**
Mother only	441 (29.17)	207 (15.27)	
Father only	239 (15.81)	122 (9.00)	
Both	21 (13.96)	77 (5.68)	
Parental Wheeze, %	626 (43.90)	307 (41.60)	0.306

**Values are means (SD) or percentages (absolute numbers). Differences were tested by using the Student t test for continuous variables, and the X^2 test for categorical variables. **SES=socio economic status was defined by the family incomes, expressed in Australian dollars ($AUD), LRTI= low respiratory tract infections in the first year of life, URTI= upper respiratory tract infections in the first year of life. ***Full details of the variable definitions are reported in the Chapter 2.*

Supplementary Table 3.2. *Model selection for FEV_1, FVC and FEV_1/FVC*

FEV_1		
Polynomial	**BIC**	**AIC**
3	-5371.13	-5357.83
3 3	-5094.61	-5068.01
3 3 3	-5028.05	-4988.14
3 3 3 3	-4996.05	-4942.84
1 3 3 3	**-4990.38**	-4942.49
3 3 3 3 3	-4998.33	-4931.82

FVC		
Polynomial	**BIC**	**AIC**
3	-5432.57	-5419.27
3 3	-5222.18	-5195.58
3 3 3	-5190.94	-5151.03
3 3 3 3	-5190.93	-5137.72
1 1 2 3	**-5190.65**	**-5153.74**
3 3 3 3 3	-5201.39	-5134.87

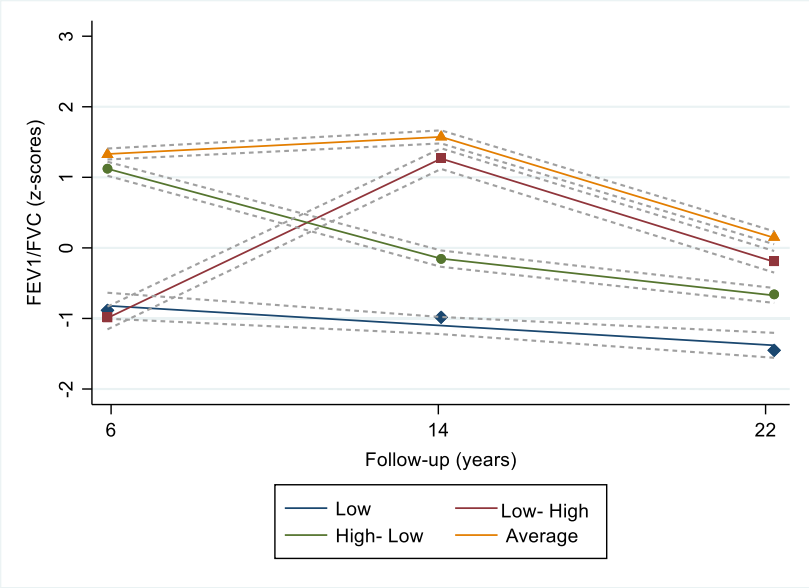
FEV_1/FVC		
Polynomial	**BIC**	**AIC**
3	-5778.60	-5765.30
3 3	-5663.34	-5636.74
3 3 3	-5566.71	-5526.80
3 3 3 3	-5471.34	-5431.43
1 3 3 3	**-5469.26**	**-5421.37**
3 3 3 3 3	-5471.22	-5404.71

Note The number of trajectory groups is represented by the number of polynomials with the corresponding type: 1=linear, 2=quadratic, 3=cubic*

***Supplementary Table 3.3**. Average posterior probabilities for each group within FEV_1, FVC, and FEV_1/FVC trajectories.*

	FEV_1 n (%)				**FVC n (%)**				**FEV_1/FVC n (%)**			
	Trajectories				**Trajectories**				**Trajectories**			
	Very Low	**Low**	**Average**	**Above Average**	**Very Low**	**Low**	**Average**	**Above Average**	**Very Low**	**Low-High**	**Average**	**High-Low**
Average posterior probabilities	0.81	0.84	0.79	0.83	0.72	0.77	0.72	0.79	0.84	0.82	0.79	0.85

Supplementary Table 3.4. *FEV_1/FVC trajectories in participants with spirometry measurements at age 14 and age 22 (might also have spirometry measurements at age 6) (n=869)*



Note The 95% confidence intervals are represented by dashed lines*

Supplementary Table 3.5. *Individual distribution of FEV_1/FVC lung function trajectories in the Raine Study participants with two or more spirometry measurements and spirometry measurements at 14 and 22 years of age.*

	Two or more spirometry measurements				
14 & 23 spiro (might have also at age 5)	Low	Low-high	High-low	Average	Total
Low	74		5		
Low-High		100		6	
High-Low		1	311	37	
Average				335	
Total					**869**

Note n=1512 participants had two or more spirometry measurements between 6, 14 and 22 years of age, while n=869 participants had spirometry measurements at 14 and 22 years of age.*

Chapter 4
Air pollution exposure in the Raine participants

4.1 Introduction

Environmental air pollution is a major health problem affecting everyone in low, middle, and high-income countries (Tong, 2019). *In utero* and early-life environmental air pollution exposure may affect optimal lung development and promote respiratory deficit during childhood that tracks into adulthood (Kim et al., 2018). Cross-sectional and longitudinal studies (Götschi et al., 2008; Mölter et al., 2013; Gehring et al., 2013) have reported declining lung function in children with a short and long-term exposure to air pollution, such as particulate matter (PM), and nitrogen dioxide (NO_2), as well as $PM_{25Absorbance}$ that is a marker for black carbon. However, the findings are controversial. Some studies showed associations with FEV_1 or FVC, others with both lung volume and expiratory flow, while some studies did not find any association at all. Although some researchers have shown that *in utero and* exposure in early life were associated with respiratory diseases, the long-term consequences of early-life exposure to air pollution on lung function are poorly understood.

Land Use Regression models are increasingly being used to estimate exposure to ambient air pollution for current or recent periods in which direct measurements of air pollution are available. In these models, data are usually collected during a dedicated environmental sampling periods at a defined number of monitoring stations, with the aim to develop a prediction model using characteristic variables, such as land use, traffic, and physical environment. Most of the existing LUR models have been used for the prediction of air pollutants in Western Europe and North America (Hoek et al., 2008; Jedynska et al., 2014; Eeftens et al., 2012) and none was available for Western Australia, since Dirgawati and colleagues (2016) developed and validated LUR models in 2012 for the Metropolitan Area of Perth. Despite their advantage in being easily applicable by researchers, LUR have some limitations. In fact, they require availability of a large number of monitoring sites, that together with a high number of variables can otherwise increase the risk of "overfitting" LUR models. Further, land use regression models require availability of accurate

historical air pollution measurements when transferring current or recent LUR models to earlier years, via back-extrapolation.

A method for the back-extrapolation of NO_2, NO_x, $PM_{2.5}$, PM_{10}, $PM_{2.5Absorbance}$ and PM_{coarse} estimated concentrations has been proposed by the European Study of Cohort for Air Pollution Effects (ESCAPE) project (www.escapeproject.eu). This approach estimates air pollution concentrations at historical addresses using historical air pollution measurements usually derived from the national monitoring network. The estimates are then assigned as individual exposure for the cohort participants in order to investigate the association between exposures and a particular health outcome. Previous studies have proven that the correlation between air pollution concentration of different years is high, even over a period of more than 35 years (Gulliver, 2013), but there are often insufficient and low reliable available data of past measurements, thus the back-extrapolation of LUR models might represent a challenging task (Levy et al., 2015). Further, whether a trend can be estimated over a time longer than 10 years is area-specific and depends on whether there have been large changes in road network, emission sources, or land use (ESCAPE manual)

The aim of this study was to conduct the back-extrapolation of the LUR models developed in 2012 for the Metropolitan area of Perth and estimate historical concentrations of NO_2, $PM_{2.5}$, and $PM_{2.5Absorbance}$ for earlier years, assigning individual air pollution exposure at residential addresses in the Raine participants from three to 22 years of age.

In this Chapter, I defined individual exposure to main air pollutants in the Raine participants from childhood to young adulthood, using a preexisting and validated Land Use Regression model. The results from this study will be applied in Chapter 5, where air pollution exposure in early life serve as predictors for the lung function trajectories identified in Chapter 3.

4.2. Methods

4.2.1. Area of study and population study

The study area consists of the Perth Metropolitan Region, capital of Western Australia, that extends in 6,418 km^2 with more than 2 million inhabitants in 2019. The city is characterized by an

Australian climate with a hot and dry summer, and a rainy season occurring between May and October (Nguyen et al., 2020). The participants included in this study were participants from the Raine Study with residential address history in early life between three and five years of age (1993-1995) (Figure 4.1)

4.2.2 Air quality data

Daily air pollution data across Perth were provided by the Department of Water and Environmental Regulation in Perth from 1991 to 2012 for $PM_{2.5}$, $PM_{2.5Absorbance}$, and NO_2, that operates air quality monitoring network in Perth (Supplementary Table 4.1, 4.2)

4.2.2.1 Back-extrapolation air pollution data

We back-extrapolated air pollution data using LUR models that were carried out and validated for the Perth metropolitan area in 2012 (Dirgawati et al., 2016). We first estimated annual air pollution concentrations for the period in which $PM_{2.5}$, $PM_{2.5Absorbance}$ and NO_2 were measured from the fixed stations between 1995 and 2012. Then, we applied the same standardized procedure to estimate NO_2 and $PM_{2.5Abs}$ from 1991 to 2012 as the historical measurements of these pollutants started earlier in time.

Following the Escape guidelines (http://www.escapeproject.eu) we back- extrapolated LUR-based predicted concentration of $PM_{2.5}$ and NO_2 by using 1) the predicted annual average concentrations from LUR models for the year 2012, and 2) the annual average of measured concentrations from the eligible air monitoring stations that covered the year preceding the baseline period of our study (1994), baseline period of our study (1995), the years of follow-up until the LUR model development (2012). The annual concentrations for the whole period between 1994 and 2012 were averaged. This provided annual average background concentrations for the entire Perth metropolitan area and for each predicted year from 1995 to 2012 (C-routine). We calculated for each study participant the average concentration based on the year before and the year after the age six for the baseline date for the routine monitoring site (C-routine baseline). A correction factor (Ratio) between the average one year before and one year after the baseline date and the year of 2012 when LUR models was developed. The back-extrapolated concentrations for each current residential address and each predict year (C-extrapolated) was calculated by multiplying the LUR-

based predicted annual average specific-year concentration for each home address with the corresponding year-specific correction factor.

$$C_{routine-baseline} = baseline\ date +/-1\ year$$

$$Ratio = C_{routine-baseline}/C_{routine-Escape}$$

$$C_{extrapolated=}C_{Escape} * Ratio$$

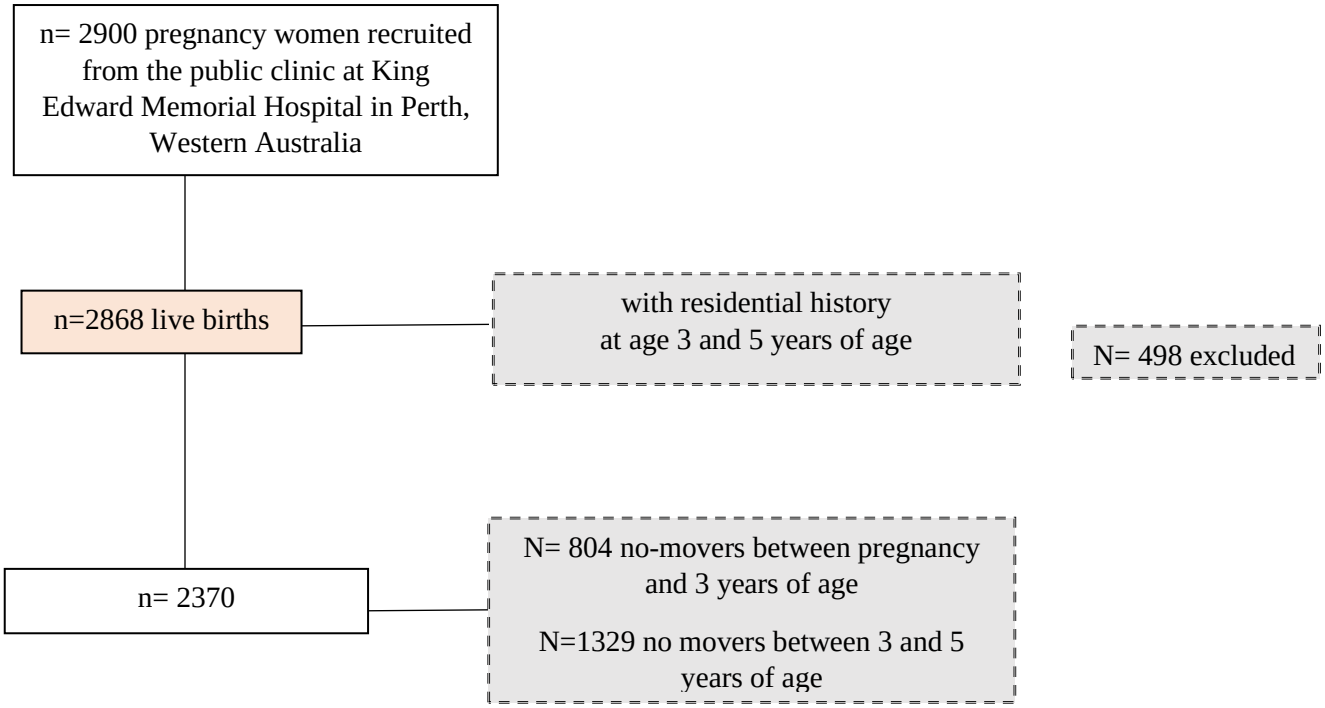
As fixed air monitoring stations data were not available for $PM_{2.5Absorbance}$, temporal NO_2 concentrations were used as a proxy for temporal change in $PM_{2.5Absorbance}$ concentrations. All statistical analysis was performed with STATA (version 16.0; StataCorp, College Station, TX).

Each participant's exposure to $PM_{2.5}$, $PM_{2.5Absorbance}$ and NO_2 was estimated at pregnancy, birth and at 1, 2, 3, 5, 8, 10, 13, 16, and 22 years of follow-up on the basis of the last residential address available, using geographical information system (GIS, version). We estimated the annual $PM_{2.5}$, $PM_{2.5Absorbance}$ and NO_2 average concentrations from the LUR model as previously described. Then, each participant's exposure to $PM_{2.5Absobance}$ and NO_2 was estimated in early life when children were aged between 3 and 4 years of age, using their exact date of birth and their year-specific address.

4.3. Results

Out of the 2,370 participants included in this study, 1329 did not move between three and five years of age, while 804 did not move between the gestational period of the mother and three years and 557 did not move for the whole period from gestational period and five years.

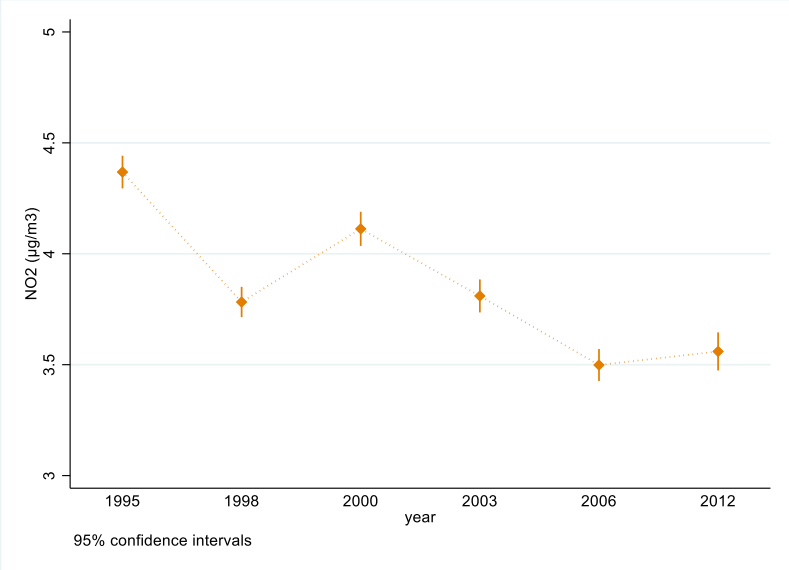
Figure 4.1. *Flow chart of participants involved in this study from the Raine Study*

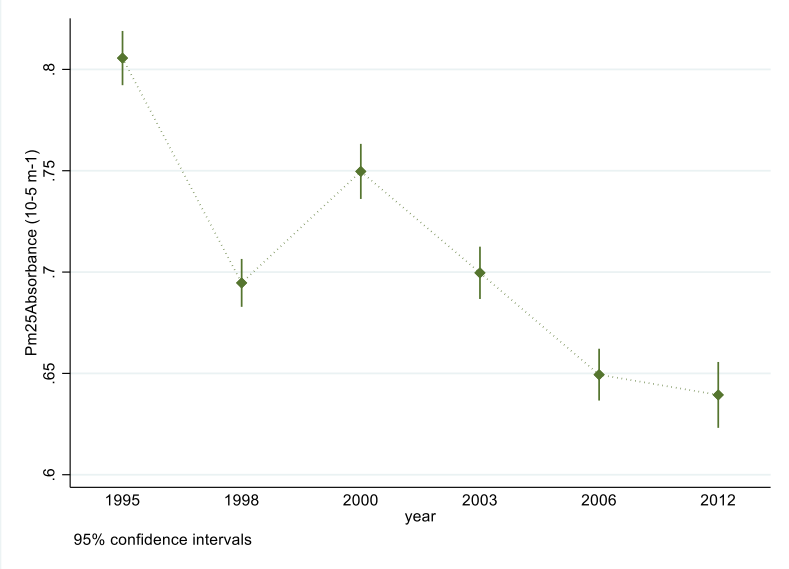


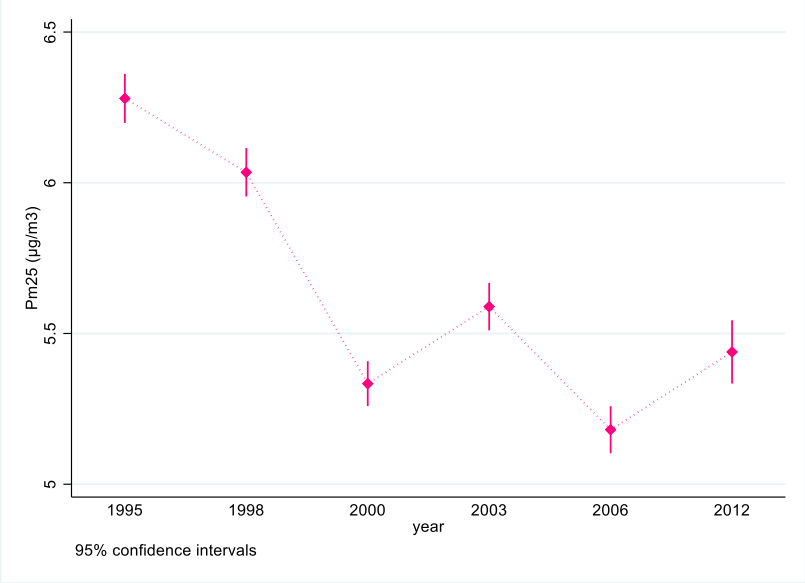
4.3.1. Individual air pollution exposure in the Raine participants

Based on LUR model developed for the Perth Metropolitan Area, we estimated exposure to air pollutants at geocodes based on each participant's home street address. Annual average concentrations of $PM_{2.5,}$ $PM_{2.5Abs}$ and NO_2 from 1995 to 2012 in the Raine participants are shown in Figure 4.2 A, B and C. Due to the availability of two monitoring sites for NO_2 across Perth (Caversham and Perth CBD) earlier then 1995, we back-extrapolated data and estimate individual exposure back to 1990 for $PM_{2.5Abs}$ and NO_2 (Figure 4.3 A and B).

Figure 4.2 A, B, C. Annual average concentration of $PM_{2.5}$, $Pm2.5_{Absorbance}$, and NO_2 in the Raine participants between 1995 and 2012.







In 1995, the annual mean concentration in the Raine participants was 6.28 µg/m^3 (SD± 1.89) for $Pm_{2.5}$, 0.86 ·10^{-5} m^{-1} (SD ± 0.33) for $Pm_{2.5Abs}$ and 4.37 µg/m^3 (SD ± 2.17) for NO_2 (Table 4.1 A, B, C). Early-life exposure to air pollutants when children were aged between three and four years of age was calculated for NO_2 and $PM_{2.5Abs}$ as the historical data for these pollutants started earlier than $PM_{2.5}$. The average concentrations of $PM_{2.5Absorbance}$ and NO_2 at home address for the first year of life and the lifetime average concentrations from the first year of life to 22 years (1991-2012) are presented in Supplementary Table 4.3 A, B. The spatial distribution of annual average concentration of NO_2, $PM_{2.5}$ and $PM_{2.5Absorbance}$ in 1995 are shown in Supplementary Figures 4.2, 4.3 and 4.4. The central busines district (CBD) is the area in which the concentrations were higher for all the three air pollutants across the Metropolitan area of Perth, while the surrounding areas had the lowest concentrations.

Table 4.1 A, B, C. *Descriptive summary of $PM_{2.5}$, $PM_{2.5Absorbance}$ and NO_2 at baseline and during the years of follow-up in the Raine participants (using last address)*

$Pm_{2.5}$	**Mean ($\mu g/m^3$)**	**Std Dev**	**Min**	**Max**
1995	6.28	±1.82	0.06	10.70
During the years of follow up (1995-2012)	5.80	±1.52	0.39	10.67

$Pm_{2.5}$ Absorbance	**Mean (10^{-5} m^{-1})**	**Std Dev**	**Min**	**Max**
Early-life (3.5-4.5 years)	1.00	±0.38	0.02	2.99
1995	0.86	±0.30	0.04	2.29
During the years of follow up (1995-2012)	0.73	±0.24	0.03	2.29

NO_2	**Mean ($\mu g/m^3$)**	**Std Dev**	**Min**	**Max**
Early-life (3.5- 4.5 years)	5.20	±2.08	0.11	12.19
1995	4.38	±1.65	0.08	10.37
During the years of follow up (1995-2012)	3.86	±1.34	0.27	9.70

4.4. Discussion

Together with other risk factors, environmental exposure to air pollution might lead to lung function deficits that may track into adulthood (Kim et al., 2018). Although the association between early life exposure of air pollution and declined lung function has been reported, findings are controversial and respiratory outcomes later in life might vary for different pollutants. In addition, a wide range of studies demonstrated the linked between the adverse effects of air pollution on lung function cross-sectionally, while less is known about the long-term consequences of air pollutant concentrations. Thus, whether the impact of early post-natal environmental air

pollution endures into young adulthood is still unknown. In this study we estimated historical air pollution exposure for 2,370 Raine Study participants, from early-life to young adulthood. By using land use regression models developed for the Metropolitan Area of Perth in 2012, we back-extrapolated data back in time for $PM_{2.5,}$ $PM_{2.5Absorbance}$ and NO_2.

The air pollution exposure models were modelled based on monitoring stations across the metropolitan area of Perth in 2012 and were used to estimate exposures in early life between 1993 and 1995. Currently, individual exposure estimates often assume that the concentrations estimated from the LUR model are valid for long period of time, and few studies have proven the stability of land use regression models, even over a period of more than 35 years (Beelen et al., 2007; Gulliver, 2013). In a study conducted in the Netherlands, Eeftens and colleagues (2011) compared two set of NO_2 measurements at the same location to test the stability over time, and they found good agreement between measured spatial contrast of measured concentration of NO_2 over a period of eight years. In another study Wang and colleagues (2013) showed that LUR model provided reliable estimates over a period of seven years in Metro Vancouver, in Canada, without loss of applicability of the model. Gulliver and colleagues (2013) evaluated back-extrapolation of LUR models for annual mean NO_2 concentration developed in Great Britain for a period of up to 18 years, demonstrating a valid reliability. In our cohort sample, we back-extrapolated air pollution data for eighteen years of follow-up. We used the ratio method proposed by ESCAPE project and we applied it to NO_2, $PM_{2.5}$ and $PM_{2.5Absorbance}$ to back-extrapolate LUR models.

Firstly, we back-extrapolate data for 1995 for NO_2, $PM_{2.5}$ and $PM_{2.5Absorbance}$ and we calculated the annual average exposure to these pollutants for the whole period of follow-up. We found that the annual mean concentrations in 1995 for NO_2, $PM_{2.5}$ and $PM_{2.5Abs}$ for the participants were 6.28 $\mu g/m^3$ for $Pm_{2.5}$, 0.81 $\cdot 10^{-5}$ m^{-1} for $Pm_{2.5Abs}$ and 4.38 $\mu g/m^3$ for NO_2, with a slight decrease over the years for all the three pollutants. Secondly, because the historical measurement for NO_2 started earlier in time, we were able to calculate the early-life exposures for NO_2 and $PM_{2.5Absorbance,}$ considered as the period in which children were aged between three and four years of age. During all the periods investigated the concentrations were below the limit suggested by the WHO guidelines corresponding to 10 $\mu g/m^3$ for $Pm_{2.5}$ and 40 $\mu g/m^3$ for NO_2 (WHO, 2006), while no guidelines for $PM_{2.5Absorbance}$ with a recommended annual mean limit have been found.

