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The impact of household risk factors and their interactions with antioxidative stress genes on respiratory health

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BScN, MPH

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2019

Allergy and Lung Health Unit, Centre for Epidemiology and Biostatistics

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The University of Melbourne

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Thesis abstract

Background: Asthma affects people of all ages and is associated with a substantial impact on both the individual and the community, yet there are few effective strategies to prevent its development or persistence. This is because the aetiology of asthma and factors influencing its progression or remission are currently poorly understood. Asthma primarily affects lung function. Progressive lung function decline associated with asthma is well-recognised but has not been commonly investigated as an outcome in asthma studies. Knowledge on the critical factors influencing maximal development of lung function in childhood is scarce.

The development of asthma and allergic diseases is very complex and believed to be influenced by multiple genetic and environmental factors that may influence asthma risk from pre-conception through to adult life. A range of strategies modifying environmental exposures have been trialled to potentially reduce the risk of asthma, with many studies finding no evidence of substantial benefit, or only limited benefit, from the interventions. Additionally, although some genes are associated with increased asthma risk, the associations are small and only explain a small percentage of the large asthma burden. There is evidence of some gene-environmental interactions, but these relationships are varied and complex and have not yet been fully investigated or understood. Elucidating the interactions between environmental risk factors and genetic factors and their associations with respiratory outcomes in both childhood and adulthood may help identify asthma prevention strategies.

The main aim of my PhD work was to investigate one group of gene-environment interactions; the relationship between oxidative exposures and antioxidant genes on asthma and lung function. Specifically, I investigated the associations between various household environmental oxidant exposures and respiratory outcomes from childhood to middle age and examined whether those associations were modified by Glutathione s-Transferase (GST) genes, which are known buffers of oxidative stress.

Methods: I used a range of methods to address my research aims, including a systematic literature review and data analyses of two longitudinal cohort studies: the Melbourne Atopy Cohort study (MACS), and the Tasmanian Longitudinal Health Study (TAHS).

MACS began as a randomised controlled trial investigating the effects of infant formulas at weaning and, has subsequently become a prospective birth cohort. Initially, 620 pregnant women were recruited between 1990 and 1994. The MACS children have now been followed up to 18 years of age. The TAHS is a population-based study of respiratory disease spanning from childhood to adulthood. The study began in 1968, recruiting 8,583 Tasmanian school children born in 1961 (approx. 98.8% of the Tasmanian population for 1961 births). Extensive follow-ups of initial participants were conducted in 2002 and 2012, respectively, when participants were in their 40s and 50s.

Both the MACS and the TAHS collected a substantial amount of exposure and outcome data at multiple ages over the lifespan through questionnaires and clinical testing, providing a great opportunity to address questions concerning gene-environment interactions and respiratory health outcomes.

I investigated the following oxidative exposures: early life tobacco smoke, early life paracetamol, heating and cooking facilities, mould, long term and current active and passive tobacco smoke. Previous evidence suggests that these common home environmental exposures are associated with increased oxidative stress, and therefore, their impacts are likely to be modified by GST genes. So, in conjunction with these exposures, I investigated potential interactions with GST gene polymorphisms at two significant developmental stages: during lung function growth in childhood and adolescence, and during lung function decline in middle age.

Research Gaps: Firstly, I systematically reviewed the current evidence on interactions between indoor air pollution and GST genes in association with allergies, asthma and lung function (**Aim 1: Chapter 3**). I then conducted a study to examine the interactions between early life tobacco smoke exposure and GST genes on asthma and lung function in childhood and adolescence using Melbourne Atopy Cohort Study (MACS) data (**Aim 2: Chapter 5**). Another study on the potential interactions between paracetamol use before 2 years of age and GST genes in association with asthma and lung function in later life was performed using MACS data (**Aim 3: Chapter 6**). In this study, I adjusted for early life exposure to respiratory infections, an important confounding factor which has not been adjusted for by many previous studies. Finally, I conducted a study using data from the TAHS to investigate similar associations in middle-age (**Aim 4: Chapter 7**). In this analysis, I identified seven longitudinal exposure profiles using Latent Class Analysis (LCA) methods with common household air exposures including heating and cooking types, mould exposure, passive and active smoking. I then determined the associations between these risk profiles and respiratory outcomes, and whether these profiles were modified by GST genes. Linear and logistic regression were used to investigate these associations.

Results:

Aim 1. My systematic review of the relationship between indoor air pollution, GST genes and asthma identified 22 eligible studies, with 15 finding some evidence of gene-environment interactions. Overall, carriers of GSTM1/T1 null and GSTP1 Val105 genotypes exposed to indoor air pollution, were more susceptible to developing asthma and reduced lung function. However, these findings were more consistent in studies of children compared to studies of adults (**Chapter 3**).

Aim 2. I found evidence that early life tobacco smoke exposure was associated with an increased risk of asthma, reduced lung function growth between 12 and 18 years, and reduced lung function at 18 years, with girls appearing to be more susceptible than boys (master research program). I also found an interaction between early life tobacco smoke exposure and GST genotypes on lung function at both 12 and 18 years. Carriers of GST null mutations and GSTP1 Ile/Ile alleles were more susceptible to tobacco smoke in early life, and these associations were not found in carriers of other GST genotypes (**Chapter 5**).

Aim 3. I found some evidence that early life paracetamol use may be associated with impaired lung function and increased risk of asthma in adolescence after adjustment for the frequency of early life respiratory tract infections. Interaction analysis suggested that these associations were only for carriers of GSTM1 null and GSTP1 Ile/Ile genotypes, I did not find evidence for carriers of other GST genotypes (**Chapter 6**).

Aim 4. I found that the exposure profiles of “Wood heating”, “All gas”, “Wood heating/smoking” and “Wood heating/gas cooking” were associated with persistent asthma and greater lung function decline by age 53 years. Carriers of GSTP1 Ile/Ile genotypes had a greater risk of asthma at age 53 years with exposure to “All gas” and “Wood heating/smoking” compared to a reference group

(reverse cycle air conditioning, electric cooking and no smoking). Carriers of GSTM1 null and GSTP1 Ile/Ile genotypes had a greater risk of accelerated lung function decline when exposed to "Wood & gas heating/gas cooking/smoking" and "Wood heating/gas cooking" compared this reference group. These associations were not observed in carriers of other genotypes (**Chapter 7**).

Conclusions: My work identified several household risk factors associated with the progression of asthma, limited maximal lung function development in childhood, or accelerated lung function decline in adulthood. Additionally, this work provides evidence on interactions between household exposures and GST polymorphisms and has contributed significantly to our understanding of gene-environment interactions in relation to respiratory health. These findings highlight the importance of considering potential gene-environment interactions in studies that investigate exposures associated with lung oxidative stress. These findings have the potential to inform guidelines and preventive strategies, especially for people who are at increased risk.

Declaration

This is to certify that

I. The thesis comprises only my original work towards the PhD, except where indicated in the acknowledgements.
II. Due acknowledgement has been made in the text to all other material used.
III. The thesis is less than 100, 000 words in length, exclusive of tables, bibliographies and appendices.

Xin Dai

Preface

Within this thesis, I have included two published papers, one manuscript in revision following peer review and one manuscript submitted for publication (all with multiple co-authors) as thesis chapters. I am the first author for all four papers.

I wrote the initial draft of all the four papers included in this thesis and was responsible for the literature search and statistical analyses in each of the papers. I helped with data collection and analytical design, interpretation of the results and editing of manuscript drafts for the additional papers that I co-authored during my PhD. As such, I have provided authorisation forms from the co-authors of each published manuscript, as well as declarations of collaborative work from my principal supervisor. All the work included in this thesis was carried out after my enrolment as a higher degree research student.

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

Xin Dai

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Acknowledgement

My sincere thanks go to my supervisors and in particular, my principal supervisor, Dr. Caroline Lodge for her endless support, patience and constant encouragement throughout the 4 years of my candidature. I also express my thanks to my co-supervisors: Professor Shyamali Dharmage, Associate Professor Adrian Lowe, Dr. John Burgess and Associate Professor Aung Win. Each has been remarkably generous in making time available to critically evaluate my work and in giving freely of their expertise. I would like to extend my gratitude to Associate Professor Adrian Lowe for his assistance with paracetamol exposure definitions and categories used in the analyses in Chapter 5. I would also like to thank Professor Michael Abramson for his guidance, support, and critical input with analytical designs and manuscript writing for my four PhD papers.

I was also fortunate to be part of a cohort of very friendly and supportive postgraduate students, postdoctoral researchers, and research assistants. In particular, I would like to acknowledge Dr. Gayan Bowatte, Dr. Dinh Bui, Dr. Jennifer Perret, Dr. Nilakshi Waidyatillake, Ms Sophie Yorkston, Ms. Raisa Cassim, Ms Brittany Campbell and Dr. Danial Tan for always being very helpful.

I acknowledge the founders of the cohort studies in which my PhD was based: MACS (Dr. David Hill, Dr. Cliff Hosking and Dr. John Thorburn) and TAHS (in 1968, H. Gibson, B. Gandevia and H. Silverstone), as well as all the current and past MACS and TAHS investigators. I would also like to acknowledge all the funding bodies that supported the follow-ups of both cohorts including the Australian National Health and Medical Research Council (NHMRC).

I thank the NHMRC for providing funding for my postgraduate scholarship for the first 3 years of my candidature. I would also like to acknowledge the Murdoch Children's Research Institute (MCRI) and Professor Shyamali Dharmage, for arrangement of flexible work hours for my last half of the 4th year, which provided me with financial support and enabled me to complete my thesis.

Finally, I would like to thank Yuntao Yu, my husband for his always believing in me and supporting me during the tough times in my life. Lastly and most importantly, I would like to thank my parents and friends, who were with me at every step of my PhD and for their loving encouragement that kept me moving forward during my PhD candidature.

最后，我要感谢我的丈夫俞云涛，谢谢他一如既往的相信我支持我。我要感谢我的家人和朋友，没有他们的鼓励和支持，我不会有勇气在艰难的博士生涯中，苦中作乐地向前走。长风破浪会有时，直挂云帆济沧海；剧辛乐毅感恩分，输肝剖胆效英才。

(This is the translation of the last paragraph into Chinese).

Publications and presentations

The publications including conference abstracts and conference presentations resulting from this thesis are listed below. In addition, I have listed other publications including the ones that I co-authored during the course of my PhD.

Fist author publications arising from this thesis

1. Dai, X, Dharmage, S.C., Bowatte, G., Waidyatillake, N., Perret, J., Hui, J., & …Lodge, C.J (2019). Interaction of Glutathione S-Transferase M1, T1, and P1 Genes with early life tobacco smoke exposure on lung function in adolescents. *Chest, 155*(1), 94-102.

2. Dai, X., Bowatte, G., Lowe, A. J., Matheson, M. C., Gurrin, L. C., Burgess, J. A., & ... Lodge, C. J. (2018). Do Glutathione S-Transferase Genes Modify the Link between Indoor Air Pollution and Asthma, Allergies, and Lung Function? A Systematic Review. *Current Allergy And Asthma Reports*, (3), 1. doi:10.1007/s11882-018-0771-0

3. Dai, X., Dharmage, S., Abramson, M., Erbas, B., Bennett, C., Svanes, C., … & … Lodge, C.J. Associations between acetaminophen exposure up to 2 years and lung function at 18 years are modified by Glutathione s-Transferase genes in a prospective birth cohort (Submitted for publication to *Journal of Allergy and Clinical Immunology on 14/08/2019*).

4. Dai, X., Bui., D., Perret, J.L., Lowe, A., Frith, P.A., Bowatte, G., …& …Lodge, C.J. Exposure to household air pollution over 10 years is related to asthma and lung function decline. (In revision following peer review by *American Journal of Respiratory and Critical Care Medicine*).

Other co-authored publications during the course of this PhD:

1. Doherty, A. M., Lodge, C. J., Dharmage, S. C., Dai, X., Lowe, A. J., & Bode, L. (n.d). Human Milk Oligosaccharides and Associations With Immune-Mediated Disease and Infection in Childhood: A Systematic Review. *Frontiers In Pediatrics, 6*
2. Tan, D.J., Bui, D.S., Dai, X., Lodge, C.L., Lowe, A.J., Thomas, P.S., … & …Dharmage, S. C..Dose the use of inhaled corticosteroids in asthma benefit lung function in the long-term? A systematic review and meta-analysis. (In revision following peer review by *Lancet Respiratory medicine*).

Conference abstracts:

1. Dai, X., Dharmage, S.C., Abramson, M., Erbas, B., Waidyatillake, N., Lowe, A J., Lodge, C.J. GST genotypes modify the association between paracetamol use in early life and lung function at 18 years. European Respiratory Society (ERS) International Congress 2018 in Paris, France.
2. Dai, X., Dharmage, S.C., Walters, E.H., Lowe, A., Burgess, J., Win, A., & …Lodge, C.J. Household exposures, Glutathione S-Transferase genes & asthma risk in middle age. The Joint Annual Meeting of the International Society of Exposure Science and the International Society for Environmental Epidemiology (ISES-ISEE 2018) in Ottawa, Canada.
3. Dai.X, Dharmage, S., Bowtte, G., Waidyatillake, N., Perret, J,. Hui, J., & …Lodge, C. J. Glutathione S-Transferase (GST) genes modify the adverse impact of early life tobacco

smoke on lung function. The 29th Annual Scientific Conference of the International Society of Environmental Epidemiology (ISEE 2017) in Sydney, Australia.

4. Dai, X., Bowatte, G., Lowe, A. J., Matheson, M. C., Gurrin, L. C., Burgess, J. A., & ... Lodge, C. J. Do variants in GST genes modify the adverse impacts of indoor pollutants on allergies, asthma and lung function? Cleaner Air, Better Health: CAR Annual Symposium 2016 in Sydney, Australia.

Conference presentations and talks:

1. Dai, X., Dharmage, S.C., Abramson, M., Erbas, B., Waidyatillake, N., Lowe, A J., Lodge, C.J. Paracetamol use in infancy is linked to increased risk of asthma in some teenagers: Specific variants in the GST genes seems to play a role in greater susceptibility to lung problems. Invited presenter for Official ERS International Congress press briefing 2018, Paris France.
2. Dai, X., Dharmage, S.C., Abramson, M., Erbas, B., Waidyatillake, N., Lowe, A J., Lodge, C.J. GST genotypes modify the association between early life paracetamol use and respiratory health at 12 and 18 years: Specific variants in the GST genes may play a role in greater susceptibility to lung problems. Invited presenter for Session "Recent insights into childhood asthma and wheezing" in ERS Paris 2018 International Congress, France.
3. Dai, X., Bowatte, G., Lowe, A. J., Matheson, M. C., Gurrin, L. C., Burgess, J. A., & ... Lodge, C. J. Do Glutathione S-Transferase Genes Modify the Link between Indoor Air Pollution and Asthma, Allergies, and Lung Function? A Systematic Review and meta-analysis". Cleaner Air, Better Health: CAR Annual Symposium 2016 in Sydney, Australia.

Press releases:

1. Genotypes linked to increased breathing issues following early tobacco exposure: GST null mutations, GSTP1 Ile/Ile alleles more susceptible to asthma and decreased lung function. Published in official website of American College of Chest Physicians in 4 January, 2019.
2. Paracetamol use in infancy is linked to increased risk of asthma in some teenagers: Specific variants in the GST genes seem to play a role in greater susceptibility to lung problems. Published in Official website of ERS in 17 September, 2018.

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1. National Health and Medical Research Council (NHMRC) postgraduate scholarship, Australia, 2015-2018
2. Population Health Investing in Research Students' Training travel award (PHIRST), Melbourne School of Population and Global Health, University of Melbourne, Australia, 2018.

Manuscript Reviews for journals:

1. Dai, X., Manuscript reviewer for *Journal of asthma* in 2016.
2. Dai, X., Manuscript reviewer for *American Journal of Respiratory and Critical Care Medicine* in 2019.

Training workshop

1. Completed school subject POPH90111 Genetic Epidemiology with grade H2A.

Abbreviations

AD:	Atopic Dermatitis
ALSPAC:	Avon Longitudinal Study of Parents and Children
BD:	Bronchodilator
BHR:	Bronchial Hyperresponsiveness
CAMP:	Childhood Asthma Management Program
CHS:	Children's Health Study
CI:	Confidence Interval
CO_2:	Carbon Dioxide
COPD:	Chronic Obstructive Pulmonary Disease
DAG:	Directed Acyclic Graph
DALY:	Disability-Adjusted Life Years
ECRHS:	European Community Respiratory Health survey
ELSPAC:	European Longitudinal study of Pregnancy and Childhood
ETS:	Environmental Tobacco Smoke
FeNO:	Fractional exhaled Nitric Oxide
FEV_1:	Forced Expiratory Volume in 1 second
FVC:	Forced Vital Capacity
GINA:	Global Initiative for Asthma
GLI:	Global Lung Function Initiative
GST:	Glutathione S-Transferase
GSTM1:	Glutathione S-transferase mu 1
GSTP1:	Glutathione S-transferase pi 1
GSTT1:	Glutathione S-transferase theta 1
GWAS:	Genome-Wide Association Study
HAP:	Household Air Pollution
HMOX-1:	Heme Oxygenase 1
IgE:	Immunoglobulin E
ISAAC:	International Survey of Asthma and Allergies in Childhood

LCA:	Latent Class Analysis
LHS:	Lung Health Study
LRTI:	Lower Respiratory Tract Infections/symptoms
MACS:	Melbourne Atopy Cohort Study
MEF:	Mid-Expiratory Flow
Moba:	Mother and Child Cohort study
NAPQI:	N-acetyl-p-benzo-quinone imine
NDI:	National Death Index
NO_2:	Nitrogen Oxides
NOS:	Newcastle-Ottawa Quality Assessment Scale
NQO1:	NAD(P)H Quinone Dehydrogenase 1
O_3:	Ozone
OM:	Otitis Media
OR:	Odds Ratio
PC20:	Provocative Concentration causing a 20% decline
PCAAS:	Perth Childhood Acute Asthma Study
PCR:	Polymerase Chain Reaction
PEFR:	Peak expiratory flow rate
PIAMA:	Prevention and Incidence of Asthma and Mite Allergy
PM:	Particulate Matter
$PM_{2.5}$:	Particulate Matter <2.5µm in diameter
PM_{10}:	Particulate Matter <10µm in diameter
RCT:	Randomized Controlled Trial
RNS:	Reactive Nitrogen Specie
ROS:	Reactive Oxygen Specie
RTI:	Respiratory Tract Infection
SAPALDIA:	Swiss Study on Air Pollution and Lung Diseases in Adults
SD:	Standard Deviation
SE:	Standard Error
SES:	Social-Economic Status

SNP:	Single Nucleotide Polymorphisms
SO_2:	Sulphur Oxidase
URTI	Upper Respiratory Tract Infection

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Chapter 1 INTRODUCTION

1.1 Overall aim

The aim of my doctoral research was to investigate if common environmental exposures associated with oxidative lung stress were associated with an increased risk of subsequent asthma and/or decreased lung function in later life, and whether those associations were modified by glutathione S-transferases (GST) genes. I have described the rationale for my doctoral work and the specific objectives below.

1.2 Rationale

Oxidative stress is a pathophysiological condition resulting from an imbalance between oxidants and antioxidants. The lungs, being constantly exposed to the external environment, are at high risk of oxidative stress. It is a significant factor in asthma development and exacerbations and is associated with ongoing airway inflammation, airway damage and lung function decline. There are several common sources of oxidant production in the environment to which people are routinely exposed. These sources include tobacco smoke, outdoor air pollution (ozone, carbon dioxide (CO_2), Sulphur oxidase (SO2), Nitrogen oxides (NO2) and volatile organic compounds (VOC)), household pollutants (heating, cooking, mould, etc.) and pharmaceutical agents.

Evidence concerning outdoor air pollution and its relationship with oxidative stress and subsequent asthma has been the subject of recent research, but there is currently a lack of evidence relating to indoor pollution and pharmaceutical agents.

To manage the impacts of oxidative stress, the human lungs are equipped with a wide range of enzymatic antioxidant defences including GSTs which are coded for by the GST gene superfamily and involved in the detoxification of reactive oxygen species (ROS). Complex interactions between these individual genetic capabilities and environmental exposures have been implicated in the pathogenesis of asthma. However, the relationship between polymorphic variants of GST genes and

environmental factors on respiratory health, especially in early life, have not been well explored.

1.2.1 What is asthma?

Asthma is a disease of the lungs characterized by airway inflammation and reversible airway obstruction. People with asthma often experience respiratory symptoms like breathlessness, wheezing, tightness in the chest and cough, which may affect their quality of life. Asthma is characterized by chronic inflammation of the lung airways (bronchi, bronchioles), with repeated attacks in which the airways are suddenly blocked, partially or completely, by inflammation [1, 2].

Asthma is usually episodic; however, some severe cases may have continuous symptoms or frequent asthma attacks, even when high dose medication is used regularly and correctly. Severe asthma is likely to be related to several factors, for instance personal smoking [3] and genetic susceptibility [4].

Inflammatory episodes commonly occur in response to allergen exposures in sensitized individuals. Although these allergens may be harmless for people without asthma, sensitized people may react to allergen exposure with airway inflammation in allergic asthma. Sensitized individuals commonly have elevated serum levels of Immunoglobulin E (IgE) antibodies which can be specific to various allergens. This characteristic (increased specific IgE), often associated with asthma, is defined as *atopy*. In particular, childhood asthma is often associated with atopy.

"Asthma" is an umbrella term for a number of multifactorial diseases resulting in multiple heterogeneous clinical phenotypes. Besides airway inflammation, reversible airflow obstruction and reduced lung function are also characteristic features of asthma. Many people with chronic and/or severe asthma experience an accelerated and progressive loss of lung function over time and their lung function parameters progress from reversible to irreversible airway obstruction [5]. Lung function tests are important as these tests measure how well the lungs are working and how asthma may be affecting a patient's breathing. If people have, or are suspected to have, asthma, lung function testing provides objective data to assist in

the diagnosis of asthma and monitor the course of the disease or response to treatment over time.

1.2.2 Asthma is a significant public health problem

Asthma is the one of the most prevalent chronic non-communicable diseases, affecting an estimated 330 million people of all ages globally [6]. Over the past 20 years, the prevalence of asthma has been increasing significantly in developing countries. However, in developed countries with an already high prevalence, it appears to have plateaued and remained high [6]. The prevalence of asthma in Australia is high in global terms, with 9.9% of the population (2.4 million) reporting current doctor-diagnosed asthma in 2015, and the most recent asthma expenditure data, from 2008-2009, estimated the cost of asthma to be $655 million, accounting for 0.9% of the total health expenditure for Australians [7]. Asthma also has substantial impacts on quality of life, such that individuals with asthma are more likely to report fair or poor health compared to individuals without this condition [8].