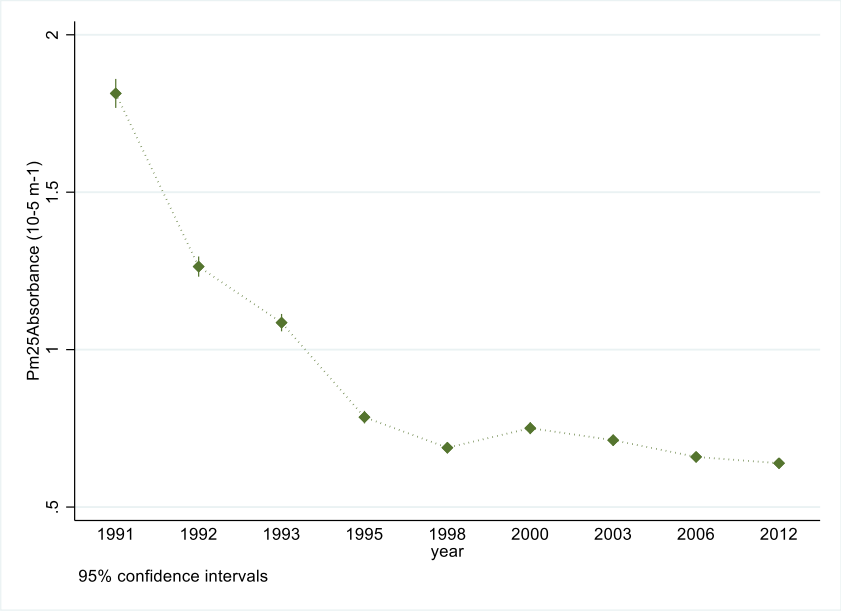
Our main interest in this study was to estimate the individual early-life exposure to the main air pollutants, such as NO_2, $PM_{2.5}$ and $PM_{2.5Absorbance}$ in the Raine Study participants. Intrauterine and early years of life are considered vulnerable periods because the lungs are still developing and ideally, we would back-extrapolated land use regression models since these periods. However, modelling historic air pollution exposure depends on the availability of monitored concentrations. Thus, one limitation of this study is that we were not able to back-extrapolate air pollution data to cover the antenatal period and in the first three years of life because monitoring measurements for $PM_{2.5}$ and NO_2 were consistently available starting from 1995 for $PM_{2.5}$ and from 1993 for NO_2. Our early life exposure was defined when children were aged between three and four years of age. Further, potential errors might be occurred with changing address in early life. Despite a wide range of studies reported adverse effect of air pollution on lung function, only few studies have investigated long-term exposure, and this can be mostly due to the limited data available from the monitoring stations. Another consideration is that air pollution measurements across monitoring stations were scarce in 1991 and 1992 comparing to the following years, so the accuracy of our data for those years were not completely reliable, resulting in high concentrations level in those earlier years (Supplementary Figure 4.1). Another limitation is that the land use regression model used for our analysis was developed and validated for the Metropolitan area of Perth in 2012. Thus, to estimate early-life air pollution exposure we back-extrapolated data for a period longer than 17 years. Although various studies had demonstrated the stability of land use regression models over a period of 35 years, one of the assumptions when utilizing this approach is that the spatial pattern remains constant in time. However, it might be possible that from 1993 to 2012, Perth has undergone changes in the urban environment, such as the increasing of the road that can have overestimate the exposure at residential address. Further, we could not evaluate the accuracy of our air pollution estimates, as the LUR model that we used was the first and the only one developed for the area of Perth.

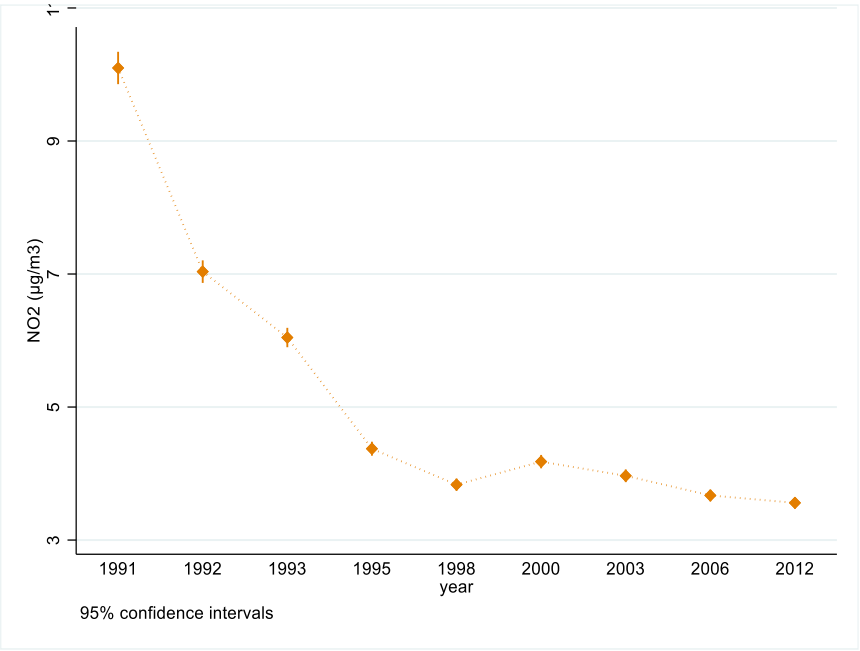
On the other hand, this study has several strengths, such as the use of a longitudinal study with more than 22 years of follow-up with complete data on the residential address history for each participant. With the available air pollution data for each participant's residential address, we were able to define different time frame of exposure (in early-life, in 1995, and lifetime average

exposure). Although most studies have assessed air pollution exposure using central site monitors, this study provides reliable exposure measurements as we had complete address histories from birth into young adulthood and we assigned exposures in early life according to the residential address through this period. The individual early life exposure to $PM_{2.5}$, $PM_{2.5Absorbance}$ and NO_2 would serve as predictors in the analysis conducted on the next Chapter, to investigate the early-life factors associated with the lung function trajectories previously identified.

4.5. Supplementary material

Supplementary Figure 4.1. *Annual average concentration of $Pm_{2.5Abs}$, and NO_2 in the Raine participants between 1991 and 2012.*




NO2 (µg/m3)
3
5
7
9
1991
1992
1993
1995
1998
2000
2003
2006
2012
year
95% confidence intervals

Supplementary Table 4.1. *Available NO_2 data obtained by Department of Environment (DER)*

	NO_2								
year	**Caversham**	**Duncraig**	**N Rockingam**	**PerthCBD**	**QuinnsRock**	**Rockingam**	**SouthLake**	**Swanbourne**	**Wattleup**
1989									
1990	From August			X					
1991	X			X					
1992	X			X	From October			From September	
1993	X			X	X			X	
1994	X			X	X			X	
1995	X	From July			Only December			X	
1996	X	X	X		X			X	
1997	X	X	X		X			X	
1998	X	X	X		X			X	
1999	X	X	X		X			X	
2000	X	X	X		X		From March	X	
2001	X	X	X		X		X	X	
2002	X	X	X		X		X	X	
2003	X	X	X		X		X	X	
2004	X	X	X		X		X	X	
2005	X	X	X		X		X	X	

2006	X	X	X		X		X	X	
2007	X	X	X		X		X	X	
2008	X	X	X		X		X	X	
2009	X	X	X		X		X	X	
2010	X	X	X		X		X	X	
2011	X	X	X		X		X	X	
2012	X	X	X		X		X	X	
2013	X	X	X		X		X		
2014	X	X	X		X		X		

Supplementary Table 4.2. *Available $PM_{2.5}$ data obtained by Department of Environment (DER)*

	PM_{25}								
year	**Caversham**	**Duncraig**	**N Rockingam**	**PerthCBD**	**QuinnsRock**	**Rockingam**	**SouthLake**	**Swanbourne**	**Wattleup**
1989									
1990									
1991									
1992									
1993									
1994	From March	From November						From June	
1995	X	X							
1996	X	X							
1997	X	X							
1998	x	X							
1999	X	X							
2000	X	X							
2001	X	X							
2002	X	X							
2003	X	X							
2004	Only January	X							
2005		X							

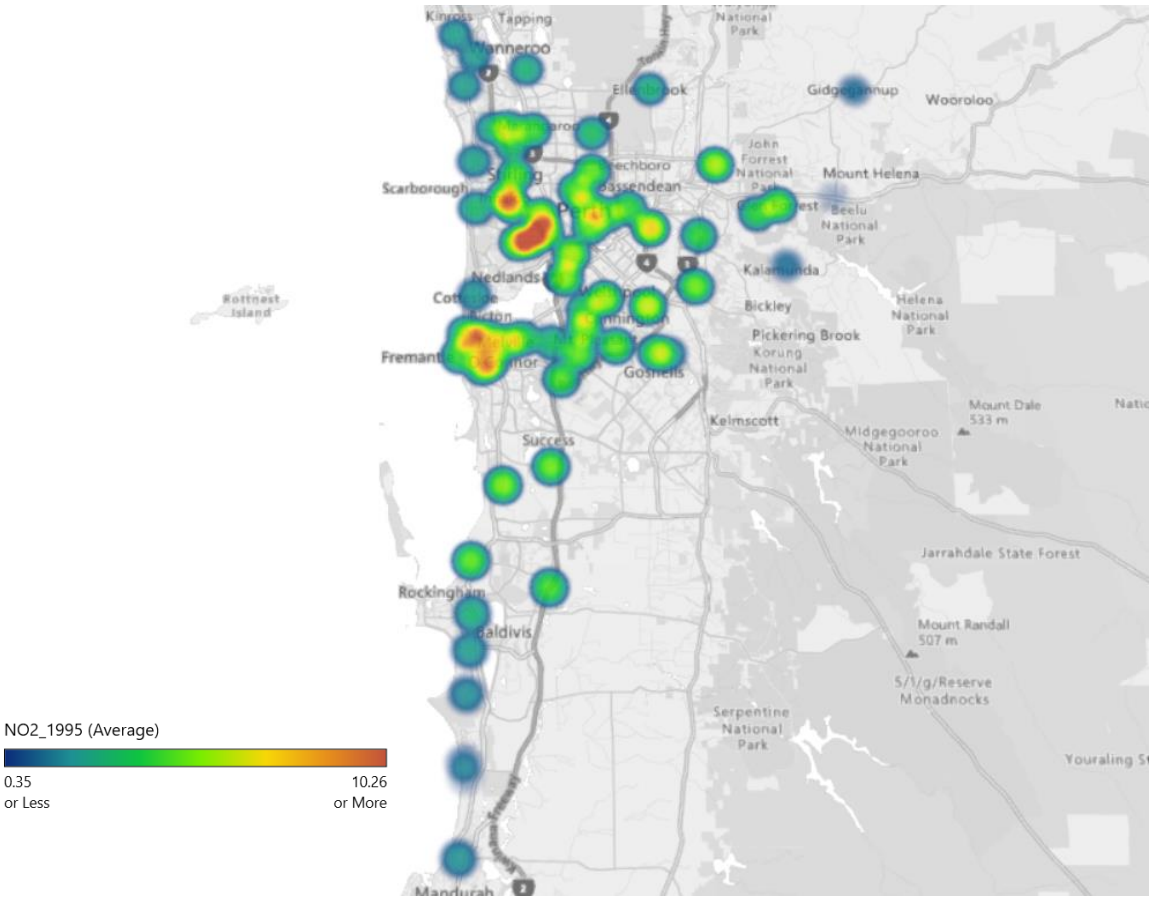
2006	From May	X			From June		From March		
2007	X	X			X		X		
2008	X	X			X		X		
2009	X	X			X		X		
2010	X	X			X		X		
2011	X	X			X		X		
2012	X	X			X		X		
2013	X	X			X		X		
2014	X	X			X		X		

Supplementary Table 4.3 A, B. *Descriptive summary of $PM2.5_{Absorbance}$ and NO_2 at home address for the first year of life and the lifetime average between the first year of life and 22 years of age in the Raine participants (using the last address)*

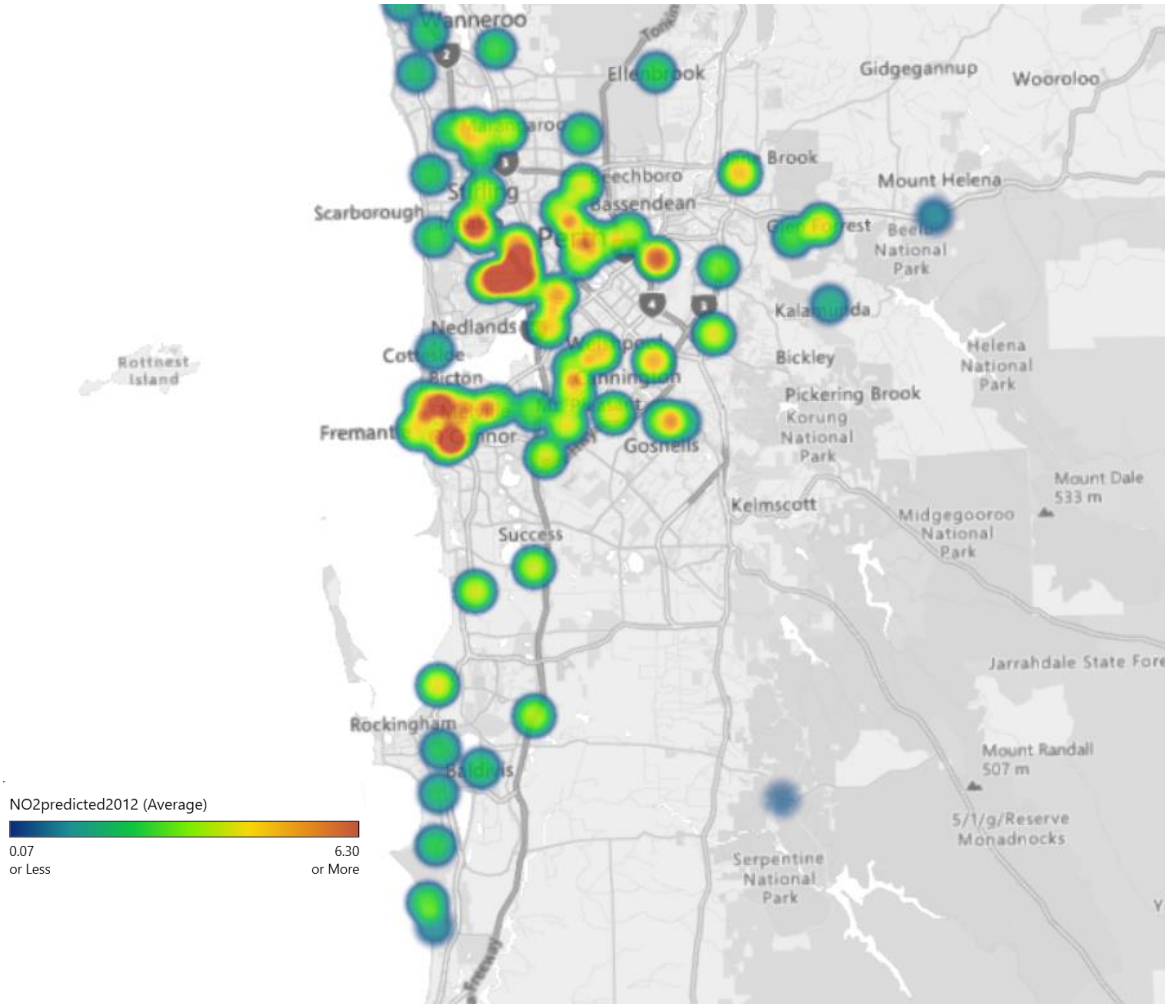
$Pm_{2.5}$ Absorbance	**Mean (10^{-5} m^{-1})**	**Std Dev**	**Min**	**Max**
1991	1.96	±0.70	0.12	5.29
1991- 2012	0.98	±0.36	0.15	3.58
During the years of follow up (1993-2012)	0.81	±0.27	0.03	2.73

NO_2	**Mean (µg/m^3)**	**Std Dev**	**Min**	**Max**
1991	10.73	±3.81	0.14	21.49
1991- 2012	5.28	±1.91	0.12	17.9
During the years of follow up (1993-2012)	4.35	±1.46	0.09	12.03

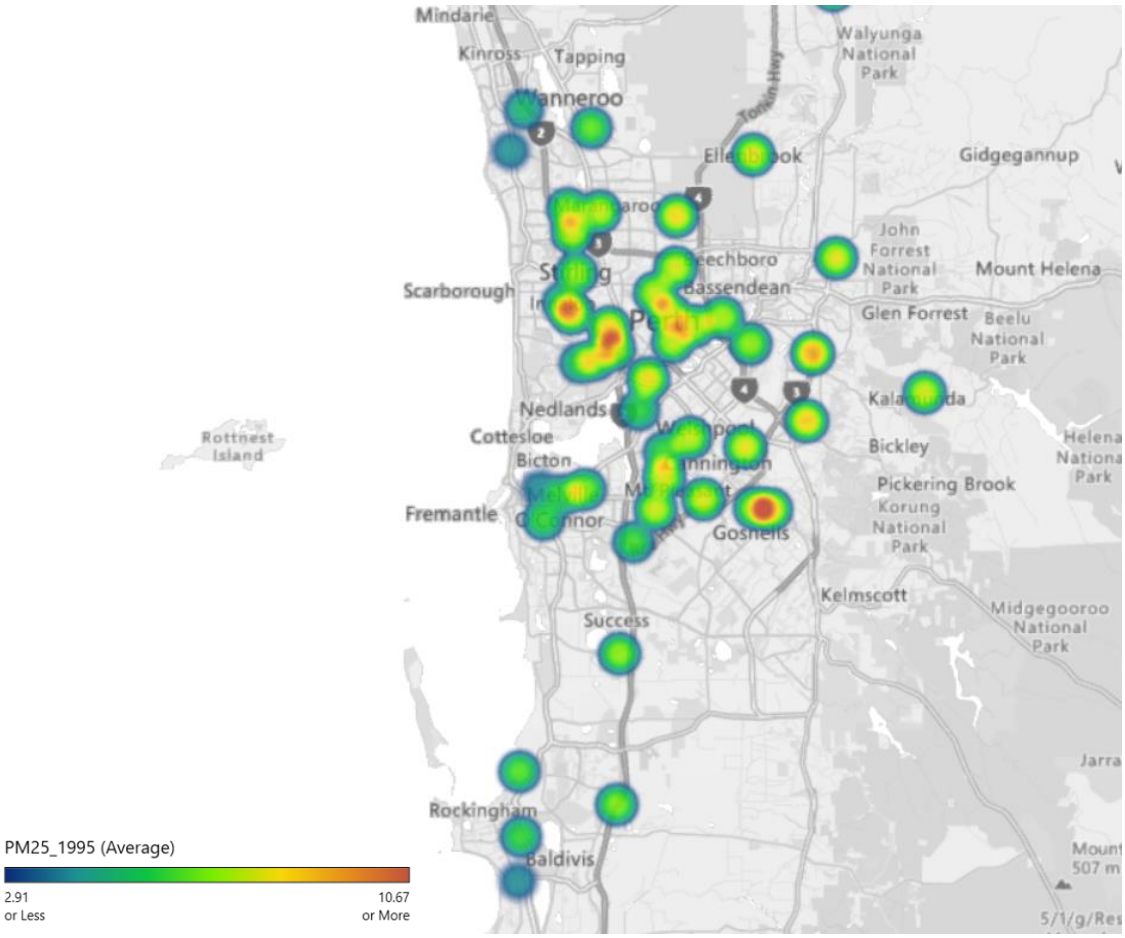
Supplementary Figure 4.4. *Spatial distribution of predicted annual concentration of NO_2 in Perth in 1995*



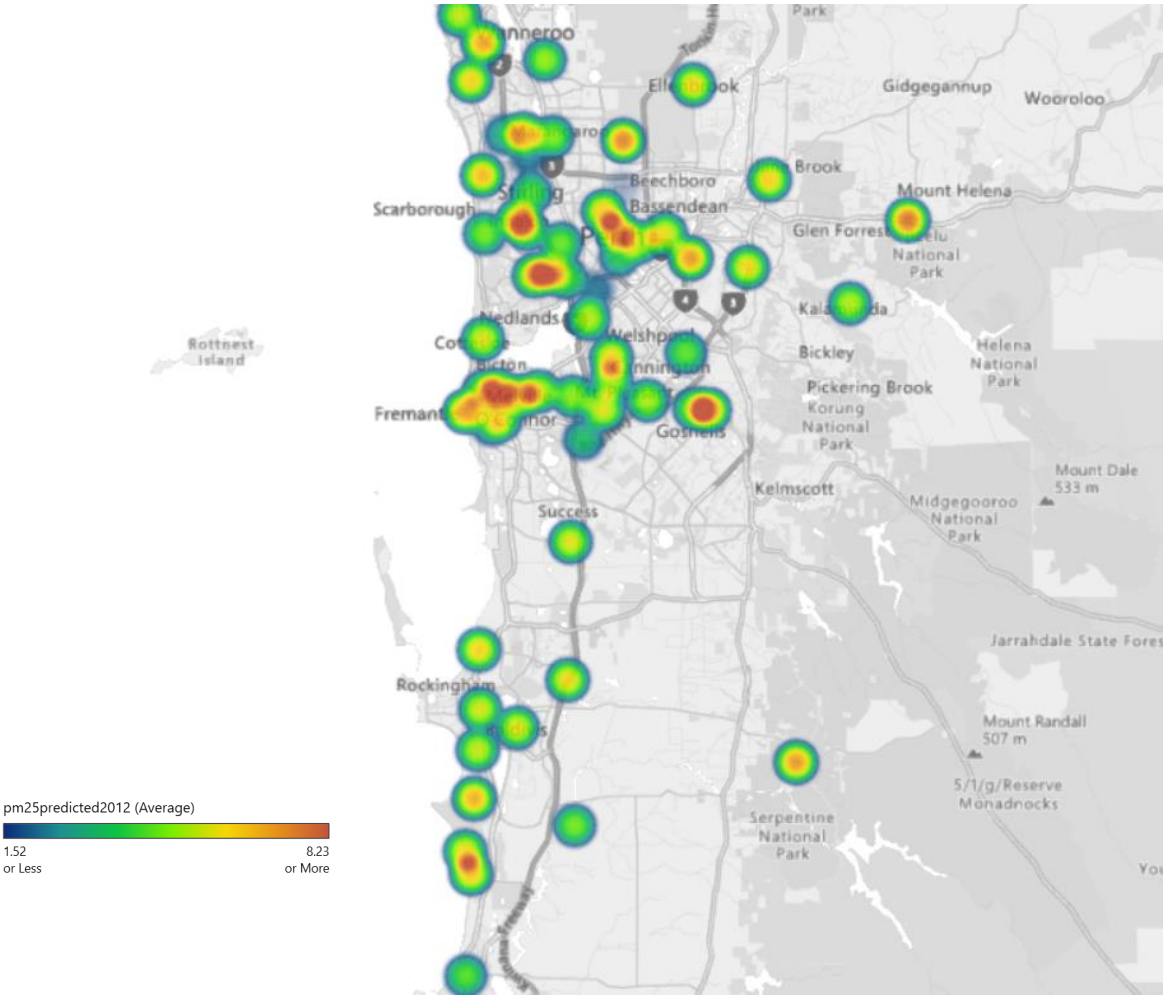
Supplementary Figure 4. 5. *Spatial distribution of predicted annual concentration of NO_2 in Perth in 2012*



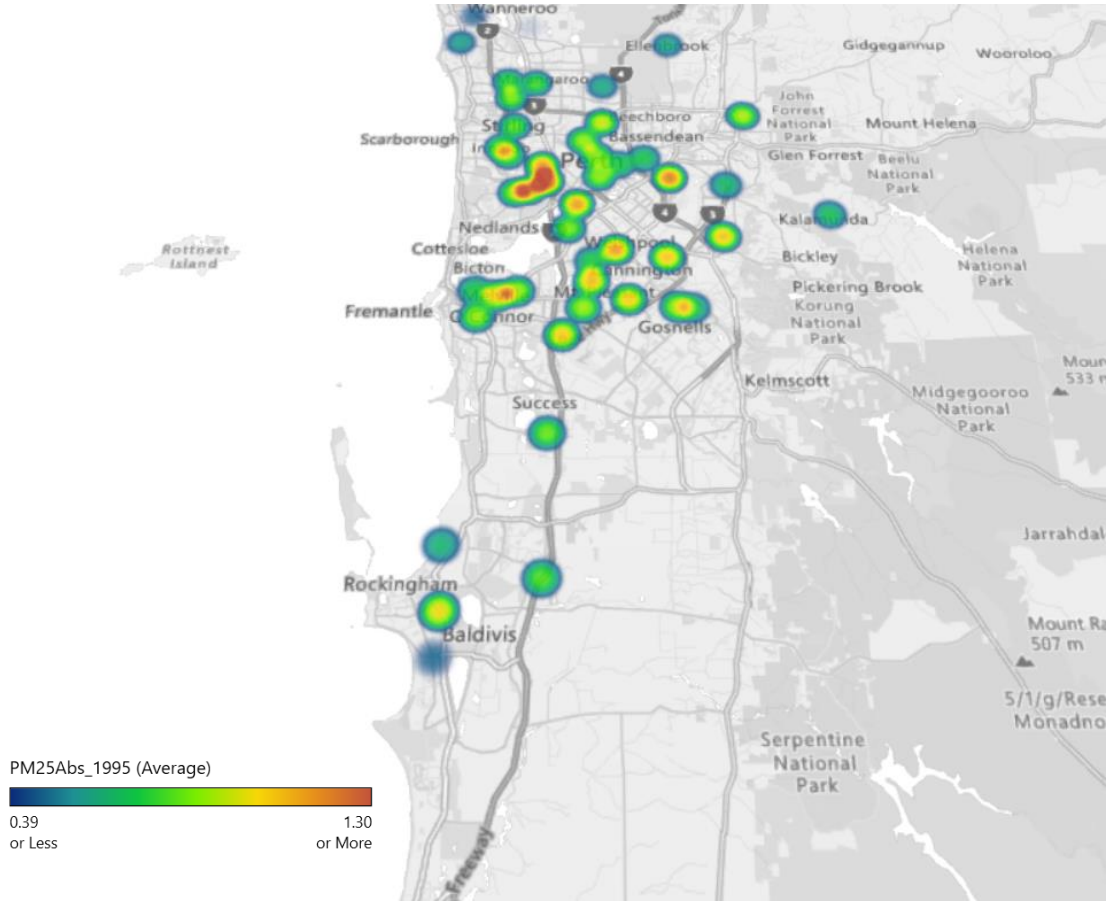
Supplementary Figure 4. 6. *Spatial distribution of predicted annual concentration of $PM_{2.5}$. in Perth in 1995*



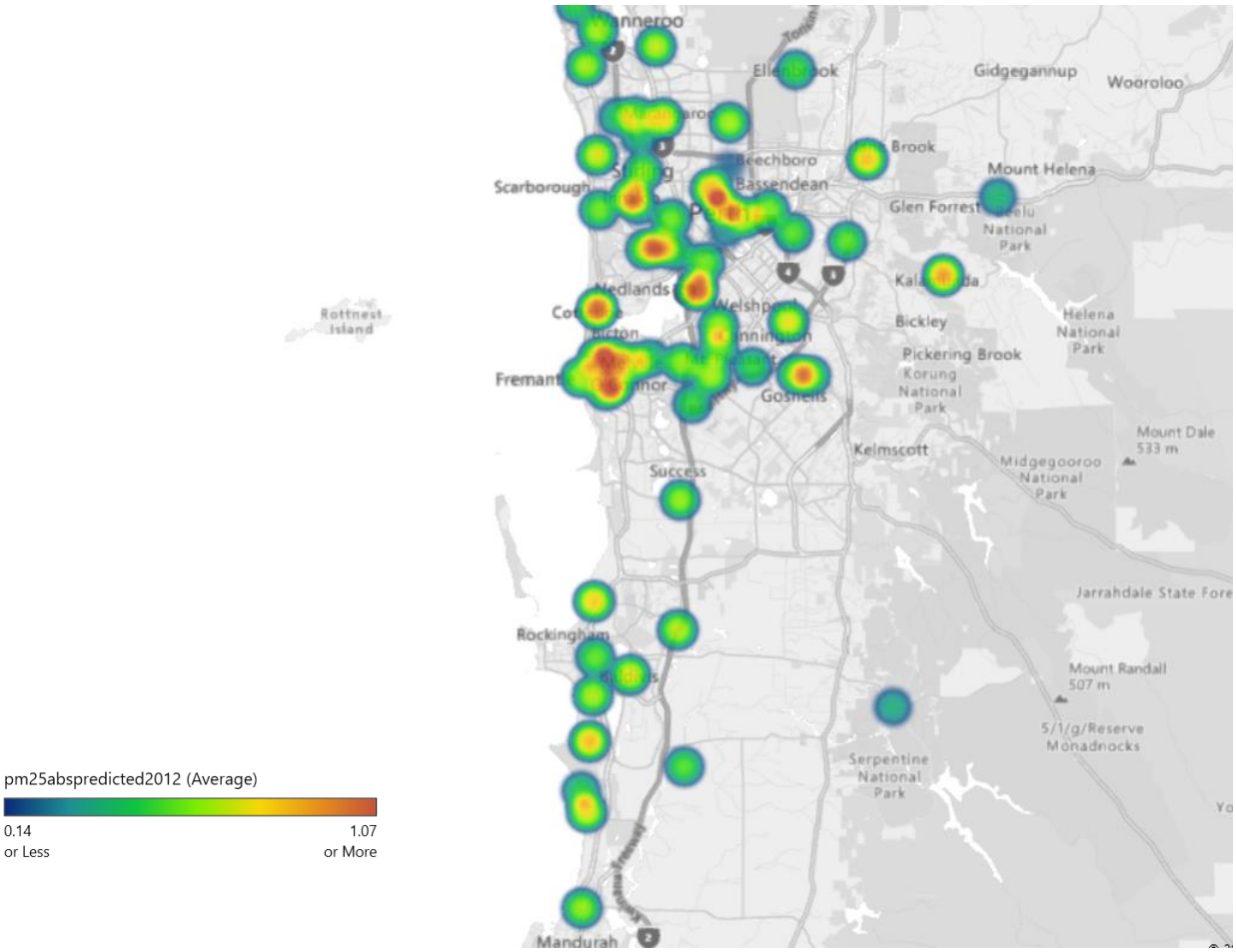
Supplementary Figure 4. 7. *Spatial distribution of predicted annual concentration of $PM_{2.5}$. in Perth in 2012.*



Supplementary Figure 4. 8. *Spatial distribution of predicted annual concentration of $PM_{2.5Abs}$ in Perth in 1995*



Supplementary Figure 4. 9. *Spatial distribution of predicted annual concentration of $PM_{2.5Abs}$ in Perth in 2012*



Chapter 5
Early-life risk factors on lung function trajectories in the Raine Study participants

5.1 Introduction

A variety of factors associated with both the environment and lifestyle that are present from the fetal period into adulthood may present during important window of opportunities leading to development of respiratory diseases (Stocks, Hislop & Sonnappa, 2013). They can impact the natural lung function growth resulting in abnormal lung function trajectories that can track into adulthood.