Asthma creates substantial health and economic burdens for individuals, families and communities worldwide, and particularly locally, in Australia. Hence, asthma is recognised as a significant public health problem and it has been a National Health Priority in Australia since 1999 [9]. Understanding the causes of, and mechanisms for, the development of asthma is crucial for effectively producing preventive strategies for managing the increasing and ongoing burden of asthma.

1.2.3 Why study the role of oxidative exposures in asthma?

The causes of asthma are not fully understood, although harmful environmental exposures have long been considered as risk factors for impaired respiratory health. There is some evidence that exposure to common household factors, including commonly used pharmaceuticals associated with oxidative stress, may influence lung health, particularly when exposure to these risk factors occurs during vulnerable periods such as early life [10-14]. However, findings on the relationship

between some of these exposures and respiratory health outcomes have been inconsistent.

One possible reason for these inconsistent findings is the lack of long-term longitudinal studies to investigate these relationships. Early life exposures may influence childhood lung development, potentially leading to diminished airway function that is subsequently associated with the occurrence of asthma and respiratory illness, even into adulthood [15]. Individuals prevented from achieving maximal levels of lung function in early adulthood due to adverse childhood exposures, are possibly at an increased risk of non-reversible air flow obstruction later in life (e.g. Chronic Obstructive Pulmonary Disease (COPD)). However, some studies have not found evidence to support these links [16-18].

Apart from cigarette smoke, many indoor air exposures have not been longitudinally studied to investigate their potentially adverse effects on respiratory health. In our daily life, common indoor air risk factors for asthma are constantly present in the air we breathe. Moreover, this is a significant route of exposure as we spend around 90% of our time indoors. These exposures have the potential to cause acute asthma attacks or exacerbate existing asthma.

1.2.4 Why study gene-environment interactions in asthma?

A further reason for the inconsistent findings between common oxidative household exposures and respiratory health could be the failure to account for potential gene-environment interactions. One key gene family proposed to be important in modifying the effects of environmental exposures on respiratory health through its actions as part of the antioxidative stress pathway is the GSTs super gene family [19, 20]. GSTs defend tissues against oxidative stress by conjugating reactive oxygen species with glutathione to assist in detoxification and elimination. GST polymorphisms associated with reduced enzymatic activity are therefore of interest in relation to asthma susceptibility. Although the independent effect of GST genes may be very small, their interactions with specific environmental exposures can lead to much larger effects.

Over the last decade, there has been increasing evidence to suggest that gene-environment interactions are likely to be involved in the development, severity, and age-of-onset of many complex diseases including asthma [2, 21]. It is possible that individuals with risk variants of GST genes may be more susceptible to environmental risk factors and more likely to develop asthma, compared to those without risk genes, even when exposed to the same environmental factors. However, few long-term studies have focused on GST gene-environment interactions and their associations with asthma and lung health in adolescence through to adult life, and the findings are inconsistent [16, 22]. The majority of these existing studies have been carried out in children and have only short follow-up periods. In order to further our knowledge on the aetiology of asthma and establish strategies to prevent its development and persistence, there is an urgent need to explore the complex mechanisms behind environmental and genetic determinants.

1.3 Context of my doctoral work

For my doctoral work, I investigated the associations between various environmental oxidant exposures and respiratory outcomes from childhood to middle-age and determined whether these associations were modified by common polymorphisms of selected GST genes. I utilised the Melbourne Atopy Cohort Study (MACS) to examine these associations and interactions in childhood and adolescence, and the Tasmanian Longitudinal Health Study (TAHS) to explore these associations into middle-age. My PhD work focused on common household exposures and their potential interactions with GST genes on two respiratory outcomes: the occurrence of asthma and, reduced lung function. I chose these outcomes because asthma is one of the most important chronic respiratory diseases and impaired lung function is a predictor of future morbidity and mortality [23].

1.4 Objectives

My PhD work involved four research objectives. I performed a systematic review to address the first objective and original data analyses for the remaining three.

Specifically, the four topics that I addressed within this thesis are:

1. To determine if GST genotypes modified the association between indoor air pollution and allergies, asthma and lung function, through conducting a systematic review of existing evidence.
2. To investigate the relationship between early life tobacco smoke exposure and lung health in adolescence and determine if this relationship is modified by genetic differences in GSTM1 null, GSTT1 null and GSTP1 genotypes.
3. To investigate the relationship between paracetamol exposure during early life and subsequent asthma and lung function up to 18 years and determine if this relationship is modified by genetic differences in GSTM1 null, GSTT1 null and GSTP1 genotypes.
4. To determine whether ten-year longitudinal environmental exposures such as heating and cooking facilities, mould, active and passive smoking, along with specific GST polymorphisms, are related to increased risk of asthma in middle age.

1.5 Thesis statement

This thesis is based on the original research I conducted during my PhD candidature. Data from the MACS have been used to examine the interaction between early life exposures and GST genotypes on the risk of asthma and decreased lung function in childhood and adolescence. The TAHS, another longitudinal study, was used to explore the interaction between environmental exposures and GST genes on the risk of asthma over a ten-year period in middle-age. The strengths of this doctoral research include the utilisation of substantial exposure data, including early life factors, at multiple time points over the life of the cohorts and large sample sizes to investigate complex gene-environment interactions in relation to asthma and lung function.

1.6 Thesis overview

Chapter 1: Summary of the rationale, objectives and overview of my thesis.

Chapter 2: Literature review on the associations and interplay between environmental exposures, polymorphisms of GST genes and risk of asthma and reduced lung function.

Chapter 3: Published systematic review titled, “Do Glutathione S-Transferase Genes modify the link between indoor air pollution and asthma, allergies, and lung function? A systematic review.”

Chapter 4: Description of the methods used in this thesis and a description of the MACS and TAHS, outcome and exposure definitions, genotyping methods and statistical methods.

Chapter 5: Published research article titled, “Interaction of Glutathione S-Transferase M1, T1, and P1 genes with early life tobacco smoke exposure on lung function in adolescents”.

Chapter 6: Submitted original research article titled, “Early life acetaminophen exposure, Glutathione s-Transferase genes and development of adolescent asthma”.

Chapter 7: In revision following peer review original research article titled, “Exposure to household air pollution over 10 years is related to asthma and lung function decline”.

Chapter 8: Overall discussion of my research in this thesis, the strengths and limitations and future recommendations and directions based on my findings.

Chapter 2 LITERATURE REVIEW

My literature review provides a comprehensive background for my thesis research. In this chapter, I provide an overview of asthma including the definitions of asthma used in research, descriptions of the pathology and pathophysiology of asthma, delineation of the burden of asthma (global and Australian) and a summary of the existing epidemiological evidence concerning the relationships between environmental exposures and asthma. This review covers a range of common risk exposures for asthma including air pollution, tobacco smoke, nutrition, respiratory infection and medication use.

This chapter also discusses the importance of lung function in health. I describe the natural history and measurement of lung growth and decline and lung function in healthy people, as well as asthma in relation to reduced lung function in childhood and its long-term impact on lung health.

I further discuss these relationships with reference to interactions with genetic predisposition. Although some studies have examined the interactions between environmental exposures and genetics in relation to asthma, the findings are inconsistent and limited studies have reported lung function outcomes together with asthma.

Additionally, I discuss oxidative stress and its potential role as an important mechanism related to asthma development and exacerbation. Using this paradigm, I discuss how indoor environmental exposures may cause asthma and other respiratory diseases in susceptible individuals with impaired ability to manage oxidative stress. I focus on the- Glutathione s-Transferase (GST) gene superfamily, reviewing current evidence on associations between GST genes and respiratory health. Additionally, I specifically review the literature on whether GST genes modify the associations between environmental exposures and risk of asthma and reduced lung function. Finally, I outline the current knowledge gaps in this area that require attention.

2.1 Asthma

2.1.1 Definition of asthma

Key messages in this section

Asthma can be defined in the following ways:

- Clinically by cough, chest tightness, shortness of breath and wheeze.
- Pathologically, by bronchial inflammation with prominent eosinophil infiltration.
- Physiologically, by bronchial hyper-reactivity with fluctuations in lung function.
- Many different definitions are used in epidemiological studies. As well as the previous definitions. It is common to use categorizations or phenotypes of asthma such as atopic asthma, early onset asthma, and multi-trigger wheeze.

2.1.1.1 Clinical symptoms

Asthma is defined by The International Consensus Report on the Diagnosis and Treatment of Asthma as

> *"A chronic inflammatory disorder of the airways in which many cells play a role, including mast cells and eosinophils. In susceptible individuals, this inflammation causes symptoms that are usually associated with widespread but variable airflow obstruction that is often reversible, either spontaneously or with treatment, and causes an associated increase in airway responsiveness to a variety of stimuli." [24]*

Asthma is a complex and heterogeneous disease that is not easy to diagnose clinically. A diagnosis of asthma is generally based on the presence of several indicative symptoms along with a personal and family history and clinical tests to support the diagnosis. Clinically, people who are affected by asthma may experience chest tightness, breathlessness, wheezing and coughing [1]. Other common symptoms include trouble sleeping, reduced activity due to cough, frequent respiratory infections, frequent episodes of "flare-ups", anxiety, rapid heartbeat,

rapid breathing and throat irritation. However, there are no definitive criteria to decide whether people with such symptoms have asthma or not. All current asthma definitions require the presence of respiratory symptoms (wheezing, breathless, chest tightness and/or cough), and spirometric lung function can be used to identify airflow obstruction and aid in asthma diagnosis [25, 26]. However, more recent asthma definitions have also included airway hyperresponsiveness and airway inflammation as non-essential components of the disease [26].

2.1.1.2 Pathology

During normal breathing, air is drawn into the nose and mouth, then into the pharynx, larynx, trachea and bronchi, before entering the lungs. In the lungs, the air moves through progressively smaller divisions of the bronchial tree to reach the bronchioles and then flows into alveoli, where gas exchange occurs[1]. The bronchioles are encircled with involuntary smooth muscle and the air/body interface is lined by columnar epithelium and mucous secreting cells which secrete mucous into the airway lumen [27]. People with asthma tend to have very sensitive airways that may react to seemingly innocuous substances in the environment. When this happens, several events may take place inside the airway, including: contraction of bronchial smooth muscle causing airway narrowing; inflammation and consequent swelling in the walls of the bronchioles; increased mucous production filling the airway lumen. These pathological processes all cause narrowing or blocking of the lumen of the airways and result in impaired breathing. The main symptoms caused by this process are described as “wheezing” (the high-pitched sound made when air travels through a narrowed airway), “shortness of breath” and “chest tightness”. Apart from asthma symptoms as mentioned above, asthma attacks may also lead to other adverse respiratory outcomes including collapsed lungs, lung infections, chronic dilation of the bronchioles (bronchiectasis), rib fractures, a permanently enlarged chest cavity and respiratory failure [1].

Although rare, asthma attacks may also cause death. In individuals who have died from acute asthma, autopsies have revealed the lung airways (small, medium and large) to be completely blocked with thick tenacious mucus, causing pulmonary

collapse [28]. Fortunately, in most cases, asthma symptoms can be managed with bronchodilator and corticosteroid medications [29, 30]. However, there is still limited published evidence from existing studies to determine whether current asthma therapies help to prevent the progressive loss of lung function seen in asthma [31]. The most recent meta-analysis (unpublished) found that long-term inhaled corticosteroids in asthma are only associated with modest improvements in lung function in adults, but not in children [32]. Furthermore, it is not known if these improvements are maintained in the long term or prevent excess lung function decline, fixed airway obstruction, or airway remodelling.

2.1.1.3 Pathophysiology

One of the major inflammatory responses in asthma results in the activation and degranulation of mast cells in the airway [33]. One important pathophysiological feature of atopic asthma is elevated immunoglobulin E (IgE) [1]. In atopic asthmatics, IgE production is triggered by foreign antigens (allergens) in the airways. When allergens interact with the mucosal airway surface, the immune system may be stimulated to produce IgE to specifically target the foreign allergen [1]. This specific IgE is bound to mast cell surfaces. On re-exposure to the same allergen, binding of the allergen to two or more IgE molecules (cross-linking) on the mast cells causes them to degranulate, releasing the pro-inflammatory mediators including histamine, proteases, tissue necrosis factors, platelet-activating factors, chemokines and cytokines [34]. The chemokines and cytokines released recruit and activate eosinophils [34]. When these activated eosinophils in-turn degranulate, they cause bronchoconstriction and inflammation in people with asthma [33, 34]. Elevated numbers of eosinophils are often found in the sputum, airways and blood of asthmatic patients [28] and are characteristic of atopic asthma. Ongoing inflammation results in airway remodelling including epithelial damage, sub-basement membrane thickening, and hypertrophy of bronchial smooth muscle, resulting in bronchial reactivity and airflow obstruction [33].

Allergic triggers may include dust, pollens, mould, animal dander and other substances. People with allergic asthma often have other allergies like eczema,

dermatitis, hay fever and/or food allergies [35]. By contrast and definition, non-atopic/non-allergic asthma is not associated with increased IgE antibodies, and people who suffer from non-allergic asthma have hyperactive airways that may react to other irritant exposures including exercise, cold air, tobacco smoke and air pollution [1].

2.1.1.4 Defining asthma in epidemiological studies

Despite the known clinical and pathophysiological features of asthma [33], no standardised definition is used in epidemiological studies. Defining asthma in epidemiological studies is commonly based on: self-report of asthma, doctor's diagnosis of asthma, self-report of doctor's diagnosis of asthma, self-report of respiratory symptoms typical of asthma, respiratory tests demonstrating typical asthmatic findings, or biomarkers consistent with asthma. Non-standardized asthma definitions mean that the results from different studies may not be comparable. For instance, in the Childhood Asthma Management Program (CAMP), diagnosis of asthma was based on methacholine hyperreactivity [PC20 (provocative concentration causing a 20% decline in forced expiratory volume in 1 s (FEV1)) >12.5 mg/ml] plus respiratory symptoms [36]; in the Perth Childhood Acute Asthma Study (PCAAS) confirmed asthma status was based on Emergency department medical records [37]; and the Taiwanese Children's Health Study (CHS) used self-reported asthma status. However, most epidemiological studies have used questionnaires asking about typical asthma symptoms to define asthma, (e.g. the presence of recent wheeze (usually in last 12 months) to define current asthma), because of the benefits of low cost, convenience and the ability to recruit a relatively larger sample. The most commonly used core questionnaire to define current wheeze is from the International Study of Asthma and Allergies in Childhood (ISAAC) [38], "Have you had wheezing or whistling in the chest in the past 12 months?" [39]. Participants who answer "yes", can then be defined as having "current wheeze" or "current asthma symptoms". This is a highly sensitive definition that tends to detect most cases of current asthma. Although there is likely substantial overlap in the individuals identified by each definition, it is important to recognize the variety of

methodology used in epidemiological studies as this may explain some inconsistency in the results and help us to better understand the relationship between asthma and potential risk factors in different settings.

The best method for identifying asthma is also relevant to the study population of interest. In older children or adults, the presence of current wheeze is often considered to mean current asthma, whereas wheeze in younger children (<2 years) may not develop into asthma. Around 50% of children who wheeze in the first two years of life are symptom-free before entering primary school [40]. In many people who don't have asthma, especially young children, wheeze may be caused by an intercurrent infection (viral respiratory tract infection) [41]. This wheeze resolves with resolution of the infection and is not asthma. Additionally, most asthma symptoms improve or disappear with asthma medication, so questionnaires also ask about asthma treatments or medications, as they can also be indicators of current non-symptomatic asthma. Given the limitations described above, objective measurements can be used in conjunction with reported symptoms to more accurately identify asthma. A thorough history and clinical examination by a physician with measurements of airway function such as bronchial hyperresponsiveness (BHR) testing and lung function testing would be ideal.

Asthma phenotypes

The underlying causes, inflammatory processes and clinical symptoms for asthma can vary from patient to patient, and even change over time in the same patient. For this reason, it is widely believed that "asthma" is an umbrella term describing a collection of respiratory diseases with different phenotypes, rather than a single disease. Delineating asthma into discrete phenotypes increases our understanding of the underlying pathobiology and allows us to develop phenotype-specific strategies for targeted asthma prevention and management. Identification of asthma phenotypes also enhances our ability to link characteristic genetic traits with the specific pathobiological features involved this complex disease. Many studies have used specific asthma phenotypes as outcomes. In this section, I will

briefly introduce several commonly used phenotypes categorized by age of onset, symptoms, and biomarkers.

Defined by sensitization to allergens and other immune response markers: Allergic and non-allergic asthma phenotypes/Atopic or non-atopic phenotypes

These are amongst the most frequently investigated phenotypes. Allergic (or atopic) asthma is common in children and linked to family history and poorer lung function outcomes. Allergic asthma accounts for slightly more than 50% of the patients with asthma [42]. Allergic asthma is characterized by a range of manifestations in the airways, including increased eosinophils in bronchial mucosa, and elevated T helper 2 (Th2)-type cells, IL-4/ IL-5, and specific IgE (sIgE) along with positive skin prick tests to specific allergens (commonly dust, pollens, foods, moulds, insects and animals) [43]. Allergic asthma can be identified using tests for allergic sensitisation. Skin prick tests and/or sIgE levels are typically used to identify allergic sensitisation and determine atopic status in asthmatics and define the allergic (or atopic) asthma phenotype. Conversely, non-allergic asthma is defined by having asthma but no sensitization and usually shows no clinical evidence of sIgE-mediated allergy to common environmental agents [43]. However, some authors believe that non-atopic asthmatics also show evidence of an immune response with increasing infiltration of eosinophils, Th2, IL-4 or IL-5 in the bronchial mucosa. It is possible that non-atopic asthmatics may actually be atopic/allergic but the allergen has yet to be identified [43]. In these cases, IgE production appears to be local rather than generalized, as it is in atopic asthmatics [43]. There is also evidence that non-atopic asthma may be associated with female gender, older age, nasal polyps and aspirin sensitivity [42].

Defined by clinical features – Age of onset: Though asthma may begin at any time of life, categorized phenotypes based on the age of asthma onset (early-onset asthma and late-onset asthma) are often used in epidemiological studies. Early onset asthma usually describes asthma that starts in early childhood, and late onset asthma is usually considered starting after adolescence, however age cut-offs vary between studies [44]. In general, the characteristic features of early onset asthma

and late onset asthma are quite different. Atopy and a family history of asthma and/or other allergic disease are more commonly seen in early onset asthma [45]. There is also evidence that children with early-onset asthma are more likely to have persistent respiratory symptoms into adulthood [46] and that early onset asthma has a greater impact on adult lung function than late onset asthma [45]. Late-onset asthma is usually associated with nutrition, environment and diseases, such as obesity, occupational exposures, psychological status (stress, depression, dementia, etc) and multi-comorbidities [47]. Aging itself has a significant effect on immunity and airway hyper-reactivity [47]. The above risk factors may increase the risk of developing asthma symptoms for the first time in adulthood. However, as definitions of early-onset and late-onset asthma may be retrospective after many years of follow-up, they are principally useful as research classifications, and are less relevant to clinicians.

The term asthma is less likely to be used for children under the age of 5, as it is difficult to make this diagnosis in this age group as they are unable to perform spirometry or reliably answer questions about symptoms[48]. Alternatively, preschool childhood wheeze (≤7 or 8 years) phenotypes have been defined by several birth cohort studies that identified distinct patterns of childhood wheeze over time. The phenotypes were similar between studies. One study, the MACS, identified 5 wheeze phenotypes for high-risk children up to the age of 7 years. These phenotypes were: early persistent wheeze (appeared in the first 6 months), intermediate-onset wheeze (appeared around 18 months), late-onset wheeze (appeared after age 4 years), early transient wheeze (appeared in the first 12 months but resolved around age 3 years) and never wheeze [49]. The multicentre Dutch Prevention and Incidence of Asthma and Mite Allergy (PIAMA) also identified 5 very similar wheezing phenotypes for children up to 8 years. [50]. However, the population-based Avon Longitudinal Study of Parents and Children (ALSPAC) found 6 wheeze phenotypes in children up to the age of 8 years. There were 5 profiles similar to those identified in MACS and PIAMA and one additional profile. This additional profile, “Prolonged early wheeze”, was characterized by wheeze that

"appeared in the first 12 months but resolved after 4 years" [50]. Childhood wheezing phenotypes appear to have differing associations with other allergic diseases and impacts on long-term respiratory health. For instance, many atopic characteristics are associated with an increased risk of belonging to the intermediate-onset wheeze phenotype [50]. As atopic people are more sensitive to environmental exposures, there is also evidence that this phenotype may be associated with greater lung function decline in later life and an increased risk of COPD [49].

Defined by patterns of wheezing: multi-trigger wheeze versus episodic (viral) wheeze

Classifications using patterns of wheeze are more commonly used in clinical practice, like multi-trigger wheeze and episodic (viral) wheeze. Many young children wheeze in response to numerous triggers, e.g. tobacco smoke and allergen exposure, thus clinicians describe this type of wheeze as multi-trigger wheeze [48]. Thus, the term multi-trigger wheeze is used to indicate a high possibility of asthma because multi-trigger wheeze in young children is linked to increased risk of asthma and chronic respiratory disease in adulthood. Episodic (viral) wheeze is defined as wheezing in discrete episodes caused by viral respiratory infection. While children with multi-trigger wheeze typically experience symptoms between episodes, those with episodic wheeze are usually symptoms free between episodes [48].

Accurate definitions and phenotyping of asthma is critical to understand and prevent the disease. Moreover, harmonizing definitions and recognizing phenotypes may help to explain the inconsistencies between findings from different epidemiological studies. The ideal study definition of asthma would include both accurately characterised asthma phenotypes and clinical measurements (such as lung function). However, clinical assessments and examinations are expensive and often unfeasible for large-scale studies.

2.1.2 Prevalence and burden of asthma

Key messages in this section

- Using standardized methodologies international surveys have demonstrated geographical variation in the prevalence of asthma between countries. In general, asthma is more common in the English-speaking countries of the world.
- Asthma is more common in children than adults. The highest prevalence (≥20%) was also generally observed in the English-speaking countries.
- Asthma is the cause of a substantial burden of disease, including direct economic cost, reduced quality of life and premature death in people of all ages.

2.1.2.1 Prevalence of asthma

Asthma in adults

Asthma is a common chronic disease that affects people in all parts of the world. Based on the World Health Organization's (WHO) World Health Survey in 2002 and 2003, the global prevalence of doctor diagnosed asthma in adults aged 18-45 years was estimated to be 4.3% from the 70 participating countries. However, there is a huge geographical variation in prevalence, ranging from 0.2% in China to 21.0% in Australia [51]. Asthma still appears to be most common in English-speaking countries, eg. The UK, US, Canada, New Zealand and Australia (Figure 2.1). High asthma prevalence was also observed in Northern and Western Europe and Brazil[6]. The earliest survey from the European Community Respiratory Health Survey (ECRHS) also found similar patterns of asthma prevalence in 1988-1994 for adults- a generally lower asthma prevalence in northern, central and southern Europe and higher in the British Isles, New Zealand, Australia and the United States. This survey was conducted in 48 centres from 41 countries, but predominantly in Western Europe [52]. Establishing these large-scale global measurements of asthma prevalence requires the implementation of standardized measurements and surveys across many countries. The most recent survey on the global prevalence of

asthma was carried out around 15 years ago and there have been no updates since 2003. Though information from large-scale global surveys is difficult to obtain, smaller regional studies have provided information on changing asthma prevalence during the last decade. While the overall global asthma prevalence remains high, there has been some reduction in high prevalence countries, particularly in north-western Europe [53] and the Western pacific region[16]. However, in Asia and eastern Europe, which previously had a lower baseline prevalence, there have been significant increases in asthma prevalence[54]. Increased urbanization and industrialization may be the reason for the quick growth of asthma prevalence in developing countries transitioning to a more developed economic status [33]. The Global Asthma report found that there were 235 million people with current asthma based on analyses from 2000-2002 [55]. Though there is no current global study regarding prevalence, the Global Burden of Disease Study (GBD) from 2016 estimates that there may be as many as 339.4 million people affected by asthma [46] and that this number will increase to 400 million by 2025 [6].

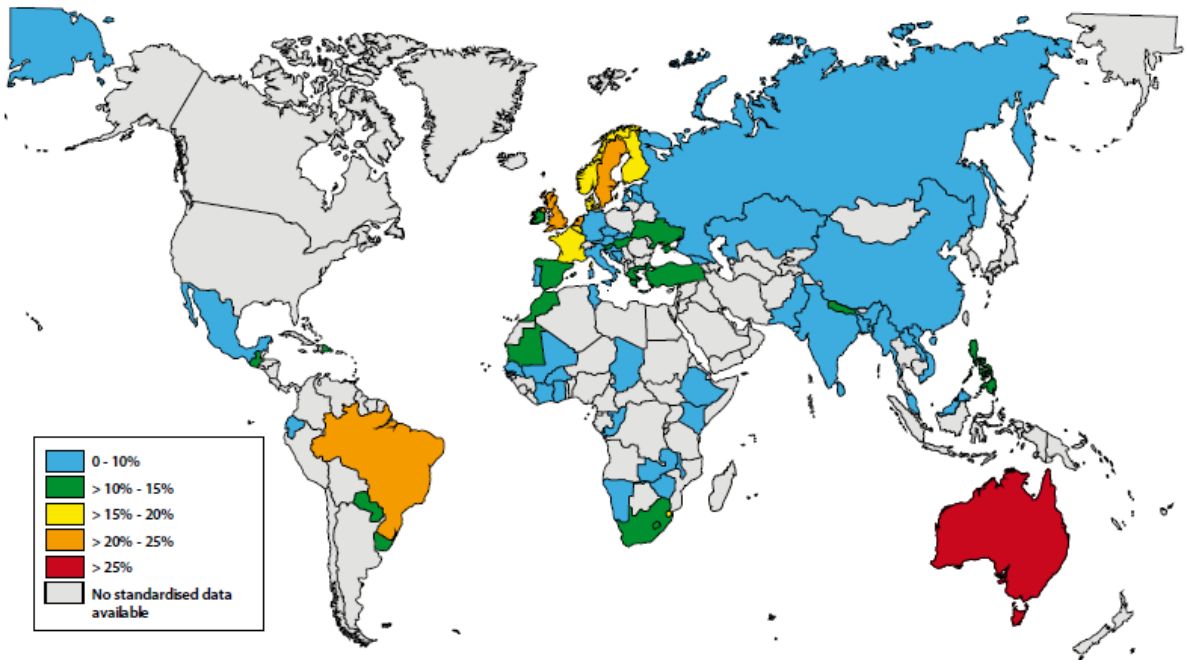

Figure 2.1 Worldwide prevalence of symptoms of asthma in the past 12 months among persons aged 18-45 years from World Health Survey 2002-2003 [51]

Asthma in children

ISAAC is the first study to assess the global prevalence of asthma in children and found similar prevalence patterns to that found in adults. ISAAC phase one included 700,000 children from 56 countries from 1993-1995 and utilised simple questionnaires to collect the worldwide prevalence of asthma symptoms, allergic rhinoconjunctivitis and eczema [56]. In 1998, phase two studied 53,383 children in 30 study centres across 22 countries with more-detailed questionnaires, some objective physiological variables and indoor exposures measurements [56]. Finally, ISAAC phase three surveyed a representative sample of 798,685 adolescents aged 13-14 years in 233 centres in 97 countries between 2000 and 2003. This survey indicated that 14% of the world's children experience asthma symptoms. The highest prevalence of recent wheeze (>20%) was generally observed in the English-speaking countries of Europe, North America, and Australia as well as parts of Latin America; the lowest prevalence (<5%) was observed in the Asia-Pacific region, the Eastern Mediterranean region, and in Northern and Eastern Europe [57]. Compared to adults, asthma is more common in children globally according to the ISAAC surveys (Figure 2.2). However, the prevalence of childhood asthma also varies widely by country as it does in adults. In adolescents aged 13-14 years, the prevalence of current wheeze (occurring in past 12 months) varied from 32.6% in Wellington, New Zealand to 0.8% in Tibet, China. In children aged 6-7 years, parent-reported current wheeze ranged from 37.6% in Costa Rica to 2.4% in Jodhpur, India [58]. From Phase one to Phase three, there was a pattern of increasing asthma prevalence for most centres [56]. A few countries showed a decreased prevalence, but this was more commonly in areas with a previously high prevalence, such as Western Europe [56].

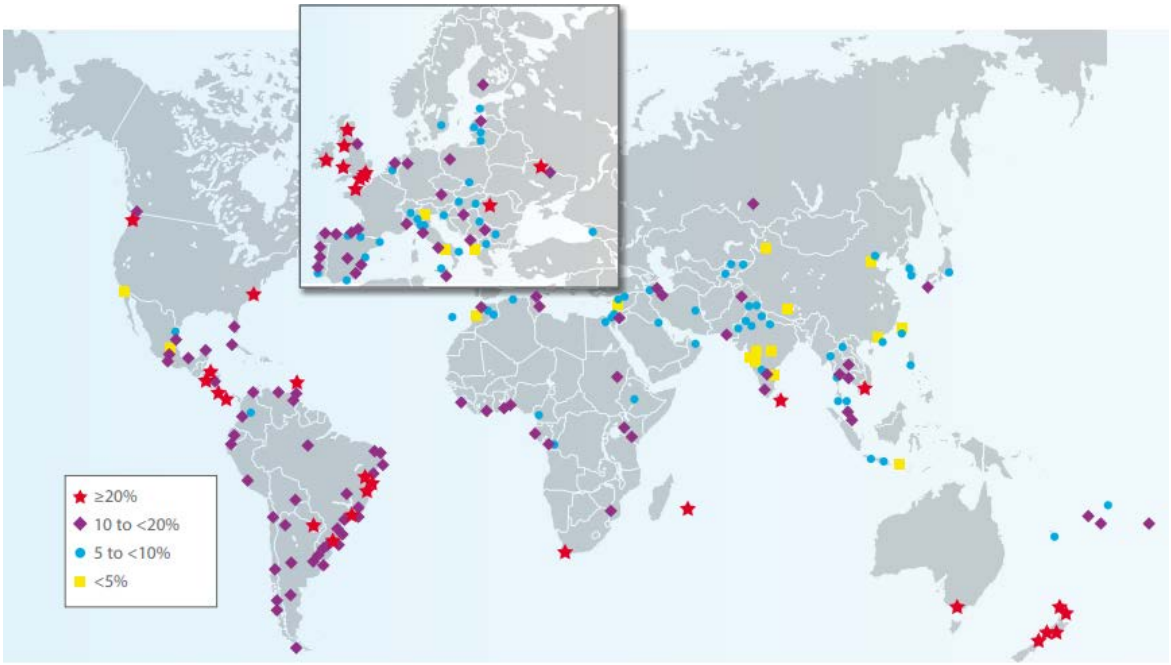

Figure 2.2 Prevalence of asthma symptoms among 13-14 years old (ISAAC) [58]

Asthma in Australia

Australia is among the countries with the highest asthma prevalence for both adults and children worldwide, and as mentioned above, WHO data ranks Australia first for highest asthma prevalence [16]. Moreover, ECRHS reported Australia to have the second highest prevalence of self-reported wheeze in adults 20-44 years from the 41 countries surveyed [59]. Around 10% of Australians have asthma, amounting to around 2 million people [60].

2.1.2.2 Health impact of asthma

The high prevalence of asthma ensures a substantial burden of disease, including both premature death and reduced quality of life for people of all ages. Asthma was the cause of death for approximately 420,000 people globally in 2016 [46].

For adults, asthma is also a major cause of absence from work in many countries including Australia, Sweden, the US and the UK [33]. Quality of life parameters are also affected significantly in those who suffer from asthma. WHO has estimated that 15 million disability-adjusted life years (DALYs) are lost each year due to asthma, which is 1% of the total global disease burden [33]. Moreover, the number of Years

of Life lost (YLL) due to asthma was 148.5 per 100,000 persons in 2016, the second highest number of years lost from respiratory disease following COPD [61].

Asthma is also a significant health burden for children. This burden is greatest for children aged 10-14, and asthma is the most common chronic disease in this age group [6]. Asthma is also recognized as the leading cause of non-attendance at school [1]. In the US, more than 7.1 million children under the age of 15 years have asthma, and this leads to thousands of annual hospitalisations and 774,000 emergency room visits per year due to severe asthma attacks [62]. A Canadian study also found that 25% of the paediatric study population had been hospitalized for asthma in the previous 12 months [63].

Asthma also has huge health impact for Australians. As Australia has such a high prevalence of asthma, the health burden is correspondingly high. A survey in 2007 found that 19.4% of adults and 29.7% of children had visited their GP or an emergency department or spent a night in hospital due to an acute attack of asthma in the past 12 months. This survey also indicated that 22.5% of adults and 20.4% of children woke up during the night in the past 4 weeks due to asthma [64]. Based on the Australian Institute of Health and Welfare data from 2009, among children 0 to 14 years, asthma is the leading cause of disease burden, responsible for 61% [65]. As a response to this huge burden, asthma has been identified as a National Health Priority area in Australia since 1999 [9].

2.1.2.3 Economic burden of asthma

Asthma also carries a high economic burden. A recent survey in US estimated that the total cost of asthma to society was $56 billion USD in 2007 amounting to $3,259 per person per year [66]. A European study of 11 countries in 2011 estimated the total cost of asthma to be €19.3 billion in that year among people aged 15 to 64 years [67]. A 2010 national Portuguese study estimated a cost of €150 million per year for childhood asthma, which equated to approximately 1% of the Portuguese health expenditure in that year [68]. Asthma also carries a considerable cost burden in Australia. Based on the Asthma Australia annual report in 2015-2016, the burden

of asthma is estimated at $28 billion AUD per annum. This figure includes both direct economic costs ($3.3 billion including doctors, hospitalizations and pharmaceuticals) and "hidden costs" ($24.7 billion in lost productivity, quality of life and premature deaths) [69]. A recent Australian survey in 2017 found that people with asthma and COPD reported 3.97 times more out-of-pocket healthcare expenditure compared to people without chronic medical conditions, amounting to a cost of to $1,642 AUD per year. This out of pocket expense was the higher than other chronic medical conditions including mental health (2.34 times higher), diabetes (1.14 times higher), heart disease (3.93 times higher) and hypertension (2.12 times higher) [70].

2.2 Lung function

Key messages in this section

- Progressive decline in lung function in asthma has been well recognised. Measurements of lung function can assist with asthma diagnosis and evaluate asthma treatment and outcomes.
- Normal lung growth and decline is made up of four distinct stages: gestational, lung function growth, plateau and decline periods. Asthma can interfere with these stages, impacting on growth and decline.

2.2.1 Why is lung function important in asthma?

Asthma may affect lung function in both the short- and long-term. Measurements of lung function are useful not only for diagnosing asthma, but also for documenting asthma progression and long-term deterioration of lung function. They also help with evaluating the effectiveness of asthma medication or treatment. Levels of lung function in those with asthma are predictors of future morbidity and mortality [15]. Between treated acute asthma attacks, many people with asthma can retain normal or close to normal lung function throughout life. Although lung function measures are below normal at the time of an attack, following resolution of the asthmatic episode, lung function usually returns to normal levels. Conversely, some asthmatics seem to have "hard to control" disease with continuous symptoms or

frequent severe episodes, and their lung function may remain abnormal, even between asthma episodes. These people have persistent airway obstruction despite the usual recommended treatments of inhaled bronchodilators and steroids and oral corticosteroids [71]. A recent meta-analysis found a clinical benefit for anti-interleukin 13 monoclonal antibodies for uncontrolled asthma, which is encouraging for exploring newer treatments [72]. Despite current therapies and relatively normal lung function for most people with asthma in between attacks, there is mounting evidence that long-term and severe asthma causes progressive loss of lung function and even life-threatening medical conditions like end-stage respiratory failure or fibrosis if their asthma is not effectively controlled [71]. In order to understand more about the relationship between asthma and lung function, it is important to understand the natural history of lung growth and decline in healthy people.

2.2.2 Measurements of lung function from spirometry

In terms of measuring lung growth and decline, spirometry is the most commonly used lung function test as it is safe, simple and non-invasive. This test is performed by a machine called a spirometer and conducted either in a respiratory laboratory or as a portable test by doctors, respiratory scientists, research assistants or other trained personnel. Whilst connected to a spirometer by a breathing tube or mask, the individual being tested is asked to take a full inspiratory breath and then to exhale that breath as quickly and completely as possible. Participants must have the correct posture and technique, as observed and instructed by the respiratory scientist, and the test is repeated until 3 acceptable blows have been measured. Using this technique, the amount of air inhaled and exhaled from the lungs can be measured over different periods of the respiratory cycle. These measurements help medical professionals diagnose various lung diseases including asthma and COPD. Spirometry is a simple and uncomplicated test that is feasible for most adults, children over 6 years of age and ninety percent of elderly people over 65 years (Figure 2.3) [73] .

Figure 2.3 Spirometry tests [74]

Two of the most commonly used measurements from spirometric testing are the forced expiratory volume in 1 second (FEV_1) and the forced vital capacity (FVC). FEV_1 is the volume of air that can be forcibly exhaled in the first second of expiration after a maximal inspiration. FVC is the total volume of air which can be forcibly exhaled from the lungs after a maximal inspiration. The normal values of FEV_1 and FVC change according to age, sex, height and ethnicity. The ratio of FEV_1/FVC can be diagnostically important for obstructive respiratory diseases like asthma. For FEV_1/FVC, the lower limit of normal is around 70-75% (typically normal should be >80%) and the exact lower limit is dependent on age and sex [75]. Another commonly used parameter from spirometry is the Mid-Expiratory Flow (MEF), which measures the rate of expiratory air-flow in litres per second at the midpoint of expiration following a maximal inspiration and expiration. This parameter can also be used to identify obstructive airway disease. Peak Expiratory Flow (PEF) is the maximal expiratory flow rate achieved and it occurs very early in the forced expiratory manoeuvre. Measurements of PEF are generally reduced in obstructive airway diseases, and the PEF trend over time is particularly useful to quantifying the response to asthma treatments and identifying triggers [75].

Spirometry can also measure the degree of reversibility of airflow obstruction and this parameter assists in diagnosing asthma. To obtain this measurement, the participant performs spirometry twice in the one session, both before and 10-15

minutes after administering an inhaled dose of bronchodilator (BD) medication such as salbutamol or terbutaline. The absolute and percentage change in FEV_1 and/or FVC after BD administration measures the degree of airway reversibility. Healthy people usually have only a small amount of reversibility (less than 8% change), whereas people with reversible airway obstruction typical of asthma have more than an 8% response to an inhaled BD. Diagnosis of asthma from bronchodilator reversibility (American Thoracic Society and European Respiratory Society guidelines) usually requires a ≥12% increase and ≥ 200ml increase in FEV_1 or FVC from the baseline measurement [76, 77]; the British Thoracic Society, recommend over 12% reversibility of FEV_1 from baseline or PEF after BD administration [78].

2.2.3 Natural history of lung growth and decline

Current physiological knowledge divides the natural history of lung growth and decline into four distinct stages: gestational, lung function growth, plateau and decline periods.

Early phases of human lung development commence from the embryonic period (day 26 to 52 of gestation) and continue to the pseudoglandular phase (day 52 to 16 weeks of gestation) [79]. During the embryonic phase, conductive airways start to be formed and acinar cells can be recognised. Initial lung architecture is formed in this phase. During the pseudoglandular phase, lung cells start to differentiate, and neuroendocrine, ciliated, goblet, cartilage, and smooth muscle cells appear. At 8 weeks, fetal breathing movements can be identified. Air exchange units start to develop in the subsequent canalicular phase (17-26 weeks of gestation) [79]. At this point, small branching airways appear, the number of peripheral blood vessels increases, and epithelial cells differentiate into type I and type II cells, which can produce surfactant. The saccular phase (24-36 weeks of gestation to term) is characterised by continuous lung growth and further maturation of the surfactant system before birth [79].

Although a newborn's lung is able to function at the time of birth, the lung is still quite immature, as the alveoli (the site of gas exchange) are not completely formed.

Alveoli start to form after 36 weeks of gestation and grow rapidly in number and size until the child is 2 years old in order to enlarge the gas-exchange surface area. The number of alveoli increases from an average of 55 million at birth to 200 - 600 million at full development [80]. There is evidence that from age 8 to 74 years, the number of alveoli remain very constant [81], but there are changes in gas-exchange surface area as the formed alveoli continuously grow and expand, holding increasing volumes of air. The number of conducting airways is complete at birth thus they only increase in size (length and diameter) after this time until the plateau phase. Capillary networks including those surrounding alveoli for gas exchange are also formed before the age of 2 years.

Simple lung growth then starts after 2 years, as single lung weight increases from 60g to 750g, and the remaining volume of air in the lungs after maximal expiration (functional lung residual capacity) may increase from about 80ml at birth to 3000ml in early adulthood [80]. In children and early adolescents, lung function is related to standing height only and there is no sex difference. However, maximal height is reached by around 16 years (range 8-17) in women but continues to increase at a slower pace until the mid-20s in men. Therefore, lung growth for FVC is largely complete in late-adolescent girls, and by contrast, FVC in adolescent boys continues to increase well into the mid-20s [82]. This continued change is also thought to be due to an increase in the strength and effectiveness of respiratory muscles [80]. Generally, adult males have larger tracheae, bronchi and higher flows than adult females who have the same lung volumes. Beside gender differences, a non-smoking healthy male twin study suggested that lung volumes are highly related to anatomic features such as body size, indicating genetic factors may also be a significant determinant of lung growth [83].

During growing period, FEV_1 and FVC measured from spirometry both increases with age, but FVC grows quicker than FEV_1, thus the ratio of FEV_1/FVC normally falls during childhood but this trend reverses in adolescence [84]. In young adulthood, lung growth is completed, and maximal lung function levels can be measured with spirometry. The predicted maximal values of lifetime in adults (for all heights and

≥18 years) is 83.9% for males at 20 years, and 87% for females at 18 years based on spirometry guidelines from the Thoracic Society of Australia and New Zealand [75]. Following this, lung function has a plateau period with minor annual loss of lung function before 40 years before moving into the decline phase with greater loss of lung function. Both FEV_1 and FVC, measured from spirometry, decreases slowly with age from the 20s (Figure 2.1). The annual decrease in FEV_1 is approximately 20 ml in people aged 25-39 years, and increases to 38 ml in people aged over 65 years [73] . FVC also decreases over time, but not uniformly with FEV_1, thus the ratio of FEV_1/FVC reduces with aging. The ratio of FEV_1/FVC decreases to 70-75% of the maximal adulthood value in females aged >55 and males aged >60 years [73] .

Despite these spirometry figures, the most significant physiological changes in lung function decline with aging include decreasing elastic recoil of the lung, reduced chest wall compliance and reduced respiratory muscle strength. Aging lungs may have unfavourable respiratory mechanics associated with decreased expiratory flows and gas exchange rates coupled with increased residual volumes after complete exhalation and increased smallest airways volumes collapse during expiration [85]. This normal ageing physiology may also interact with existing lung diseases to worsen lung function and amplify disease manifestations in older individuals [85].

It is noted that though spirometrically-measured respiratory performance decreases with aging, this results in very few respiratory symptoms over most of the lifespan. This is primarily because of the huge capacity for gas exchange attained at the time of maximal lung growth, allowing for gradual gas exchange loss over life with minimal symptoms before a critical threshold is reached. In fact, even the elderly can preserve a large amount of gas exchange and increase ventilatory function through regular training, even with reduced alveolar surface area. However, individuals experiencing this normal decline can become symptomatic if lung function is further compromised by disease.

2.2.4 Lung function in asthmatics

Measurements of lung function are very useful in diagnosing asthma, monitoring lung function over time, and assessing the effects of asthma therapies. The typical findings on pre- BD spirometry in people with asthma are normal or reduced FEV_1, normal FVC and normal or reduced FEV_1/FVC. As asthmatic patients may have normal lung function, the demonstration of reversible airflow obstruction (post-BD spirometric improvement) is often necessary for evaluating asthma status. Conversely, some people with asthma may show no reversibility, or fixed obstruction, on spirometry. A review provided evidence that over time, lung function obstruction may become irreversible as a consequence of asthma evolvement or progression [86], but it may initially showed reversible obstruction. A study in the Netherlands found that 16% of asthmatics had irreversible airway obstruction in adulthood, which was defined as FEV_1 <80% of predicted with reversibility <9% of predicted [87]. Asthma mortality is associated with lower FEV_1, less reversibility, increasing age and treatment requirements [71]. However, there is evidence that patients who receive regular treatment are less likely to develop irreversible airway obstruction [71].

There is also evidence that asthma patients have accelerated decline in lung function compared to the general population. The Copenhagen City Heart Study of 17,506 adult subjects over 15 years found that 1,095 participants with asthma had an average of 38 mLs annual FEV_1 decline compared to 22 mls in those without asthma, irrespective of sex, age and smoking status [88]. Adverse exposures in adulthood can further increase the rate of lung function decline. There is little doubt that smoking is a major risk factor for accelerated lung function decline, particularly for people with asthma. A 10-year US study among young adults aged 18-30 years found that lung function decline (in terms of FEV_1 measurement) was 8.5% over the 10 year period in never-smokers without asthma, 10.1% in never-smokers with asthma, 11.1% in non-asthmatic smokers and increased to 17.8% in asthmatic smokers (p for interaction=0.04) [89].