Several birth-cohort studies have shown that there is a distribution of different lung function trajectories in the population (Turner et al., 2014; Lødrup Carlsen et al., 2014; Belgrave et al., 2014; Bui et al., 2018; Karmaus et al., 2019) and individuals have unique lifetime lung function trajectories based on their own life experiences. Together with respiratory infections, maternal tobacco use, preterm birth and allergens, air pollution is also a risk factor for respiratory health where children are considered more vulnerable than adults. Although the full implications of this finding are unknown, it is postulated that this increased vulnerability may be associated with relatively larger lung surface area in children (Kim et al., 2018). A birth-cohort study (Rice et al., 2018) suggested that exposure to ambient pollution at relatively low levels may reduce lung function and increase risk of lung function deficits in mid-childhood. To date, there is an urgent need to further investigate lung function over the lifespan and identify groups at higher risk of lung disease and modifiable exposures.

In our study, we hypothesized that insults in early life and exposure to ambient pollution are associated with low lung function trajectories. This study aimed to identify early-life, parental, and environmental predictors associated with the lung function trajectories identified. Only conditions determined before age six were considered as risk factors. In this Chapter, I investigated early life,

parental, and environmental predictors for the FEV_1, FVC, FEV_1/FVC lung function trajectories identified in Chapter 3. Full details regarding the environmental exposure were reported in Chapter 4.

5.2. Methods

5.2.1. Study population

We included participants from the Raine Study with two or more spirometry measurements from 6 to 22 years. The cohort description was fully reported in Chapter 3.

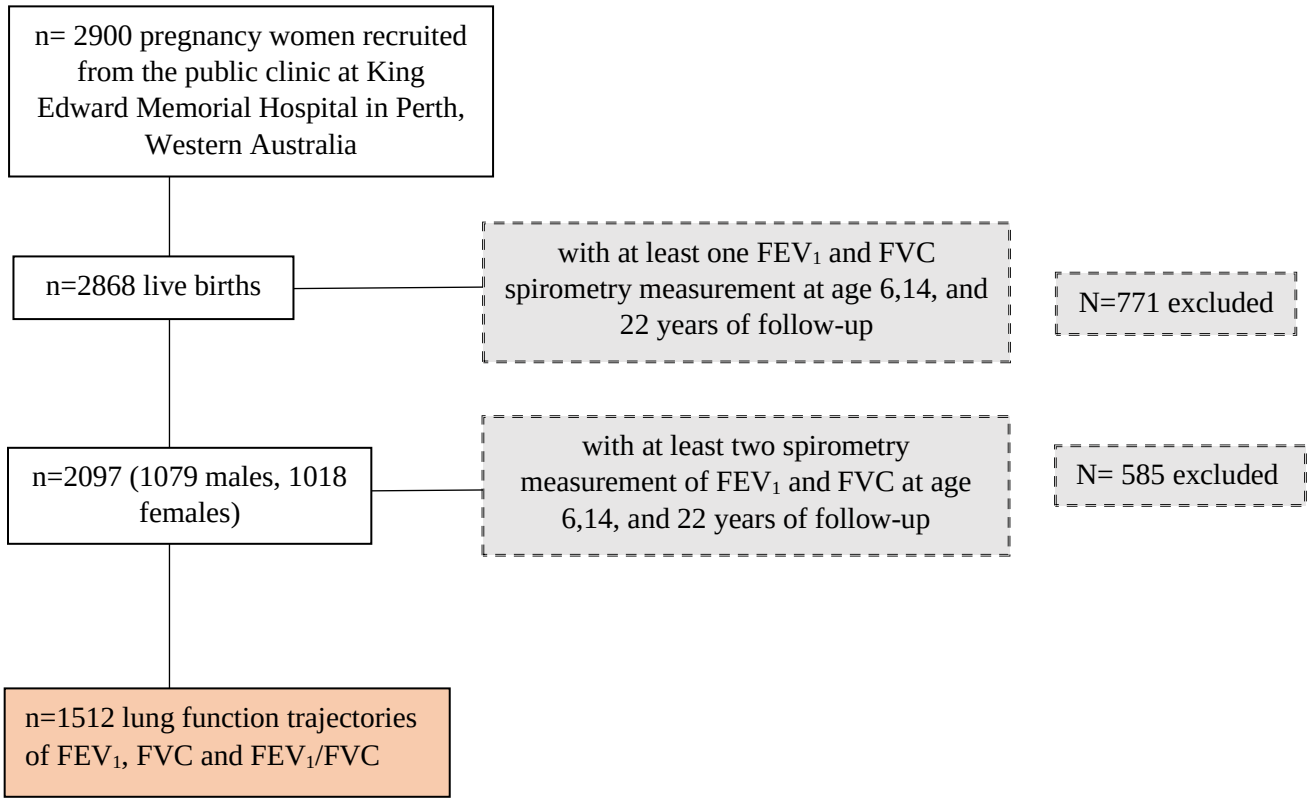
5.2.2. Statistical analysis

The distribution of participants' characteristics by lung function trajectories were summarized using percentages, and Person's chi-squared tests were used to estimate differences between groups. Significance of the associations between lung function trajectories and potential risk factors were first evaluated by unadjusted multinomial logistic regression. Variables and all the childhood, parental and environmental predictors associated with lung function trajectories with $p<0.1$ were used to build the full model. The associations between early life and parental predictors with lung function trajectory membership were estimated through relative risk ratios (RRR) with 95% confidence intervals (95% CI) obtained by multinomial logistic regression models. We used the lung function trajectories as the dependent variable and childhood, parental and environmental predictors associated with lung function trajectories as independent variables. The likelihood ratio test was used to compare the fit of the models. The final multinomial regression model was adjusted for gender, socioeconomic status, preterm birth, childhood asthma, childhood respiratory infections, childhood atopy, maternal smoking, parental asthma, parental hay fever, parental eczema, and early life exposure to NO_2 and $PM_{2.5Absorbance}$. Because wheeze was highly correlated with asthma, we removed this variable from the final model. However, we conducted a further analysis adjusting for childhood and parental wheeze instead of childhood and parental asthma (Supplementary Table 5.2). Further, as the sample size within individual lung function trajectory groups was low, we performed a sensitivity analysis dichotomizing or removing those variables with fewer participants (Supplementary Table 5.3.).

5.3 Results

To identify early-life and parental predictors associated with low lung function trajectories, we included data from 1512 participants (Figure 5.1).

Figure 5.1. *Flow chart of participants in this study from the Raine Study*



Childhood wheeze was prevalent in the very low trajectory of FEV_1 compared with the average trajectory (29.2% vs 18.6%, p=0.042). No statistically significant association was found between childhood asthma and asthma ever with different FVC trajectories. The prevalence of asthma was higher in the very low trajectory of FEV_1/FVC. Specifically, as compared with those in the average trajectory, participants in the very low trajectory were more likely to have early onset asthma (13.0 % vs 7.3%, p=0.010), persistent asthma (4.6% vs 0.9%, p=0.010), and late-onset asthma (8.4% vs, 6.3% p=0.010). We found a significantly higher percentage of participants with childhood wheeze

(32.6%) and parental wheeze (59.2%) in the very low trajectory of FEV_1/FVC compared with the average trajectory. Among the trajectories, preterm birth was prevalent in the low trajectories of FEV_1/FVC compared to the average trajectory (9.6% vs 5.0%). Prevalence of childhood and parental characteristics within lung function trajectories are reported in Table 5.1.

Table 5.1. *Childhood and parental characteristics in participants with two or more spirometry measurements within FEV_1, FVC and FEV_1/FVC lung function trajectory groups*

	FEV_1					FVC					FEV_1/FVC				
Variable	**Very low n (%)**	**Low n (%)**	**Average n (%)**	**Above average n (%)**	**P-value**	**Very low n (%)**	**Low n (%)**	**Average n (%)**	**Above Average n (%)**	**P-value**	**Very low n (%)**	**Low- High n (%)**	**High- Low n (%)**	**Average n (%)**	**P-value**
Female	19 (37.2)	375 (50.3)	302 (49.2)	48 (46.2)	0.302	24 (39.3)	431 (50.4)	225 (49.2)	64 (46.0)	0.331	61 (46.6)	109 (55.1)	240 (45.4)	334 (51.1)	0.069
SES															
≤16.000	3 (6.4)	69 (10.0)	57 (10.1)	8 (8.4)	0.211	3 (5.1)	81 (10.3)	36 (8.6)	17 (12.8)	0.454	11 (8.8)	20 (10.8)	54 (11.1)	52 (8.6)	0.078
16000-40000	29 (61.7)	288 (41.6)	246 (43.5)	46 (48.4)		32 (54.2)	340 (43.0)	180 (43.2)	57 (42.9)		60 (48.0)	62 (33.5)	217 (44.7)	270 (44.8)	
≥40000	15 (31.9)	335 (48.4)	262 (46.4)	41 (43.2)		24 (40.7)	369 (46.7)	201 (48.2)	59 (44.4)		54 (43.2)	103 (55.7)	215 (44.2)	281 (46.6)	
Asthma															
Never	32 (72.7)	560 (80.3)	493 (83.1)	88 (87.1)	0.133	43 (81.1)	662 (81.4)	360 (82.6)	108 (81.2)	0.905	82 (62.6)	150 (75.8)	400 (75.6)	541 (82.7)	**0.010**
Early-onset	3 (6.8)	76 (10.9)	46 (7.8)	7 (6.9)		5 (9.4)	74 (9.1)	43 (9.9)	10 (7.5)		17 (13.0)	17 (8.6)	50 (9.5)	48 (7.3)	
Persistent	2 (4.6)	12 (1.7)	8 (1.4)	1 (1.0)		0 (0.0)	13 (1.6)	7 (1.6)	3 (2.3)		6 (4.6)	3 (1.52)	8 (1.51)	6 (0.9)	
Late-onset	7 (15.9)	49 (7.0)	46 (7.8)	5 (5.0)		5 (9.4)	64 (7.9)	26 (6.0)	12 (9.0)		11 (8.4)	16 (8.1)	39 (7.4)	41 (6.3)	
Current wheeze at age 6	14 (29.2)	173 (24.3)	109 (18.6)	18 (18.8)	**0.042**	16 (26.7)	182 (22.2)	86 (19.9)	30 (22.6)	0.599	42 (32.6)	42 (21.7)	116 (23.6)	114 (18.1)	**0.002**
Atopic status at age 6															
Never	30 (58.8)	426 (57.1)	346 (56.6)	51 (49.0)	0.062	35 (57.4)	498 (58.6)	251 (54.9)	69 (49.6)	0.094	72 (55.0)	120 (60.6)	300 (56.7)	361 (55.2)	0.239
Food Allergy only	5 (9.8)	49 (6.6)	38 (6.2)	4 (3.9)		4 (6.6)	53 (6.2)	34 (7.4)	5 (3.6)		15 (11.5)	7 (3.5)	33 (6.2)	41 (6.3)	
Aeroallergens only	1 (2.0)	64 (8.6)	36 (5.9)	5 (4.8)		2 (3.3)	66 (7.7)	31 (6.8)	7 (5.04)		6 (4.6)	15 (7.6)	33 (6.2)	52 (8.0)	
Both	15 (29.4)	207 (27.8)	191 (31.3)	44 (42.3)		20 (32.8)	238 (27.9)	141 (30.9)	58 (41.7)		38 (29.0)	56 (28.3)	163 (30.8)	200 (30.6)	
Maternal smoke															
Never	25 (62.5)	454 (67.2)	379 (66.8)	68 (73.9)	**0.018**	31 (58.5)	533 (68.7)	273 (65.3)	89 (69.5)	**0.022**	67 (54.9)	126 (69.6)	307 (64.9)	426 (71.1)	**0.005**
Smoking in pregnancy only	2 (5.0)	24 (3.6)	31 (5.5)	9 (9.8)		3 (5.7)	30 (3.9)	22 (5.3)	11 (8.6)		7 (5.7)	8 (4.4)	29 (6.1)	22 (3.7)	
Smoke at 5 only	0 (0.0)	56 (8.3)	47 (8.3)	3 (3.3)		5 (9.4)	72 (9.3)	25 (6.0)	4 (3.1)		8 (6.6)	12 (6.6)	34 (7.2)	52 (8.7)	
Both	13 (32.5)	142 (21.0)	110 (19.4)	12 (13.0)		14 (26.4)	141 (18.2)	98 (23.4)	24 (18.8)		40 (32.8)	35 (19.3)	103 (21.8)	99 (16.5)	

Respiratory infections															
Never	26 (51.0)	345 (46.3)	263 (43.0)	42 (40.4)	0.080	32 (52.5)	380 (44.4)	206 (45.1)	58 (41.7)	0.532	62 (47.4)	84 (42.4)	240 (45.4)	290 (44.3)	0.928
URTI only	9 (17.7)	233 (31.2)	221 (36.2)	40 (38.5)		14 (23.0)	283 (33.1)	152 (33.3)	54 (38.9)		39 (29.8)	69 (34.9)	170 (32.1)	225 (34.4)	
LRTI or both	16 (31.4)	168 (22.5)	127 (20.8)	22 (21.2)		15 (24.6)	192 (22.5)	99 (21.7)	27 (19.4)		30 (22.9)	45 (22.7)	119 (22.5)	139 (21.3)	
Preterm birth	3 (6.4)	56 (7.9)	36 (6.1)	6 (6.0)	0.649	6 (10.0)	57 (7.0)	30 (6.9)	8 (5.9)	0.776	6 (4.7)	18 (9.6)	46 (9.1)	31 (5.0)	**0.015**
Parental Asthma															
No	25 (64.1)	446 (69.9)	376 (71.4)	69 (79.3)	0.485	29 (58.0)	523 (71.6)	272 (70.1)	92 (74.8)	0.677	72 (65.5)	135 (76.3)	288 (65.2)	421 (74.9)	0.064
Mother only	10 (25.6)	104 (16.3)	90 (17.1)	11 (12.6)		13 (26.0)	115 (15.8)	67 (17.3)	20 (16.3)		21 (19.1)	25 (14.1)	88 (19.9)	81 (14.4)	
Father only	4 (10.3)	66 (10.3)	49 (9.3)	5 (5.8)		6 (12.0)	72 (9.9)	37 (9.5)	9 (7.3)		14 (12.7)	13 (7.3)	50 (11.3)	47 (8.4)	
Both	0 (0.00)	22 (3.5)	12 (2.3)	2 (2.3)		2 (7.3)	20 (2.7)	12 (3.1)	2 (1.6)		3 (2.7)	4 (2.3)	16 (3.6)	13 (2.3)	
Parental Eczema															
No	32 (80.0)	453 (71.3)	379 (71.6)	69 (77.5)	0.399	36 (69.2)	541 (73.9)	265 (68.3)	91 (75.2)	0.399	69 (63.9)	136 (76.4)	317 (72.1)	411 (72.5)	0.265
Mother only	3 (7.5)	113 (17.8)	96 (18.2)	11 (12.4)		9 (17.3)	115 (15.7)	79 (20.4)	20 (16.5)		23 (21.3)	24 (13.5)	74 (16.8)	102 (18.0)	
Father only	4 (10.0)	54 (8.5)	33 (6.2)	6 (6.7)		6 (11.5)	57 (7.8)	27 (7.0)	7 (5.8)		14 (13.0)	11 (6.2)	36 (8.2)	36 (6.4)	
Both	1 (2.5)	15 (2.4)	21 (4.0)	3 (3.4)		1 (1.9)	19 (2.6)	17 (4.4)	3 (2.5)		2 (1.9)	7 (3.9)	13 (3.0)	18 (3.2)	
Parental Hay Fever															
No	26 (51.0)	325 (43.6)	229 (37.5)	41 (39.4)	0.444	24 (39.3)	384 (44.9)	167 (36.5)	46 (33.1)	0.052	56 (42.8)	75 (37.9)	217 (41.0)	273 (41.7)	0.771
Mother only	14 (27.5)	214 (28.7)	185 (30.3)	28 (26.9)		19 (31.2)	242 (28.3)	138 (30.2)	42 (30.2)		32 (24.4)	60 (30.3)	150 (28.4)	199 (30.4)	
Father only	6 (11.8)	111 (14.9)	104 (17.0)	18 (17.3)		11 (18.0)	117 (13.7)	86 (18.8)	25 (18.0)		26 (19.9)	34 (17.2)	88 (16.6)	91 (13.9)	
Both	5 (9.8)	96 (12.9)	93 (15.2)	17 (16.35)		7 (11.5)	112 (13.1)	66 (14.4)	26 (18.7)		17 (13.0)	29 (14.7)	74 (14.0)	91 (13.9)	
Parental Wheeze	27 (57.4)	306 (43.6)	252 (43.5)	41 (42.3)	0.298	28 (48.3)	345 (42.8)	190 (44.6)	63 (46.7)	0.708	74 (59.2)	78 (40.4)	226 (46.2)	248 (40.1)	**0.001**

*Note. *All values are given as frequencies with percentages in parentheses. **Significance of differences was evaluated by chi-square test and are given in bold. ***SES= socio economic status, LRTI= low respiratory tract infections in the first year of life, URTI= upper respiratory tract infections in the first year of life ****The average trajectory of FEV_1, FEV_1/FVC and FVC were used as reference category.*

Associations between childhood, parental, environmental predictors, and lung function trajectories are shown in Table 5.2. The final model was selected based on the predictors with a p-value <0.1 in the unadjusted analysis (Supplementary Table 5.1)

Participants in the very-low trajectory of FEV_1 and FVC were more likely to have late-onset asthma (RR=6.52, 95% CI: 1.17- 36.23, p=0.032 and RR=6.75, 95% CI: 1.57- 28.93, p=0.010, respectively) when compared with the reference group. Participants in the above average trajectory of FVC were less likely to be exposed to maternal smoke compared with those in the average trajectory (RR=0.42, 95% CI: 0.18- 0.96, p=0.040). Having food allergies during childhood was associated with the very low trajectory of FEV_1/FVC (RR= 3.11, 95% CI: 1.16- 8.34, p= 0.025). Participants in the very low trajectory of FEV_1/FVC were more likely to be exposed to maternal smoke in pregnancy (RR=3.80, 95% CI: 1.01- 14.27, p= 0.048) or to be exposed to both pre- and post-natal maternal smoking (RR=3.52, 95% CI: 1.64- 7.57, p=0.001). The high-low trajectory was associated with maternal smoke when children was aged five (RR=2.16, 95% CI: 1.06-4.40, p=0.035). Participants in the very low trajectory of FEV_1/FVC had 3.18 times the risk to have paternal eczema, compared with those in the average trajectory (95% CI: 1.09-9.28, p= 0.034). An association between early-life exposure to air pollutants and the trajectories of FEV_1/FVC was found for $PM_{2.5Abs}$ and NO_2 (RR=0.25, 95% CI: 0.07-0.82, p= 0.022 and RR=1.27, 95% CI:1.03-1.57, p= 0.022, respectively). Although childhood and parental wheeze were associated with lung function trajectories in the unadjusted analysis, we did not include them in the full model as these variables were included in the definition of asthma ("Have you wheezed in the last 12 months?"). A further analysis was carried out adjusting for childhood and parental wheeze instead of childhood and parental asthma (Supplementary Table 5.2), in which food allergies, maternal smoke, father eczema and air pollution exposure were no longer significant on the FEV_1/FVC lung function. In the sensitivity analysis (Supplementary Table 5.3.) we observed that late-onset and persistent asthma combined were not associated with low FEV_1 or FVC lung function trajectories. Further, participants whose mothers smoked in pre- or post-natal life had a lower risk to be in the high-low trajectory, while no association was observed with low lung function trajectories.