Lower lung function in adults often originates from low lung function and/or reduced lung function growth in childhood. A birth cohort study in Dunedin, New

Zealand found that adolescents with persistent wheezing had a lower post-BD FEV_1/FVC ratio (over 2 standard deviations (SD) below the mean values) than non-asthmatic and non-smoking peers, and when lung function was measured again in adulthood, the degree of reversibility decreased further [90]. Therefore, any risk factors or adverse exposures during early life, or even preconception, may impair the ability to attain maximal lung function during early adulthood or accelerate decline in later life through reducing baseline lung function at birth and/or changing the natural course of lung function growth in adolescence [91-93]. A prospective cohort study from the first to the sixth decade of life indicated that detrimental early life exposures such as maternal smoking, accelerated lung function decline and increased COPD risk in middle age [92]. Additionally, when sub-maximal lung function tracks over the usual plateau and decline phases in adult life, respiratory disease like COPD may occur at an earlier age. Nevertheless, the Lovelace smokers Cohort highlighted the important role of maximally attained lung function in adulthood [88]. People who had an FEV_1 lower than 80% of the predicted values before 40 years of age, had a much higher prevalence of COPD after 22 years follow-up, compared to those with normal FEV_1 (P for difference<0.001). However, not all people with lower FEV_1 developed COPD; in this study 75% of people with a low maximally attained FEV_1 did not develop COPD. Moreover, the people with normal lung function below the age of 40 still had a risk of developing COPD, and of those developing airflow limitation, half had normal lung function growth in early adulthood [94]. Figure 2.4 illustrates different lung function growth patterns including a normal lung function growth and decline pattern (curve D), reduced lung function growth before early adulthood but normal decline (curve B), normal lung function growth before early adulthood but rapid decline (curve C) and reduced lung function growth from childhood (curve A). There are currently no universally determined factors contributing to the different patterns of lung function growth and decline except personal smoking, although this is an area of current interest. The most commonly studied risk factors to date include maternal smoking, allergic diseases, childhood respiratory infection and family asthma history [92, 95]. My PhD

thesis focussed on the pattern with reduced lung function growth from childhood (curve A) and its possible contributors from the household environment.

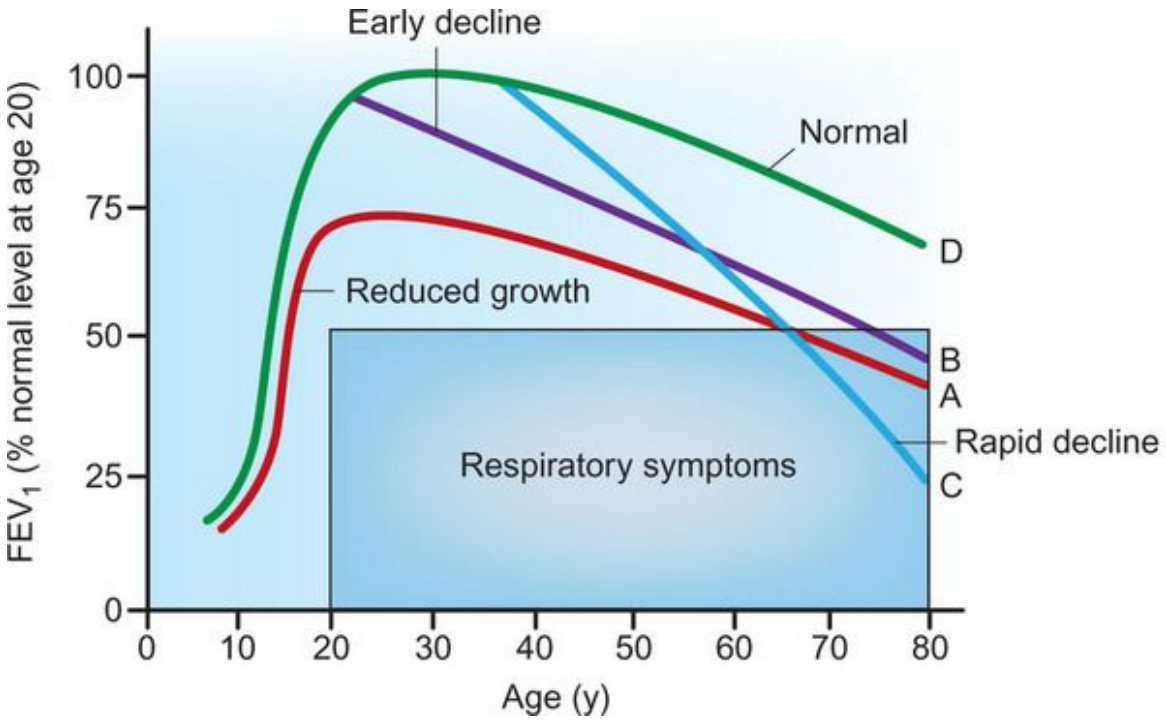

Figure 2.4 Pulmonary function varies by age [95].

The growth of FEV_1 as a percentage of the maximal value shown is shown as a function of age. Curve A depicts reduced growth but normal decline; Curve B depicts premature decline in FEV_1; Curve C depicts normal growth, no premature decline but accelerated decline; Curve D represents the optimal and ideal lung function, with normal growth and normal decline for age in a healthy individual.

Currently, the aetiology of asthma and the early life factors influencing its development and persistence are poorly understood and knowledge on the factors determining maximal development of lung function in adolescence and early adulthood is scarce. However, there is some inconsistent evidence that environmental factors including household exposures (e.g. gas heating and cooking, mould, cigarette smoking) and drugs (paracetamol) may influence lung health [16, 92, 96-100]. One of the likely reasons for these inconsistencies is failure to account for gene-environment interactions, which may be linked to insufficiencies in dealing with oxidative stress generated from harmful environment exposures; also, there

are few long-term studies that have sufficient follow-up from early life to adolescence and adulthood to explore these questions. Currently published work may not have enough power to explore the relationship between early life exposures and later life respiratory health. To fill these knowledge gaps, I investigated the relationship between household exposures and lung health in long-term longitudinal cohorts at multiple time points over the lifespan while taking into account potential gene-environment interactions.

2.2.5 Other lung tests

There are several other ways to measure lung function and early inflammatory signs in the airways. These tests also help to improve asthma monitoring and management. Though the tests described in this section were not used in my analysis, they are used in other relevant research.

Fractional exhaled nitric oxide (FeNO): FeNO is the amount of nitric oxide (NO) in an exhaled breath. The breath NO level can be measured non-invasively by using specifically designed equipment with an electrochemical sensor. People with asthma often have increased FeNO levels due to eosinophil-mediated inflammation in the central and peripheral airways [101]. FeNO testing can assist in diagnosing asthma when people have respiratory symptoms indicative of asthma, however, this test does not assess airway obstruction. FeNO may also be useful as a measure of ongoing airway inflammation to guide anti-inflammatory treatments (for instance, determining the likelihood of steroid responsiveness [101] and the effects of monoclonal antibody treatment [102]).

Bronchial hyperresponsiveness (BHR): Bronchial challenge testing can be used to measure the degree of airway responsiveness to an inhaled stimulus, such as increasing doses of methacholine or histamine, administered to induce bronchospasm. People with asthma have a lower threshold for response, indicating increased airway sensitivity, thus the dose-response curve is significantly different between asthmatic and normal subjects[103]. BHR has long been recognized as a hallmark of inflammation in asthma and is related to disease severity [104]. A

substantial proportion of people with asthma have persistent BHR despite good clinical control [105, 106]. Moreover, BHR is another commonly used outcome when assessing gene-environment interactions in relation to lung health. ECRHS found evidence that increased BHR was associated with gas cooking among subjects with the GSTM1 null genotype [107].

2.3 Oxidative stress in the lungs and its relationship with asthma

Though the aetiology of asthma is unknown, many authors have found evidence that asthma is associated with increased oxidative stress in the airways; evidenced by the presence of increased oxidative biomarkers [108]. The next 2 sections deal with how oxidative stress occurs and body's mechanisms for dealing with oxidative stress.

Key messages in this section

- Asthma is associated with increased oxidative stress in the airways. Overproduction of oxidants (ROS and RNS) may lead to acute and chronic inflammation in asthma. There are many exogenous sources of oxidants, such as smoking, air pollution, allergens and medications.
- The human body can detoxify environmental oxidants using antioxidant enzyme systems. Decreased activity of antioxidant enzymes may lead to the accumulation of oxidative damage without sufficient repair.

2.3.1 Oxidative stress in the lungs

Oxidative stress reflects the imbalance between the overproduction of oxidants in terms of reactive oxygen species (ROS) and reactive nitrogen species (RNS) and underproduction of antioxidant defence mechanisms in the body [108]. There are many exogenous sources of ROS, such as cigarette smoke, air pollutants, metals, mould, and some medications. These ROS play an important role in the pathogenesis of inflammatory conditions. After exposure to environmental triggers, normal physiological antioxidant defence mechanisms are usually able to eliminate ROS and repair damage. However, an imbalance between oxidants and antioxidants can have significant functional and structural consequences associated with

excessive airway inflammation and without sufficient repair may lead to accumulated oxidative changes until this process reaches the "threshold" for asthma [108]. This mechanism is a long-term continuous and dynamic process and may explain the development and persistence of asthma [108]. Due to the fact that environmental exposures are the sources of ROS, the competence of genes that code for antioxidant defence enzymes could modify the balance between oxidative stress and asthmatic inflammation.

The environmental exposures of outdoor and indoor air pollution are important contributors to airway oxidative stress. Outdoor air pollution is a major source of oxidative stress as the lungs are exposed to several thousand litres of air per day that contains a large number of compounds that could potentially cause oxidative damage including: carbon oxides, nitrogen oxides, sulphur oxides, volatile organic compounds, pollen, and particulate matter. Larger particles (aerodynamic diameter over 5 µm) can be cleared by the nose and upper airways, but fine particles (aerodynamic diameter of 5µm or less) can easily access the lower airways and lead to oxidative stress [109]. Outdoor air pollution stimuli can cause direct oxidative lung injury and promote airway inflammation [110]. Oxidative stress also contributes to other respiratory effects such as reduced lung function, slower lung growth and less sensitivity to asthma medications [110]. A Canadian study of 182 asthmatic children showed that increased concentrations of SO_2, NO_2 and Particulate matter <2.5µm in diameter ($PM_{2.5}$) were associated with immediate increased oxidative stress biomarkers (thiobarbituric acid reactive substances and 9-isoprostane) and decreased lung function [111].

Compared to outdoor air pollution, there are fewer studies investigating the associations between indoor air pollution and oxidative stress. A few indoor exposures have been associated with oxidative stress and respiratory health. For instance, both active and passive tobacco smoke constitute one of the most significant exposures for increasing airway oxidative stress [110]; biomass fuel smoke has also been shown to be linked with systemic and airway oxidative stress that may play an important role in the development of asthma [112]. Fewer studies

have investigated other oxidant exposures like cooking and heating facilities and pharmaceutical exposures, and even for household exposures that have been investigated, these studies were not conducted in long-running cohorts. Many respiratory health risk factors are still unknown. These household exposures, in terms of smoking, heating and cooking facilities, and early life medication use are further detailed below in section 2.4 as they are the focus of my thesis.

2.3.2 How do lungs deal with oxidative stress?

The lungs are equipped with a range of antioxidant enzymes for managing the constant exposure to a wide range of oxidants. These enzymes include superoxide dismutase (SOD) and glutathione peroxidase, which can detoxify a wide range of environmental oxidants.

Decreased activity or dysfunction of normal antioxidant enzyme defences can be identified in asthmatic airways. Glutathione is an important antioxidant enzyme in humans which can be found in the alveolar epithelial lining fluid and it protects against airway peroxidation [110]. People with mild asthma have been shown to have a high glutathione level, reflecting an adaptive response to increased ROS, compared to healthy people. However, in severe asthma, glutathione levels have been shown to be significantly lower when compared to people with mild to moderate asthma or healthy controls [110]. This reduced glutathione level may impair the ability to detoxify ROS.

During the antioxidative response, glutathione is converted to glutathione disulphide, a molecule that is effective for reducing oxidative stress. Many exposures may delay or prevent this conversion, for example, tobacco smoke reduces the rate of conversion from glutathione to glutathione disulphide, resulting in an impaired antioxidant response and further inflammation and worsening oxidative stress.

The presence of antioxidant enzymes such as GST enzymes can facilitate compounds like glutathione to neutralize ROS. These antioxidant enzymes are important in the bio-activation of glutathione. They assist by moving glutathione from cells via

membrane-based glutathione conjugate pumps and also facilitate detoxification of various exogenous toxic compounds [113]. Additionally, GST genes regulate a complex network of antioxidant defences involving many other antioxidant compounds such as arginine, citrulline, taurine, creatine, selenium, zinc and vitamins A, C, D and E, and antioxidant enzymes [21]. Thus, GST genetic variations may alter the influence of many household exposures on respiratory health through oxidative stress pathways. In the next section, I will introduce the impact of a few household exposures that may be modified by antioxidative genes in relation to asthma outcomes.

2.4 Common household exposures increasing oxidative stress and risk of asthma

As people spend much of their time indoors, exposure to numerous household risk factors including indoor environmental pollution has been hypothesized to be particularly relevant to the risk of asthma and impaired lung function. Though results are inconsistent, exposure to a number of diverse household factors, particularly during the *in-utero* period, infancy and early childhood, have been implicated in asthma pathogenesis, including environmental tobacco smoking (ETS), indoor air pollution from cooking and heating sources, nutrition, commonly used medications and more. As no single factor can account for asthma development and/or exacerbations, this could be one of the major reasons for inconsistency in the literature. Many gene-environment interactions, gene-gene interactions and multiple environmental exposures likely play a role in the pathogenesis of asthma, via oxidative stress pathogenesis.

Key messages in this section

Though the causes of asthma are unknown, many household sources of oxidative stress that may be associated with risk of asthma have been identified in the literature.

- There is a clear causal relationship between **tobacco smoke** and lung diseases, but the longitudinal impact of early life smoke exposure on lung health is still not well characterized.
- Many **other household air pollutants** may contribute to the risk of asthma, but the evidence is inconsistent.
- **Early life paracetamol use** may be associated with lung health in later life, but there is no solid evidence for a causal relationship. Early life respiratory infections may also confound these associations.
- **Nutrition** is also related to antioxidative stress functions and is important for lung health.

2.4.1 Smoking

There is irrefutable evidence that tobacco smoke exposure has adverse effects on respiratory health, and the association between smoking and lung disease is universally acknowledged, even by the tobacco industry. Active smoking leads to airway inflammation which is central to the pathophysiology of asthma [114]. It has previously been reported from studies of young asymptomatic smokers with normal spirometry that central airway inflammation is found in almost all participants with signs of distal airway inflammation in up to 50% [115]. Over 80% of young smokers have evidence of mucosal damage within their major bronchi [116] [117] and 26% of young smokers have chronic bronchitis [118]. The severity of asthma symptoms is also affected by active smoking. Asthmatic smokers were found to have an increase in attacks of wheezing and breathlessness and increased night-time wakening compared with non-smokers [119]. Additionally, the mortality rates following a near-fatal asthma attack were found to be 3.6 fold higher among smokers than non-smokers [120].

The evidence for an association between early life smoke exposure and later life respiratory health is shown in Table 2.1. *In-utero* exposure to maternal tobacco smoking is associated with increased rates of asthma and wheezing in later life [15]. Nicotine has been shown to have detrimental effects on foetal lung development as growth of the lung, including airways, alveoli and blood vessels, advances rapidly from late pregnancy to 2 years after birth [80, 121]. The Mater University Study of Pregnancy (n=7,223) showed that girls who were exposed to heavy maternal smoking, in utero and postnatally, had an increased risk of asthma symptoms at age 14 years [122]. Another large cohort study in Western Norway (n=3,786) extended these findings into adults from 15 to 70 years regardless of gender and found that pre- and post-natal tobacco smoke exposure carried a substantial risk for developing adult asthma and respiratory symptoms [123]. However, it is noteworthy to mention that findings from longitudinal studies investigating the relationship between tobacco smoke exposure and respiratory health in later life have been inconsistent. For instance, a large longitudinal study in Denmark (n=11,144) reported that maternal smoking during the 36th week of gestation did not contribute to the development of asthma for children aged 14-18 years [124]. The lack of exploration into gene-environment interactions may explain the inconsistencies in previous literature. Because antioxidant genes may be involved in detoxification of tobacco smoke, people lacking full function of antioxidant genes may be more susceptible to asthma and other respiratory symptoms compared to people with full antioxidant enzyme activity. The CHS investigated 2,950 children and found that previous exposure to *in-utero* smoking and current ETS was not associated with asthma outcomes. However, children who had the GSTM1 null genotype were at greatest risk of adverse respiratory health effects when exposed to *in-utero* tobacco smoke [125].

Table 2.1 The association between early life tobacco smoke exposure and respiratory outcomes in cohort studies

Reference	Exposure age	Outcome age	Results
Tager et al. (1983) [126]	5-9 years	5-9 years	Reduced lung growth
Cummingham et al. (1994) [93]	Prenatal only and current smoking	8-12 years	Prenatal smoking associated with reduced lung function; Current smoking was not associated with reduced lung function.
Wang et al. (1994) [127]	The first 5 years	6-18 years	Reduced lung function
Gilliland et al. (2003) [128]	*In-utero* to postnatal maternal smoking	7-18 years	Reduced lung function
Rizzi et al. (2004) [129]	During pregnancy and current smoking	Mean age 16 years	Reduced lung function
Jedrychowski et al. (2005) [130]	Maternal smoking postnatally	9 years	Reduced lung function
Magnusson et al. (2005) [124]	The 36th week of gestation	14-28 years	Increased risk of wheezing and hay fever
Alati et al. (2006) [122]	Last trimester, 6 month and 5 years	14 years	*In-utero* smoking increased risk of asthma in girls, but postnatal smoking (only) only did not increase the risk of asthma; No associations were seen in boys.
Prabhu et al. (2010) [131]	11 weeks, 20 weeks, and 32 weeks of gestation	2 and 5 years	Maternal smoking throughout pregnancy associated with reduced lung function; children whose mothers smoked during the first trimester were at increased risk of asthma at age 2 years, but had normal lung function.
Chen et al. (2011) [132]	*In-utero* and ETS before 5 years	12-14 years	In utero smoking associated with early onset asthma; ETS before 5 years associated with late onset asthma.
Grabenhenrich et al. (2014) [133]	*In-utero* smoking	20 years	Increased risk of asthma
Dai et al. (2016) [134]	Early life smoking (mean time: 4 days before birth)	12 and 18 years	Reduced lung function, lung growth and increased risk of asthma seen in girls, not in boys.
Thacher et al. (2018) [135]	*In-utero* smoking, during fetal life, infancy, childhood, adolescence.	14-16 years	*In-utero* smoking was associated with increased risk of asthma and rhinoconjunctivitis; tobacco smoking after birth, was not associated with adolescent-onset asthma or rhinoconjuctivitis.
Huang et al. (2018) [136]	ETS before 3 years	8 years	Increased risk of asthma

2.4.2 Other household air pollution

Exposure to air pollution, including both household and outdoor, is believed by some experts to be the main cause of allergies and asthma [137]. It is reported that exposure to outdoor airborne pollutants such as ozone (O_3) [138], NO_2 [139, 140], SO_2 [140] and particulates[139] has adverse effects on immune cells and airway responsiveness. Evidence concerning outdoor air pollution and its relationship with oxidative stress and subsequent asthma has been well studied in recent research, however, there is currently a lack of enough evidence concerning this relationship for household air pollution. I have reviewed some positive and negative evidences that whether household air pollution increase risk of asthma.

Many household air pollutants are produced indoors from heating and cooking facilities, although their concentrations may be very different depending on the of source and house ventilation [141]. Moreover, other contributors to household air pollution (eg. Dust mite allergens, pet allergens, mould, etc) can also act as irritants and make allergic symptoms worse [141]. Home dust mites are microscopic parasites that can travel through the air and contribute to poor indoor air quality [141, 142]. House dust mite allergens are also known to play an important role in inducing IgE-mediated sensitization and asthma [143]. Home dust mites can release exogenous proteases that react with cell-surface receptors in the airways to generate leukocyte infiltration and amplify an inflammatory response [144]. Mould is another important indoor air pollutant that can cause allergic reactions. A meta-analysis has addressed the relationship between exposure to indoor dampness and mould and asthma outcomes, finding a significantly increased risk (30-50%) in those exposed for a variety of respiratory and asthma-related health outcomes [145]. Carpet is also at high risk of mould colonization. A US cross-sectional study indicated that *Alternaria* concentration was significantly higher in kitchens with carpet than in kitchens without carpet ($p=0.001$), and carpet has also been shown to be a reservoir for fungi in bedrooms, with the measured levels of fungi found in the bed being much lower than the levels in carpet [146]. Fungi and mould can disperse through the air by producing numerous spores during growth and germination [147]. The cell wall of most fungi contains

glucans with inflammatory properties, which may enhance airway inflammation after inhalation [148]. Pet dander and skin flakes can cause allergic reactions in sensitized individuals; additionally, animal hair can also act as a medium to transmit pollen, dust, mould and other allergens [149]. Previous reviews have also provided some evidence that these indoor exposures are associated with an increased risk of asthma (Table 2.2).

Table 2.2 The associations between indoor air pollution and asthma based on published review articles

Reference	Risk factors	Association
Lin et al. (2013) [150]	Gas cooking	Increased risk (OR 1.32, 95%CI 1.18, 1.48)
Kim et al. (2011) [151]	Household biomass fuels including wood or coal fire	Increased risk
Erwin et al. (2005) [152]	Cat and dust mite	No association
Jie et al. (2011) [153]	Coal and biomass fuels	Increased risk but some studies found negative associations. These findings may be confounded by rurality – those using coal were generally living in rural area.
	Dust mite	Increased risk
	Cat and dog allergens	No association, but some studies indicated that cat appeared to be more beneficial than dog.
	Fungal allergens	Increased risk
Chen et al. (2010) [154]	Cat and dog	No associations.
Fisk et al. (2007) [145]	Dampness and mould	Increased risk
Quansah et al. (2012) [155]	Dampness and mould	Increased risk (OR 1.29, 95% CI 1.04, 1.60)
Becher et al. (2018) [156]	Carpet	Increased risk

However, given the heterogeneity between studies and inconsistent results for a small number of studies, there are many uncertainties regarding whether indoor pollution is a cause of asthma. Concerning indoor heating types, there is still conflicting evidence on whether wood smoke has an impact on respiratory health. A population-based study of over 4,000 school-aged children in 12 Southern California communities found that exposure in the first year of life to wood or oil smoke was associated with the occurrence of asthma by 5 years of age (OR 2.39, 95% CI 1.17, 4.89) [157]. Whilst in Finland, another large cohort study (n=10,667) found no evidence for an association between childhood wood stove exposure and subsequent asthma at 18-25 years but a negative association for the odds of developing allergic rhinitis (OR 0.61, 95%CI, 0.61, 0.91) [158]. Another example of inconsistent evidence is related to carpet use, which has been associated with increased allergen exposure. However, in Sweden, where there has been a substantial reduction in carpet use in houses and replacement with wooden flooring, there has been no evidence of a subsequent reduction in asthma prevalence [33].

Though household pollutions, such as ozone, particulates, dust mites, metals and other pollutants are important exogenous sources of ROS, oxidative stress only occurs when there is an imbalance between ROS and antioxidant defence mechanisms in the body. In the case of asthmatics, they may have decreased activity/dysfunction of antioxidant defences, thus exposure to air pollution may lead to chronic inflammation accompanied by overproduction of ROS, causing asthmatic symptoms. One of the aims of my thesis is to investigate the relationship between household pollution and respiratory health, moreover, to determine if the relationship is modified by genetic differences in oxidative stress genes.

2.4.3 Early life Infections and medication use

Both bacterial and viral infections are capable of modulating the immature immune system, and therefore early life infections may play a significant role in asthma induction [159, 160]. Many epidemiological studies have provided evidence of a close relationship between early life respiratory infection and wheeze, and these infections have long been associated with the subsequent development of asthma. In a cohort

of high-risk Australian children, early life rhinovirus infection was associated with a 10-fold increased asthma risk in childhood, making it the most significant viral predictor of asthma, even stronger than respiratory syncytial virus [161]. The Tennessee Asthma Bronchiolitis Study (TABS) of over 95,000 children reported a dose-response relationship between the severity of viral bronchiolitis in infancy and increased risk of childhood asthma [162]. Other than viral infections, early life bacterial infections may also be associated with increased asthma risk. A recent study of bacterial pathogens identified asymptomatic *Streptococcus pneumoniae* colonisation in lower airways in the first month of life as a strong predictor of asthma[163].

Antibiotics and paracetamol are the most commonly used medications administered to treat and reduce the fever and pain from infections globally. Several antibiotics have adverse effects on gut microbiota, and through this dysbiosis and its potential effects on educating the immune system, antibiotics may influence neonatal immune development and the development of asthma [14]. Paracetamol is used to relieve fever and pain and is recommended as one of the safest medicines for this use for children by WHO's List of Essential Medicines [164]. However, there is accumulating evidence of a possible association between paracetamol use and increased risk of asthma[99, 165, 166].

There are several findings that contribute evidence towards the pathophysiological mechanism behind paracetamol exposure and increased asthma risk. Glutathione is an important antioxidant that exists in the respiratory tract lining fluid. Evidence from several studies suggests that glutathione is implicated in reducing lung inflammation [110, 167]. Glutathione has been detected at lower levels in the exhaled breath condensate of asthmatic children during exacerbations compared to healthy children [168]. Glutathione peroxidase activity was also lower in platelets and whole blood in asthmatic and atopic patients [169, 170]. Paracetamol consumes glutathione therefore reducing the amount available to deal with other ROS exposures [169, 171]. It has been shown that paracetamol at therapeutic doses can reduce the serum antioxidant capacity for 14 days [172]. However, in conjunction with paracetamol exposure, the specific genotype of antioxidant stress genes may also be important in

regulating antioxidant stress function. One study found that exposure to paracetamol before 1 year of age was associated with wheezing at the age of 5 years, and this risk was modified by GSTP1 [173]. Though scant, this evidence suggests the existence of a glutathione pathway mechanism underlying the relationship between paracetamol and asthma risk, and there is a need to investigate this relationship in other studies.

As summarised in Table 2.3, the evidence from existing studies is currently inconclusive on the association between paracetamol during infancy and the risk of subsequent asthma. In 2015, a systematic review and meta-analysis regarding the relationship between early life paracetamol use and childhood asthma suggested that the associations found in some studies likely reflected confounding by indication. That is to say, in many of these studies, paracetamol was administered in infancy or early childhood to treat fever and discomfort from respiratory tract infections, and these infections have a known association with increased risk of asthma. Therefore, the observed relationships may represent confounding rather than a causal association [13]. Whether there is an association between paracetamol and asthma is still under debate [171]. My thesis will explore the novel relationship between early life paracetamol intake and respiratory health in adolescence by considering the effect of early life respiratory infection and determine whether any observed relationships are modified by antioxidant genes.

Table 2.3 Associations between paracetamol use during infancy and respiratory outcomes in cohort studies.

Reference	Exposure age	Outcome age	Results
Lowe et al. (2010) [174]	Up to 2 years	7 years	No association
Schnabel et al. (2010) [175]	From 6 months to 2 years	6 years	No association
Shaheen et al. (2010) [176]	Up to 6 months	7 years	No association
Wickens et al. (2010) [177]	Up to 15 months	5-6 years	No association
Bakkeheim et al. (2011) [178]	Up to 6 months	10 years	Increased risk of allergic sensitization and asthma in girls, not in boys
Kreiner-Moller et al. (2012) [179]	Up to 12 months	0-3 years	Increased risk of any troublesome lower lung symptoms
Magnus et al. (2016) [180]	Up to 6 months	3 and 7 years	Increased risk of asthma at both ages
Piler et al. (2018) [165]	Up to 18 months	3, 5, 6, and 11 years	Increased risk of at least one record of asthma

2.4.4 Nutrition

Nutrients, especially vitamins, have antioxidant properties and may play a role in asthma pathogenesis. Vitamin D is known to have properties as a lung immunomodulator and Vitamins A, C, E can act as antioxidants reducing airway inflammation [181]. Other dietary antioxidants which may help modulate susceptibility to ROS include lycopene, γ-tocopherol, α-tocopherol, selenium, carotenoids and flavonoids [182]. In several studies, reduction in these antioxidants has been associated with increased asthma risk, and adequate levels may have a role in prevention of asthma development. One case control study found evidence that carotenoid levels in whole blood plasma were lower in asthmatic adults compared to healthy controls [183]. Maternal nutrition during pregnancy is also associated with asthma symptoms in their children. A mother-child pairs pre-birth study found evidence that low maternal dietary vitamin D intake in pregnancy was related to recurrent wheeze in their children. Children from mothers with the highest quartile of dietary intake had a 62% reduction in the risk of recurrent wheeze at the age of 3 years [184]. This is biologically plausible as it is known that vitamin D can cross the placenta and enter the foetal bloodstream, assisting foetal growth [182].

Though some household exposures may be important in the development and exacerbation of asthma, not all people who are exposed to these factors will develop asthma. Some people are born with the capacity to tolerate these household exposures whilst others are more susceptible due to their genetic capability. I will review another important determinant of asthma – genes in next section.

2.5 Antioxidant genes and asthma

Key messages in this section

- There are no genes showing strong independent associations with asthma.
- Antioxidative defence genes determine the antioxidant enzyme levels available to detoxify environmental exposures, potentially influencing asthma susceptibility in exposed individuals.
- GSTs are a major class of antioxidative genes. However, current evidence does not support a substantial role of GST genes on respiratory health.

Hundreds to thousands of genetic markers have been assessed for their relationships with asthma by large-scale genome-wide association studies (GWAS). However, very few strong associations have been found regarding the variants and asthma, and these studies have concluded that this is likely to indicate that asthma is a multifactorial disease. Complex gene-gene interactions and gene-environment interactions are involved in multiple disease phenotypes, resulting in null findings for a simple genome-wide test without consideration of these interactions. This section will focus on the antioxidative stress genes that can potentially influence the respiratory system. GST genes, as a major class of antioxidative stress gene, were not directly associated with respiratory health based on current evidence. However, they may affect asthma susceptibility if exposure interactions are taken into account.

2.5.1 Overview of genetic factors

Asthma can be inherited: A family history of asthma or atopy is an important risk factor for the development of asthma in an individual, indicating that genetic factors and shared environmental or familial factors are important in the aetiology of asthma. It has been found that people whose parents are asthmatic are more likely to have asthma and allergies [160, 185]. A study indicated that people who had a father with asthma were 2.3 times more likely to have asthma and those who had a mother with asthma were 1.8 times more likely to have asthma [186]. These findings are consistent with the existence of, but not proof of, the role of inherited genetic factors in the development of asthma and allergy.

Possible asthma related genes: So far, over 70 variants in candidate genes have been found to be associated with phenotypes of asthma and atopy [33], including the β_2-Adrenergic Receptor, IL-4, IL4 Receptor α, CD14, β chain of the high-affinity IgE receptor, TNF-α and the α_1-antitrypsin gene [187]. The main gene regions where these variants have been found are on chromosomes 2q, 5q, 6p, 11q, 12q, 16q and 17q [33]. The hope was that identification of genes associated with asthma would help us to target high risk populations for prevention and therapy. However, the process of gene identification is complex. It involves linkage studies that allow identification of broad regions of the human genome that are linked to a phenotype and identifying DNA

sequence variations in the genes within the linked loci. Moreover, it requires proof of disease-causing mutations which depend on the strength of association between specific gene polymorphisms and disease [187]. Currently, asthma genetic studies are at the stage of identifying loci that are associated with the disease in ethnically diverse populations and phenotypic subgroups [188].

Genes alone cannot determine asthma: Though genetic predisposition (asthmatic parents) appears to be important in asthma aetiology, a review regarding the association between positive family history and childhood asthma found that family history alone only identifies a small number of children at risk [189]. Monozygotic twins provide evidence that only 50-60% of atopic diseases and only 26% of asthma are inherited [33]. In fact, asthma is believed to be a multifactorial disease, rather than being purely genetically driven and environmental exposures cannot be ignored as they may also alter asthma risk along with genetic factors. A large cross-sectional study in Southern Californian found that parental history of asthma was associated with early-onset asthma. They also found that a sibling history of asthma was strongly associated with early-onset persistent asthma among children without a parental history of asthma [185]. This finding of sibling history of asthma as an independent risk factor indicated either the importance of early-life environment or an interaction between genetics and the early environment.

2.5.2 Antioxidant defence genes and asthma susceptibility

The importance of antioxidant genes: GWAS have been searching for asthma related genes, however, the findings are limited. Some genetic variants have shown significance, but the associations were relatively moderate. Many genes that may have an association with asthma may not be detected in these studies as they may be related to asthma risk only in combination with other genes or environmental factors. Antioxidant genes are not usually directly associated with asthma, but they may interact with oxidative exposures to influence the risk of asthma. For instance, GSTP1 was initially considered as a candidate gene by asthma linkage studies for its potential antioxidative mechanism that could influence the development of asthma [190]. However, the increased risk of disease independently associated with this individual

GST gene is relatively low [191]. A meta-analysis found that GSTM1 null alleles were associated with a slightly increased risk of asthma outcomes (OR 1.19, 95% CI 1.03, 1.38), and GSTT1 null alleles were associated with 1.39 folds (95%CI 1.09, 1.77) increased odds of asthma outcomes. People with specific GST genetic polymorphisms are more susceptible to asthma when exposure to environmental risk factors. These findings reflect the multifactorial aetiology of asthma, and a GWAS approach investigating direct effects of individual genes may not be an appropriate approach to determine the causes of this complex disease. There are several antioxidant genes that have been identified as potentially important for lung health and have been more commonly studied including,: NAD(P)H dehydrogenase [quinine] 1 (NQO1), GSTs, and heme oxygenase 1 (HMOX-1) [2]. The effect of each antioxidant gene individually may be very small, hard to detect and dependent on the presence of specific environmental exposures. The studies investigating these interactions between antioxidant genes and environmental exposures have greatly increased in the past decade for many complex diseases [21].

The importance of gene-environment interactions: Studies of genetic susceptibility greatly improve our understanding of the pathophysiological mechanisms of environmental exposures leading to asthma. Current studies have unexpectedly shown relatively small effects for various environmental exposures with biological plausibility to impact asthma risk. This may be because individual differences in susceptibility to exposures (determined by genetic capacity) were ignored. Environmental exposures like air pollution and ETS have been shown to induce oxidative stress, however, individuals differ in their ability to deal with an oxidant burden, and these differences may be partially due to genetic variants. Antioxidant genes, regulating antioxidant defences, are likely to modulate the response to pollutants. Thus, the effects of environmental exposures on respiratory health may be found only for individuals with a high-risk genetic profile who are also exposed to a high-risk environment. This hypothesis has been investigated by several studies, although the evidence is still not clear. For example, a review of gene-environment interactions suggests that three common antioxidant genetic variants (NQO1, HMOX-1 and GSTs) alter individual susceptibility to the effects of air pollution on respiratory

health [2]. Based on these hypotheses, preventive strategies can be targeted to individuals with high-risk genetic profiles in order to increase antioxidant defences through methods such as antioxidant vitamin supplementation [21].

2.5.3 GST genes

Though there were many antioxidant genes, my PhD thesis is concerned with specific antioxidant genes, GSTs, and their potential to modify associations between oxidant exposures and lung health. There is increasing evidence that GST polymorphisms may influence susceptibility to asthma, atopy and airway hyperresponsiveness via oxidative stress pathways [190]. Understanding the GST genes that are associated with asthma development and specific phenotypes will provide insight into mechanisms of asthma severity and disease progression, as well as help to develop personalized therapies for susceptible people.

2.5.3.1 Overview of GST genes

Why are GSTs important: GSTs are a supergene family coding for enzymes that act to detoxify ROS with glutathione, thus defending tissues against oxidative stress [2, 190]. The level of GST gene expression is particularly important in determining the resultant enzyme levels available to assist with detoxification of chemicals and xenobiotic compounds [113]. In the process of detoxification, glutathione firstly binds with ROS or xenobiotics, and then GST enzymes catalyse the conjugation of glutathione with ROS and xenobiotics, in order to convert them to less reactive and more water-soluble compounds. Finally, the detoxified compound is eliminated (Figure 2.4) [113, 167]. GST enzymes have been widely studied as potential biomarkers for oxidative stress related to environmental exposures[113, 167].

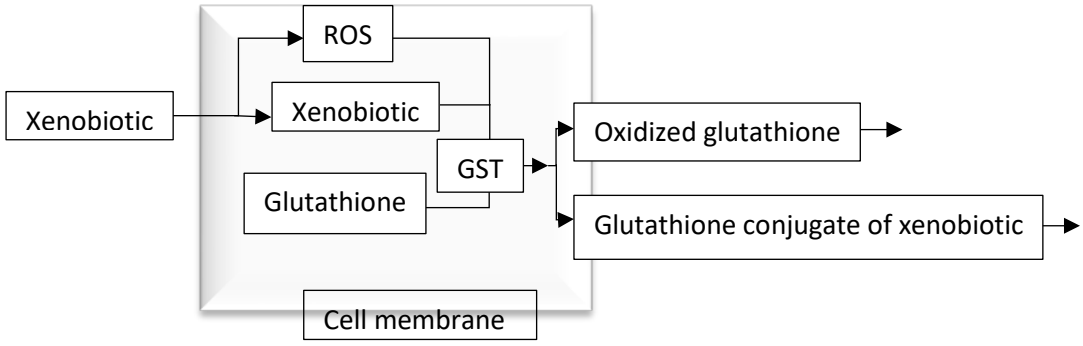

Figure 2.5 Glutathione neutralizes xenobiotics and ROS with the help of GSTs

There are several classes of GSTs, but the three main classes are: pi (GSTP), mu (GSTM), and theta (GSTT).

Overview of GSTP1: There is only one isoform identified for GSTP, called GSTP1, which is located on chromosome 11q13. GSTP1 is the most common form of GST found in the respiratory tract lining fluid, responsible for over 90% of GST enzyme activity [2]. GSTP1 has complex genetic variation and haplotypes in terms of 4 alleles identified as GSTP1*A (Ile105-Ala114), GSTP1*B (Val105-Ala114), GSTP1*C (Val105-Val114) and GSTP1*D (Ile105-Val114) [192]. Current studies have focussed on the Ile105-Val105 substitution rather than haplotypes because enzymes with Ala114val have lower allele frequencies [192, 193] and there is no evidence of association with respiratory health in previous studies [37, 194]. In terms of the amount of active GST enzymes produced from polymorphisms of GSTP1 (Ile/Val 105), the homozygous wild genotype (Ile/Ile) has the highest level of enzyme activity, whilst the homozygous mutant genotype (Val/Val) has the lowest activity [195]. However, some studies unexpectedly found GSTP1 with Ile/Ile genotypes to be associated with increased risk of asthma [196]. The reason for this association is not clear but may be because only single SNPs but not common haplotypes (including multiple SNPs) were being considered.

Overview of GSTM1 and GSTT1: The GSTM class is located at a 97-kb region on chromosome 1p13. There are five isoforms within the GSTM family. Though all GSTM genes have specific functions on each isoform and should be considered separately for health outcomes, only the isoform GSTM1 polymorphism has been well studied so far. The GSTT class is located on chromosome 22q11 and there is only one isoform called GSTT1. Unlike GSTP1, the GSTM1 and GSTT1 polymorphisms are usually evaluated in terms of the presence or absence of homozygous deletion (present or null genotype). People with null genotypes have complete loss of enzyme activity resulting in the reduction of antioxidant capacity [2]. There is some evidence that people with GSTM1 null and GSTT1 null genotypes exposed to various environmental oxidants are more likely to develop adverse respiratory outcomes [197, 198].

Prevalence of GST genes: These variations of GST genes are common in the general population, which increases the public health importance of studying GST

susceptibility. The frequency of GST variants differs across ethnic groups. The frequency of Val alleles in GSTP1 varies from 14% (95% CI 9, 19) in Asians to 32% (95%CI 31, 33) in Europeans [2]; The prevalence of GSTM1 null genotypes varies from 18% to 66% overall (median 50%) [2]. Although there is typically a similar prevalence across ethnicities, it is slightly higher in Asian populations 48%-63%, than for people of African descent (32% to 53%), Hispanic descent (40%-53%) and Europeans (35% to 62%). GSTT1 null genotypes are also higher in Asians, ranging from 38%-58%, than among those of European ancestry (15%-31%) and African descent (22%-29%). The prevalence of GSTT1 null is lowest in Hispanic populations (10%-12%) [199].

GSTs detoxify different substances: The different classes of GST enzymes preferentially detoxify different ROS products. For instance, GSTMs can utilize quinone metabolites of catecholamines (dopachrome), but conversely, the same function has not been seen in GSTT1 and GSTP1 [200]. GSTT1 utilizes oxidized lipid and DNA, whilst GSTP1 detoxifies the base propenal that arise from DNA oxidation [200]. Both GSTM1 and GSTT1 (but not GSTP1) can also work with phospholipid hydroperoxide [200]. Therefore, some GSTs may not alter the influence of particular exposures, and this could contribute to some of the inconsistency between findings.

GSTs influence many diseases: Particular genotypes of these GST polymorphisms have clinical significance because they are associated with an increased susceptibility to poor health outcomes. Apart from their association with asthma [108], polymorphisms of GSTP1, GSTM1 and GSTT1 have been reported to be associated with various diseases including cancers [78], atherosclerosis[201], allergies [20], Alzheimer's [202] and Parkinson's disease [203].

2.5.3.2 Evidence for an association between GSTs and respiratory health

Although there is a good biological rationale for a relationship between GST genes and respiratory health, the current evidence is still weak or inconsistent (Table 2.4). The most studied GST gene in relation to lung function and asthma outcomes is GSTP1, but the evidence for GSTP1 risk alleles is inconsistent. As the GSTP1 with Ile/Ile genotypes has the highest enzyme activity, carriers of GSTP1 with any Val alleles would be expected to have a higher risk of respiratory symptoms. There is some support for this

hypothesis. A case control study in Turkey found that subjects with GSTP1 homozygous Val/Val genotypes had a 3.55 fold increased risk of atopic asthma in adulthood (95%CI 1.10, 12.56) compared to those with homozygous Ile/Ile genotypes [204]. A Spanish population-based birth cohort study found that 6-year-old children with GSTP1 Val/Val genotypes had an increased risk of wheezing compared to those with Ile/Ile genotypes (OR 5.01, 95%CI 1.72, 14.58). The risk appeared to be dose dependent with respect to the number of Val genotypes, with an intermediate risk for children with GSTP1 Ile/Val genotypes (2.32 95%CI 1.03, 5.23) [205]. However, a greater number of studies have found the opposite relationship. A UK study conducted on adults reported that having the GSTP1 homozygous Val variant was associated with a sixfold lower risk of asthma compared with homozygous Ile carriers [200]. A case-control study in Pakistan also found that carriers of GSTP1 with Val/Val genotypes were more frequently found in the non-asthmatic population than in asthmatic patients [206]. Other studies have also found a protective effect of GSTP1 homozygous Val/Val on asthma phenotypes in Egyptian children [207], the Swiss general population [196] and Japanese patients with COPD [194]. Moreover, not all studies found an association between the GSTP1 genotype and respiratory health. A German study did not find evidence that GSTP1 polymorphisms played a major role in the development of bronchial asthma in children [208].

Overall, null GSTM1 and GSTT1 genotypes seem to be associated with adverse asthma outcomes. A case control study in Turkey conducted on patients hospitalized for asthma and healthy volunteers (controls) found that asthma patients had a higher prevalence of both GSTM1 null genotypes (OR 3.55, 95%CI: 1.10, 12.56), and GSTT1 null genotypes (OR 2.34, 95%CI: 1.31, 4.20) than controls [204]. Similar case control studies were also undertaken in Russia [209], Iran [210]and China [211] to identify potentially adverse effects of GSTM1 and GSTT1 null genotypes on asthma outcomes. Although all these studies found positive associations between null genotypes and asthma risk, they were all small studies with sample sizes of less than 300 participants. In contrast, two large cohort studies, the Swiss Study on Air Pollution and Lung Diseases in Adults (SAPALDIA) [196] (n=4,422), and ALSPAC [205] in the UK (n=7,262), did not find evidence of associations between GSTM1 or GSTT1 genotypes and asthma.