Table 5.2. *Association between childhood, parental factors, air pollution exposure and FEV_1, FVC and FEV_1/FVC lung function trajectories in Raine participants with two or more spirometry measurements (adjusted for gender, socioeconomic status, preterm, childhood asthma, childhood respiratory infections, childhood atopy, maternal smoking, parental asthma, parental hay fever, parental eczema, early life exposure to NO_2 and $PM_{2.5Absorbance}$).*

	FEV_1 (n=1512)				**FVC (n=1512)**		**FEV_1/FVC (n=1512)**		
Variable	**Very low**	**Low**	**Above average**	**Very low**	**Low**	**Above average**	**Very Low**	**Low-High**	**High-Low**
Females	0.42 (0.12- 1.51)	0.89 (0.63- 1.26)	1.30 (0.71- 2.41)	0.81 (0.31- 2.15)	**0.63 (0.44- 0.92)**	0.68 (0.39- 1.21)	1.05 (0.56- 1.98)	0.76 (0.46- 1.26)	0.75 (0.51-1.11)
SES									
16000-40000	3 participants	1.06 (0.53- 2.14)	0.87 (0.26- 2.94)	32 participants	0.76 (0.35- 1.67)	0.55 (0.18- 1.72)	1.84 (0.37- 9.19)	0.58 (0.21- 1.60)	0.73 (0.34- 1.56)
≥40000	15 participants	0.97 (0.48- 1.95)	0.68 (0.21- 2.27)	24 participants	0.69 (0.32- 1.51)	0.54 (0.18- 1.65)	2.44 (0.49- 12.23)	1.05 (0.39- 2.83)	0.64 (0.30- 1.36)
Preterm	3 participants	1.43 (0.73- 2.80)	0.34 (0.04- 2.73)	0.73 (0.08- 6.88)	1.35 (0.63- 2.88)	0.67 (0.17- 2.61)	0.69 (0.13- 3.59)	2.06 (0.80- 5.30)	**2.36 (1.09- 5.09)**
Respiratory Infections									
URTI only	0.32 (0.05- 2.09)	0.92 (0.63- 1.34)	1.45 (0.75-2.80)	0.47 (0.15- 1.49)	0.84 (0.56- 1.26)	0.96 (0.52- 1.78)	0.89 (0.45- 1.78)	0.96 (0.55- 1.66)	0.94 (0.62-1.43)
LRTI or both	2.72 (0.66-11.19)	1.45 (0.90- 2.32)	1.45 (0.60- 3.54)	0.96 (0.28- 3.32)	1.36 (0.80- 2.29)	1.40 (0.63- 3.09)	0.85 (0.34- 2.13)	1.68 (0.89- 3.17)	1.25 (0.75-2.10)
Asthma									
Early-onset	2.47 (0.24- 25.60)	1.15 (0.63- 2.10)	1.09 (0.34- 3.49)	1.40 (0.26- 7.42)	1.06 (0.54- 2.07)	1.53 (0.58- 3.99)	2.43 (0.89- 6.67)	1.48 (0.64- 3.40)	1.02 (0.51- 2.07)
Persistent	2 participants	1.49 (0.37- 6.07)	1.73 (0.17- 18.04)	No participants	1.69 (0.32- 9.06)	3.19 (0.39- 26.18)	6.17 (0.87- 43.72)	1.41 (0.13- 15.10)	2.19 (0.44- 10.85)
Late-onset	**6.52 (1.17- 36.23)**	0.90 (0.45-1.83)	0.31 (0.04- 2.44)	**6.75 (1.57- 28.93)**	1.67 (0.68- 4.09)	2.93 (0.90- 9.53)	2.24 (0.56- 8.93)	2.59 (1.00- 6.72)	2.19 (0.97- 4.92)
Atopy (IgE) at age 6									
Food Allergy (only)	2.94 (0.62-13.87)	0.96 (0.48-1.91)	1.20 (0.37- 3.92)	1.42 (0.31- 6.42)	0.77 (0.38- 1.57)	0.80 (0.24- 2.65)	**3.11 (1.16- 8.34)**	0.31 (0.07- 1.38)	1.06 (0.51- 2.21)
Aeroallergens (only)	1 participant	1.82 (0.93- 3.57)	1.41 (0.37- 5.41)	0.56 (0.06- 5.13)	1.31 (0.62- 2.78)	1.33 (0.42- 4.22)	0.44 (0.09- 2.09)	0.63 (0.24- 1.66)	0.67 (0.32- 1.40)
Both	15 participants	0.87 (0.59- 1.29)	1.49 (0.77- 2.91)	0.54 (0.16- 1.82)	0.77 (0.50- 1.17)	1.51 (0.82- 2.80)	1.12 (0.54- 2.31)	0.83 (0.47- 1.45)	0.87 (0.56- 1.35)

Maternal Smoke									
Smoke in pregnancy only	4.28 (0.55- 33.31)	0.40 (0.14- 1.10)	1.23 (0.37- 4.12)	2.71 (0.45-16.39)	0.50 (0.18- 1.36)	1.66 (0.53-5.25)	**3.80 (1.01- 14.27)**	1.11 (0.28- 4.40)	1.49 (0.54- 4.02)
Smoke at 6 only	No participants	1.01 (0.53- 1.91)	0.41 (0.09- 1.88)	5 participants	0.81 (0.41- 1.60)	0.40 (0.11-1.50)	2.93 (0.93- 9.29)	1.70 (0.62- 4.68)	**2.16 (1.06- 4.40)**
Both	1.34 (0.27- 6.61)	1.05 (0.67- 1.63)	0.42 (0.15- 1.15)	0.95 (0.29- 3.05)	0.66 (0.41- 1.07)	**0.42 (0.18- 0.96)**	**3.52 (1.64- 7.57)**	1.79 (0.95- 3.39)	1.45 (0.87- 2.42)
Parental Asthma									
Mother Asthma only	2.21 (0.49- 10.05)	0.98 (0.59- 1.62)	0.59 (0.19- 1.85)	2.80 (0.88- 8.91)	1.13 (0.65- 1.96)	0.52 (0.19- 1.40)	0.67 (0.24- 1.86)	0.82 (0.38- 1.76)	1.31 (0.76- 2.25)
Father Asthma only	2.64 (0.36-19.3)	0.84 (0.45- 1.60)	0.58 (0.15- 2.21)	1.67 (0.29- 9.70)	1.01 (0.50- 2.03)	0.65 (0.21- 2.05)	1.04 (0.32- 3.35)	0.72 (0.26- 1.97)	1.49 (0.74- 2.98)
Both	No participants	1.41 (0.44- 4.58)	1.04 (0.11- 10.37)	3.26 (0.27- 38.91)	1.06 (0.30- 3.82)	0.41 (0.04- 4.25)	No participants	0.50 (0.05- 4.61)	1.66 (0.51- 5.42)
Parental Hayfever									
Mother Hayfever	0.24 (0.04- 1.50)	0.84 (0.56-1.27)	1.06 (0.50- 2.24)	1.09 (0.33- 3.61)	0.76 (0.48- 1.19)	1.04 (0.52- 2.10)	0.58 (0.25- 1.33)	0.68 (0.36- 1.27)	1.12 (0.71- 1.76)
Father Hayfever	0.43 (0.06- 3.03)	0.81 (0.49- 1.33)	1.21 (0.51- 2.89)	1.18 (0.29- 4.85)	0.70 (0.41- 1.20)	1.11 (0.50- 2.47)	1.48 (0.66- 3.33)	1.02 (0.51- 2.04)	1.10 (0.63- 1.94)
Both	0.49 (0.08- 3.03)	0.80 (0.47-1.36)	0.98 (0.38- 2.52)	1.42 (0.35- 5.80)	1.00 (0.55- 1.80)	1.44 (0.61- 3.42)	0.68 (0.23-2.05)	1.54 (0.75- 3.16)	1.38 (0.76- 2.48)
Parental Eczema									

Mother Eczema only	1.28 (0.27- 5.99)	1.03 (0.66- 1.62)	0.66 (0.27- 1.62)	1.46 (0.49- 4.35)	0.62 (0.38- 1.01)	0.83 (0.40- 1.72)	1.83 (0.84- 3.96)	0.59 (0.28- 1.22)	0.91 (0.55- 1.49)
Father Eczema only	**9.73 (1.19- 79.53)**	1.69 (0.85- 3.38)	0.63 (0.13- 2.97)	4.40 (0.92- 21.0)	1.09 (0.52- 2.31)	0.90 (0.27- 3.04)	**3.18 (1.09- 9.28)**	0.98 (0.33- 2.88)	1.77 (0.83- 3.76)
Both	1 participant	0.79 (0.31- 2.04)	3 participants	1 participant	0.63 (0.23- 1.71)	0.32 (0.04- 2.80)	0.95 (0.11- 8.51)	0.96 (0.23- 4.08)	1.33 (0.46- 3.86)
$PM_{2.5Absorbance}$ (early life)	1.28 (0.13- 12.45)	0.70 (0.39-1.25)	0.85 (0.31- 2.38)	2.57 (0.52- 12.70)	1.05 (0.56- 1.96)	1.18 (0.46- 3.06)	**0.25 (0.07- 0.82)**	0.47 (0.20- 1.11)	0.68 (0.36- 1.28)
NO_2 (early life)	0.87 (0.58- 1.30)	0.98 (0.88- 1.09)	1.01 (0.83- 1.23)	0.80 (0.59-1.09)	0.98 (0.87- 1.11)	0.96 (0.80- 1.16)	**1.27 (1.03- 1.57)**	1.04 (0.89- 1.21)	1.00 (0.89- 1.13)

*Note. *Relative Risk Ratios (95% confidence intervals) and significance differences from the average trajectory were derived from a multinomial logistic regression. **Bold values indicate a significant association at α= 0.05. ***SES=socio economic status, LRTI= low respiratory tract infections in the first year of life, URTI= upper respiratory tract infections in the first year of life. ****The average trajectory of FEV_1, FEV_1/FVC and FVC were used as reference category*

5.4 Discussion

Important early-life predictors of low lung function trajectories were asthma, atopy, maternal smoke, paternal eczema, and early life exposure to air pollutants. In addition, childhood wheeze was associated with the very low trajectory of FEV_1 and females were more likely to belong to the low trajectory of FVC compared with males. Regarding other potential risk factors, parental asthma and parental hay fever were not significantly associated with lung function trajectories in this study cohort.

The present study showed that asthma was an important predictor for belonging to low trajectories of FEV_1 and FVC. In fact, having late-onset asthma was associated with a more than 6- fold increased risk of having a very low lung function trajectory compared to an average trajectory, suggesting that participants in the low trajectory have an increased risk of asthma in later life, or it might be that risk factors associated to low lung function trajectories across childhood are also associated with late-onset asthma. We also found that the prevalence of early-onset, persistent and late-onset asthma was higher in the very low trajectory of FEV_1/FVC compared with the average trajectory. Childhood asthma was a significant predictor of low lung function trajectories of FEV_1 and FEV_1/FVC in the unadjusted multivariate analysis but was no longer significant in the full adjusted model.

A limited number of previous studies have investigated childhood asthma in relation to lung function trajectories. In the Tucson Children's Respiratory Study cohort, Berry et al (2016) found that the prevalence of children with asthma was more than 3-times higher in the low trajectory of FEV_1/FVC compared with the normal trajectory (26.4 vs 7.7%, $p<0.001$). Childhood asthma was found to be associated with the low lung function trajectories identified in the Tasmanian Longitudinal Health Study (TAHS) cohort (Bui, 2019), while other studies reported the same association in males, but not in females (Karmaus et al., 2019). Although we defined late-onset, it should not be confused with adulthood asthma, as in our study late-onset might occur after five years of age. To the best of our knowledge this is the first study to investigate asthma phenotypes in all the three lung function outcomes such as FEV_1, FVC and FEV_1/FVC.

Participants in the above average trajectory of FVC were less likely to be exposed to maternal smoke compared with those in the average trajectory. An association was also seen between the very low trajectory of FEV_1/FVC and maternal smoking both in pregnancy or when the child was aged five years – but not if they only have smoked when the child was five years of age. The effect of maternal smoking during pregnancy and post-natal life has been widely investigated but its role on lung function trajectories is not well elucidated as very few studies have been conducted. Our findings are in line with previous research in which maternal smoking was associated with lower FEV_1 in adulthood (Bui et al., 2019; Svanes et al., 2010). However, in the Isle of Wight birth cohort in the UK (Karmaus et al., 2019) being exposed to smoke during pregnancy was not considered as a risk factor of being in the low lung function trajectories. There are several potential explanatory mechanisms for the influence of maternal smoking on lung function over life, mostly derived by animal studies (Xiao et al., 2011; Spindel et al., 2016). *In utero* exposed mice showed that nicotine receptors mediate most of the effect of maternal smoking on lung development (Xiao et al., 2011). This was confirmed by analysis on airway size and diameter, showing an increased number of airways of small diameter with nicotine treatment. Another mechanism that appears to mediate the effect of nicotine exposure on lung development and the effect of maternal smoking during pregnancy was the oxidative mechanisms. Multiple studies demonstrated that prenatal nicotine exposure increases markers of oxidative damage. In fact, the lungs are smaller in offspring, with larger alveoli, and a reduction in alveolarization (Maritz & Harding, 2011). Our results suggest that maternal smoking affects the lung capacity of their children, making the volume that is associated with the FVC smaller compared with children whose mother did not smoke during pregnancy or in the first years of children's life. Even if we have not found an association with FEV_1, parental smoke seems to be associated with an increased risk of airway obstruction, as defined by a low FEV_1/FVC trajectory.

Our findings suggest that early life exposure to NO_2 was associated with the very low lung function trajectories of FEV_1/FVC, A study conducted in Australia (Salimi et al., 2018) showed that low levels of NO_2 were associated with more hospitalizations for asthma, suggesting that even concentration levels lower than those recommended by the WHO might have an effect on respiratory health. Unexpected, our results showed that early life exposure to $PM_{2.5Absorbance}$ was negatively associated with the very low trajectory of FEV_1/FVC (RR=0.25, 95% CI: 0.07- 0.82).

Despite plausible biological mechanisms, the role of ambient air pollution in the development of respiratory diseases through life is still uncertain. To the best of our knowledge, this is the first study on lung function trajectories of FEV_1, FVC and FEV_1/FVC that also considered the impact of early-life exposure to air pollutants. Our results need to be interpreted carefully. Firstly, because we back-extrapolated air pollution data for nineteen years of follow-up and we might overestimate the results, as significant alterations in the urban landscape will have occurred over this period. Some, but not all, areas in Perth may have undergone significant re-development or expansion that might have led to prediction error from our models. Although other studies (Beelen et al., 2007; Gulliver, 2013) have suggested that LUR are stable even over a period of 35 years, the performance of the model would be weaker and we were not able to verify the accuracy of our estimation, due to the fact that the LUR we used in our analysis was the only one developed for the Perth area. Secondly, the availability of monitoring station during the early 90's was limited. Further, we were not able to measure air pollution in the gestational and the first years of life in which lung morphology changes dramatically. More studies should be conducted both in low and high-level concentrations settings in order to further our understanding of the long-term effects of air pollution exposure at specific periods, especially before and after birth.

In our study, the history of paternal eczema was associated with the very low trajectory of FEV_1/FVC. The Copenhagen Prospective Study on Asthma in Childhood, neonatal lung function was not affected by parental- mother or father- atopic diseases, such as eczema or allergic rhinitis (Bisgaard et al., 2009). However, there is no previous evidence reporting the relationship between paternal eczema and lung function trajectories. We observed an association between childhood food allergies and the very-low trajectory of FEV_1/FVC. These findings are in line with a study conducted within the Chicago Food Allergy cohort, in which having food allergies was a risk factor for greater small airway airflow obstruction among children. Other studies (Friedlander er al., 2013) reported that individuals with asthma with multiple food allergies had significantly decreased FEV_1 and FEV_1/FVC compared to individuals with asthma but without any food allergies. However, this study included only participants with asthma. Food allergy is a reaction of the immune system triggered by the ingestion of a food protein antigen. The exposure to allergenic food can lead to clinical symptoms, including airway inflammation. It is yet unclear if

having food allergies in childhood is an independent risk factor for decreasing lung function despite the asthma status.

Males were more likely to belong to the low lung function trajectory of FVC, while preterm children were more likely to belong to the high-low trajectory of FEV_1/FVC. Socioeconomic status, respiratory infections, and parental hay fever were not found to be associated with the lung function trajectories of FEV_1, FVC or FEV_1/FVC. Previous research showed a lack of association between children's respiratory health and socioeconomic status (Gehring et al., 2006), while others supported the hypothesis of the association between social economic status and respiratory outcomes in children, such as the diagnosis of asthma (Gong et al., 2014) with different effects depending on race and ethnicity (Thakur et al., 2013).

When we conducted the sensitivity analysis, late-onset and persistent asthma combined was no longer associated with low lung function trajectories, along with maternal smoking. Discrepancies with our main results may be partly due because the model was not adjusted for all the confounders, such as preterm and socio-economic status as they consist of few participants within trajectories. Another explanation is that the combination in categories might have reduced accuracy and we might have lost part of the information, such as the association of low lung function trajectories as a function of asthma phenotypes. Therefore, we have chosen to present the results from the first approach.

The main limitation of this study is that although we investigated the early-life exposure to $PM_{2.5Absorbance}$ and NO_2 for each participant, we were not able to include exposure during other important susceptible periods before five years of age, such as pregnancy and the very first years of life as the earliest available measure was when children were aged between 3.5 and 4.5 years. A strength of the study was that the longitudinal design from a large cohort allowed us to investigate the early life factors associated with lung function trajectories defined for an Australian cohort. Demographics and clinical information of children and their parents were available from pregnancy and for the years of follow up, including repeated spirometry measurements from childhood to young adulthood.

To conclude, taken together, these results provide further insight into the emerging knowledge of lung function trajectories for different respiratory markers. We identified childhood atopy, asthma, maternal smoke, paternal eczema, and early life exposure to air pollution as risk factors for low lung function trajectories in this cohort study.

5.5. Supplementary material

Supplementary Table 5.1*. Association between childhood, parental factors, air pollution exposure and FEV1, FVC and FEV1/FVC lung function trajectories in Raine participants with two or more spirometry measurements (unadjusted)*

	FEV_1 (n=1512)				**FVC (n=1512)**		**FEV_1/FVC (n=1512)**		
Variable	**Very low**	**Low**	**Above average**	**Very low**	**Low**	**Above average**	**Very Low**	**Low-High**	**High-Low**
Females	0.61 (0.34- 1.10)	1.03 (0.84- 1.28)	0.88 (0.58- 1.33)	0.67 (0.39- 1.15)	1.05 (0.84-1.32)	0.88 (0.60- 1.29)	0.83 (0.57- 1.22)	1.17 (0.85- 1.61)	***0.80 (0.63-1.00)***
SES									
16000-40000	2.24 (0.66- 7.61)	0.98 (0.65- 1.43)	1.33 (0.59-2.98)	2.13 (0.62- 7.35)	0.84 (0.54-1.29)	0.67 (0.35- 1.28)	1.05 (0.52-2.13)	**0.60 (0.33-1.07)**	0.77 (0.51-1.18)
≥40000	1.09 (0.30- 3.88)	1.06 (0.72-1.55)	1.11 (0.50-2.51)	1.43 (0.41- 5.01)	0.82 (0.53- 1.25)	0.62 (0.32- 1.19)	0.91 (0.45- 1.85)	0.95 (0.54- 1.67)	0.74 (0.48 1.12)
Preterm	1.04 (0.31- 3.51)	1.30 (0.84- 2.00)	0.97 (0.40- 2.37)	1.51 (0.60- 3.79)	1.03 (0.65- 1.62)	0.85 (0.38-1.90)	0.94 (0.38- 2.30)	***2.03 (1.11- 3.71)***	1.92 (1.20- 3.08)
Respiratory Infections									
URTI only	***0.41 (0.19- 0.90)***	0.80 (0.63- 1.03)	1.13 (0.71- 1.81)	0.59 (0.31- 1.15)	1.01 (0.78- 1.31)	1.26 (0.82- 1.93)	0.81 (0.52- 1.25)	1.06 (0.74- 1.52)	0.91 (0.70- 1.19)
LRTI or both	1.27 (0.66- 2.46)	1.01 (0.76- 1.34)	1.08 (0.62- 1.89)	0.98 (0.50- 1.88)	1.05 (0.78- 1.41)	0.97 (0.58- 1.62)	1.01 (0.62- 1.63)	1.12 (0.74- 1.69)	1.03 (0.77- 1.39)
Asthma									
Early-onset	1.00 (0.30- 3.41)	**1.45 (0.99-2.13)**	0.85 (0.37-1.94)	0.97 (0.37- 2.59)	0.94 (0.63- 1.39)	0.78 (0.38-1.60)	***2.34 (1.28-4.26)***	1.28 (0.71-2.29)	**1.41 (0.93- 2.14)**
Persistent	**3.85 (0.79-18.9)**	1.32 (0.54- 3.26)	0.70 (0.87- 5.67)	No participants	1.01 (0.40- 2.55)	1.43 (0.36- 5.62)	***6.58 (2.08-20.94)***	1.80 (0.45- 7.30)	1.80 (0.62- 5.24)
Late-onset	**2.34 (0.98- 5.61)**	0.94 (0.62- 1.43)	0.61 (0.24-1.56)	1.61 (0.59- 4.41)	1.34 (0.83- 2.15)	1.54 (0.75- 3.15)	1.77 (0.87-3.59)	1.41 (0.77- 2.58)	1.29 (0.81- 2.03)
Wheeze at age 6	**1.81 (0.94-3.48)**	***1.40 (1.07-1.84)***	1.01 (0.58- 1.76)	1.46 (0.79- 2.72)	1.15 (0.86- 1.53)	1.17 (0.73- 1.88)	***2.18 (1.43-3.32)***	1.25 (0.84- 1.86)	***1.39 (1.04-1.87)***

Atopy (IgE) at age 6									
Food Allergy (only)	1.52 (0.56- 4.14)	1.05 (0.67- 1.64)	0.71 (0.24- 2.08)	0.84 (0.28-2.52)	0.79 (0.50- 1.24)	0.53 (0.20- 1.42)	**1.83 (0.96- 3.49)**	**0.51 (0.22- 1.18)**	0.97 (0.60- 1.57)
Aeroallergens (only)	0.32 (0.04- 2.42)	**1.44 (0.94- 2.22)**	0.94 (0.35- 2.51)	0.46 (0.10- 2.01)	1.07 (0.68- 1.69)	0.82 (0.37- 1.95)	0.58 (0.24- 1.40)	0.87 (0.47-1.60)	0.76 (0.48- 1.21)
Both	0.91 (0.48- 1.72)	0.88 (0.69- 1.12)	***1.56 (1.01- 2.43)***	1.02 (0.57- 1.83)	0.85 (0.66- 1.10)	***1.50 (0.99- 2.24)***	0.95 (0.62- 1.46)	1.46 (0.59- 1.21)	0.98 (0.76- 1.27)
Maternal Smoke									
Smoke in pregnancy only	0.98 (0.22- 4.32)	0.65 (0.37- 1.12)	1.62 (0.74- 3.55)	1.20 (0.34- 4.24)	0.70 (0.40- 1.23)	1.53 (0.72-3.29)	2.02 (0.83- 4.92)	1.23 (0.53-2.83)	**1.83 (1.03- 3.24)**
Smoke at 5 only	No participants	0.99 (0.66-1.50)	**0.36 (0.10- 1.18)**	1.76 (0.63- 4.93)	1.48 (0.91- 2.38)	0.49 (0.17- 1.45)	0.98 (0.44- 2.15)	0.78 (0.40-1.51)	0.91 (0.57- 1.43)
Both	1.79 (0.89-3.62)	1.08 (0.81- 1.43)	0.61 (0.31- 1.16)	1.26 (0.64- 2.46)	***0.74 (0.55- 0.99)***	0.75 (0.45- 1.25)	***2.57 (1.64- 4.02)***	1.20 (0.77- 1.84)	***1.44 (1.06- 1.97)***
Parental Asthma									
Mother Asthma only	1.67 (0.77- 3.60)	0.97 (0.71- 1.33)	0.67 (0.34- 1.31)	**1.82 (0.90- 3.69)**	0.89 (0.64- 1.25)	0.88 (0.51- 1.53)	1.52 (0.88-2.60)	0.96 (0.59- 1.57)	***1.59 (1.13- 2.22)***
Father Asthma only	1.23 (0.41-3.68)	1.14 (0.77- 1.68)	0.56 (0.21-1.45)	1.52 (0.59- 3.91)	1.01 (0.66- 1.54)	0.72 (0.33- 1.55)	**1.74 (0.91-3.33)**	0.86 (0.45- 1.64)	***1.56 (1.02- 2.38)***
Both	No participants	1.55 (0.75- 3.16)	0.91 (0.20- 4.15)	1.56 (0.33- 7.34)	0.87 (0.42- 1.80)	0.49 (0.11- 2.24)	1.35 (0.38- 4.85)	0.96 (0.31-2.99)	1.80 (0.85-3.80)
Parental Hayfever									
Mother Hayfever	0.67 (0.34- 1.31)	0.82 (0.63-1.06)	0.85 (0.50- 1.42)	0.96 (0.50- 1.82)	**0.76 (0.58- 1.01)**	1.10 (0.69- 1.78)	0.78 (0.49- 1.26)	1.10 (0.75-1.61)	0.95 (0.72- 1.25)

Father Hayfever	0.51 (0.20- 1.27)	**0.75 (0.55-1.03)**	0.97 (0.53- 1.76)	0.89 (0.42- 1.90)	***0.59 (0.42- 0.82)***	1.06 (0.61- 1.83)	1.39 (0.83- 2.35)	1.36 (0.85- 2.18)	1.22 (0.86-1.71)
Both	**0.47 (0.18-1.27)**	0.73 (0.52-1.01)	1.02 (0.55- 1.89)	0.74 (0.30- 1.80)	**0.74 (0.52- 1.05)**	1.43 (0.82- 2.50)	0.91 (0.50-1.65)	1.16 (0.71- 1.89)	1.02 (0.72- 1.46)
Parental Eczema									
Mother Eczema only	0.37 (0.11- 1.23)	0.98 (0.73-1.34)	0.63 (0.32- 1.24)	0.84 (0.39- 1.82)	***0.71 (0.52- 0.98)***	0.74 (0.43- 1.27)	1.34 (0.80- 2.26)	0.71 (0.44-1.16)	0.94 (0.67- 1.31)
Father Eczema only	1.44 (0.47- 4.31)	1.37 (0.87-2.15)	1.00 (0.40-2.47)	1.64 (0.63- 4.23)	1.03 (0.64- 1.67)	0.75 (0.32- 1.79)	***2.32 (1.19- 4.52)***	0.92 (0.46- 1.86)	1.30 (0.80- 2.11)
Both	0.56 (0.07- 4.33)	0.59 (0.30- 1.17)	0.78 (0.23- 2.70)	0.43 (0.06- 3.35)	**0.55 (0.27- 1.07)**	0.51 (0.15- 1.79)	0.66 (0.15- 2.92)	1.18 (0.48- 2.87)	0.94 (0.45- 1.94)
Parental Wheezing	**1.76 (0.96- 3.21)**	1.01 (0.81-1.26)	0.95 (0.62- 1.47)	1.16 (0.67- 2.01)	0.93 (0.73- 1.18)	1.09 (0.74-1.60)	***2.17 (1.47- 3.21)***	1.01 (0.72- 1.41)	***1.29 (1.01- 1.63)***
$PM_{2.5Absorbance}$ (early life)	***0.29 (0.08-0.91)***	0.80 (0.57-1.14)	1.18 (0.64-2.20)	0.79 (0.31-2.00)	0.99 (0.68- 1.45)	1.06 (0.59- 1.92)	0.63 (0.34- 1.17)	**0.59 (0.35- 1.01)**	***0.57 (0.39- 0.85)***
NO_2 (early life)	***0.81 (0.69-0.96)***	0.96 (0.91-1.02)	1.07 (0.97-1.19)	0.92 (0.80- 1.06)	1.03 (0.97-1.09)	1.05 (0.95-1.16)	0.96 (0.87- 1.06)	***0.92 (0.84-0.99)***	***0.93 (0.87-0.98)***

*Note. *Relative Risk Ratio (95% confidence intervals) and significance differences from the average trajectory were derived from a multinomial logistic regression. **Bold values indicate a significant association at α= 0.1, while bold and Italic at <0.05 ***The model was unadjusted for covariates. ****SES=socio economic status, LRTI= low respiratory tract infections, URTI= upper respiratory tract infections *****The average trajectory of FEV_1, FEV_1/FVC and FVC were used as reference category.*

***Supplementary Table 5.2**. Association between childhood, parental factors, air pollution exposure and FEV1, FVC and FEV1/FVC lung function trajectories in Raine participants with two or more spirometry measurements (adjusted for gender, socioeconomic status, preterm, childhood respiratory infections, childhood wheeze, childhood atopy, maternal smoking, parental hay fever, parental eczema, parental wheezing, early life exposure to NO_2 and $PM_{2.5Absorbance}$).*

	FEV_1 (n=1512)			**FVC (n=1512)**			**FEV_1/FVC (n=1512)**		
Variable	**Very low**	**Low**	**Above average**	**Very low**	**Low**	**Above average**	**Very Low**	**Low-High**	**High-Low**
Females	**0.28 (0.09-0.86)**	0.88 (0.64- 1.23)	1.29 (0.71- 2.35)	0.60 (0.26- 2.40)	**0.68 (0.48- 0.97)**	0.78 (0.45- 1.35)	0.89 (0.50- 1.58)	0.82 (0.51- 1.31)	0.76 (0.53- 1.09)
SES									
16000-40000	29 participants	1.07 (0.57- 2.00)	1.57 (0.43- 5.78)	2.65 (0.31-22.83)	0.38 (0.38- 1.54)	0.85 (0.27- 2.67)	2.70 (0.60- 12.85)	0.56 (0.22- 1.38)	0.74 (0.37- 1.47)
≥40000	15 participants	1.05 (0.56- 1.97)	1.18 (0.32- 4.34)	1.54 (0.17-13.78)	0.80 (0.39- 1.61)	0.89 (0.29- 2.77)	3.18 (0.67- 15.16)	1.00 (0.41- 2.40)	0.67 (0.34- 1.33)
Preterm	3 participants	1.21 (0.64- 2.28)	0.58 (0.13- 2.65)	1.12 (0.22-5.59)	1.09 (0.55- 2.18)	0.58 (0.16- 2.18)	0.54 (0.11- 2.57)	2.07 (0.84- 5.14)	**2.49 (1.22- 5.10)**
Respiratory Infections									
URTI only	0.89 (0.25- 3.12)	0.90 (0.63- 1.29)	1.23 (0.65- 2.35)	0.72(0.27- 1.88)	0.81 (0.55- 1.19)	0.79 (0.43- 1.45)	0.86 (0.45- 1.62)	0.91 (0.54- 1.54)	0.99 (0.66- 1.48)
LRTI or both	2.85 (0.82-9.92)	1.42 (0.92- 2.19)	1.38 (0.60-3.17)	0.90 (0.30- 2.70)	1.08 (0.67- 1.74)	0.85 (0.39-1.82)	1.00 (0.47- 2.13)	1.38 (0.76- 2.51)	1.18 (0.73- 1.91)
Childhood wheeze	2.67 (0.79- 8.94)	1.18 (0.77-1.79)	0.75 (0.31-1.80)	1.63 (0.58-4.55)	1.13 (0.72-1.80)	1.11 (0.53- 2.29)	**2.32 (1.20-4.48)**	1.06 (0.57-1.96)	1.14 (0.67- 1.48)