Table 2.4 Associations between GST genes and asthma

Reference	Outcomes	Outcome age	Main results
			Case-control studies
Fryer et al. (2000) [192]	Atopic asthma	Adults	GSTP1 with Val/Val alleles associated with reduced risk of asthma compared to Ile/Ile (OR 0.16, 95%CI 0.05, 0.55); No evidence for GSTM1 and GSTT1.
Chung et al. (2002) [212]	Physician diagnosed asthma	Adults	No evidence for GSTM1, GSTT1, or GSTP1.
Sideleva et al. (2002) [213]	Atopic asthma	Adults and children	GSTM1 (OR 3.49, 95%CI 1.91, 6.38) and GSTT1 (OR 6.66, 95% CI 3.55, 12.52) null genotypes were associated with risk of asthma.
Vavilin et al. (2002) [209]	Physician diagnosed asthma	Children	No evidence GSTM1 or GSTT1 null genotypes were associated with risk of asthma.
Safronova et al. (2003) [214]	Bronchial asthma	Children	No evidence for GSTP1.
Aynacioglu et al. (2004) [215]	Physician diagnosed asthma	Adults	GSTP1 with Val/Val alleles were associated with decreased risk of asthma compare to those with Ile/Ile (OR 0.31, 95%CI 0.14, 0.69).
Saadat et al. (2004) [216]	Physician diagnosed asthma	Adults and children	GSTM1 (OR 3.17, 95%CI 1.65, 6.12) and GSTT1 (OR 2.62, 95%CI 1.41, 4.87) null genotypes were associated with risk of asthma.
Tamer et al. (2004) [204]	Physician diagnosed asthma	Adults	GSTM1 null genotypes (OR 2.51, 95%CI 1.43, 4.42) and GSTP1 with Val/Val alleles (OR 4.27, 95%CI 1.71, 10.69) were associated with increased risk of asthma; No evidence for GSTT1.
Zhang et al. (2004) [211]	Physician diagnosed asthma	Adults	GSTM1 (OR 10.39, 95% CI 4.42, 24.46) and GSTT1 (OR 19.15, 95%CI 7.28, 50.41) null genotypes were associated with increased risk of asthma.
Lee et al. (2005) [217]	Parent reported asthma	Children	GSTP1 with Ile/Val genotypes (OR 0.51, 95%CI 0.28, 0.93) were associated with decreased risk of asthma compared to those with Ile/Ile; No evidence for GSTM1.
Nickel et al. (2005) [218]	Physician diagnosed asthma	Children	No evidence for GSTP1.
Oh et al. (2005) [219]	Physician diagnosed asthma	Adults and children	No evidence for GSTP1.
Arbag et al. (2006) [220]	Physician diagnosed asthma	Adults and children	No evidence for GSTM1 and GSTT1.
Ercan et al. (2006) [221]	Physician diagnosed asthma	Children	No evidence for GSTM1, GSTT1, or GSTP1.

Holla et al. (2006) [222]	Physician diagnosed asthma	Adults and children	No evidence for GSTM1 or GSTT1.
Plutecha et al. (2006) [223]	No defined asthma	Adults	No evidence for GSTP1.
Abdel-Alim et al. (2007) [224]	Physician diagnosed Asthma	Children	No evidence for GSTP1.
Hanene et al. (2007) [225]	No defined asthma	Children	GSTM1 null genotypes (OR: 2.35, 95% CI 1.48, 3.75) were associated with increased risk of asthma, GSTP1 with Val/Val alleles (OR: 0.39, 95%CI 0.19, 0.80) were associated with decreased risk of asthma compared with those with Ile/Ile genotypes. No evidence of GSTT1.
Kamada et al. (2007) a [226]	Physician diagnosed asthma	Adults and children	No evidence for GSTM1.
Kamada et al. (2007) b [226]	Physician diagnosed asthma	Children	No evidence for GSTM1.
Mak et al. (2007) [227]	Physician diagnosed asthma	Adults	No evidence for GSTM1, GSTT1 or GSTP1.
Babusikova et al. (2009) [228]	Physician diagnosed asthma	Children	No evidence for GSTT1.
Castro-Giner et al. (2009) [229]	Self-reported asthma	Adults	No evidence for GSTM1, GSTT1 or GSTP1.
Mahmoud et al. (2011) [230]	GINA criteria of asthma	Adults	No evidence for GSTP1.
Tatarskyy et al. (2011) [231]	Physician diagnosed asthma	Adults and children	GSTP1 with any Val genotypes (OR 0.39, 95% CI 0.20, 0.77) was associated with decreased risk of asthma compared to Ile/Ile genotypes; No evidence for GSTM1 and GSTT1.
Dar et al. (2017) [232]	Allergic asthma	Children	No evidence for GSTM1 and GSTT1.
		Cohort studies	
Imboden et al. (2007) [233]	Self-reported asthma	Adults	No evidence for GSTM1, GSTT1 and GSTP1.
Schroer et al. (2009) [22]	Parent-reported wheezing	Children	No evidence for GSTP1.
Turner et al. (2018) [234]	Asthma attack	Children	No evidence for GSTM1, GSTT1 and GSTP1.

Cross-sectional studies			
Salam et al. (2007) [235]	Parent-reported asthma	Children	Marginal association between GSTM1 null genotypes (OR 1.18, 95% CI 0.97, 1.13) and increased risk of asthma; No evidence for GSTT1 and GSTP1.
Gilliland et al. (2002) [125]	Parent-reported wheezing	Children	No evidence for GSTM1.
Kabesch et al. (2004) [236]	Parent-reported asthma	Children	No evidence for GSTM1 and GSTT1.

In 2013, a meta-analysis was conducted on 43 papers, including studies in both adults and children, and found no evidence for associations between GSTM1 or GSTP1 and asthma susceptibility. However, there was evidence that GSTT1 null genotypes were associated with increased risk of asthma (pooled OR 1.33, 95%CI 1.10,1.60), but high heterogeneity was identified in these analyses [237]. Another review and meta-analysis, conducted in 2010, summarised the evidence for an association between GST genes and asthma phenotypes and did not support a substantial independent role of GST genes in the development of asthma [205]. There are several possible reasons for this lack of evidence to support associations. Firstly, people may have overlapping GSTM1, GSTT1 and GSTP1 de-functional and beneficial polymorphisms, such that a deficiency in one isoform may be compensated for by another. Conversely, the combination of multiple GST risk genotypes may have an additive effect on respiratory health outcomes. To explain this, the same meta-analysis included 14 additional studies on adults and found a higher risk of asthma for people with GSTM1 null and GSTT1 null together (OR 2.15, 95%CI 1.21, 3.71) compared to those with two non-null genotypes, but no evidence of an association in those with only one null GSTM1 or GSTT1 gene [205].

Failure to account for environmental exposures may also partially explain the lack of consistent, strong evidence for an association between GST genes and asthma. Both meta-analyses suggested that future studies of large sizes should focus on GST genes in conjunction with environmental oxidative exposures [205, 237]. The presence of gene-environment interactions may help to explain the weak or undetectable genetic main effects found in face of the strong biological plausibility. Thus, the main aim of my thesis is to determine if GST genetic differences modify the association between various household exposures and respiratory outcomes.

2.6 GST genes, household oxidative exposures and asthma, allergies and lung function

Though the evidence in the literature does not support a substantial independent role of GST genes on respiratory health, it is highly likely that household exposures interact with GST genes involved in the antioxidant pathway. I have summarized the

evidence on GST genes as modifiers of the relationship between indoor exposures and asthma, allergies and lung function below.

Key messages in this section

- Carriers of GSTM1/T1 null genotypes are more susceptible to household oxidative exposures, and have a higher risk of asthma, allergies and lung function deficits.
- Evidence related to the GSTP1 risk genotype is inconsistent, but most studies suggest that carriers of GSTP1 Val genotypes have an increased risk of asthma and lung function deficits when exposed to household oxidative exposures.

2.6.1 Evidence of GSTM1 polymorphisms on the relationship between common household exposures and asthma, allergies and lung function

Evidence for an interactive effect of GSTM1 on the association between household exposures and adverse respiratory outcomes is summarized in Table 2.5. As mentioned above, the overall evidence supports a relationship between GSTM1 null genotypes and increased risk of respiratory issues in individuals exposed to various environmental factors.

Table 2.5 Influence of GSTM1 polymorphisms on the relationship between household environmental exposures and asthma, allergies and lung function

Reference	Household exposures	Respiratory/allergic outcomes	Outcome age	Results (interaction)
Gilliland et al. (2002) [125]	*In-utero* maternal smoking	Ever/current asthma, medication for asthma, early onset asthma, persistent asthma, ever wheezing, wheeze with/without cold, persistent wheeze.	Over 8 years (range 8-14 years)	Evidence for GSTM1 interaction ($p<0.05$ for a range of asthma and wheezing outcomes).
He et al. (2002) [238]	Active smoking during last 5 years	FEV_1 %predicted	35-60 years	No evidence of interaction
He et al. (2004) [239]	Smoking history (pack-year)	High lung function and low lung function group	35-60 years	No evidence of interaction
Kabesch et al (2004) [236]	*In-utero* /current ETS	Current/ever wheeze, current wheeze.	9-11 years	Evidence for GSTM1 interaction (p values ranged from (0.05-0.77).
Palmer et al. (2006) [197]	ETS	PEFR %predicted, FEV_1, and FVC	2 groups: 3-12 years and 13-21 years	Evidence of interaction in older asthmatic children.
Imboden et al. (2007) [233]	Ever smoking/never smoker/persistent smoker	Difference in mean annual change in FEV_1, FVC and FEF_{2575} over 11 years	Age at baseline 40.8±11.5 years	No evidence of interaction.
Lee et al. (2007) [240]	Household ETS	Ever/current wheeze	Cases: 11.8 ±1.7 years Controls: 12.1±1.8 years	No evidence of interaction.
Li et al. (2009) [38]	ETS	Ever asthma	Grade 1-6	Borderline evidence for GSTM1 interaction (no p value reported)
Murdzoska et al (2009) [241]	*In-utero* smoking	Maximal flow at functional residual capacity ($Vmax_{FRC}$)	1, 6 and 12 months	No evidence of interaction.
Rogers et al. (2009) [36]	*In-utero* and postnatal ETS	Current asthma, FEV_1 %predicted, FVC %predicted, FEV_1/FVC	8.8 years ±2.1	Evidence of GSTM1 interaction for in utero ($p=0.02$) and ETS ($p=0.08$)

				for asthma outcomes, and p<0.05 for lung function.
Shaheen et al. (2010) [176]	Prenatal and infant paracetamol exposure	Asthma/wheezing	7 years	No evidence of interaction.
Wang et al (2010) [242]	*In-utero* smoking	Atopic dermatitis	2 years	Evidence of interaction (no p value provided).
Gerbase et al (2011) [243]	Second-hand smoking	Allergic rhinitis	Mean age 50 years	No evidence of interaction.
Wang et al. (2011) [244]	Incense burning smoke in past 12 months	Ever/current asthma, ever/current wheeze, use of asthma medication, exercise wheeze	12.26 ±0.50 years	No evidence of interaction.
Hersoug et al. (2012) [16]	ETS, use of wood stove/candles during wintertime, active smoking	Current wheeze, FEV1 %predicted	18-69 years	No evidence of interaction (p values ranged from 0.3 to 0.9).
Malling et al. (2012) [198]	Current active smoker/ever smoker	FEV_1 %predicted, $FEV_1/height^2$	22-44 years	Evidence of GSTM1 interaction (p=0.03).
Wu et al. (2014) [245]	*In-utero*/family member smoked	Ever asthma, dermatitis rhinitis	6 years	Evidence of interaction for ever asthma in girls. No associations found for dermatitis and rhinitis.

Asthma: One of the earliest studies that investigated GSTM1 interactions, conducted in the US in 2002, found an association between *in-utero* exposure to maternal smoking and increased risk of childhood asthma and wheezing occurrence at school age, that was only restricted to children with GSTM1 null genotypes (exact interaction p values were not provided but marked with asterisk when < 0.05). In this study, *in-utero* exposure to smoking was widely associated with a range of wheezing outcomes but not with asthma outcomes when there was no interaction considered. Moreover, GSTM1 genotype or current maternal smoking were not found to be individually associated with any asthma or wheezing outcomes [246]. Another German study on school age children extended these findings into current exposure to environmental tobacco smoke (ETS) in childhood. The authors found evidence that children lacking the GSTM1 allele who were living in a household with a heavy smoker (>20 cigarettes per day) had increased risk of current asthma, current wheeze, ever wheeze and shortness of breath, compared to those with GSTM1 present alleles, however, interaction p values were not significant (p values = 0.77, 0.71, 0.56 and 0.15, respectively) [247]. In contrast, a Taiwanese study provided conflicting evidence of a borderline protective association between GSTM1 null genotypes and decreased risk of asthma when exposed to ETS (p=0.0573) [38].

Allergies and lung function: There have been a limited number of studies investigating the interactions between GSTM1 polymorphisms and environmental exposures on the risk of allergies and lung function, and the findings are inconsistent. In Taiwan, there was evidence that children with GSTM1 null genotypes were more likely to develop atopic dermatitis by 2 years of age when they were exposed to *in-utero* smoking, compared to those with GSTM1 present genotypes [242]. A Swiss study conducted in adulthood did not identify an interaction between GSTM1 and active smoking on rhinitis risk, however, people with GSTM1 null variants had an increased risk of having a high total IgE level if they continuously smoked for 5 years during the follow-up when compared with the continuously smoking GSTM1 present group [243]. Three studies found that carriers of GSTM1 null variants who were exposed to smoking, had an increased risk of impaired lung function in terms of FEV_1 %predicted [197, 198],

FVC %predicted and FEV_1/FVC ratio compared to those with GSTM1 present variants [36].

2.6.2 Evidence of GSTT1 polymorphisms on the relationship between common household exposures and asthma, allergies and lung function

Asthma and allergies: The evidence regarding an interactive effect of GSTT1 on the relationship between indoor exposures and adverse respiratory outcomes is presented in Table 2.6. Overall, there was less evidence for an interaction in relation to asthma or allergy outcomes. Of the 5 papers, 2 found evidence of an interaction. These two cross-sectional studies both investigated asthma in school age children. One study found that the frequency of incense burning was associated with an increased risk of current asthma, asthma medication use, lifetime wheeze, nocturnal wheeze and exercise wheeze in children with GSTT1 null genotypes. These associations were not seen in those with GSTT1 present genotypes [244]. The other study also found evidence that GSTT1 null children had an increased risk of ever wheeze with ETS exposure, compared with GSTT1 present children not exposed to ETS [236]. Two other studies investigated allergic outcomes and did not find evidence of interactions. The Taiwan Birth Panel Cohort study investigated cotinine levels as a biomarker of smoking exposure, finding no evidence that GSTT1 null genotypes were related to atopic dermatitis in children at 2 years regardless of blood cotinine levels [242]. Another European study did not identify an interaction between GSTT1 and current smoking on rhinitis on adulthood [243]. A Copenhagen study even considered an expanded number of common indoor air pollutants like the use of woodstoves and candles on wheeze outcomes, however, no evidence of an interaction was found (all interactions $p>0.2$) [16].

Table 2.6 Influence of GSTT1 polymorphisms on the relationship between household environmental exposures and asthma, allergies and lung function

Reference	Household exposures	Respiratory/allergic outcomes	Outcome age	Results (interaction)
He et al. (2002) [238]	Active smoking during last 5 years	FEV_1 %predicted	35-60 years	No evidence of interaction.
He et al. (2004) [239]	Smoking history (pack-year)	High lung function and low lung function group	35-60 years	Evidence of GSTT1 interaction (p=0.038)
Kabesch et al (2004) [236]	*In-utero* /current ETS	Current/ever wheeze, current wheeze.	9-11 years	Evidence for GSTT1 interaction (p values ranged from (0.05-0.77).
Imboden et al. (2007) [233]	Ever smoking/never smoker/persistent smoker	Difference in mean annual change in FEV_1, FVC and FEF_{2575} over 11 years	Age at baseline 40.8±11.5 years	In male, evidence of GSTT1 interaction on FEV_1 decline in ever smoker (p<0.001) and persistent smoker (0.029).
Murdzoska et al (2009) [241]	*In-utero* smoking	Maximal flow at functional residual capacity ($Vmax_{FRC}$)	1, 6 and 12 months	Evidence of GSTT1 interaction (p=0.008)
Shaheen et al. (2010) [176]	Prenatal and infant paracetamol exposure	Asthma/wheezing	7 years	No evidence of interaction.
Gerbase et al (2011) [243]	Second-hand smoking	Allergic rhinitis	Mean age 50 years	No evidence of interaction.
Wang et al. (2011) [244]	Incense burning smoke in past 12 months	Ever/current asthma, ever/current wheeze, use of asthma medication, exercise wheeze	12.26 ±0.50 years	Evidence of GSTT1 interaction (p<0.05).
Hersoug et al. (2012) [16]	ETS, use of wood stove/candles during wintertime, active smoking	Current wheeze, FEV_1 %predicted	18-69 years	No evidence of interaction (p values ranged from 0.3 to 0.9).
Malling et al. (2012) [198]	Current active smoker/ever smoker	FEV_1 %predicted, $FEV_1/height^2$	22-44 years	No evidence of interaction.

Lung function: Only one of the studies that investigated the relationship with lung function found evidence of an interaction. This study investigated infant lung function, finding that in a group of infants who were exposed to tobacco smoke *in utero*, GSTT1 present infants had increased VFRCmax in the first year of life compared with GSTT1 null infants, and no evidence of associations were seen in the groups (GSTT1 present and absent) not exposed to *in utero* tobacco smoke (interaction p=0.037) [241]. Another two studies conducted on adults investigated an interaction between GSTT1 genotype and pack-years on lung function in smokers, though neither found evidence of an interaction [233, 239].

2.6.3 Evidence of GSTP1 polymorphisms on the relationship between common household exposures and asthma, allergies and lung function

Asthma and allergies: The evidence regarding a potential interactive effect of GSTP1 on the relationship between indoor exposures and adverse respiratory outcomes is presented in Table 2.7. Though GSTP1 is the most commonly studied of the three mentioned GST polymorphisms, particularly in conjunction with more varied exposures (more indoor air pollution and pharmaceuticals), the findings with respect to risk alleles are more conflicting. There are four papers providing evidence that GSTP1 with Val alleles is associated with increased risk of asthma/wheeze/allergy outcomes in childhood with exposure to *in utero* smoking [240, 242, 248, 249]. However, another study did not identify an interaction between GSTP1 and ETS on asthma severity score, and it provided conflicting evidence on GSTP1 risk alleles on atopy (defined by positive skin prick test). In this study, people with Ile/Ile genotypes had increased risk of atopy (OR=9.02, p=0.05) when assessed by genotype alone, this risk increased to 13.66 fold (p=0.029) when ETS was included with an interaction p value of 0.08, but the results may be limited by the small sample size (n=221) [37]. Additionally, a Korean study conducted three-way interactions between GSTP1, vitamin A and ETS, and found evidence that school-age children with GSTP1 homozygous Ile genotypes with low vitamin A levels had an increased risk of asthma diagnosis (OR 4.44, 95% CI 1.58, 12.52) compared to children who did not have these two risk factors [39]. Finally, three other studies showed no evidence of an interaction for GSTP1 genotypes on indoor air exposures. A birth cohort study did not find

interactions between GSTP1, ETS, and mould exposures on persistent wheezing for infants at both 12 and 24 months of age [22]. A separate study also failed to identify interactions related to rhinitis and smoking in adulthood [243]. Lastly, a case-control study in Northern Mexico also found that associations between GSTP1 and childhood asthma were similar between groups with and without exposure to tobacco smoke and no significant differences were observed [250].

Table 2.7 Influence of GSTP1 polymorphisms on the relationship between household environmental exposures and asthma, allergies and lung function

Reference	Household exposures	Respiratory/allergic outcomes	Outcome age	Results (interaction)
He et al. (2002) [238]	Active smoking during last 5 years	FEV_1 %predicted	35-60 years	No evidence of interaction.
He et al. (2004) [239]	Smoking history (pack-year)	High lung function and low lung function group	35-60 years	No evidence of interaction
Imboden et al. (2007) [233]	Ever smoking/never smoker/persistent smoker	Difference in mean annual change in FEV_1, FVC and FEF_{2575} over 11 years	Age at baseline 40.8±11.5 years	No evidence of interaction.
Lee et al. (2007) [240]	Household ETS	Ever/current wheeze	Cases: 11.8 ±1.7 years Controls: 12.1±1.8 years	Evidence of GSTP1 interaction (p=0.01 for ever wheeze, p=0.004 for current wheeze).
Li et al (2008) [248]	*In-utero* smoking	Ever asthma, current wheeze, early/late onset asthma, medication for wheeze	8-18 years	Evidence for interaction on outcome current wheeze (p=0.035), and medication for wheezing (p=0.043).
Murdzoska et al (2009) [241]	*In-utero* smoking	Maximal flow at functional residual capacity (VFRCmax)	1, 6 and 12 months	No evidence of interaction.
Perzanowski et al. (2009) [251]	Prenatal paracetamol exposure	Wheezing	5 years	Evidence of GSTP1 interaction (p=0.009). Children with at least one Val allele were at increased risk of wheezing.
Schroer et al (2009) [22]	Household ETS	Wheezing at 12 and 24 months, persistent wheezing	12 and 24 months	No evidence of interaction (p>0.2).
Panasevich et al. (2010) [249]	Early life maternal smoking (pregnancy and early childhood)	Current asthma, early/late onset wheeze, transient wheeze	4 years	Insignificant evidence of GSTP1 on early onset wheeze (p=0.17).
Schultz et al. (2010) [37]	ETS	Asthma severity, Atopy	2-16 years	No evidence of interaction.
Shaheen et al. (2010) [176]	Prenatal and infant paracetamol exposure	Asthma/wheezing	7 years	No evidence of interaction.
Wang et al (2010) [242]	*In-utero* smoking	Atopic dermatitis	2 years	Evidence of interaction.
Gerbase et al (2011) [243]	Second-hand smoking	Allergic rhinitis	Mean age 50 years	No evidence of interaction.

Wang et al. (2011) [244]	Incense burning smoke in past 12 months	Ever/current asthma, ever/current wheeze, use of asthma medication, exercise wheeze	12.26 ±0.50 years	No evidence of interaction.
Malling et al. (2012) [198]	Current active smoker/ever smoker	FEV_1 %predicted, FEV_1/height^2	22-44 years	No evidence of interaction.
Munoz et al. (2012) [250]	ETS	Asthma (no clear definition)	6-9 years	No evidence of GSTP1
Lee et al. (2015) [39]	ETS	Current asthma	7-12 years	No evidence of GSTP1 interaction, but 3-way interaction were seen for those with Ile/Ile genotype for exposed children plus low vitamin A.