Atopy (IgE) at age 6									
Food Allergy (only)	1.69 (0.42-6.81)	0.94 (0.49-1.82)	1.04 (0.32- 3.35)	1.44 (0.40- 5.19)	0.68 (0.34- 1.34)	0.62 (0.19- 2.04)	2.26 (0.90- 5.68)	0.26 (0.06- 1.14)	1.02 (0.51-2.06)
Aeroallergens (only)	5 participants	1.88 (0.97- 3.65)	1.39 (0.37- 5.25)	0.46 (0.05- 3.94)	1.39 (0.66- 2.92)	1.45 (0.46- 4.57)	0.59 (0.16- 2.18)	0.60 (0.23- 1.60)	0.60 (0.29-1.24)
Both	15 participants	0.93 (0.64- 1.34)	1.47 (0.77- 2.81)	0.47 (0.16- 1.38)	0.83 (0.56- 1.25)	1.54 (0.85- 2.82)	1.02 (0.53- 1.97)	0.78 (0.46- 1.33)	0.79 (0.52-1.19)
Maternal Smoke									
Smoke in pregnancy only	2.16 (0.34- 13.67)	0.49 (0.19- 1.28)	1.92 (0.63- 5.91)	2.84 (0.64-12.70)	0.50 (0.18- 1.35)	2.17 (0.72-6.52)	3.04 (0.83- 11.04)	1.09 (0.28- 4.28)	1.62 (0.64-4.10)
Smoke at 5 only	No participants	1.20 (0.66- 2.20)	0.70 (1.2- 2.49)	5 participants	0.87 (0.46- 1.64)	0.40 (0.11-1.43)	2.29 (0.81- 6.51)	2.40 (1.02- 5.63)	1.85 (0.94-3.67)
Both	1.19 (0.34- 4.11)	1.17 (0.76- 1.79)	0.44 (0.16- 1.21)	1.22 (0.44-3.38)	0.72 (0.45- 1.13)	**0.43 (0.18- 0.99)**	**2.56 (1.28-5.09)**	1.75 (0.94- 3.27)	1.55 (0.95-2.52)
Parental Wheeze	2.09 (0.71-6.16)	0.89 (0.63-1.26)	0.91 (0.48-1.72)	0.97 (0.40-2.39)	1.10 (0.75-1.60)	1.06 (0.59- 1.91)	1.66 (0.92-3.00)	0.93 (0.56- 1.54)	0.89 (0.61-1.32)
Parental Hayfever									
Mother Hayfever	0.38 (0.10- 1.45)	0.86 (0.63-1.26)	0.90 (0.43- 1.86)	1.25 (0.45- 3.49)	0.73 (0.48- 1.11)	0.92 (0.46- 1.82)	0.65 (0.32- 1.35)	0.67 (0.37- 1.23)	1.22 (0.80-1.88)

Father Hayfever	0.48 (0.11- 2.12)	0.80 (0.49- 1.29)	1.07 (0.46- 2.50)	1.19 (0.35- 4.03)	0.68 (0.41- 1.14)	1.13 (0.52- 2.47)	1.39 (0.65- 2.95)	1.14 (0.58- 2.23)	1.27 (0.74- 2.19)
Both	0.40 (0.07- 2.35)	0.88 (0.53-1.47)	0.82 (0.33- 2.05)	1.31 (0.34- 5.03)	1.00 (0.57- 1.78)	1.30 (0 56- 3.03)	0.66 (0.25-1.76)	1.68 (0.84- 3.35)	1.66 (0.94- 2.93)
Parental Eczema									
Mother Eczema only	0.84 (0.270- 3.48)	1.01 (0.66- 1.54)	0.61 (0.25- 1.46)	1.47 (0.56- 3.88)	0.58 (0.37- 0.91)	0.71 (0.35- 1.44)	1.22 (0.60- 1.23)	0.55 (0.28- 1.11)	0.93 (0.58- 1.49)
Father Eczema only	5.12 (0.87- 30.08)	1.66 (0.82- 2.97)	0.84 (0.23- 3.05)	2.81 (0.67- 11.80)	1.04 (0.52- 2.10)	0.95 (0.31- 2.87)	**2.73 (1.03- 7.25)**	0.98 (0.36- 2.66)	1.95 (0.97- 3.93)
Both	Only one participant	0.62 (0.25- 1.54)	0.42 (0.05-3.50)	1 participant	0.39 (0.16-11.0)	0.20 (0.02- 1.67)	1.05 (0.21- 5.36)	0.62 (0.15- 2.48)	0.93 (0.34- 2.56)
$PM_{2.5}$Absorbance (early life)	1.59 (0.24- 10.44)	0.74 (0.42-1.29)	0.85 (0.31- 2.34)	1.69 (0.42- 6.84)	1.03 (0.56- 1.91)	1.05 (0.41- 2.70)	0.43 (0.15- 1.23)	0.52 (0.23- 1.18)	0.64 (0.34- 1.19)
NO_2 (early life)	0.82 (0.59- 1.14)	0.97 (0.88- 1.08)	1.02 (0.85- 1.24)	0.87 (0.67-1.14)	0.99 (0.88- 1.10)	0.99 (0.83- 1.18)	1.08 (0.90- 1.29)	1.01 (0.87- 1.17)	1.00 (0.89- 1.18)

*Note. *Relative Risk Ratio (95% confidence intervals) and significance differences from the average trajectory were derived from a multinomial logistic regression. **Bold values indicate a significant association at α= 0.1, while bold and Italic at <0.05 ***SES=socio economic status, LRTI= low respiratory tract infections, URTI= upper respiratory tract infections ****The average trajectory of FEV_1, FEV_1/FVC and FVC were used as reference category.*

Supplementary Table 5.3. *Association between childhood, parental factors, air pollution exposure and FEV1, FVC and FEV1/FVC lung function trajectories in Raine participants with two or more spirometry measurements (adjusted for gender, childhood respiratory infections, childhood asthma, maternal smoking, parental asthma, parental hay fever, parental eczema, early life exposure to NO_2 and $PM_{2.5Absorbance}$).*

	FEV_1 (n=1512)				**FVC (n=1512)**		**FEV_1/FVC (n=1512)**		
Variable	**Very low**	**Low**	**Above average**	**Very low**	**Low**	**Above average**	**Very Low**	**Low-High**	**High-Low**
Females	**0.63 (0.20- 1.96)**	0.93 (0.67- 1.29)	1.25 (0.69- 2.28)	1.60 (0.93- 2.72)	1.22 (0.66- 2.26)	1.13 (0.68- 1.90)	1.39 (0.74- 2.62)	1.21 (0.73- 1.99)	1.28 (0.89-1.86)
Respiratory Infections									
URTI only	0.33 (0.06- 1.70)	0.86 (0.59- 1.24)	1.24 (0.50-3.05)	1.41 (0.76- 2.63)	1.56 (0.77- 3.13)	1.49 (0.42- 2.71)	1.07 (0.43- 1.76)	1.07 (0.61- 1.89)	1.06 (0.70-1.60)
LRTI or both	2.31 (0.64-18.28)	1.39 (0.88- 2.20)	1.39 (0.73- 2.66)	0.91 (0.45- 1.83)	0.86 (0.80- 2.29)	1.07 (0.55- 2.09)	0.73 (0.30- 1.76)	1.27 (0.66- 2.44)	0.91 (0.55-1.50)
Asthma									
Early-onset	0. 82 (0.09- 7.21)	1.01 (0.56- 1.84)	0.73 (0.21- 2.61)	0.73 (0.36- 1.49)	1.00 (0.32- 3.12)	1.04 (0.41- 2.63)	1.79 (0.67- 4.91)	1.20 (0.50- 2.91)	0.83 (0.42- 1.65)
Persistent OR late-onset	3.41 (0.80- 15.36)	0.99 (0.52- 1.88)	0.53 (0.11- 2.4)	0.82 (0.32- 2.12)	0.91 (0.31- 2.61)	0.56 (0.24- 1.45)	1.04 (0.35- 3.10)	1.13 (0.48- 2.62)	**0.45 (0.21- 0.92)**
Maternal Smoke									
Smoke in pregnancy OR at a 6	1.31 (0.25- 6.91)	0.69 (0.40- 1.17)	0.74 (0.29- 1.90)	1.07 (0.45-2.49)	1.16 (0.44- 3.01)	0.71 (0.31-1.64)	1.55 (0.64- 3.74)	0.75 (0.34- 1.67)	**0.50 (0.28- 0.90)**
Both	1.05 (0.25- 4.31)	1.04 (0.68- 1.61)	0.46 (0.17- 1.24)	0.81 (0.43- 1.54)	0.69 (0.32- 1.51)	0.58 (0.31- 1.10)	1.77 (0.84- 3.71)	1.06 (0.57- 1.99)	**0.62 (0.37- 0.99)**
Parental Asthma									

Mother Asthma only	2.69 (0.68- 10.60)	0.96 (0.58- 1.60)	0.57 (0.19- 1.78)	0.73 (0.36- 1.49)	**0.26 (0.10- 0.71)**	0.53 (0.26- 1.06)	0.66 (0.24- 1.81)	0.90 (0.43- 1.88)	0.77 (0.44- 1.35)
Father Asthma only	1.86 (0.31-11.15)	0.80 (0.43- 1.50)	0.58 (0.16- 2.07)	0.82 (0.31- 2.12)	0.41 (0.12- 1.37)	0.84 (0.34- 2.09)	0.49 (0.15- 1.60)	0.41 (0.14- 1.16)	0.59 (0.30- 1.13)
Parental Hayfever									
Mother Hayfever	0.27 (0.05- 1.35)	0.89 (0.59- 1.34)	1.08 (0.51- 2.26)	1.26 (0.64- 2.46)	1.25 (0.57- 2.73)	1.23 (0.65- 2.33)	0.53 (0.22- 1.22)	0.78 (0.42- 1.46)	0.97 (0.62- 1.53)
Father Hayfever	0.39 (0.07- 2.08)	0.76 (0.47- 1.22)	1.16 (0.50- 2.69)	1.16 (0.56- 2.38)	1.29 (0.57- 2.94)	0.59 (0.28- 1.20)	1.20 (0.54- 2.69)	1.03 (0.50- 2.10)	0.94 (0.55- 1.61)
Both	0.49 (0.09- 2.67)	0.78 (0.46-1.33)	0.94 (0.36- 2.44)	1.25 (0.52- 2.97)	1.44 (0.54- 3.90)	1.11 (0.48- 2.56)	0.62 (0.21-1.84)	0.96 (0.45- 2.08)	0.81 (0.45- 1.45)
Parental Eczema									
Mother Eczema only	1.19 (0.28- 4.96)	1.05 (0.67- 1.64)	0.76 (0.31- 1.84)	0.68 (0.35- 1.30)	1.01 (0.48- 2.13)	0.49 (0.26- 0.94)	1.66 (0.76- 3.65)	0.55 (0.26- 1.17)	1.05 (0.64- 1.71)
Father Eczema only	3.31 (0.56- 19.99)	1.54 (0.79- 3.02)	0.58 (0.13- 2.65)	0.59 (0.22- 1.64)	0.64 (0.19- 2.09)	0.62 (0.23- 1.63)	1.93 (0.70- 5.36)	0.64 (0.22- 1.86)	0.69 (0.33- 1.45)
$PM_{2.5Absorbance}$ (early life)	1.01 (0.14- 7.62)	0.74 (0.42-1.31)	0.87 (0.31- 2.42)	0.64 (0.26- 1.55)	0.78 (0.28- 2.16)	0.66 (0.28- 1.55)	0.43 (0.13- 1.42)	0.78 (0.32- 1.92)	1.50 (0.80- 2.81)
NO_2 (early life)	0.85 (0.58- 1.23)	0.95 (0.85- 1.05)	0.98 (0.81- 1.19)	1.18 (0.99-1.41)	1.10 (0.91- 1.35)	1.11 (0.94- 1.31)	**1.19 (1.01- 1.46)**	1.00 (0.85- 1.17)	1.00 (0.88- 1.12)

*Note. *Relative Risk Ratio (95% confidence intervals) and significance differences from the average trajectory were derived from a multinomia1 logistic regression. **Bold values indicate a significant association at α= 0.1, while bold and Italic at <0.05 ***LRTI= low respiratory tract infections, URTI= upper respiratory tract infections ****The average trajectory of FEV_1, FEV_1/FVC and FVC were used as reference category.*

Chapter 6.
General discussion

6.1 Discussion of findings

There is a growing body of evidence that a proportion of chronic lung disease in adults has its roots in childhood. This is in line with the Development Origins of Health and Disease (DoHaD) theory first proposed by Barker and colleagues in 1989 (Barker et al., 1989). According to this concept, our health is influenced by the early-life environment in which we live, and by the events occurring *in utero* and in early childhood.

Normal lung function development is characterized by a growth phase in which lung function grows, reaching a peak during early adulthood, a plateau phase, and a steady decline due to aging (GOLD, 2017). Normal ageing of the lung is associated with structural remodeling due to cell senescence resulting in reduced respiratory function. However, disadvantageous conditions during the intrauterine period and the first years of life might influence the lifetime lung function trajectory of an individual. The factors that may be implicated in poor lung health include respiratory infections, environmental tobacco smoke, adverse dietary intake, premature birth, air pollution and asthma (Stocks & Sonnappa, 2013). The contribution of this research is to identify people who follow an abnormal lung function growth trajectory as early as possible and investigate specific early-life factors associated with abnormal lung function.

In this thesis, I aimed to establish lung function trajectories from childhood to young adulthood within the Raine Study cohort (Western Australia) and to explore childhood and parental risk factors as well as environmental pollution predictors associated with the low lung function trajectories identified. In Chapter 3, I identified lung function

trajectories using spirometry measurements that have been undertaken at 6, 14, and 22 years of age. By using a finite mixture modelling, I have identified lung function trajectories for FEV_1, FVC, and FEV_1/FVC, respectively. In Chapter 5, I aimed to explore early-life predictors associated with the low lung function trajectories identified. Within the scope of this study, I have carried out the back-extrapolation of LUR models and estimated the individual exposure to environmental air pollutants, such as NO_2, $PM_{2.5}$, and $PM_{2.5Absorbance}$ for the study participants, as reported in Chapter 4.

To the best of our knowledge, this study described for the first time lung function trajectories from childhood to young adulthood for three respiratory outcomes, FEV_1, FVC and FEV_1/FVC in z-scores, and explored early life predictors associated with the trajectories identified, including individual early-life exposure to ambient air pollution. Four distinct trajectories were found for FEV_1, FVC and FEV_1/FVC from 6 to 22 years of age in the Raine study participants. Trajectories of FEV_1 and FVC were similar in shape and were labelled as "very low", "low", "average", and "above average". The shape of the trajectories of FEV_1/FVC differed from FEV_1 and FVC and were characterized as "low", "low-high", "high-low", and "average" trajectories. Asthma, maternal smoke and parental asthma were identified as risk factors for low lung function trajectories in this cohort study, as well as early exposure to NO_2 – but not $PM_{2.5Absorbance}$.

Up to now, only a few studies have investigated lung function trajectories over time, with FEV_1 being the most common respiratory outcome reported (Bui et al., 2018; McGeachie et al., 2016). Only one study explored FEV_1, FVC, and FEV_1/FVC trajectories and compared them using these different markers. Unlike our study, the respiratory outcomes were not measured in z-scores, hypothesized to be the most clinical meaningful approach (Vaz Fragoso et al., 2016) and their trajectories did not include the childhood period as the trajectories were modelled between 10 and 26 years of age (Karmaus et al., 2019). Similar to our study, FEV_1/FVC differed from other

trajectories in that they showed a stable decline from puberty to young adulthood. Indeed, both FEV_1 and FVC trajectories seem to gradually increase from 6 to 22 years of age. In the Tasmanian Longitudinal Health Study (TAHS), Bui and colleagues identified six trajectories in individuals followed from 5 to 53 years of age, of which two (the early below average, accelerate decline and early low, accelerate growth, normal decline) were previously unknown. This difference from our study most likely due to the age range in TAHS being wider and participants being older than our study population. Thus, we could not identify these two trajectories in our cohort.

Results from our analysis showed that our population was clustered into discrete trajectories from childhood to adulthood suggesting that events in the first years of life might have a role in influencing the lung function trajectories and that they may be partly established before six years of age.

Data from this thesis revealed associations between childhood, parental and environmental factors and the trajectories identified. Specifically, asthma, maternal smoking during gestational period or childhood, and parental asthma were found to be risk factors for low lung function trajectories in this cohort study. Other respiratory symptoms, such as childhood wheeze was prevalent in the very low trajectory of FEV_1 compared with the average category. Further, all the three asthma phenotypes, such as early-onset asthma, persistent and late-onset asthma were prevalent in the very low trajectory of FEV_1/FVC, compared with the average trajectory. Early-life exposure to low concentration of NO_2 was found to be associated with low lung function trajectories of FEV_1/FVC in our cohort- but not with FEV_1 or FVC. A wide range of studies demonstrated the linked between the adverse effects of air pollution on lung function cross-sectionally, while this study adds to relatively small body of literature investigating the impact of early post-natal environmental air pollution into young adulthood. Perth is an area with relatively low concentrations of pollutants such as $PM_{2.5}$, $PM_{2.5Abs}$ and NO_2, that were also below the WHO guidelines and some limitations in the use of LUR such as its development in a subsequent period were addressed. Further, we were not able to capture other potential crucial periods, such as

the gestational and the first years of life in which lung morphology undergoes dramatic changes. Indeed, one of the limitations encountered is that we could not back-extrapolate air pollution data to pregnancy and in the first three years of life as monitoring measurements for $PM_{2.5}$ and NO_2 were consistently available only from 1995 for $PM_{2.5}$ and from 1993 for NO_2. We defined early life exposure when children were aged between three and four years of age. More studies should be conducted both in low and high-level concentrations in order to further our understanding of the long-term effects of air pollution exposure at specific periods, especially before and after birth.

All these findings together, provide further insight into the emerging knowledge of lung function trajectories for different respiratory markers. The association between childhood/parental/environmental predictors and low lung function trajectories contributed to strengthen the hypothesis that early-life factors might influence adult respiratory health. Knowing the early-life risk factors associated with the lung function trajectories might serve as a purpose to prevent and tackle deficits in later life. As it has been previously demonstrated that low lung function trajectories of FEV_1 contributed 75% to participants diagnosed with COPD in adulthood (Bui et al., 2019), special attention would be given to participants belonging to the very low trajectory of FEV_1 who might be potentially at higher risk. The practical relevance of this study is the opportunity to identify those children who have a higher risk to be in the low trajectories, and consequently prevent future respiratory deficits. As the Raine Study is still on-going, it will be worth to investigate the association between lung function trajectories and diagnosis of COPD when the participants will reach an older age.

6.2 Strengths, limitations, and future directions

One of the major strengths of this study was the availability of a longitudinal dataset from a large cohort that allowed us to investigate the early risk factors associated with the lung function trajectories defined over time. The Raine Study, established in 1989 in Perth (Western Australia), is one of the largest prospective cohorts of pregnancy,

childhood, and adolescence to be carried out anywhere in the world. Its long observation period of 22 years, compared with shorter periods in other studies, allowed us to determine the lung function trajectories from pre-school children into young adulthood. Indeed, only one study had a wider study period with lung function measured between 7 and 53 years (Bui et al., 2019). Due to the extensive period of follow up, we were also able to acquire predictor variables that were related to childhood and parental respiratory health, as well as demographics and individual addresses at each follow-up.

Further, while prior studies investigated only one respiratory health outcome, this study provided trajectories of FEV_1, FVC, and FEV_1/FVC using an unsupervised statistical method through group-based trajectory modelling. The advantage in using this novel approach, compared with other more traditional such as hierarchical modelling and latent curve analysis, is that this enabled us to identify meaningful subgroups within the population that follow distinctive trajectories that are not detectable *a priori* based on some measured characteristics (Nagin, 2014).

This study has also some limitations. Although spirometry maneuvers are considered to be reliable and acceptable in pre-school children, one of the problems encountered when performing the test is that patients might be unable to exhale completely. Indeed, in our population sample, the forced vital capacity measured at six years seem to be underestimated, resulting in an overestimation of lung function trajectories of FEV_1/FVC ratio. Another limitation we faced during this study was that we could not back-extrapolate air pollution data earlier than 1993, year in which most of our children population were aged three. Lung development starts *in utero* and the fetal and the first few years of post-natal life are considered crucial as the lung growth and development are rapid. Our aim was to investigate early life risk factors on lung function, and it was of our interest to explore the role of environmental pollution *in utero* and in the very early stage of life. However, due to the scarcity of monitoring air pollution data in the past, we were unable to back-extrapolate air pollution data before 1993 for $PM_{2.5Absorbance}$ and NO_2. Further, despite various studies having demonstrated the

stability of LUR models for periods longer than 35 years, we were not able to assess the stability of our model as the 2012 LUR model used for this study was the only one developed and validated for the metropolitan area of Perth.

6.3 Conclusions

In conclusion, this study suggests that a distinctive group of individuals demonstrate a low lung function trajectory that may be partly established before six years of age or even, before birth.

The use of an advanced novel statistical approach enabled the identification of lung function trajectories in the Raine Study cohort. We were able to identify four trajectories for FEV_1, FVC and FEV_1/FVC in an unsupervised manner. Lung function trajectories for FEV_1 and FVC were similar in shapes, being characterized by a very low, low, average, and above average trajectories, where the low trajectory was the most populated trajectory. FEV_1/FVC was characterized by a very-low, low-high, average, and high-low trajectories, based on their shape, where nearly half of participants were in the average trajectory. The FEV_1/FVC trajectories showed an early decline for all trajectories during adolescence, that might be explained by reaching a relative higher FVC than FEV_1 between 14 and 22 years of age.

While prior studies investigated one lung function outcome at time, this study provided for the first time trajectories of FEV_1, FVC, and FEV_1/FVC, expressed in z-scores in a large population sample followed from childhood to young adulthood.

We found that early-life predictors of low lung function trajectories were asthma, maternal smoke, parental asthma, and early-life exposure to NO_2. In addition, childhood wheeze was associated with the very low trajectory of FEV_1 and females were more likely to belong to the low trajectory of FVC compared with males. Other potential risk factors that we investigated, such as parental hay fever, parental eczema, and parental wheezing were not significantly associated with lung function trajectories

in this study cohort. These findings together support the hypothesis of the Development Origins of Health and Disease (Barker, 1989), in which our health is influenced by events happened in vulnerable periods, such as early childhood. Despite the risk factors that we choose to explore were potentially associated with low lung function trajectories, these are not exhaustive as there are many other potential risk factors that we did not include, such as diet, physical activity, or chemical exposure. To the best of our knowledge, this is the first study on lung function trajectories that also include individual early-life exposure to ambient air pollution.

This thesis adds to the current knowledge showing trajectories along with several predictors assessed from 6 to 22 years of age. Investigating early life risk factors on lung function trajectories from childhood to young adulthood is critically important as a first step in preventing long term lung impairments and to identify children that might be at higher risk to develop deficits of lung function later in life. Indeed, as it has been shown that lung function track into adulthood, a particular attention should be drawn to those children belonging to the low trajectories of lung function.

It is fundamentally important to develop strategies to promote optimum lung development. Indeed, prevention at an early stage of life is crucial to optimize healthy lung function after birth, in childhood into adulthood. For example, public and individual-level interventions should be put in place to avoid NO_2 exposure in early life, as well as to avoid exposure to smoke, especially in children with asthma.

Another practical implication of this study is that the generation of lung function trajectories of FEV_1, FVC, and FEV_1/FVC defined in this thesis for the Raine Study participants can also be used as an outcome for further investigations within the same cohort exploring other novel risk factors for low lung function.

Chapter 7.

References

1. Accordini, S., Calciano, L., Johannessen, A., Portas, L., Benediktsdóttir, B., Bertelsen, R. J., … Svanes, C. (2018). A three-generation study on the association of tobacco smoking with asthma. International Journal of Epidemiology, 47(4), 1106–1117. https://doi.org/10.1093/ije/dyy031
2. Aging-Related Considerations When Evaluating the Forced Expiratory Volume in 1 Second (FEV_1) Over Time. *Journals of Gerontology Series A: Biomedical Sciences and Medical Sciences*, *71*(7), 929-934. https://doi.org/10.1093/gerona/glv201
3. Agusti, A., & Faner, R. (2019). Lung function trajectories in health and disease. The Lancet Respiratory Medicine, 7(4), 358–364. https://doi.org/10.1016/S2213-2600(18)30529-0
4. Allinson, J. P., Hardy, R., Donaldson, G. C., Shaheen, S. O., Kuh, D., & Wedzicha, J. A. (2017). Combined impact of smoking and early-life exposures on adult lung function trajectories. American Journal of Respiratory and Critical Care Medicine, 196(8), 1021–1030. https://doi.org/10.1164/rccm.201703-0506OC
5. Arasi, S., Porcaro, F., Cutrera, R., & Fiocchi, A. G. (2019). Severe asthma and allergy: A pediatric perspective. Frontiers in Pediatrics, 7(FEB), 1–10. https://doi.org/10.3389/fped.2019.00028
6. Asher, M. I., & Weiland, S. K. (1998). The International Study of Asthma and Allergies in Childhood (ISAAC). ISAAC Steering Committee. *Clinical and experimental allergy: Journal of the British Society for Allergy and Clinical Immunology*, *28*, 52-66. https://doi.org/10.1046/j.1365-2222.1998.028s5052.x.
7. Australian Government, 2019

8. Barker, D. J., Winter, P. D., Osmond, C., Margetts, B., & Simmonds, S. J. (1989). Weight in infancy and death from ischaemic heart disease. *Lancet (London, England)*, *2*(8663), 577–580. https://doi.org/10.1016/s0140-6736(89)90710-1

9. Beelen, R., Hoek, G., Fischer, P., van den Brandt, P. A., & Brunekreef, B. (2007). Estimated long-term outdoor air pollution concentrations in a cohort study. *Atmospheric Environment*, *41*(7), 1343-1358. https://doi.org/10.1016/j.atmosenv.2006.10.020

10. Bekkers, M. B. M., Wijga, A. H., de Jongste, J. C., Kerkhof, M., Postma, D., Gehring, U., … Brunekreef, B. (2013). Waist circumference, BMI, and lung function in 8-year-old children: The PIAMA birth cohort study. Pediatric Pulmonology, 48(7), 674–682. https://doi.org/10.1002/ppul.22722

11. Belgrave, D. C. M., Buchan, I., Bishop, C., Lowe, L., Simpson, A., & Custovic, A. (2014). Trajectories of Lung Function during Childhood. https://doi.org/10.1164/rccm.201309-1700OC

12. Bentsen, M. H., Markestad, T., Øymar, K., & Halvorsen, T. (2017). Lung function at term in extremely preterm-born infants: A regional prospective cohort study. BMJ Open, 7(10), 1–9. https://doi.org/10.1136/bmjopen-2017-016868

13. Berry, C. E., Billheimer, D., Jenkins, I. C., Lu, Z. J., Stern, D. A., Gerald, L. B., … Martinez, F. D. (2016a). A Distinct Low Lung Function Trajectory from Childhood to the Fourth Decade of Life. https://doi.org/10.1164/rccm.201604-0753OC

14. Bisgaard, H., Loland, L., Holst, K. K., & Pipper, C. B. (2009). Prenatal determinants of neonatal lung function in high-risk newborns. *Journal of allergy and clinical immunology*, *123*(3), 651-657. https://doi.org/10.1016/j.jaci.2008.11.036.

15. Bose, S., Rosa, M. J., Mathilda Chiu, Y. H., Leon Hsu, H. H., Di, Q., Lee, A., … Wright, R. J. (2018). Prenatal nitrate air pollution exposure and reduced child lung function: Timing and fetal sex effects. Environmental Research, 167(August), 591–597. https://doi.org/10.1016/j.envres.2018.08.019

16. Brazzale, D., Hall, G., & Swanney, M. P. (2016). Reference values for spirometry and their use in test interpretation: A Position Statement from the Australian and

New Zealand Society of Respiratory Science. Respirology, 21(7), 1201–1209. https://doi.org/10.1111/resp.12855

17. Bui, D. S., Lodge, C. J., Burgess, J. A., Lowe, A. J., Perret, J., Bui, M. Q., ... & Hamilton, G. S. (2018). Childhood predictors of lung function trajectories and future COPD risk: a prospective cohort study from the first to the sixth decade of life. *The lancet Respiratory medicine*, *6*(7), 535-544. https://doi.org/10.1016/S2213-2600(18)30100-0
18. Burke, H., Leonardi-Bee, J., Hashim, A., Pine-Abata, H., Chen, Y., Cook, D. G., … McKeever, T. M. (2012). Prenatal and passive smoke exposure and incidence of asthma and wheeze: Systematic review and meta-analysis. Pediatrics, 129(4), 735–744. https://doi.org/10.1542/peds.2011-2196
19. Carlsen, K. C. L., Mowinckel, P., Hovland, V., Håland, G., Riiser, A., & Carlsen, K. H. (2014). Lung function trajectories from birth through puberty reflect asthma phenotypes with allergic comorbidity. *Journal of allergy and clinical immunology*, *134*(4), 917-923. https://doi.org/10.1016/j.jaci.2014.05.020.
20. Chen, M., & Zhang, L. (2012). Epigenetic mechanisms in developmental programming of adult disease. 16, 1007–1018. https://doi.org/10.1016/j.drudis.2011.09.008.Epigenetic
21. Collins, R. A., Parsons, F., Deverell, M., Hollams, E. M., Holt, P. G., & Sly, P. D. (2011). Risk factors for bronchial hyperresponsiveness in teenagers differ with sex and atopic status. *Journal of Allergy and Clinical Immunology*, 128(2), 301-307. https://doi.org/10.1016/j.jaci.2011.03.016
22. Cooper BG, Stocks J, Hall GL, et al. (n.d.). The Global Lung Function Initiative (GLI) Network: bringing the world' s respiratory reference values together. 13(3), 56–64. https://doi.org/10.1183/20734735.012717
23. Crapo, R. O., Hankinson, J. L., Irvin, C., MacIntyre, N. R., Voter, K. Z., Wise, R. A., ... & Veigi, G. (1995). Standardization of spirometry: 1994 update. *American Journal of Respiratory and Critical Care Medicine*, *152*(3), 1107-1136. https://doi.org/10.1164/ajrccm.152.3.7663792

24. Dirgawati, M., Barnes, R., Wheeler, A. J., Arnold, A. L., McCaul, K. A., Stuart, A. L., ... & Heyworth, J. S. (2015). Development of land use regression models for predicting exposure to NO2 and NOx in metropolitan Perth, Western Australia. *Environmental Modelling & Software*, *74*, 258-267. https://doi.org/10.1016/j.envsoft.2015.07.008
25. Doyle, L. W., Adams, A. M., Robertson, C., Ranganathan, S., Davis, N. M., Lee, K. J., … Woods, H. (2017). Increasing airway obstruction from 8 to 18 years in extremely preterm/low-birthweight survivors born in the surfactant era. Thorax, 72(8), 712–719. https://doi.org/10.1136/thoraxjnl-2016-208524
26. Eeftens, M., Beelen, R., de Hoogh, K., Bellander, T., Cesaroni, G., Cirach, M., ... & Dimakopoulou, K. (2012). Development of land use regression models for PM2. 5, PM2. 5 absorbance, PM10 and PMcoarse in 20 European study areas; results of the ESCAPE project. *Environmental science & technology*, *46*(20), 11195-11205. https://doi.org/10.1021/es301948k
27. Eeftens, M., Beelen, R., Fischer, P., Brunekreef, B., Meliefste, K., & Hoek, G. (2011). Stability of measured and modelled spatial contrasts in NO2 over time. *Occupational and environmental medicine*, *68*(10), 765-770. https://doi.org/10.1136/oem.2010.061135
28. Elliott, L., Henderson, J., Northstone, K., Chiu, G. Y., Dunson, D., & London, S. J. (2011). Prospective study of breastfeeding in relation to wheeze, atopy, and bronchial hyperresponsiveness in the Avon Longitudinal Study of Parents and Children (ALSPAC). Bone, 23(1), 1–7. https://doi.org/10.1016/j.jaci.2008.04.001
29. Fan H, Zhang X. Body mass index trajectory across childhood and subsequent risk of elevated blood pressure. *Journal of Clinical Hypertension (Greenwich).* 2020 Oct;22(10):1902-1907. https://doi.org/10.1111/jch.14001
30. Filippone, M., Bonetto, G., Cherubin, E., Carraro, S., & Baraldi, E. (2009). Childhood course of lung function in survivors of bronchopulmonary dysplasia. JAMA - Journal of the American Medical Association, 302(13), 1418–1420. https://doi.org/10.1001/jama.2009.1419

31. Fletcher, C., & Peto, R. (1977). The natural history of chronic airflow obstruction. *Br Med J*, *1*(6077), 1645-1648. https://doi.org/10.1136/bmj.1.6077.1645
32. Forno, E., Han, Y., Mullen, J., & Dame, N. (2019). Overweight, obesity, and lung function in children and adults: a meta-analysis. 6(2), 570–581. https://doi.org/10.1016/j.jaip.2017.07.010
33. Friedlander, J. L., Sheehan, W. J., Baxi, S. N., Kopel, L. S., Gaffin, J. M., Ozonoff, A., Fu, C., Gold, D. R., & Phipatanakul, W. (2013). Food allergy and increased asthma morbidity in a School-based Inner-City Asthma Study. *The Journal of Allergy and Clinical Immunology. In practice*, *1*(5), 479–484. https://doi.org/10.1016/j.jaip.2013.06.007
34. Fuertes, E., Bracher, J., Flexeder, C., Markevych, I., Klümper, C., Hoffmann, B., … Schulz, H. (2015). Long-term air pollution exposure and lung function in 15 year-old adolescents living in an urban and rural area in Germany: The GINIplus and LISAplus cohorts. International Journal of Hygiene and Environmental Health, 218(7), 656–665. https://doi.org/10.1016/j.ijheh.2015.07.003
35. Gehring, U., Gruzieva, O., Agius, R. M., Beelen, R., Custovic, A., Cyrys, J., ... & Hoffmann, B. (2013). Air pollution exposure and lung function in children: the ESCAPE project. *Environmental health perspectives*, *121*(11-12), 1357-1364.
36. Gehring, U., Pattenden, S., Slachtova, H., Antova, T., Braun-Fahrländer, C., Fabianova, E., ... & Luttmann-Gibson, H. (2006). Parental education and children's respiratory and allergic symptoms in the Pollution and the Young (PATY) study. European Respiratory Journal, 27(1), 95-107. https://doi.org/10.1183/09031936.06.00017205
37. Gilliland, F. D., Berhane, K., Li, Y. F., Rappaport, E. B., & Peters, J. M. (2003). Effects of early onset asthma and *in utero* exposure to maternal smoking on childhood lung function. American Journal of Respiratory and Critical Care Medicine, 167(6), 917–924. https://doi.org/10.1164/rccm.200206-616OC
38. Global Initiative for Asthma. Global Strategy for Asthma Management and Prevention, 2020.Gorlanova, O., Appenzeller, R., Mahmoud, Y. S., Ramsey, K. A.,

Usemann, J., Decrue, F., … On, B. (2020). Effect of breastfeeding duration on lung function, respiratory symptoms and allergic diseases in school-age children. Pediatric Pulmonology, 55(6), 1448–1455. https://doi.org/10.1002/ppul.24733

39. Global Initiative for Chronic Obstructive Lung Disease. (2017). Global strategy for the diagnosis, management, and prevention of chronic obstructive lung disease. https://doi.org/10.1164/rccm.201701-0218PP
40. Gong, T., Lundholm, C., Rejnö, G., Mood, C., Långström, N., & Almqvist, C. (2014). Parental socioeconomic status, childhood asthma and medication use--a population-based study. *PloS one*, *9*(9), e106579. https://doi.org/10.1371/journal.pone.0106579
41. Götschi, T., Heinrich, J., Sunyer, J., & Künzli, N. (2008). Long-term effects of ambient air pollution on lung function: A review. Epidemiology, 19(5), 690–701. https://doi.org/10.1097/EDE.0b013e318181650f
42. Graham, B. L., Steenbruggen, I., Barjaktarevic, I. Z., Cooper, B. G., Hall, G. L., Hallstrand, T. S., … Thompson, B. R. (2019). Standardization of spirometry 2019 update an official American Thoracic Society and European Respiratory Society technical statement. American Journal of Respiratory and Critical Care Medicine, 200(8), E70–E88. https://doi.org/10.1164/rccm.201908-1590ST
43. Guilbert, T. W., Stern, D. A., Morgan, W. J., Martinez, F. D., & Wright, A. L. (2007). Effect of breastfeeding on lung function in childhood and modulation by maternal asthma and atopy. *American journal of respiratory and critical care medicine*, *176*(9), 843-848. https://doi.org/ 10.1164/rccm.200610-1507OC.
44. Gulliver, J., de Hoogh, K., Hansell, A., & Vienneau, D. (2013). Development and back-extrapolation of NO2 land use regression models for historic exposure assessment in Great Britain. *Environmental science & technology*, *47*(14), 7804-7811. https://doi.org/10.1021/es4008849
45. Hall, G. L., Thompson, B. R., Stanojevic, S., Abramson, M. J., Beasley, R., Coates, A., … Swanney, M. P. (2012). The Global Lung Initiative 2012 reference values reflect contemporary Australasian spirometry. Respirology, 17(7), 1150–1151. https://doi.org/10.1111/j.1440-1843.2012.02232.x

46. Hayatbakhsh, M. R., Sadasivam, S., Mamun, A. A., Najman, J. M., Williams, G. M., & O'Callaghan, M. J. (2009). Maternal smoking during and after pregnancy and lung function in early adulthood: A prospective study. Thorax, 64(9), 810–814. https://doi.org/10.1136/thx.2009.116301
47. Ho, T., Cusack, R. P., Chaudhary, N., Satia, I., & Kurmi, O. P. (2019). Under-and over-diagnosis of COPD: a global perspective. *Breathe*, *15*(1), 24-35. https://doi.org/10.1183/20734735.0346-2018
48. Hoek, G., Beelen, R., De Hoogh, K., Vienneau, D., Gulliver, J., Fischer, P., & Briggs, D. (2008). A review of land-use regression models to assess spatial variation of outdoor air pollution. Atmospheric environment, 42(33), 7561-7578. https://doi.org/10.1016/j.atmosenv.2008.05.057
49. Hollams, E. M., Deverell, M., Serralha, M., Suriyaarachchi, D., Parsons, F., Zhang, G., ... & Rowe, J. (2009). Elucidation of asthma phenotypes in atopic teenagers through parallel immunophenotypic and clinical profiling. *Journal of allergy and clinical immunology*, *124*(3), 463-470. https://doi.org/10.1016/j.jaci.2009.06.019
50. http://www.escapeproject.eu
51. Iliodromiti, Z., Zygouris, D., Sifakis, S., Pappa, K. I., Tsikouras, P., Salakos, N., Daniilidis, A., Siristatidis, C., & Vrachnis, N. (2013). Acute lung injury in preterm fetuses and neonates: mechanisms and molecular pathways. The journal of maternal-fetal & neonatal medicine : the Official Journal of the European Association of Perinatal Medicine, the Federation of Asia and Oceania Perinatal Societies, the International Society of Perinatal Obstetricians, 26(17), 1696–1704. https://doi.org/10.3109/14767058.2013.798284
52. Ito, M., Tamura, M., & Namba, F. (2017). Role of sex in morbidity and mortality of very premature neonates. Pediatrics International, 59(8), 898–905. https://doi.org/10.1111/ped.13320
53. Jackson, D. J., & Lemanske, R. F. (2010). The Role of Respiratory Virus Infections in Childhood Asthma Inception. Immunology and Allergy Clinics of North America, 30(4), 513–522. https://doi.org/10.1016/j.iac.2010.08.004

54. Jedynska, A., Hoek, G., Wang, M., Eeftens, M., Cyrys, J., Keuken, M., ... & Cirach, M. (2014). Development of land use regression models for elemental, organic carbon, PAH, and hopanes/steranes in 10 ESCAPE/TRANSPHORM European study areas. Environmental science & technology, 48(24), 14435-14444. https://doi.org/10.1021/es502568z
55. Jeon J, Jung KJ, Jee SH. Waist circumference trajectories and risk of type 2 diabetes mellitus in Korean population: the Korean genome and epidemiology study (KoGES). *BMC Public Health*. 2019 Jun 13;19(1):741. https://doi.org/10.1186/s12889-019-7077-6
56. Jones, B. L., & Nagin, D. S. (2013). A note on a Stata plugin for estimating group-based trajectory models. *Sociological Methods & Research*, *42*(4), 608-613. https://doi.org/10.1177/0049124113503141
57. Joseph-Bowen, J., de Klerk, N. H., Firth, M. J., Kendall, G. E., Holt, P. G., & Sly, P. D. (2004). Lung function, bronchial responsiveness, and asthma in a community cohort of 6-year-old children. *American Journal of Respiratory and Critical Care Medicine*, 169(7), 850-854. https://doi.org/10.1164/rccm.200304-556OC
58. Karmaus, W., Mukherjee, N., Janjanam, V. D., Chen, S., Zhang, H., Roberts, G., … Arshad, H. (2019). Distinctive lung function trajectories from age 10 to 26 years in men and women and associated early life risk factors - A birth cohort study. Respiratory Research, 20(1), 1–13. https://doi.org/10.1186/s12931-019-1068-0
59. Kim D, Chen Z, Zhou LF, Huang SX. Air pollutants and early origins of respiratory diseases. Chronic Dis Transl Med. 2018 Jun 7;4(2):75-94 https://doi.org/10.1016/j.cdtm.2018.03.003
60. Knibbs, L. D., Cortés de Waterman, A. M., Toelle, B. G., Guo, Y., Denison, L., Jalaludin, B., … Williams, G. M. (2018). The Australian Child Health and Air Pollution Study (ACHAPS): A national population-based cross-sectional study of long-term exposure to outdoor air pollution, asthma, and lung function. Environment International, 120(August), 394–403. https://doi.org/10.1016/j.envint.2018.08.025

61. Künzli, N., Perez, L., & Rapp, R. (2010). *Air quality and health*. European Respiratory Society.
62. Lange, P., Celli, B., Agustí, A., Boje Jensen, G., Divo, M., Faner, R., … Vestbo, J. (2015). Lung-Function Trajectories Leading to Chronic Obstructive Pulmonary Disease. New England Journal of Medicine, 373(2), 111–122. https://doi.org/10.1056/NEJMoa1411532
63. Latzin, P., Röösli, M., Huss, A., Kuehni, C. E., & Frey, U. (n.d.). Air pollution during pregnancy and lung function in newborns: a birth cohort study. https://doi.org/10.1183/09031936.00084008
64. Lemanske, R. F., Jackson, D. J., Gangnon, R. E., Evans, M. D., Li, Z., Shult, P. A., … Gern, J. E. (2005). Rhinovirus illnesses during infancy predict subsequent childhood wheezing. Journal of Allergy and Clinical Immunology, 116(3), 571–577. https://doi.org/10.1016/j.jaci.2005.06.024
65. Lessler, J., Reich, N. G., Brookmeyer, R., Perl, T. M., Nelson, K. E., & Cummings, D. A. (2009). Incubation periods of acute respiratory viral infections: a systematic review. *The Lancet infectious diseases*, *9*(5), 291-300. https://doi.org/10.1016/S1473-3099(09)70069-6
66. Leung, T. F., Chan, I. H., Liu, T. C., Lam, C. W., & Wong, G. W. (2013). Relationship between passive smoking exposure and urinary heavy metals and lung functions in preschool children. *Pediatric pulmonology*, *48*(11), 1089-1097. https://doi.org/10.1002/ppul.22801
67. Levy, I., Levin, N., Schwartz, J. D., & Kark, J. D. (2015). Back-extrapolating a land use regression model for estimating past exposures to traffic-related air pollution. *Environmental Science & Technology*, 49(6), 3603-3610. https://doi.org/10.1021/es505707e
68. Lødrup Carlsen, K. C., Jaakkola, J. J. K., Nafstad, P., & Carlsen, K. H. (1997). *In utero* exposure to cigarette smoking influences lung function at birth. European Respiratory Journal, 10(8), 1774–1779. https://doi.org/10.1183/09031936.97.10081774

69. Lopez Bernal, J. A., Upton, M. N., Henderson, A. J., Dedman, D., McCarthy, A., Davey Smith, G., & Ben-Shlomo, Y. (2013). Lower respiratory tract infection in the first year of life is associated with worse lung function in adult life: Prospective results from the Barry Caerphilly Growth study. Annals of Epidemiology, 23(7), 422–427. https://doi.org/10.1016/j.annepidem.2013.05.006
70. Losacco, C., & Perillo, A. (2018). Particulate matter air pollution and respiratory impact on humans and animals. Environmental Science and Pollution Research, 25(34), 33901–33910. https://doi.org/10.1007/s11356-018-3344-9
71. Lowe, L. A., Woodcock, A., Murray, C. S., Morris, J., Simpson, A., & Custovic, A. (2004). Lung function at age 3 years: effect of pet ownership and exposure to indoor allergens. *Archives of pediatrics & adolescent medicine*, *158*(10), 996-1001. https://doi.org/ 10.1001/archpedi.158.10.996
72. Maritz, G. S., & Harding, R. (2011). Life-long programming implications of exposure to tobacco smoking and nicotine before and soon after birth: Evidence for altered lung development. International Journal of Environmental Research and Public Health, 8(3), 875–898. https://doi.org/10.3390/ijerph8030875
73. McEvoy, C. T., & Spindel, E. R. (2017). Pulmonary effects of maternal smoking on the fetus and child: effects on lung development, respiratory morbidities, and life long lung health. *Paediatric respiratory reviews*, *21*, 27-33. https://doi.org/10.1016/j.prrv.2016.08.005
74. McGeachie, M. J., Yates, K. P., Zhou, X., Guo, F., Sternberg, A. L., van Natta, M. L., … Strunk, R. C. (2016). Patterns of growth and decline in lung function in Persistent Childhood Asthma. New England Journal of Medicine, 374(19), 1842–1852. https://doi.org/10.1056/NEJMoa1513737
75. Meel, E. R. van, Jong, M. de, Elbert, N. J., Dekker, H. T. den, Reiss, I. K., Jongste, J. C. de, … Duijts, L. (2020). Duration and exclusiveness of breastfeeding and school-age lung function and asthma. Annals of Allergy, Asthma & Immunology, 733206(2017), 1–8. https://doi.org/10.1016/j.anai.2017.05.002
76. Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, Crapo R, Enright P, van der Grinten CP, Gustafsson P et al.; ATS/ERS Task Force.

Standardisation of spirometry. Eur. Respir. J. 2005; 26: 319–38. https://doi.org/10.1183/09031936.05.00034805

77. Mölter, A., Agius, R. M., de Vocht, F., Lindley, S., Gerrard, W., Lowe, L., … Simpson, A. (2013). Long-term exposure to PM10 and NO2 in association with lung volume and airway resistance in the MAAS birth cohort. Environmental Health Perspectives, 121(10), 1232–1238. https://doi.org/10.1289/ehp.1205961
78. Moore, V. C. (2012). Spirometry: Step by step. Breathe, 8(3), 233–240. https://doi.org/10.1183/20734735.0021711
79. Morgenstern, V., Zutavern, A., Cyrys, J., Brockow, I., Gehring, U., Koletzko, S., … Heinrich, J. (2007). Respiratory health and individual estimated exposure to traffic-related air pollutants in a cohort of young children. Occupational and Environmental Medicine, 64(1), 8–16. https://doi.org/10.1136/oem.2006.028241
80. Morten, M., Collison, A., Murphy, V. E., Barker, D., Oldmeadow, C., Attia, J., ... & Gibson, P. G. (2018). Managing Asthma in Pregnancy (MAP) trial: FENO levels and childhood asthma. *Journal of Allergy and Clinical Immunology*, *142*(6), 1765-1772. https://doi.org/10.1016/j.jaci.2018.02.039
81. Mortimer, K., Neugebauer, R., Lurmann, F., Alcorn, S., Balmes, J., & Tager, I. (2008). Air pollution and pulmonary function in asthmatic children: effects of prenatal and lifetime exposures. *Epidemiology*, 550-557. https://doi.org/10.1097/EDE.0b013e31816a9dcb. PMID: 18520616.
82. Nagin, D. S. (1999). Analyzing developmental trajectories: a semiparametric, group-based approach. *Psychological Methods*, 4(2), 139. https://doi.org/10.1037/1082-989X.4.2.139
83. Nagin, D. S., Jones, B. L., Passos, V. L., & Tremblay, R. E. (2018). Group-based multi-trajectory modeling. *Statistical Methods in Medical Research*, 27(7), 2015-2023. https://doi.org/ 10.1177/0962280216673085.
84. Nagin, D. S. (2014). Group-based trajectory modeling: an overview. In *Handbook of quantitative criminology* (pp. 53-67). Springer, New York, NY. https://doi.org/10.1159/000360229

85. Nagin, D. S., & Land, K. C. (1993). Age, criminal careers, and population heterogeneity: Specification and estimation of a nonparametric, mixed Poisson model. *Criminology*, *31*(3), 327-362. https://doi.org/10.1016/j.chiabu.2007.04.011
86. Nagin, D. S., & Odgers, C. L. (2010). Group-based trajectory modeling in clinical research. *Annual review of clinical psychology*, *6*, 109-138. https://doi.org/10.1146/annurev.clinpsy.121208.131413
87. Narang, I., Rosenthal, M., Cremonesini, D., Silverman, M., & Bush, A. (2008). Longitudinal evaluation of airway function 21 years after preterm birth. American Journal of Respiratory and Critical Care Medicine, 178(1), 74–80. https://doi.org/10.1164/rccm.200705-701OC
88. National Asthma Council Australia, 2018
89. Nelson, H. S. (2000). The importance of allergens in the development of asthma and the persistence of symptoms. Journal of Allergy and Clinical Immunology, 105(6 II), S628–S632. https://doi.org/10.1067/mai.2000.106154
90. Newnham, J. P., Evans, S. F., Michael, C. A., Stanley, F. J., & Landau, L. I. (1993). Effects of frequent ultrasound during pregnancy: a randomised controlled trial. *Lancet* (London, England), 342(8876), 887–891. https://doi.org/10.1016/0140-6736(93)91944-h
91. Nguyen, T. T., Barber, P., Harper, R., Linh, T. V. K., & Dell, B. (2020). Vegetation trends associated with urban development: The role of golf courses. PloS one, 15(2), e0228090. https://doi.org/10.1371/journal.pone.0228090
92. Nicolai, T., Carr, D., Weiland, S. K., Duhme, H., Ehrenstein, O. von, Wagner, C., & Mutius, E. von. (2003). Urban traffic and pollutant exposure related to respiratory outcomes and atopy in a large sample of children. 956–963. https://doi.org/10.1183/09031936.03.00041103
93. Oddy, W. H. (2017). Breastfeeding, Childhood Asthma, and Allergic Disease. Annals of Nutrition and Metabolism, 70(2), 26–36. https://doi.org/10.1159/000457920
94. Ogbuanu, I. U., Karmaus, W., Arshad, S. H., Kurukulaaratchy, R. J., & Ewart, S. (2009). Effect of breastfeeding duration on lung function at age 10 years: a

prospective birth cohort study. *Thorax*, *64*(1), 62-66. https://doi.org/10.1136/thx.2008.101543

95. Papi, A., Brightling, C., Pedersen, S. E., & Reddel, H. K. (2018). Asthma. The Lancet, 391(10122), 783–800. https://doi.org/10.1016/S0140-6736(17)33311-1
96. Peristera, P., Platts, L. G., Magnusson Hanson, L. L., & Westerlund, H. (2020). A comparison of the B-spline group-based trajectory model with the polynomial group-based trajectory model for identifying trajectories of depressive symptoms around old-age retirement. Aging & Mental Health, 24(3), 445-452. https://doi.org/10.1080/13607863.2018.1531371
97. Quanjer, P. H., Stanojevic, S., Cole, T. J., Baur, X., Hall, G. L., Culver, B. H., ... & Stocks, J. (2012). Multi-ethnic reference values for spirometry for the 3–95-yr age range: the global lung function 2012 equations. https://doi.org/10.1183/09031936.00080312
98. Rice, M. B., Rifas-Shiman, S. L., Litonjua, A. A., Oken, E., Gillman, M. W., Kloog, I., Luttmann-Gibson, H., Zanobetti, A., Coull, B. A., Schwartz, J., Koutrakis, P., Mittleman, M. A., & Gold, D. R. (2016). Lifetime Exposure to Ambient Pollution and Lung Function in Children. *American journal of respiratory and critical care medicine*, *193*(8), 881–888. https://doi.org/10.1164/rccm.201506-1058OC
99. Rona, R. J., Gulliford, M. C., & Chinn, S. (1993). Effects of prematurity and intrauterine growth on respiratory health and lung function in childhood. British Medical Journal, 306(6881), 817–820. https://doi.org/10.1136/bmj.306.6881.817
100. Salimi, F., Morgan, G., Rolfe, M., Samoli, E., Cowie, C. T., Hanigan, I., ... & Marks, G. B. (2018). Long-term exposure to low concentrations of air pollutants and hospitalisation for respiratory diseases: A prospective cohort study in Australia. *Environment international*, *121*, 415-420. https://doi.org/10.1016/j.envint.2018.08.050.
101. Scichilone, N., Messina, M., Battaglia, S., Catalano, F., & Bellia, V. (2005). Airway hyperresponsiveness in the elderly: Prevalence and clinical implications. European Respiratory Journal, 25(2), 364–375. https://doi.org/10.1183/09031936.05.00080204

102. Silva, G. E., Sherrill, D. L., Guerra, S., & Barbee, R. A. (2004). Asthma as a risk factor for COPD in a longitudinal study. *Chest*, *126*(1), 59-65. https://doi.org/10.1378/chest.126.1.59

103. Simpson, S. J., Turkovic, L., Wilson, A. C., Verheggen, M., Logie, K. M., Pillow, J. J., & Hall, G. L. (2018). Lung function trajectories throughout childhood in survivors of very preterm birth: a longitudinal cohort study. The Lancet Child and Adolescent Health, 2(5), 350–359. https://doi.org/10.1016/S2352-4642(18)30064-6

104. Sly, P. D. (2011). The early origins of asthma: who is really at risk?. *Current opinion in allergy and clinical immunology*, *11*(1), 24-28. https://doi.org/10.1097/ACI.0b013e328342309d

105. Spathopoulos, D., Paraskakis, E., Trypsianis, G., Tsalkidis, A., Arvanitidou, V., Emporiadou, M., ... & Chatzimichael, A. (2009). The effect of obesity on pulmonary lung function of school aged children in Greece. *Pediatric pulmonology*, *44*(3), 273-280. https://doi.org/10.1002/ppul.20995

106. Spindel, E. R., & McEvoy, C. T. (2016). The role of nicotine in the effects of maternal smoking during pregnancy on lung development and childhood respiratory disease. Implications for dangers of e-cigarettes. *American journal of respiratory and critical care medicine*, *193*(5), 486-494. https://doi.org/https://doi.org/10.1164/rccm.201510-2013PP

107. Stanojevic, S., Quanjer, P., Miller, M. R., & Stocks, J. (2013). The Global Lung Function Initiative: dispelling some myths of lung function test interpretation. *Breathe*, *9*(6), 462-474. https://doi.org/ 10.1183/20734735.012113

108. Stern, D. A., Morgan, W. J., Halonen, M., Wright, A. L., & Martinez, D. (2010). Adulthood: a Longitudinal Birth-Cohort Study. 372(9643), 1058–1064. https://doi.org/10.1016/S0140-6736(08)61447-6.