Recently, there were two studies published investigating a possible interaction between paracetamol use in infancy and GST genes on respiratory outcomes, though the results were inconsistent. One cross-sectional study (n=986) investigated interactions between paracetamol use and asthma outcomes in preschool children and found that the adverse relationship between paracetamol use during the 1st year of life and current asthma was only seen in those with GSTP1 Ile/Ile and Ile/Val genotypes, not for those with Val/Val genotypes [173]. ALSPAC (n=14,541) did not identify any interaction between infant paracetamol and GSTP1 genes on childhood asthma at 7 years [176], but they collected exposure on paracetamol intake across 6 months only. As the critical time for infant lung growth continues until the age of 2 years, the data on paracetamol use that could affect respiratory health in later life may not have been complete.

Lung function: One study, supporting the presence of a GSTP1 interaction, provided evidence on the potential adverse role of GSTP1 val105 on lung function when exposed to second-hand tobacco smoke. This childhood study found that older children (13-21 years vs 3-12 years) homozygous for GSTP1 val105 and exposed to smoking households, were more likely to have a lower Peak Expiratory Flow Rate (PEFR % predicted) compared to the group carrying GSTP1 with any Ile allele [252]. A Swiss study found that heterozygosity for GSTP1 heterozygous Ili105Val was associated with slower decline in FVC in persistent female smokers compared to those with homozygous Ile genotypes, while no association was observed in the female never-smokers' group [233]. However, female smokers with GSTP1 with homozygous Val genotypes did not show an increased decline compared to those with a homozygous Ile genotype. These associations were not seen in males [233]. Another study nested in the Lung Health Study (LHS) in North America further investigated the potential interaction of GST with smoking history on lung function, but they found neither of the GSTP1 polymorphisms tested, nor a combination of the two, provided evidence of a significant interaction [253].

2.7 Knowledge gaps in the literature

Key messages in this section

There are several factors that may contribute to the inconsistency in evidence for gene-environment interactions:

- No accurate assessment of exposures
- Lack of long-term studies
- Lack of objective outcome measurements
- Failure to adequately account for confounders

Moreover, household oxidative exposures were less likely investigated.

Inconsistent findings from gene-environment studies: Though findings from several studies support the hypothesis that GST polymorphisms modify the associations between environment exposures and asthma and respiratory diseases, these findings have not been consistent across studies. Additionally, there are several contradictory findings. There are many possible reasons for the inconsistent findings on gene-environment interactions. More studies are needed to carefully document environmental exposures and examine their relationship with the risk of asthma and reduced lung function, as heterogeneity in the measurement or definitions of exposures and outcomes, as well as study populations, may contribute to the inconsistency between studies. For instance, many studies have investigated tobacco smoke by self-report from questionnaires, not from objective exposures like urine cotinine levels. Lung function, as an objective outcome measure and continuous variable, has more power to find evidence for an association when compared with binary outcomes (like asthma). Furthermore, many studies are not conducted over long enough follow up periods for relevant outcomes to be seen, while others are cross-sectional in design and do not have the ability to establish temporality of associations. Respiratory outcomes for asthma status and lung function change over time, and studies designed to investigate these outcomes need to cover a fairly long period, ideally the entire period of lung growth during childhood up to adulthood, with sufficient power to completely assess the longitudinal effects of early life exposures on respiratory health outcomes such as maximal lung function, change of lung function and asthma incidence.

Knowledge gap 1: Perinatal tobacco smoke exposure is a key exposure of interest as there is still conflicting evidence on whether early life smoke exposure has an impact on adolescent respiratory health, as findings have been inconsistent for outcomes measured at different ages. There is a critical need for studies that can provide solid evidence related to early life smoke exposures and interactions with genetic determinants using data from a long-term cohort with frequent follow-ups and objective markers of disease.

Knowledge gap 2: Another novel area of concern is whether the observed association between early life paracetamol use and adverse respiratory outcomes is casual in nature and if this relationship is modified by GST genes. There are a number of studies investigating the association between paracetamol and asthma, however, most have been limited by crude measurements of paracetamol use, short term follow-up and incomplete adjustment for the occurrence of respiratory tract infections which may induce confounding by indication. No study has investigated associations between early life paracetamol exposure and lung function outcomes. Regarding GST interactions on the associations between paracetamol and respiratory health, only two studies have investigated interactions relating to GSTP1, paracetamol and asthma. Both of these studies had some critical limitations, for instance, small numbers of participants with "Val/Val genotypes" and limited measurement of paracetamol use, along with failure to adjust for respiratory tract infections or provide statistical evidence (no interaction p values). GSTM1 and GSTT1 have not been investigated in this context.

Knowledge gap 3: The impact of indoor air pollution on respiratory outcomes has received limited attention. The most studied indoor exposure is tobacco smoke defined as current ETS and active smoke exposure, but most studies have focused these exposures in early life, particularly in utero, and childhood, but few have measured the effect of ETS exposure in adulthood. Current studies of indoor air pollution are mainly based on the short-term relationship between tobacco smoke exposure in childhood using cross-sectional study designs. However, many other under-studied sources of indoor pollution including heating/cooking facilities and

mould may also be associated with the risk of asthma and impaired lung function throughout life. A better understanding of the effects of long-term exposures to other indoor pollution in adulthood is of paramount importance, given the large proportion of our time spent in indoor environments.

My thesis includes three chapters of original data analyses addressing the following knowledge gaps: 1) Tobacco smoke and GST interactions on lung health in adolescents [Chapter 5], 2) Paracetamol and GST interactions on lung health in adolescents [Chapter 6] and 3) Household air pollution and GST interactions on lung health in middle-aged adults [Chapter 7].

Through my PhD work, conducted with data from two well-designed longitudinal studies, I have contributed to a better understanding of key knowledge gaps on the influence of various household exposures and gene-environment interactions on respiratory diseases and lung health in children and adults. The findings from my work have the potential to inform public health policy for susceptible populations with specific gene polymorphisms.

Chapter 3 GST GENES, INDOOR AIR POLLUTION AND LUNG HEALTH: A SYSTEMATIC REVIEW

3.1 Chapter introduction

This chapter includes my published first author paper of a systematic review investigating the interaction of GST genes on the associations between indoor air pollution and asthma, allergies and lung function. This chapter also includes a data supplement containing meta-analyses, which were not included in the publication due to high heterogeneity. This chapter (with the data supplement) provides evidence that GST genes may modify the relationship between indoor air pollution and allergy/lung function. Specifically, carriers of GSTM1/GSTT1 null and GSTP1 Val genotypes were more susceptible to respiratory effects of indoor air pollution exposures.

3.2 Research aims

3.2.1 Research questions

Do Glutathione S-Transferase genes modify the link between indoor air pollution and asthma, allergies and lung function?

3.2.2 Research objectives

To investigate the influence of GST genes on the relationship between indoor air pollution and allergy/lung function.

3.3 Publication

3.3.1 Main document

Dai, X., Bowatte, G., Lowe, A. J., Matheson, M. C., Gurrin, L. C., Burgess, J. A., & ... Lodge, C. J. (2018). Do Glutathione S-Transferase Genes Modify the Link between Indoor Air Pollution and Asthma, Allergies, and Lung Function? A Systematic Review. *Current Allergy And Asthma Reports*, (3), 1. doi:10.1007/s11882-018-0771-0

3.3.2 Supplemental material (Unpublished)

Meta-analysis on the GSTM1 null polymorphism

There was extreme heterogeneity between studies for GST genes, allergic outcomes and specific lung function measurements. Therefore, meta-analysis was only performed for 3 studies (n=6405) investigating the GSTM1 deletion polymorphism. The reference group for these three studies was participants with GSTM1 present (G+) and not exposed to tobacco smoke (TS-). The studies all investigated the odds of wheezing symptoms in three different exposure groups: GSTM1 present and tobacco smoke exposed (G+N+); GSTM1 null and not tobacco smoke exposed (G-TS-) and; GSTM1 null and tobacco smoke exposed (G-TS+) Meta-analyses were performed separately for each of these (G+TS+, G-TS-, G-TS+). The random effects pooled OR (G-TS+) found that individuals carrying GSTM1 null when exposed to tobacco smoke had an increased risk of current wheeze (OR 2.15, 95% CI 1.07-4.34; I2 63.1%), and ever wheeze (OR 1.74 95% CI 1.17-2.59 I2 39.3%). Adverse outcomes were only observed when both exposures (GSTM1 null and smoking exposure) were evaluated jointly. Neither GSTM1 null nor tobacco smoke exposure alone was associated with wheeze on meta-analysis when compared to those with GSTM1 non-null, and not tobacco exposed. The random effects pooled OR found that individuals carrying GSTM1 null when exposed to tobacco smoke (G-TS+) had an increased risk of current wheeze (Figure 3.E1) and ever wheeze (Figure 3.E2). Table 3.E1 also shows the departure from the expected effect based on no interaction, and p-values support that the departures are statistically significant. Details of discussion of meta-analysis are in Chapter 8 (overall discussion, implications and conclusion).

Newcastle-Ottowa quality score (NOS)

The rating criteria of NOS is listed in Appendix 1. Of 5 studies determined to be of high quality from NOS [38, 125, 242-244], 4 support the existence of GST-indoor pollution interactions [38, 125, 242, 244]. All 4 of these were conducted in childhood whilst the paper that did not identify an interaction was conducted in adulthood. Interactions were found more commonly in childhood compared to adulthood. These findings for high-quality papers are consistent with the findings including all papers.

Figure 3.S1 Random effects meta-analysis of associations between tobacco smoke exposure and current wheeze based on GSTM1 (present vs. null)

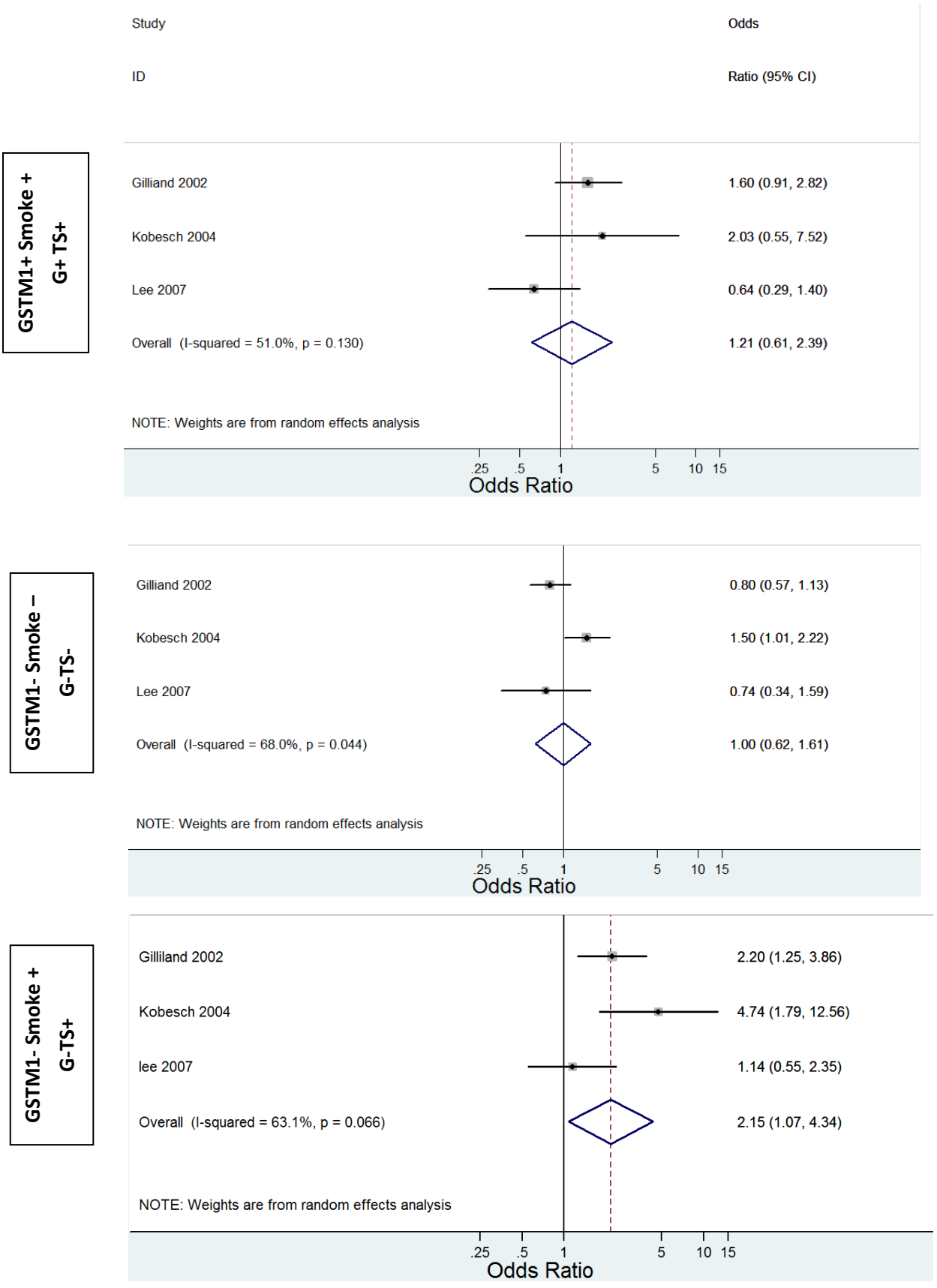

Figure 3.S2 Random effects meta-analysis of associations between tobacco smoke exposure and ever wheeze based on GSTM1 (present vs. null)

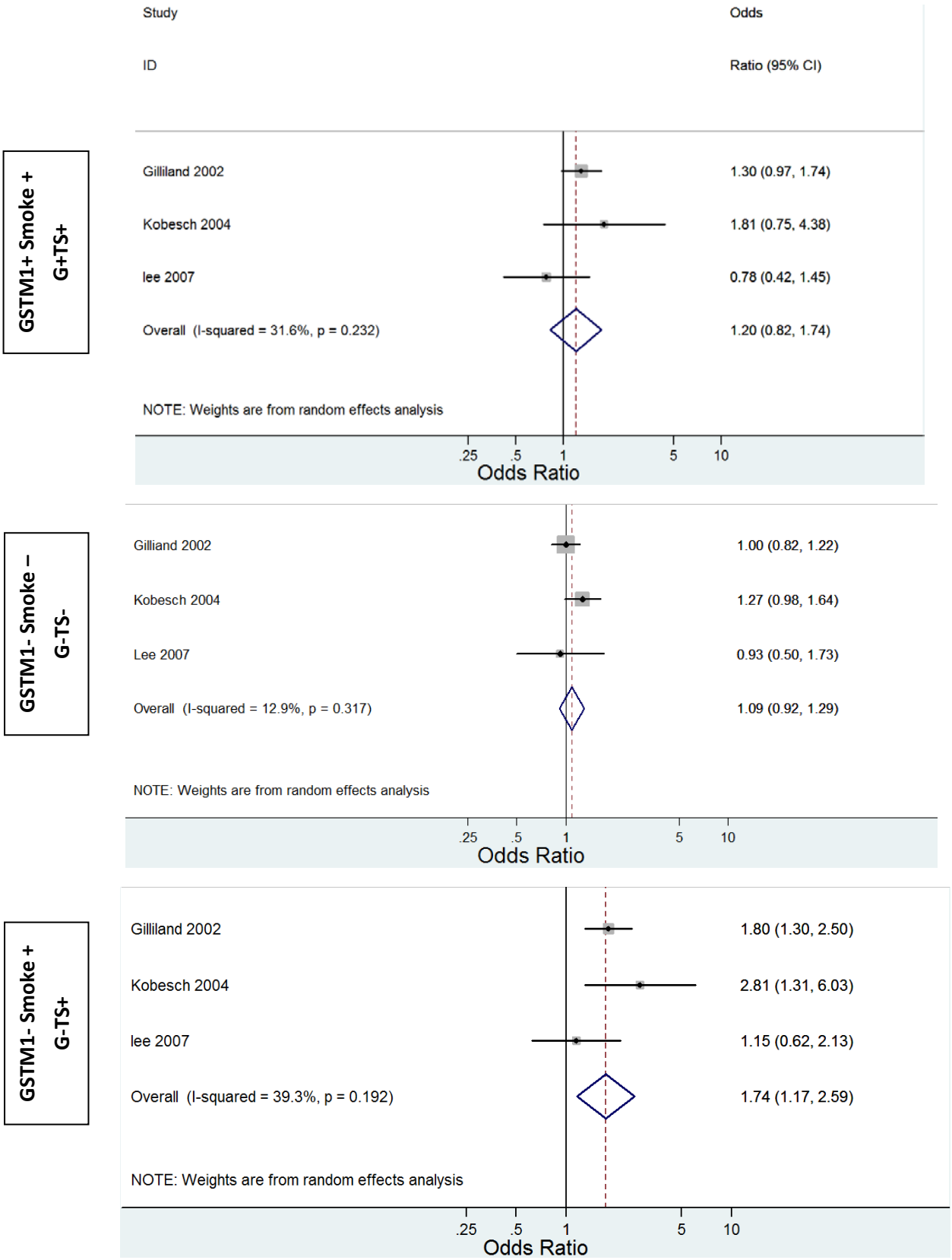

Table 3.S1 Meta-analysis of associations between nicotine smoke exposure, GSTM1 and asthma

Tobacco smoke	GSTM1	Outcomes	No. of studies	Test of association			Test of heterogeneity		
				OR	95% CI	P value	Model	P value	I^2
-	+	Current wheeze	3	Reference					
		Ever wheeze	3	Reference					
+	+	Current wheeze	3	1.07	0.55-2.10	0.836	R	0.04	69.0
		Ever wheeze	3	1.20	0.82-1.74	0.334	R	0.232	31.6
-	-	Current wheeze	3	0.83	0.61-1.13	0.239	R	0.382	0.0
		Ever wheeze	3	1.02	0.85-1.23	0.834	R	0.421	0.0
+	-	Current wheeze	3	2.15	1.07-4.34	0.032	R	0.066	63.1
		Ever wheeze	3	1.74	1.17-2.59	0.006	R	0.192	39.3

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ALLERGIES AND THE ENVIRONMENT (M HERNANDEZ, SECTION EDITOR)

Do Glutathione S-Transferase Genes Modify the Link between Indoor Air Pollution and Asthma, Allergies, and Lung Function? A Systematic Review

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Abstract

Purpose of Review Glutathione S-transferase (GST) genes are involved in oxidative stress management and may modify the impact of indoor air pollution. We aimed to assess the influence of GST genes on the relationship between indoor air pollution and allergy/lung function.

Recent Findings Our systematic review identified 22 eligible studies, with 15 supporting a gene-environment interaction. Carriers of GSTM1/T1 null and GSTP1 val genotypes were more susceptible to indoor air pollution exposures, having a higher risk of asthma and lung function deficits. However, findings differed in terms of risk alleles and specific exposures. High-exposure heterogeneity precluded meta-analysis.

Summary We found evidence that respiratory effects of indoor air pollution depend on the individual's GST profile. This may help explain the inconsistent associations found when gene-environment interactions are not considered. Future studies should aim to improve the accuracy of pollution assessment and investigate this finding in different populations.

Keywords Epidemiology · Genetics · Rhinitis · Asthma · Atopic dermatitis · Reviews

Abbreviations

AD	Atopic dermatitis
CI	Confidence interval
ETS	Environmental tobacco smoke
FEV_1	Forced expiratory volume in 1 s
FVC	Forced vital capacity
GSTs	Glutathione s-transferases
$VFRC_{max}$	Maximal flow at functional residual capacity
NOS	Newcastle-Ottawa quality assessment scale
OR	Odds ratio
PEFR	Peak expiratory flow rate
NQO1	Quinone oxidoreductase-1
ROS	Reactive oxygen species
SES	Socio-economic status
SE	Standard error

Prior presentation This work was presented in Cleaner Air, Better Health: CAR Annual Symposium 2016 in Sydney, Australia, by Xin Dai in 05/05/2016.

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Introduction

The global prevalence of allergic diseases has increased steadily for more than 50 years [1], initially due to increasing prevalence in the developed countries and more recently in developing countries [2]. The etiology of these conditions is believed to be complex [3, 4]. Exposure to indoor air pollution has been hypothesized to be particularly relevant to allergic disease pathogenesis as people spend much of their time indoors [5]. Recognized indoor air pollutants include tobacco

smoke [6, 7], dust [8, 9], and pollutants generated from cooking and heating [10]. However, studies have failed to find consistent associations between indoor air pollutants and allergic disease [11, 12]. The reason for these inconsistent findings may be partly related to failure to account for potential gene-environment interactions.

One key group of genes proposed to modify the impact of air pollutants on asthma and allergies are the Glutathione S-transferases (GSTs) genes [13, 14]. The GSTs are major phase II detoxification enzymes found mainly in the cytosol and include the Mu (GSTM1), Theta (GSTT1), and Pi (GSTP1) classes [15]. These enzymes provide the first line of defense against oxidative stress through their ability to protect cells from reactive oxygen species (ROS) [16•, 17]. ROS are active participants in complex biological processes, including allergic sensitization and allergic asthma [18]. ROS can cause airway inflammation, which may result in airway dysfunction [19, 20]. The GSTM1 and GSTT1 genes have null alleles resulting in partial or complete loss of the enzyme activity [16•, 17, 21–23]. Despite the biological plausibility of a relationship between GST polymorphisms and allergies and poorer lung function, the supporting evidence is scarce and inconsistent. A 2010 systematic review investigated the role of GST genes in asthma, finding no direct association [24•]. The authors postulated that the null findings may be due to not including exposure data. Failure to take gene-environment interactions into account in this review may explain the absence of consistently reported observable effects of the GST genes. There have been no previous systematic syntheses of the literature focusing on the interaction between indoor environmental exposures and GST genes on outcomes of allergic disease and lung function.

Establishment of this potential gene-environment interaction may help to explain the inconsistencies found where these factors are investigated in isolation, and help identify an important subpopulation requiring intervention to prevent respiratory morbidity. We aimed to address this through a systematic review and meta-analysis of the existing literature investigating the influence of GST genes on the relationship between indoor air pollution and allergy/lung function.

Methods

We performed a systematic review of published studies that reported original data, according to PRISMA guidelines. The review protocol was registered with PROSPERO (CRD42017062275).

Literature Search and Study Selection

We searched PubMed and Embase databases from inception for journal articles written in English. The final search was performed on 25/03/2017. We used search terms in PubMed to identify articles having the following three categories in common: indoor air pollution (MeSH heading "smoke," "air pollution, indoor"), and, allergy (MeSH heading "asthma," "rhinitis," "allergic," "eczema," "dermatitis;" "food allergy") or lung function (MeSH heading "respiratory function test"), and GST (MeSH heading "glutathione s-transferase"). A similar search strategy was developed for Embase (see online supplement 1 and 2). The search was performed by two independent investigators (XD and GB).