109. Stick, S. M., Burton, P. R., Gurrin, L., Sly, P. D., & LeSouëf, P. N. (1996). Effects of maternal smoking during pregnancy and a family history of asthma on respiratory function in newborn infants. Lancet, 348(9034), 1060–1064. https://doi.org/10.1016/S0140-6736(96)04446-7

110. Stocks, J., & Sonnappa, S. (2013). Early life influences on the development of chronic obstructive pulmonary disease. Therapeutic Advances in Respiratory Disease, 7(3), 161–173. https://doi.org/10.1177/1753465813479428

111. Stocks, J., Hislop, A., & Sonnappa, S. (2013). Early lung development: lifelong effect on respiratory health and disease. *The Lancet Respiratory medicine*, 1(9), 728-742. https://doi.org/ 10.1016/S2213-2600(13)70118-8.

112. Svanes, C., Sunyer, J., Plana, E., Dharmage, S., Heinrich, J., Jarvis, D., … Antó, J. M. (2010). Early life origins of chronic obstructive pulmonary disease. Thorax, 65(1), 14–20. https://doi.org/10.1136/thx.2008.112136

113. Tennant, P. W., Gibson, G. J., & Pearce, M. S. (2008). Lifecourse predictors of adult respiratory function: results from the Newcastle Thousand Families Study. *Thorax*, *63*(9), 823-830. https://doi.org/10.1136/thx.2008.096388

114. Thacher, J. D., Schultz, E. S., Hallberg, J., Hellberg, U., Kull, I., Thunqvist, P., ... & Bergström, A. (2018). Tobacco smoke exposure in early life and adolescence in relation to lung function. *European Respiratory Journal*, *51*(6), 1702111. https://doi.org/10.1183/13993003.02111-2017

115. Thakur, N., Oh, S. S., Nguyen, E. A., Martin, M., Roth, L. A., Galanter, J., Gignoux, C. R., Eng, C., Davis, A., Meade, K., LeNoir, M. A., Avila, P. C., Farber, H. J., Serebrisky, D., Brigino-Buenaventura, E., Rodriguez-Cintron, W., Kumar, R., Williams, L. K., Bibbins-Domingo, K., Thyne, S., … Burchard, E. G. (2013). Socioeconomic status and childhood asthma in urban minority youths. The GALA II and SAGE II studies. *American journal of respiratory and critical care medicine*, *188*(10), 1202–1209. https://doi.org/10.1164/rccm.201306-1016OC.

116. Tong, S. (2019). Air pollution and disease burden. *The Lancet Planetary Health*, 3(2), e49-e50. https://doi.org/10.1016/S2542-5196(18)30288-2

117. Townsi, N., Laing, I. A., Hall, G. L., & Simpson, S. J. (2018). The impact of respiratory viruses on lung health after preterm birth. European Clinical Respiratory Journal, 5(1), 1–11. https://doi.org/10.1080/20018525.2018.1487214

118. Turner, S., Fielding, S., Mullane, D., Cox, D. W., Goldblatt, J., Landau, L., & Le Souef, P. (2014). A longitudinal study of lung function from 1 month to 18 years

of age. *Thorax*, *69*(11), 1015-1020. 10.1136/thoraxjnl-2013-204931. https://doi.org/10.1136/thoraxjnl-2013-204931.

119. Um-Bergström, P., Hallberg, J., Thunqvist, P., Berggren-Broström, E., Anderson, M., Adenfelt, G., … Melén, E. (2017). Lung function development after preterm birth in relation to severity of Bronchopulmonary dysplasia. BMC Pulmonary Medicine, 17(1), 1–8. https://doi.org/10.1186/s12890-017-0441-3

120. Vasquez, M. M., Zhou, M., Hu, C., Martinez, F. D., & Guerra, S. (2017). Low lung function in young adult life is associated with early mortality. *American journal of respiratory and critical care medicine*, *195*(10), 1399-1401. https://doi.org/10.1164/rccm.201608-1561LE

121. Vaz Fragoso, C. A., McAvay, G., Van Ness, P. H., Metter, E. J., Ferrucci, L., Yaggi, H. K., ... & Gill, T. M. (2016). Aging-related considerations when evaluati Karmaus, W., Mukherjee, N., Janjanam, V. D., Chen, S., Zhang, H., Roberts, G., … Arshad, H. (2019). Distinctive lung function trajectories from age 10 to 26 years in men and women and associated early life risk factors - A birth cohort study. Respiratory Research, 20(1), 1–13. https://doi.org/10.1186/s12931-019-1068-0

122. Vollstæer, M., Røksund, O. D., Eide, G. E., Markestad, T., & Halvorsen, T. (2013). Lung function after preterm birth: Development from mid-childhood to adulthood. Thorax, 68(8), 767–776. https://doi.org/10.1136/thoraxjnl-2012-202980

123. Wadhwa, P. D., Buss, C., Entringer, S., & Swanson, J. M. (2009, September). Developmental origins of health and disease: brief history of the approach and current focus on epigenetic mechanisms. In *Seminars in reproductive medicine* (Vol. 27, No. 5, p. 358). NIH Public Access. https://doi.org/10.1055/s-0029-1237424

124. Wang, B., Chen, H., Chan, Y. L., Wang, G., & Oliver, B. G. (2020). Why Do Intrauterine Exposure to Air Pollution and Cigarette Smoke Increase the Risk of Asthma? Frontiers in Cell and Developmental Biology, 8(February), 1–13. https://doi.org/10.3389/fcell.2020.00038

125. Wang, R., Henderson, S. B., Sbihi, H., Allen, R. W., & Brauer, M. (2013). Temporal stability of land use regression models for traffic-related air pollution. *Atmospheric Environment*, *64*, 312-319. https://doi.org/10.1016/j.atmosenv.2012.09.056

126. Weinmayr, G., Schulz, H., Klenk, J., Denkinger, M., Duran-Tauleria, E., Koenig, W., ... & Rothenbacher, D. (2020). Association of lung function with overall mortality is independent of inflammatory, cardiac, and functional biomarkers in older adults: the ActiFE-study. *Scientific reports*, *10*(1), 1-9. https://doi.org/10.1038/s41598-020-68372-w

127. White, E. C., de Klerk, N., Hantos, Z., Priston, M., Hollams, E. M., James, A., ... & Hall, G. L. (2017). Mannitol challenge testing for asthma in a community cohort of young adults. *Respirology*, 22(4), 678-683. https://doi.org/10.1111/resp.12955

128. World Health Organization (2019)

129. World Health Organization. (2006). *WHO Air quality guidelines for particulate matter, ozone, nitrogen dioxide and sulfur dioxide: global update 2005: summary of risk assessment* (No. WHO/SDE/PHE/OEH/06.02). World Health Organization.

130. Wu, J.-Z., Ge, D.-D., Zhou, L.-F., Hou, L.-Y., Zhou, Y., & Li, Q.-Y. (2018). Effects of particulate matter on allergic respiratory diseases. Chronic Diseases and Translational Medicine, 4(2), 95–102. https://doi.org/10.1016/j.cdtm.2018.04.001

131. Xiao, D., Huang, X., Yang, S., & Zhang, L. (2011). Antenatal nicotine induces heightened oxidative stress and vascular dysfunction in rat offspring. *British Journal of Pharmacology*, *164*(5), 1400-1409. https://doi.org/0.1111/j.1476-5381.2011.01437.x.

132. Xing, Y. F., Xu, Y. H., Shi, M. H., & Lian, Y. X. (2016). The impact of PM2.5 on the human respiratory system. Journal of Thoracic Disease, 8(1), E69–E74. https://doi.org/10.3978/j.issn.2072-1439.2016.01.19

133. Zacharasiewicz, A. (2016). Maternal smoking in pregnancy and its influence on childhood asthma. ERJ Open Research, 2(3), 1–9. https://doi.org/10.1183/23120541.00042-2016

134. Zimmermann, M. B., Gübeli, C., Püntener, C., & Molinari, L. (2004). Detection of overweight and obesity in a national sample of 6–12-y-old Swiss children: accuracy and validity of reference values for body mass index from the US Centers for Disease Control and Prevention and the International Obesity Task Force. The American journal of clinical nutrition, 79(5), 838-843. https://doi.org/10.1093/ajcn/79.5.838

Chapter 8.

Gastritis and gastroesophageal reflux disease are strongly associated with non-allergic nasal disorders

8.1 Introduction

During my PhD from 2017 to 2020, I have been working within two research Institutes, the University of Verona (Verona, Italy) and the Telethon Kids Institute (Perth, Western Australia). The main research project reported in this thesis has been carried out during my stay in Australia and it has been fully reported from Chapter 1 to Chapter 7. In this last Chapter, I will focus on the research conducted at University of Verona, focused on the gastritis and gastroesophageal reflux disease associated with non-allergic nasal disorders. The work from this Chapter has been sent and accepted by the BMC Pulmonary Medicine Journal.

8.2. Abstract

Background: Gastroesophageal reflux disease (GERD) has been reported to be significantly associated with chronic rhinosinusitis, but the strength of the association is still debated.

Aims: To evaluate the strength of the association between gastritis/GERD and non-allergic rhinitis (NAR)/allergic rhinitis (AR)/sinusitis.

Methods: We investigated 2887 subjects aged 20-84 years, who underwent a clinical visit in seven Italian centres (Ancona, Palermo, Pavia, Terni, Sassari, Torino, Verona) within the study on Gene Environment Interactions in Respiratory Diseases, a population-based multicase-control study between 2008 and 2014. Subjects were asked if they had doctor-diagnosed “gastritis or stomach ulcer (confirmed by gastroscopy)” or “gastroesophageal reflux disease, hiatal hernia or esophagitis”. The association between NAR/AR/sinusitis and either gastritis or GERD was evaluated through relative risk ratios (RRR) by multinomial logistic regression.

Results: The prevalence of gastritis/GERD increased from subjects without nasal disturbances (22.8%=323/1414) to subjects with AR (25.8%=152/590) and further to subjects with NAR (36.7%=69/188) or sinusitis (39.9%=276/691). When adjusting for centre, sex, age, education level, BMI, smoking habits and alcohol intake, the combination of gastritis and GERD was associated with a four-fold increase in the risk of NAR (RRR = 3.80, 97% CI 2.56-5.62) and sinusitis (RRR = 3.70, 2.62-5.23) with respect to controls, and with a much smaller increase in the risk of AR (RRR = 1.79, 1.37-2.35).

Conclusion: The study confirmed the association between gastritis/GERD and nasal disturbances, which is stronger for NAR and sinusitis than for AR.

8.3 Manuscript

Gastritis and gastroesophageal reflux disease are strongly associated with non-allergic nasal disorders

Finocchio E[1], Locatelli F[1], Sanna F[1], Vesentini R[1], Marchetti P[1], Spiteri G[2], Antonicelli L[3], Battaglia S[4], Bono R[5], Corsico A[6], Ferrari M[7], Murgia N[8], Pirina P[9], Olivieri M[2], Verlato G[1]

[1]Unit of Epidemiology & Medical Statistics, Department of Diagnostics & Public Health, University of Verona, Italy, [2]Unit of Occupational Medicine, Department of Diagnostics & Public Health, University of Verona, Italy, [3]Department of Internal Medicine Ospedali Riuniti Ancona, Italy, [4]University of Palermo, Italy, [5]Department of Public Health and Pediatric Sciences, University of Torino, Italy, [6]Department of Internal Medicine and Medical Therapy, University of Pavia, Italy, [7]Unit of Respiratory Diseases, Department of Medicine, University of Verona, Italy, [8]Section of Occupational Medicine, Respiratory Diseases and Toxicology, University of Perugia, Italy, [9]Department of Clinical, Surgical and Experimental Sciences, University of Sassari, Italy

Introduction

Rhinitis is a global common problem and is defined as the presence of at least one of the following: congestion, rhinorrhea, sneezing, nasal itching, and nasal obstruction. The two major classifications are allergic (AR) and non-allergic rhinitis (NAR) [1]. NAR occurs when obstruction and rhinorrhea are related to non-allergic, non-infectious triggers such as a change in the weather, exposure to caustic odors or cigarette smoke, barometric pressure differences, etc. [2]. The prevalence of AR in adults in Europe ranged from 17% to 28.5% [3], while NAR affects up to 30% of individuals in the Western population [4].

Gastro-esophageal reflux disease (GERD) is also a worldwide prevalent condition, which is on the rise in Europe and North America [5]. Hence also esophageal and extraesophageal diseases associated with GERD are expected to increase. Some of the well-established extraesophageal manifestations are reflux-induced cough, laryngitis, asthma and dental erosion. Other manifestations, such as sinusitis, pharyngitis, idiopathic pulmonary fibrosis, and recurrent otitis media, are proposed but not established as it is unclear whether GERD is a significant causal or exacerbating factor [6].

Since both chronic rhinosinusitis and GERD are highly prevalent, it is difficult to establish a direct relation between them, as they can easily coexist independently [7]. Moreover, while the association between GERD and nasal disorders gained support in children [8, 9], in adults the evidence is still sparse. Several studies focused on the possible correlation of GERD and sinusitis [10-12], which have been reported to occur together more frequently than expected [13]. At first, several reviews did not find out a clear evidence-based relationship between Chronic rhinosinusitis and GERD [13 – 15]. However, in the last years the association between GERD and sinusitis gained support. Two European studies found that the SNOT (Sino-Nasal Outcome Test) score significantly increased among patients with GERD, suggesting a direct role of GERD in the development of chronic rhinosinusitis [10, 16]. A large cohort study based on Taiwan Health Care Utilization database by Lin et al. [17] found that the risk of

developing chronic rhinosinusitis was more than doubled in cases with newly diagnosed GERD with respect to controls matched for sex, age and comorbidities. In a population-based Brazilian survey the diagnosis of gastritis/ulcer/gastroesophageal reflux was associated with higher prevalence of rhinosinusitis symptoms in multivariable analysis [18]. An Italian study on a small series undergoing both nasal citology and esophageal manometry and 24-hour pH-impedance monitoring showed that NAR with neutrophils strongly correlated with higher acid exposure time and refluxes number [19]. On the basis of this accumulating evidence, the International Consensus Statement on Allergy and Rhinology: Rhinosinusitis [20] assigned grade B evidence to support the association between chronic rhinosinusitis and GERD, although causation could not be clearly demonstrated.

The association between GERD and AR is more questioned. The recent International Consensus Statement on Allergic Rhinitis does not even mention GERD at all as a potential risk factor [21]. The situation is complex, as several studies which found an association between GERD and nasal disorders did not distinguish between NAR and AR [22].

Since nasal disorders are highly prevalent diseases that can have a deep impact on individual life and healthcare system, it is important to identify causative and triggering factors, and their comorbidities. The present study aimed to investigate the relation between gastritis/GERD and allergic and non-allergic rhinitis in a large population-based case-control study.

Methods

Study design

The study was performed in the frame of the GEIRD (Gene-Environment Interactions in Respiratory Diseases) study, a multicase-control study on respiratory health, involving seven Italian centers, three located in Northern Italy (Verona, Pavia, Turin), two in Central Italy (Ancona, Terni) and two in the major islands (Sassari in Sardinia and Palermo in Sicily) [23]. The study comprised a screening phase and a clinical

phase. In the screening phase a screening questionnaire was mailed to random samples from the general population aged 20-84 years, while in the clinical phase subjects reporting symptoms suggestive of chronic bronchitis, asthma or rhinitis, as well as a sample of subjects without respiratory symptoms, were invited to a local Respiratory/Allergy Unit, in order to undergo interviews and clinical tests. In particular, participating subjects were administered a modified version of the ECRHS (European Community Respiratory Health Survey) clinical questionnaire, including detailed questions on socio-demographic characteristics, smoking habits and other lifestyle factors, respiratory symptoms and other disturbances, drug consumption [23; available at www.geird.org]. In each center, the GEIRD study was approved by the local ethics committee and written consent was obtained from each participant.

Fifty-nine percent (17,972 / 30,349) of selected subjects answered the screening questionnaire, while 2,945 subjects out of 7,739 participated in the clinical visit between 2008 and 2014, yielding a participation rate of 40.1%.

Nasal disorders

Subjects were classified as having "rhinitis" if they answered affirmatively to at least one of the following questions: "Do you have any nasal allergies including hay fever?", "During your lifetime have you ever had any nasal allergies including hay fever?", "Have you ever had a problem with sneezing, or a runny or a blocked nose when you did not have a cold or the flu?". Rhinitis was further classified as allergic rhinitis (AR) and non-allergic rhinitis (NAR), according to the presence or absence of atopy. Subjects were also asked whether they ever suffered from nasal polyps.

Treatments for nasal disorders were assessed by the following questions "Have you used any of the following nasal medicines (e.g. nasal sprays, inhaled powders or drops) for the treatment of your nasal disorders?" and "Have you used any of the following pills, capsules, or tablets for the treatment of your nasal disorder?".

Other respiratory disorders

Asthma was deemed present when the subject reported physician-diagnosed asthma. The disease was further classified in: current asthma if the subject took any medicine for asthma or had had an attack of asthma or reported any asthma-like symptom (wheezing, chest tightness or shortness of breath) in the previous 12 months; past asthma otherwise.

Chronic cough and phlegm was defined by a positive answer to the question: "*Have you had coughing and phlegm on most days for a minimum of 3 months a year and for at least 2 successive years*?". Doctor-diagnosed chronic bronchitis was defined by an affirmative answer to the question: "*Have you ever been told by a doctor that you have or had chronic bronchitis, chronic obstructive pulmonary disease (COPD) or emphysema*?".

Atopy

Atopy was established by a positive skin prick test in which the following panel of allergens were used: Cupressus arizonica, Dermatophagoides pteronyssinus, Artemisia vulgaris, Dermatophagoides farinae, Ambrosia artemisifolia, Alternaria tenuis, Parietaria judaica, dog dandruff, Corylus avellana, cat, Olea europea, Betula verrucosa, Cladosporium herbarum, and Phleum pratense. The result was considered positive if, after twenty minutes, the average wheal diameter was 3 mm greater than the negative control.

Gastritis/gastroesophageal reflux

Subjects were classified as having gastritis if they answered positively to the question "*Has a doctor told you having or have had gastritis or stomach ulcer (confirmed by a gastroscopy)*?" Similarly, subjects were considered having gastroesophageal reflux disease (GERD) if they answered affirmatively to the question "*Has a doctor told you having or have had gastroesophageal reflux disease, hiatal hernia or esophagitis?*"

Lifestyle factors

Subjects were classified as normal weight (BMI <25 Kg/m2), overweight (25≤BMI<30 Kg/m2), or obese (BMI ≥30 Kg/m2). They were considered active when reporting to exercise for at least 1 hour getting out of breath or sweating with a frequency of at least 2-3 times a week.

With regard to smoking habits, subjects were classified as 1) current smokers, if they reported to have smoked at least one cigarette per day or one cigar a week for as long as one year, and also in the last month; 2) ex-smokers if they had smoked the same minimum amount previously reported, but had stopped smoking for at least one month before the interview; 3) never smokers otherwise. As regards alcohol consumptions, subjects were classified as drinkers and nondrinkers.

Statistical analyses

Significance of the association between AR/NAR/sinusitis and potential risk factors was evaluated by Fisher's exact test or chi-squared test. The same statistical tests were used to evaluate the association between gastritis/gastroesophageal reflux and other risk factors.

Multivariable analysis was accomplished by a multinomial logistic regression model [24], where the response variable was nasal symptoms: 0 = no symptom (base outcome), 1 = allergic rhinitis, 2 = non-allergic rhinitis, 3 = sinusitis. Gastroesophageal reflux (none / gastritis / reflux / both gastritis and reflux) was the explanatory variable, while sex, age (per 10 year increase), age at completing full-time education (<18, 18-21, >=22 years), BMI (<25, 25-29.9, >=30 Kg/m2), smoking habits (never smoker, past smoker, current smoker), alcohol intake (nondrinker, drinker), were the potential confounders. Results were synthesized through the relative risk ratios (RRR), adjusting standard errors for intra center correlation. Analyses were performed with STATA statistical software, release 14 (StataCorp, College Station, TX, USA) and statistical significance was set at $p<0.05$.

Results

Description of controls and cases of AR/NAR/Sinusitis, as a function of main risk factors

2887 subjects participated in the clinical visit and they had a mean age (SD) of 50.1 (13.2) years. Cases of AR and sinusitis were younger (mean age±SD = 46.0±11.8 and 48.7±11.9 years, respectively) than controls and cases of NAR (52±13.7 and 54.6±14 years, respectively) ($p<0.001$). Controls and cases of NAR had a lower level of education and physical activity, a higher prevalence of obesity than cases of AR and sinusitis. Atopy, which was used to define NAR and AR, had a prevalence of 27% in controls and 58% in cases of sinusitis. Nasal polyps were rare in controls and cases of NAR, and more common in cases of AR and sinusitis. Use of antihistamines and steroids was frequent among cases of AR, and rare among cases of NAR, while the use of vasoconstrictors was similar in the two groups. Sex, smoking habits and alcohol intake did not significantly differ between cases and controls (Table 1).

Table 1. Number and percent of controls, cases of non-allergic rhinitis (NAR), allergic rhinitis (AR) and sinusitis as a function of main socio-demographic, lifestyle, and clinical characteristics.

	Controls (n=1416) n (%)	**AR (n= 592) n (%)**	**NAR (n=188) n (%)**	**Sinusitis (n=691) n (%)**	***P*-value**
Sex					0.068
Male	729 (51.5)	317 (53.5)	87 (46.3)	326 (47.2)	
Female	687 (48.5)	275 (46.5)	101 (53.7)	365 (52.8)	
Age					**<0.001**
20-35	144 (10.2)	**95 (16.1)**	12 (6.4)	70 (10.1)	
35-45	339 (23.9)	**194 (32.8)**	34 (18.1)	**218 (31.6)**	
45-55	388 (27.4)	**191 (32.3)**	**61 (32.5)**	**218 (31.6)**	

55-65	254 (17.9)	72 (12.2)	**36 (19.2)**	119 (17.2)	
≥65	**291 (20.6)**	40 (6.8)	**45 (23.9)**	66 (9.6)	
Time at stopping education (years)					**<0.001**
<18	**465 (33.1)**	141 (24)	**64 (34.8)**	190 (27.7)	
(18-22)	498 (35.5)	**222 (37.8)**	**68 (37)**	**267 (38.9)**	
≥22	440 (31.4)	**225 (38.3)**	52 (28.3)	228 (33.3)	
BMI (kg/m^2**)**					**0.030**
<25	681 (49.9)	328 (**56.6**)	94 (50.5)	355 (53.4)	
(25-30)	475 (34.8)	187 (32.2)	62 (33.3)	236 (35.5)	
≥30	208 (**15.3**)	65 (11.2)	30 (**16.1**)	74 (11.1)	
Physical activity (times/week)					**0.038**
<2-3 times	983 (69.9)	379 (64.6)	138 (74.2)	467 (67.9)	
≥2-3 times	423 (30.1)	208 **(35.4**)	48 (25.8)	221 (32.1)	
Smoking habits					0.577
No smoker	684 (49.4)	305 (52.6)	81 (43.8)	333 (49.3)	
Ex-smoker	411 (29.7)	158 (27.2)	60 (32.4)	200 (29.6)	
Current smoker	290 (20.9)	117 (20.2)	44 (23.8)	142 (21.0)	
Alcohol intake (No/Yes)					0.158
No drinker	875 (62.3)	349 (59.4)	121 (64.7)	399 (58.1)	
Drinker	529 (37.7)	239 (40.6)	66 (35.3)	288 (41.9)	
Atopy					**<0.001***
No	931 (**72.7**)	----------	188 (100.0)	268 (41.9)	
Yes	349 (27.3)	592 (100.0)	----------	372 (**58.1**)	
Nasal polyps (ever)					**<0.001**
No	1396 (98.8)	564 (95.4)	185 (98.4)	632 (91.7)	
Yes	17 (1.2)	**27 (4.6)**	3 (1.6)	**57 (8.3)**	
Asthma					**<0.001**
No	1807 (85.25)	225 (40.47)	109 (72.67)	328 (52.82)	
Yes	107 (8.39)	**238 (42.81)**	**29 (19.33)**	**213 (34.30)**	

Past	81 (6.35)	**93 (16.73)**	**12 (8.00)**	**80 (12.88)**	
Chronic chough and phlegm					**<0.001**
No	1.338 (95.1)	524 (88.81)	154 (83.24)	588 (85.59)	
Yes	69 (4.9)	**66 (11.19)**	**31 (16.76)**	**99 (14.41)**	
Doctor-diagnosed chronic bronchitis					**0.562**
No	1236 (97.78)	521 (97.38)	156 (96.89)	587 (96.71)	
Yes	28 (2.22)	**14 (2.62)**	**5 (3.11)**	**20 (3.29)**	
Steroids (ever)					**<0.001**
No	1302 (98.6)	422 (80.8)	160 (92.5)	491 (79.1)	
Yes	18 (1.4)	**100 (19.2)**	**13 (7.5)**	**130 (20.9)**	
Vasoconstrictors (ever)					**<0.001**
No	1274 (96.4)	394 (75.3)	142 (81.6)	486 (77.8)	
Yes	48 (3.6)	**129 (24.7)**	**32 (18.4)**	**139 (22.2)**	
Antihistamines (ever)					**<0.001**
No	1320 (99.7)	362 (69)	162 (93.1)	495 (78.8)	
Yes	4 (0.3)	**163 (31.1)**	**12 (6.9)**	**133 (21.2)**	

Significance of differences was computed by Fisher's exact test or chi-square test. *computed only on controls and people with sinusitis.

Description of controls and cases of gastroesophageal disorders, as a function of main risk factors

People with gastritis and/or gastroesophageal reflux were older and had attained a lower education level than people without these disorders (Table 2). Women more frequently reported gastritis and gastroesophageal reflux than men. Gastritis and gastroesophageal reflux were more common, respectively, in current smokers and overweight people.

Atopy and nasal polyps, the level of physical activity and alcohol intake did not significantly change as a function of gastritis/gastroesophageal reflux.

Table 2. Number (percentage) of controls, cases of gastritis and gastroesophageal reflux, isolated or combined, as a function of main socio-demographic, lifestyle and clinical characteristics.

	No gastritis/reflux (n=2128)	**Gastritis only (n=264)**	**Reflux only (n=310)**	**Both Gastritis and Reflux (n=278)**	***P*- value**
Sex					**0.034**
Male	1101 (51.7)	131 (49.6)	159 (51.3)	118 (42.5)	
Female	1027 (48.3)	133 (50.4)	151 (48.7)	**160 (57.**6)	
Age					**<0.001**
20-35	**282 (13.3)**	11 (4.2)	19 (6.1)	15 (5.4)	
35-45	601 (28.2)	61 (23.1)	78 (25.2)	70 (25.2)	
45-55	648 (30.5)	68 (25.8)	87 (28.1)	81 (29.1)	
55-65	323 (15.2)	46 (17.4)	**61 (19.7**)	**63 (22.7)**	
>65	274 (12.9)	**78 (29.6)**	**65 (21.0)**	**49 (17.6)**	
Time at stopping education (years)					**<0.001**
<18	578 (27.4)	**111 (42.4)**	93 (30.4)	**103 (37.5)**	
(18-22)	806 (38.2)	73 (27.9)	111 (36.3)	99 (36.0)	
≥22	727 (34.4)	78 (29.8)	102 (33.3)	73 (26.5)	
BMI					**0.021**
<25	1104 (53.8)	137 (53.5)	130 (43.6)	126 (46.8)	
(25-30)	679 (33.1)	83 (32.4)	125 (**42.0**)	102 (37.9)	

≥30	271 (13.2)	36 (14.1)	43 (14.4)	41 (**15.2**)	
Physical activity (hours/week)					0.112
<2/3	1428 (67.7)	194 (74.1)	217 (70.5)	198 (71.5)	
≥2/3	683 (32.4)	68 (26.0)	91 (29.6)	79 (28.5)	
Smoking habits					**0.006**
No smoker	1059 (50.9)	103 (40.4)	154 (50.2)	128 (47.4)	
Ex-smoker	588 (28.2)	92 (36.1)	105 (34.2)	79 (29.3)	
Current smoker	435 (20.9)	**60 (23.5)**	48 (15.6)	**63 (23.3)**	
Alcohol intake (No/Yes)					0.793
No drinker	1284 (60.9)	165 (63.2)	182 (59.1)	168 (60.4)	
Drinker	826 (39.1)	96 (36.8)	126 (40.9)	110 (39.6)	
Atopy					0.485
No	1009 (51.1)	123 (53.0)	131 (49.6)	143 (55.6)	
Yes	964 (48.9)	109 (47.0)	133 (50.4)	114 (44.4)	
Nasal polyps					0.238
No	2049 (96.6)	253 (96.2)	291 (94.5)	271 (97.5)	
Yes	73 (3.4)	10 (3.8)	17 (5.5)	7 (2.5)	

Significance of differences was evaluated by Fisher's exact test or chi-square test.

Description of controls and cases of AR/NAR/Sinusitis, as a function of gastroesophageal disorders

Gastritis/gastroesophageal reflux were strongly associated with nasal disorders. The prevalence of gastritis/reflux was 5.9% in controls, it slightly increased to 7.5% in cases of allergic rhinitis and further to 18.1% and 15.8% in cases of NAR and sinusitis, respectively (Table 3).

Table 3. Prevalence of gastroesophageal disorders (gastritis/GERD) in controls, rhinitis, allergic rhinitis and sinusitis subjects. P-values were computed by Pearson's chi-square test.

	Controls (n=1414) n(%)	**AR (n= 590) n(%)**	**NAR (n=188) n(%)**	**Sinusitis (n=691) n(%)**	**P-value**
Gastritis/reflux					**<0.001**
No gastritis/GERD	1091 (77.2)	438 (74.2)	119 (63.3)	415 (60.1)	
Gastritis/GERD	323 (22.8)	152 (25.8)	69 (36.7)	276 (39.9)	
Gastritis only	108 (7.6)	43 (7.3)	21 (11.2)	81 (11.7)	
GERD Only	132 (9.3)	65 (11)	14 (7.5)	86 (12.5)	
GERD and Gastritis	83 (5.9)	44 (7.5)	34 (18.1)	109 (15.8)	

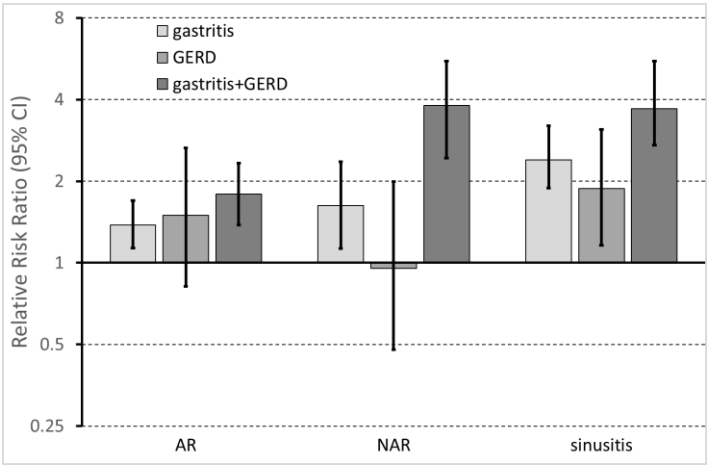
Significance of differences was evaluated by Fisher's exact test or chi-square test.

Multivariable analyses

The combination of gastritis and GERD was associated with a four-fold increase in the risk of NAR (RRR = 3.80, 97% CI 2.56-5.62) and sinusitis (RRR = 3.70, 2.62-5.23) with respect to people without these disorders, and with a much smaller increase in the risk of AR (RRR = 1.79, 1.37-2.35). The risk of nasal disorders was significantly increased, although to a smaller extent, in subjects reporting gastritis alone, while GERD was significantly associated with sinusitis but not with either NAR or AR (Figure 1).

As regards the other factors, the risk of NAR was higher in women than men (RRR=1.28, 1.19-1.37). Moreover, the risk of AR and sinusitis decreased with advancing age (RRR per 10-year increase=0.70, 0.63-0.79 and 0.79, 0.66-0.95, respectively). The risk of NAR was higher in current smokers than never smokers (RRR=1.48, 1.16-1.89), and in people with medium than low education (RRR = 1.12, 1.00-1.26). With respect to normoweight, obesity was associated with a lower risk of AR (RRR = 0.75, 0.58-0.97) and sinusitis (RRR = 0.79, 0.65-0.95). The risk of sinusitis was slightly higher in past smokers than never smokers (RRR = 1.18, 1.01-1.38) and in alcohol drinkers than non-drinkers (RRR = 1.23, 1.02-1.47).

Figure 1. Association between nasal disorders and gastritis/gastroesophageal reflux, estimated by a multinomial regression model, adjusting for sex, age, education level, BMI, smoking habits, alcohol intake. Columns are RRRs, bars are 95% Confidence Intervals.



Discussion

The main findings of the present study are:

1.Gastritis and GERD were strongly associated with non-allergic nasal diseases (NAR and sinusitis) and, to a much smaller extent, to allergic rhinitis, both in univariable and multivariable analysis.

2.The association was rather strong for the combination of gastritis and GERD, and rather weak for gastritis alone, and nearly absent for GERD alone. Subjects self-reporting both gastrointestinal diseases had probably a more severe condition than the other subjects.

3.Nasal polyps, while nearly absent in controls and cases of NAR, were found in 5% of cases of AR and 8% of cases of sinusitis. Their prevalence was not significantly affected by gastritis/GERD.

4.The risk of AR and sinusitis decreased with advancing age and in obese people. As regards lifestyle factors, NAR was associated with current smoking, and sinusitis with alcohol consumption.

The present multicase-control study showed that upper gastrointestinal disorders were strongly associated with non-allergic nasal disorders: the prevalence of gastritis and/or GERD was 22.8% in controls, only slightly increased in cases of AR (25.8%), and picked up in cases of non-allergic nasal disorders, such as NAR (36.7%) and sinusitis (39.9%). These findings were confirmed in multivariable analysis, where the combination of gastritis and GERD was associated with a nearly four-fold increase in the risk of NAR and sinusitis, while the risk of AR was less than doubled.

These findings are in agreement with the current literature. As already mentioned, the association between chronic rhinosinusitis and GERD has been acknowledged by the International Consensus Statement on Allergy and Rhinology: Rhinosinusitis [20], although with moderate evidence. On the other hand, the association between AR and GERD is not even mentioned by the recent International Consensus Statement on Allergic Rhinitis [21]. A recent study supported GERD involvement in the development of NAR, since patients with NAR displayed a high level of pepsin in saliva samples, especially in the postprandial period, compared to healthy controls [4].

Nasal polyps were not significantly related to gastritis/GERD in the present study. Accordingly, Lin et al. [17] the association between GERD and chronic rhinosinusitis was stronger in subjects without than with nasal polyps.

An interesting approach to the relation between GERD and nasal disorders consists in verifying whether treatment of GERD could improve nasal symptoms especially in chronic rhinosinusitis refractory to clinical or surgical treatment. However, a recent review assessed the effect of treatment with proton pump inhibitors (PPIs) on chronic rhinosinusitis symptoms in four longitudinal studies, and found conflicting results [25].

Pathophysiological mechanisms

Several mechanisms have been proposed to explain the relation between acid reflux and chronic rhinosinusitis. Subjects with chronic rhinosinusitis have been shown to have more proximal gastroesophageal reflux than healthy controls [26].

First of all, gastric acid exposure may exacerbate inflammation within the mucosa of the upper airways and sinuses and impair mucociliary motility, causing obstruction of sinus ostia and favouring recurrent infections [27 - 29].

A second mechanism could be vagally-mediated neuroinflammatory changes [11]. Autonomic dysfunction can lead to reflex sinonasal swelling and inflammation, leading to blockage of the ostia. Wong et al. [30] gave experimental support to this hypothesis, showing that infusion of saline with hydrochloric acid in the lower esophagus increased nasal mucus production and nasal symptom score.

Also a role of *Helicobacter pylori* (*H. pylori*) has been proposed, as the microorganism has been detected not only in the stomach but also in oral and nasal mucosa [31]. In particular, *H. pylori* has been found in nasal polyps but not in control tissue [32], and in patients who have both GERD and chronic rhinosinusitis [33]. Moreover, *H. Pylori* causes not only gastritis but also systemic inflammation, which can involve also the nasal mucosa.

Strengths and limitations

The present study has several strengths. It involved seven centres scattered from Northern to Southern Italy, 1,471 cases of nasal disorders and 1,416 controls, and information was collected by standardized methods (questionnaire and skin prick test).

However, some limitations should be acknowledged. First of all, the cross-sectional design did not allow to properly infer the cause-effect relationship between GERD/gastritis and NAR, Indeed, to prove such a cause effect relation, longitudinal studies with an adequate number of patients are needed. Second, while information on nasal disorders was based on questionnaire and objective measurement (skin prick test), information on gastritis and GERD was exclusively derived by questionnaire. Gastritis alone apparently had larger effects on AR and NAR than GERD alone. It should be reminded that the question on gastritis was probably more reliable as it involved not only medical diagnosis but also objective assessment (gastroscopy), while the question on GERD put together different diseases (GERD, hiatal hernia, or esophagitis) and did not refer to instrumentally confirmed diagnosis. Moreover, subjects who reported both gastritis and GERD probably had a more clear-cut gastrointestinal disease than those reporting only one disease. In turn, improvement in exposure definition allowed to better assess association with nasal disorders.

Conclusions

According to the present study, gastritis and GERD were strongly associated with nasal disorders, in particular non-allergic ones (NAR and sinusitis). On the other hand, AR, which was defined by symptoms and positive skin prick test, has an IgE-mediated pathogenesis and is only mildly affected by irritant substances, such as acid reflux.

Nevertheless, to prove a causal effect relationship, prospective studies with a significant number of patients are needed. In particular randomized controlled trials should verify whether reflux treatment also improve concomitant nasal disorders.

Author Contributions

EF, MO, GV devised the present study. RV, PM, FL prepared the database. EF, FL, FS, GV performed statistical analyses. LA, SB, RB, AC, MF, NM, PP, MO, GV found

resources. EF, FL, FS, MO, GV drafted the preliminary manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Ethics approval was obtained in each centre involved in the GEIRD study from the appropriate ethics committee (Comitato Etico dell'Azienda Ospedaliero-Universitaria Ospedali Riuniti di Ancona; Comitato Etico dell'Azienda Ospedaliera Ospedali Riuniti Villa Sofia Cervello di Palermo; Comitato di Bioetica della Fondazione IRCCS Policlinico San Matteo di Pavia; Comitato Etico delle Aziende Sanitarie dell'Umbria di Perugia; Comitato di Bioetica dell'Azienda Sanitaria Locale di Sassari; Comitato Etico dell'Azienda Sanitaria Locale TO/2 di Torino; Comitato Etico per la Sperimentazione dell'Azienda Ospedaliera Istituti Ospitalieri di Verona).

Information on the purpose of the survey and participants' rights according to then current Italian law (Decreto Legislativo 30 giugno 2003, n. 196) were enclosed to the questionnaire administered by mail. Individuals were free to answer or not answer the questionnaire, as denoted by the non-negligible nonresponse rate. Hence, consent to participate was implied by the answer to the questionnaire, and this form of consent was approved by the local Ethics Committee.

References

1. Bachert C, van Cauwenberge P, Olbrecht J, van Schoor J. Prevalence, classification and perception of allergic and nonallergic rhinitis in Belgium. Allergy. 2006;61(6):693-8.
2. Tran NP, Vickery J, Blaiss MS. Management of Rhinitis: Allergic and Non-AllergicAllergy. Asthma Immunol Res. 2011;3(3):148-56.
3. Brożek JL, Bousquet J, Agache I, Agarwal A, Bachert C, Bosnic-Anticevich S, et al. Allergic Rhinitis and its Impact on Asthma (ARIA) guidelines-2016 revision. J Allergy Clin Immunol. 2017;140(4):950-958.

4. Wang Q, Lenham RK, Wang X, Li Y, Jiang M, Chen W, et al. A pilot study demonstrating the evidence for reflux disease in patients presenting with non-allergic rhinitis (NAR)—reflux disease in association with non-allergic rhinitis. Ann Esophagus. 2019;2:6
5. El-Serag HB, Sweet S, Winchester CC, Dent J. Update on the epidemiology of gastro-oesophageal reflux disease: a systematic review. Gut. 2014;63:871–80.
6. Vakil N, van Zanten SN, Kahrilas P, Dent J, Jones R, Globale Konsensusgruppe. The Montreal definition and classification of gastroesophageal reflux disease: a global evidence-based consensus. Am. J. Gastroenterol. 2006;101:1900.
7. Wong IWY, Omari TI, Myers JC, G Rees, Nair SB, Jamieson GG, et al. Nasopharyngeal pH monitoring in chronic sinusitis patients using a novel four channel probe. Laryngoscope. 2004;114(9):1582-5.
8. Phipps CD,Wood WE, GibsonWS, Cochran WJ. Gastroesophageal reflux contributing to chronic sinus disease in children: a prospective analysis. Arch Otolaryngol Head Neck Surg. 2000;126(7):831–6.
9. Bothwell MR, Parsons DS, Talbot A, Barbero GJ, Wilder B. Outcome of reflux therapy on pediatric chronic sinusitis. Otolaryngol Head Neck Surg. 1999;121(3):255–62.
10. Katle EJ, Hart H, Kjaergaard T, Kvaløy JT, Steinsvåg SK. Nose- and sinus-related quality of life and GERD. Eur Arch Otorhinolaryngol. 2012;269(1):121–5.
11. Loehrl TA, Samuels TL, Poetker DM, Toohill RJ, Blumin JH, Johnston N. The role of extraesophageal reflux in medically and surgically refractory rhinosinusitis. Laryngoscope. 2012;122(7):1425–30.
12. Chambers DW, Davis WE, Cook PR, Nishioka GJ, Rudman DT. Long-term outcome analysis of functional endoscopic sinus surgery: correlation of symptoms with endoscopic examination finding and potential prognostic variables. Laryngoscope. 1997;107(4):504–10.
13. Hanna BC, Wormald PJ. Gastroesophageal Reflux and Chronic Rhinosinusitis. Curr Opin Otolaryngol Head Neck Surg. 2012;20(1):15-8.

14. Lupa M, DelGaudio JM. Evidence-based practice. Otolaryngol Clin N Am. 2012;45:983-92
15. Flook EP, Kumar BN. Is there evidence to link acid reflux withchronic sinusitis or any nasal symptoms? A review of the evidence. Rhinology. 2011;49:11-6.
16. Bohnhorst I, Jawad S, Lange B, Kjeldsen J, Hansen JM, Kjeldsen AD. Prevalence of chronic rhinosinusitis in a population of patients with gastroesophageal reflux disease. Am J Rhinol Allergy. 2015;29(3):e70-4.
17. Lin YH, Chang TS, Yao YC, Li YC. Increased Risk of Chronic Sinusitis in Adults With Gastroesophgeal Reflux Disease: A Nationwide Population-Based Cohort Study. Medicine. 2015;94(39):e1642.
18. Caminha GP , Pizzichini E , Neto JFL , Hopkins C, da Silva Moreira J, Pizzichini MMM. Rhinosinusitis Symptoms, Smoking and COPD: Prevalence and Associations. Clin Otolaryngol. 2018;43(6):1560-1565.
19. Mandolesi D, Schiavon P, Ioannou A, Mancini M, Cimatti MC, Azzaroli F, et al. Chronic Non-Allergic Rhinitis With Neutrophils Is Associated With Higher Acid Exposure Time: A pH-impedance Monitoring Study. Dig Liver Dis. 2020;52(4):414-419.
20. Orlandi RR, Kingdom TT, Hwang PH, Smith TL, Alt JA, Baroody FM, et al. International Consensus Statement on Allergy and Rhinology: Rhinosinusitis. Int Forum Allergy Rhinol. 2016;6 Suppl 1:S22-209.
21. Wise SK, Lin SY, Toskala E, Orlandi RR, Akdis CA, Alt JA, et al. International consensus statement on allergy and rhinology: allergic rhinitis. Int Forum Allergy Rhinol. 2018;8(2):108–352.
22. Schiöler L, Ruth M, Jõgi R, Gislason T, Storaas T, Janson C, et al. Nocturnal GERD - A Risk Factor for Rhinitis/Rhinosinusitis: The RHINE Study. Allergy. 2015;70(6):697-702.
23. de Marco R, Accordini S, Antonicelli L, Bellia V, Bettin MD, Bombieri C, et al. The Gene-Environment Interactions in Respiratory Diseases (GEIRD) Project. Int Arch Allergy Immunol. 2010;152(3):255-63.
24. Agresti A. Categorical data analysis. New York, NY: John Wiley & Sons; 1990.

25. Sella GCP, Tamashiro E, Anselmo-Lima WT, Valera FCP. Relation between chronic rhinosinusitis and gastroesophageal reflux in adults: systematic review. Braz J Otorhinolaryngol. 2017;83(3):356-363.
26. Katle EJ, Hatlebakk JG, Grimstad T, Kvaløy JT, Steinsvåg SK. Gastro-oesophageal reflux in patients with chronic rhinosinusitis investigated with multichannel impedance – pH monitoring. Rhinology. 2017;55:27-33.
27. Holma B, Lindegren M, Andersen JM. pH effects on ciliomotilityand morphology of respiratory mucosa. Arch Environ Health. 1977;32:216-26.
28. Delehaye E, Dore MP, Bozzo C, Mameli L, Delitala G, Meloni F. Correlation Between Nasal Mucociliary Clearance Time and Gastroesophageal Reflux Disease: Our Experience on 50 Patients. Auris Nasus Larynx. 2009;36(2):157-61.
29. Hait EJ, McDonald DR. Impact of Gastroesophageal Reflux Disease on Mucosal Immunity and Atopic Disorders. Clin Rev Allergy Immunol. 2019;57(2):213-225.
30. Wong IWY, Rees G, Greiff L, Myers JC, Jamieson GG, Wormald PJ. Gastroesophageal reflux disease and chronic sinusitis: in search of an esophageal-nasal reflex. Am J Rhinol Allergy. 2010;24:255-9
31. Kurtaran H, Uyar ME, Kasapoglu B, Turkay C, Yilmaz T, Akcay A, Kanbay M. Role of Helicobacter Pylori in pathogenesis of upper respiratory system diseases. J Nat Med Assoc 2008; 100(10): 1224-30
32. Koc C, Arikan OK, Atasoy P, Aksoy A. Prevalence of Helicobacter Pylori in Patients With Nasal Polyps: A Preliminary Report. Laryngoscope. 2004;114(11):1941-4.
33. Morinaka S, Ichimiya M, Nakamura H. Detection of Helicobacter Pylori in Nasal and Maxillary Sinus Specimens From Patients With Chronic Sinusitis. Laryngoscope. 2003;113(9):1557-63.

Appendix 1. Ethical approval

The research data reported in this thesis was assessed and approved by the University of Western Australia (UWA) Human Research Ethics Committee. Approval nº RA/4/1/2100. Data from each Raine Study follow-up used in this thesis has received single scientifical approval that was specific for a given follow-up.

Appendix 2.

List of presentations, publications arising from materials in this thesis

Presentations and attendance at Conferences

Nov 2020	**Rottnest Respiratory Conference**
Oct 2020	**Early-life predictors of lung function trajectories in the Raine Study** Oral presentation at the Student Symposium, Perth, Western Australia Oral presentation at the Raine Study 13th Annual Scientific Meeting 2020, Perth, Western Australia Poster presentation at the Child Health Research Symposium, Perth, Western Australia
Sept 2020	**Childhood predictors of lung function trajectories in the Raine Study** Poster presentation at the European Respiratory Society (ERS) Congress (virtual)
2019	**Lung function trajectories from childhood to adulthood in the Raine Study** Oral presentation at the Raine Study 12th Annual Scientific Meeting, Perth, Western Australia Poster presentation at the Institute for Respiratory Health Symposium, Perth, Western Australia
July 2019	**The association between gastritis/ gastroesophageal reflux and nasal disorders** Poster presentation at the WA Combined TSANZ & ANZSRS Annual Scientific Meeting, Perth, Western Australia

Awards and Prizes

September 2019	**Respiratory Research Centre Scientific Meeting Travel Scholarships,** Perth, Western Australia
September 2019	**Respiratory Research Centre Scientific Meeting New Investigator Award,** Perth, Western Australia
July 2019	**Award for the best poster presentation, WA Combined TSANZ & ANZSRS**, Perth, Western Australia

Publications

2020	**Lung function trajectories from childhood to adulthood in the Raine study** (manuscript in preparation) Authors: **F Sanna**, F Locatelli, PD Sly, E White, GL Hall, RE Foong
2020	**Gastritis and gastroesophageal reflux disease are strongly associated with non-allergic nasal disorders** (manuscript accepted) Authors: Finocchio E, Locatelli F, **Sanna F**, Vesentini R, Marchetti P, Spiteri G, Antonicelli L, Battaglia S, Bono R, Corsico A, Ferrari M, Murgia N, Pirina P, Olivieri M, Verlato G
2020	**Characterization of lung function trajectories in the Raine Study** (Conference Paper) Authors: **F Sanna**, F Locatelli, PD Sly, E White, GL Hall, RE Foong