Eligibility Criteria

To be included, a study had to meet the following criteria: (1) human population, (2) indoor air pollution exposure (smoke, cleaning products, heating, cooking, air quality and airborne exposure), (3) investigated interaction by GST genes, (4) assessed any allergic disease (asthma, rhinitis, eczema, dermatitis and food allergy) or lung function outcome, (5) cohort study, cross-sectional, or case-control study design. Non-English language papers, unpublished studies, and studies not reporting original data (reviews, editorials, and conference abstracts) were excluded. Two authors (XD and GB) independently evaluated all articles retrieved.

Data Extraction

Two researchers (XD and GB) independently reviewed titles and abstracts, and where necessary full texts, to determine if they met the inclusion criteria as described above. Any disagreements were settled by a third author (CL). For each included study, information regarding study design, target population, time of exposure measurement, outcome measures and age, interactions investigated, covariates included, comparison, and association/findings were extracted into a standard table and checked by two authors (XD and GB). Exposure and outcome definitions and effect estimates (odd ratios (OR), relative risks, prevalence ratios, lung function parameters) with 95% confidence interval (95% CI) were extracted where available for inclusion in meta-analyses.

Quality Assessment

Two authors (XD and GB) independently assessed the quality and bias of included studies using the Newcastle-Ottawa quality assessment scale (NOS). Each study was scored using a star (*) method to report the quality based on selection of sample, comparability and the ascertainment of the exposure or outcome measures. All papers were classified into high (8–9*), medium (6–7*), low (4–5*), and unsatisfactory quality (0–3*) based on the Newcastle-Ottawa quality score (supplemental table 1). We resolved any disagreement through

consensus with a third author (CL). We aimed to perform forest plots to address potential publication bias if applicable.

Results

Search Results

From a total of 576 citations identified initially, 71 were selected for full-text review and 22 met the inclusion criteria. Of these, 16 papers evaluated allergy-related outcomes, and 8 measured lung function outcomes (2 papers measured both asthma and lung function (Fig. 1). Supplemental table 2 outlines reasons for exclusion at the full-text review stage. We were unable to perform meta-analyses due to high heterogeneity, with specific gene-pollutant-outcome combinations lacking numbers.

Characteristics of the Included Studies

Of the 16 studies with allergic disease outcomes (Table 1), 14 investigated outcomes of asthma/wheeze [5, 25–34•, 35••, 36, 37••], and the remainder investigated outcomes of rhinitis [35••, 38] and dermatitis [35••, 39]. The 8 studies [5, 26, 40–45] investigating lung function outcomes (Table 2) used a variety of measurements including maximal flow at functional residual capacity ($VFRC_{max}$), forced expiratory volume in 1 s (FEV_1)%, forced vital capacity (FVC)%, peak expiratory flow rate (PEFR)%, FEV_1, FVC, FEV_1/FVC, FEV_1/height^2, 5-year change in FEV_1%, mean annual change in FEV_1, FVC, and high/low lung function categories. The ages at which outcomes

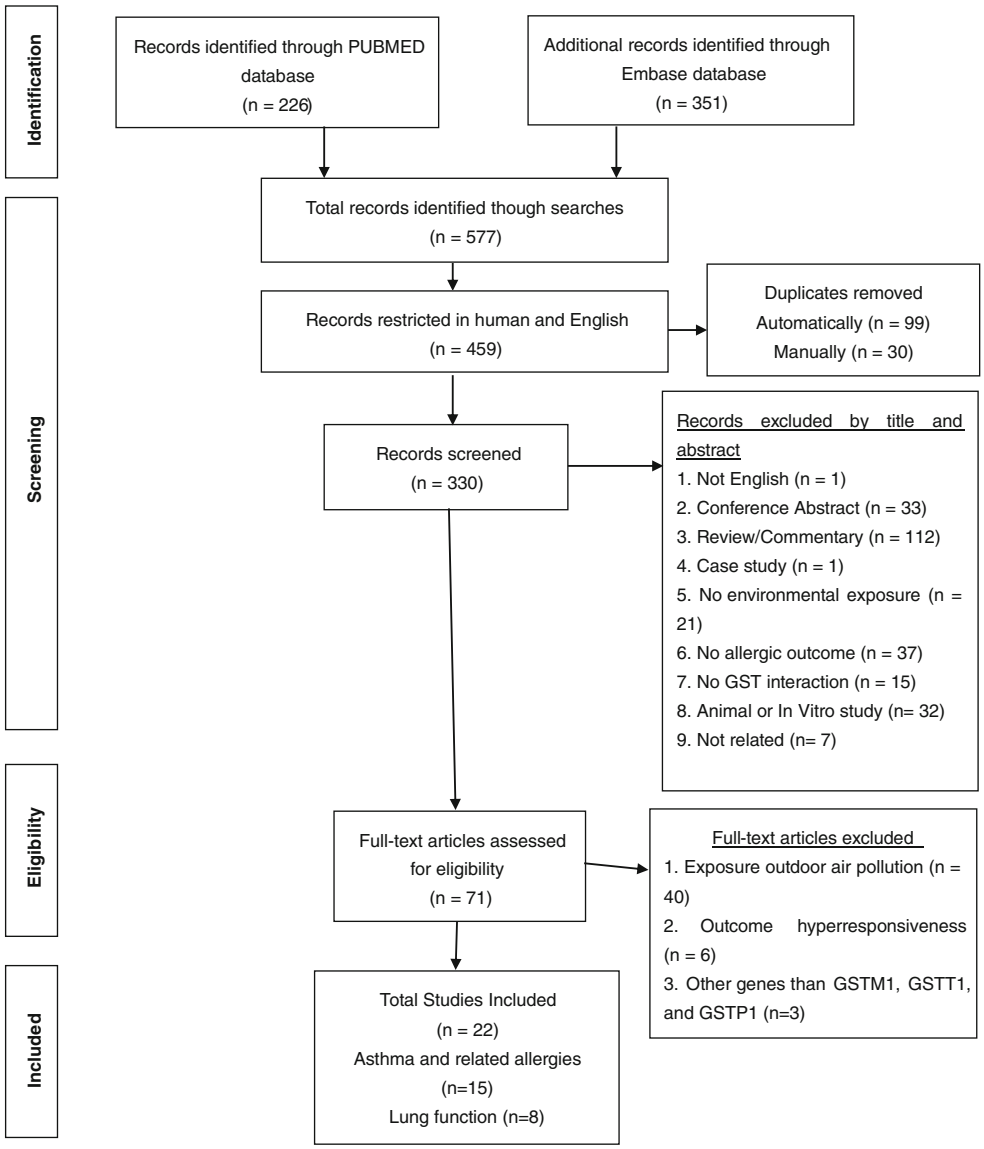

Fig. 1 PRISMA flow diagram

Table 1 Characteristics and methods of the 16 studies with allergic disease outcomes

Name of study (acronym)/ type of study/first author/date published/country	Number of participant and study dates	Exposure and age	Outcome age	Outcome measures	Interactions investigated	Association/findings	Conclusions
Birth Cohort study Wu et al. (2014) Taiwan Population based—Obstetric Clinic at Chang Gung Memorial Hospital	$N = 1848$ from 2001 to 2005, 6-year follow-up survey $N = 756$.	In-utero (3rd trimester) tobacco smoke (family member smoked at home indoors)	6 years	Ever asthma (EA) dermatitis, rhinitis	GSTM1: null and present	*EA* *OR_1 2.31 (1.33–4.00)* OR_2 1.22 (0.630–2.38) *Gender dependent* Females: *OR_1 4.107 (1.699–10.107)* OR_2 1.271 (0.469–3.448) Males: OR_1 1.510 (0.773–2.952) OR_2 1.134 (0.479–2.681) No associations found for dermatitis and rhinitis.	Evidence of interaction for GSTM1 in girls. ($p = 0.001$)
The Swiss Study on Air Pollution and Health in Adults (SAPALDIA) Population-based cohort study Gerbase et al. (2011)	$N = 9651$ in 1991, (age mean 40) $N = 8010$ in 2002. Final analysis $N = 2463$	Second-hand smoking (SHS) 3 categories exposure: 1. Never 2. Baseline 3. Baseline and Fup	Mean 50 years	Allergic rhinitis	GSTT1 and GSTM1: null and non-null; GSTP1 Ile105Val: Ile/Ile, Ile/val, val/val	No significant associations found between atopic rhinitis and GST genotypes in three categories of SHS exposure	No evidence of any GST interaction on SHS and rhinitis. (no p values given)
The Cincinnati Childhood Allergy and Air Pollution Study (CCAAPS) High Risk Birth cohort study (≥ 1 atopic parent) 7 counties Schroer et al. (2009)	$N = 570$ recruited between 2001 and 2003.	Household ETS (at least 1 smoker living in home), mold. (12 and 24 months)	12 and 24 months	Wheezing at 12 and 24 months, persistent wheezing	GSTP1, (Reference Ile/Ile) Ile105Val Ile/Ile, and Ile/val or val/val.	Carriers of Val105allele cf. Ile/Ile significantly more likely to wheeze at 12 and 24 months if ETS exposed. No significance for mold	No evidence for GSTP1 interaction ($p > 0.2$)
Childhood Asthma Management Program (CAMP) Asthma Cohort study (multi-center) Rogers et al. (2009) North American	$N = 1041$ enrolled $N = 511$ white subjects included in analysis.	In-utero smoking and ETS (postnatal until study enrolment) Both variables retrospectively recorded at study entry—mean age 8.8 years)	8.8 years ± 2.1	Current asthma (CA)	GSTM1 null or present	In smoke-exposed group, (in utero or ETS) interaction *p value = 0.03* for association between GSTM1 null and age of asthma onset (2.5 yrs. in nulls vs 4 in non-nulls) There was no evidence of a main association between GST genotype and asthma	Evidence of GSTM1 interaction ($p < 0.05$) Stronger for in-utero exposure ($p = 0.02$) compared with ETS $p = 0.08$
The Children's Health Study (CHS) Population-based cohort study—12 communities (public school children) Gilliland et al. (2002) Southern California	$N = 5925$ in 1993. Available buccal cell specimens $N = 950$	In-utero maternal smoking (retrospective at 4th, 7th, or 10th grade)	Over 8 years (range 8–> 14)	EA, CA, medication for asthma, early onset asthma, persistent asthma Ever wheezing (EW), wheeze with cold, wheeze without cold, persistent wheeze (CW).	GSTM1 null or present	Ref: GSTM1 present in non-exposed GSTM1 null in exposed: *OR_5 (EA) 1.4 (0.9–2.1);* *OR_5 (CA) 1.7 (1.1–2.8);* *OR_5 (EW) 1.8 (1.3–2.5);* *OR_5 (CW) 2.2 (1.3–4.0);* GSTM1 present in exposed: *OR_2 (EA) 0.9 (0.6–1.4);* *OR_2 (CA) 0.8 (0.5–1.3);*	Evidence for GSTM1 interaction ($p < 0.05$ for some outcomes)

Table 1 (continued)

Name of study (acronym)/ type of study/first author/date published/country	Number of participant and study dates	Exposure and age	Outcome age	Outcome measures	Interactions investigated	Association/findings	Conclusions
						OR_2 (EW) 1.3 (1.0–1.8); *OR_2 (CW) 1.6 (0.9–2.8);* GSTM1 null in non-exposed *OR_4 (EA) 1.0 (0.8–1.2);* *OR_4 (CA) 0.8 (0.6–1.1);* *OR_4 (EW) 1.0 (0.8–1.2);* *OR_4 (CW) 0.8 (0.6–1.2)*	
Cross-sectional study Lee et al. (2015) Seoul, Korea Population based—all children in single elementary school	*N* = 1111 (no year of study given)	ETS Exposure to tobacco > once/week	7–12 years	CA	GSTP1: Ile105Val AA; AA+AG AA = Ile/Ile Risk genotype Ile/Ile	No value provided for interaction between ETS, GSTP1 and CA. Increased asthma risk only seen among those with Ile/ile genotype for exposed children plus low vitamin A. (aOR, 4.44; 95% CI, 1.58–12.52)	No evidence of GSTP1 interaction. (No *p* values)
The Health 2006 Study Cross-sectional study Hersoug et al. (2012) Copenhagen Population based—drawn from civil registration system	Recruited between June 2006 to June 2008 *N* = 7931 Final analysis *N* = 3471	ETS (h/day in rooms with tobacco smoke) Use of woodstove/candles during wintertime Active smoking	18–69 years	CW	GSTM1 /T1 null copies, 1 or 2 copies	GSTM1: Interaction *p* value for: smoking status = 0.316; ETS = 0.821; woodstove = 0.245 candle use = 0.604 GSTT1: Interaction *p* value for: smoking status = 0.365; ETS = 0.321; woodstove = 0.475 candle use = 0.793	No evidence of GSTM1 and GSTT1 interaction. (*p* values ranged from 0.3 to 0.8)
Cross-sectional study Wang et al. (2011) Taiwan Population based (middle school children from public schools in 14 communities)	In 2007 Final analysis *N* = 3738.	Incense burning smoke in past 12 months 3 categories; 1) Never 2) Less than daily 3) Daily	7th grade, 12.26 ± 0.50 years	EA, CA, EW, CW, use of asthma medication exercise wheeze.	GSTT1 null, GSTM1 null, GSTP1 Ile105Val lle/lle	Ref: GSTT1 present in non-exposed. GSTT1 null in exposed, OR_5 (EA) 1.11 (0.79–1.58) OR_5 (CA) 1.56 (0.93–2.63) OR_5 (EW) 1.12 (0.84–1.49) OR_5 (CW) 1.03 (0.65–1.63) GSTT1 present in exposed, OR_2 (EA) 0.87 (0.61–1.25) OR_2 (CA) 1.09 (0.63–1.87) OR_2 (EW) 0.87 (0.65–1.17) OR_2 (CW) 0.79 (0.49–1.27) GSTM1 and GSTP1 had no interactive effect.	Evidence of GSTT1 interaction ($p < 0.05$)
The Perth Childhood Acute Asthma Study (PCAAS) Cross-sectional study in children with acute asthma presenting to ED	*N* = 221 Between July 2002 and November 2006.	ETS—any current indoor smoking in household	2–16 years	Asthma severity score (NIH) Atopy +ve SPT (3 mm) 11 allergens	GSTP1 Ile105Val (Reference Val/Val) genotypes:lle/lle, lle/val, val/val.	No significant difference in asthma severity found between genotypes and haplotypes or in combination	No evidence of GSTP1 interaction on asthma severity (no *p* values)

Table 1 (continued)

Name of study (acronym)/ type of study/first author/date published/country	Number of participant and study dates	Exposure and age	Outcome age	Outcome measures	Interactions investigated	Association/findings	Conclusions
at Princess Margaret Hospital Schultz et al. (2010)						with ETS exposure (no ORs or RRs given). Interactions between GSTP1, atopy and ETS. ($p = 0.08$)—higher risk in exposed Ile/Ile—OR 9.17 (1.42–59.12)	
Taiwanese Children's Health Study (TCHS) Cross-sectional study. Elementary school students, enriched 2 stage sampling for in-utero maternal smoking Li et al. (2009) Taiwan	$n = 1092$ in 2006 and 2008	ETS (1 or more smokers in household)	Grade 1–6	EA (ever doctor diagnosed)	GSTM1 Null or present	In exposed group: GSTM1 null vs present OR_3 0.8 (0.6–1.0). In non-exposed group GSTM1 null vs present OR_4 (EA) 1.0 (0.8–1.1) Interaction $p = 0.057$ GSTM1 genotype modified association between NQO1 polymorphism and asthma in children exposed to ETS ($p = 0.0002$).	Borderline evidence for GSTM1 interaction (no p value reported)
The Children's Health Study (CHS) Cross-sectional study. Population based—Children from public school classrooms (grades 4, 7, and 10) in 12 communities Li et al. (2008) Southern California	$N = 3082$ In 1993	In-utero smoking (maternal and household)	8–18 years	EA, CW, early/late onset Asthma (before/after 3 years age), current wheezing, medication for wheeze (MFW)	GSTP1 Ile105Val and 4 haplotype model	GSTP1 Ile105Val (baseline Ile/Ile) EA OR_2 1.1 (0.7–1.6); OR_1 1.2 (0.9–1.5); OR_5 1.4 (0.9–2.0). CW OR_2 1.2 (0.7–1.7); OR_1 1.0 (0.7–1.1); *$OR_5$1.9 (1.3–2.6)* MFW OR_2 0.9 (0.5–1.5); OR_1 1.1 (0.8–1.3); *$OR_5$1.9 (1.2–2.8)*	Evidence for GSTP1 interaction $p = 0.035$ CW $p = 0.043$ MFW
Cross-sectional study (part of ISAAC) Kabesch et al. (2004) Munich and Dresden	In 1995 and 1996, $N = 3054$	Current ETS In-utero ETS	9–11 years	CA, EW, CW	Presence or null of GSTM1 and GSTT1 gene.	GSTM1 null in exposed *OR_5 (CA) 5.48 (1.62–18.55)* *OR_5 (EW) 2.81 (1.31–6.04)* *OR_5 (CW) 4.74(1.79–12.57)* GSTM1 null in non-exposed OR_4 (CA) 1.40 (0.85–2.30) *OR_4 (EW) 2.81 (1.31–6.04)* *OR_4 (CW) 1.50 (1.01–2.22)* GSTM1 present in exposed OR_2 (CA) 2.94 (0.61–14.05)	Evidence for GSTM1 and GSTT1 interaction (p values ranged from 0.05–0.77)

Table 1 (continued)

Name of study (acronym)/ type of study/first author/date published/country	Number of participant and study dates	Exposure and age	Outcome age	Outcome measures	Interactions investigated	Association/findings	Conclusions
						OR_2 (EW) 1.81 (0.75–4.41) OR_2 (CW) 2.03 (0.55–7.54) GSTT1 null in exposed OR_5 (CA) 4.10 (0.43–39.04) OR_5 (EW) *4.37 (1.17–16.39)* OR_5 (CW) 3.30 (0.61–17.91) GSTT1 null in non-exposed OR_4 (CA) 0.73 (0.35–1.50) OR_4 (EW) *2.81 (1.31–6.04)* OR_4 (CW) 0.84 (0.60–1.19) GSTT1 present in exposed OR_2 (CA) *3.25 (1.14–9.27)* OR_2 (EW) 1.77 (0.92–3.42) OR_2 (CW) *2.61 (1.09–6.21)*	
Case control study Munoz et al. (2012) Coahiula Mexico	$N = 201$ Cases (asthma): $N = 90$ Controls: $N = 111$ No survey time was specified in paper.	ETS—3 cats 1. Not exposed 2. household exposure 3. In utero and household	6–9 years	Asthma (unable to define if it is current asthma or ever asthma)	GSTP1 Ile105Val A allele, G allele, AA genotype, AG genotype, GG genotype.	Among 5 genotyping groups in GSTP1, allelic and genotypic frequencies were similar and no significant differences were observed. No ORs or RRs were available in this study.	No evidence of GSTP1 interaction No interactions reported
Children, allergy, Milieu, Stockholm, Epidemiological Survey (BAMSE) Case control study nested in a birth cohort study Panasevich et al. (2010)	Cases (wheezers): $N = 542$ Controls: $N = 542$ Recruited in 1994–1996	Early maternal smoking (> = 1 cig/day in pregnancy or early childhood)	4 years	CA, early onset wheeze, late onset wheeze, transient wheeze.	GSTP1: Ile105val (rs1695)	Early onset wheeze GSTP1 ile, OR_2 1.5 (0.8–2.7); GSTP1 ile/val, OR_1 1.8 (1.1–3.2); GSTP1 val, OR_1 5.7 (1.4–22.2), interaction $p = 0.17$.	Evidence of GSTP1 interaction ($p = 0.17$)
Taiwan Birth Panel cohort study(TBPCS) Case control study nested in birth cohort Wang et al. (2010) Taiwan	Cases AD: $n = 34$; 4 matched (sex, age, enrolment time) Controls for each case: $n = 106$ TBPCS start 2004. This analysis in 2006	Prenatal ETS Cotinine level in cord blood Low vs high (0.1 ng/ml)	2 years	Atopic dermatitis (AD)- ISAAC	GSTM1 and GSTP null and present, GSTP1 (ref any val) Ile/val or val/val, Ile/Ile.	*GSTM1* *OR_3 5.21 (1.32–20.58)* OR_4 1.88 (0.46–7.69) GSTP1 OR_3 2.61 (0.75–9.12) *OR_4 6.63 (1.46–30.18)*	Evidence of GSTM1 and GSTP1 interaction (no p value provided)
Case control study drawn from a national cross-sectional study. Lee et al. (2007) Taiwan	In 2001. cases: $n = 216$; controls: $n = 185$	Household ETS (past 12 months) Participants with in-utero exposure or personal smoking excluded	Cases: 11.8 ± 1.7 years, controls: 12.1 ± 1.8 years	EW and CW	GSTP1-105 Ile. Ile/val, val/val GSTM1 null or present	GSTP1 *EW* *OR_5 0.85 (0.46–1.60)* *OR_1 2.22 (1.07–4.58);* *OR_4 0.43 (0.21–0.86)* *OR_2 0.67 (0.40–1.12))* *interaction p = 0.01* *CW* *OR_5 0.68 (0.31–1.46)* *OR_1 3.81 (1.34–10.84);*	Evidence of GSTP1 interaction ($p = 0.01$ for EW, $p = 0.004$ for CW)

Table 1 (continued)

Name of study (acronym)/ type of study/first author/date published/country	Number of participant and study dates	Exposure and age	Outcome age	Outcome measures	Interactions investigated	Association/findings	Conclusions
						OR_4 0.68 (0.37–1.25); OR_2 0.68 (0.37–1.25). Interaction p = 0.004	

Note: OR_1 odds of asthma/wheeze for participants with environmental exposure (EE) compared to participants without environmental exposure in carriers of the risk genotype (RG) (GSTM1null/GSTT1 null/GSTP1 ile/val or val/val), EE+/RG+ compared with EE−/RG+; OR_2 odds of asthma/wheeze for participants with environmental exposure compared to participants without exposure in carriers of the non-risk genotype (GSTM1present /GSTT1 present/GSTP1 ile/ile.) or 5 odds of asthma/wheeze in environmentally exposed without risk genotype compared to non-environmentally exposed without risk genotype, EE+/RG− compared with EE−/RG−; OR_3 odds of asthma/wheeze for participants with the risk genotype compared to those without the risk genotype in participants environmentally exposed, EE+/RG+ compared with EE+/RG−; OR_4 odds of asthma/wheeze for participants with the risk genotype compared to those without the risk genotype in participants not environmentally exposed, EE −/RG+ compared with EE−/RG−; OR_5 odds of asthma/wheeze in environmentally exposed with risk genotype compared to non-environmentally exposed without risk genotype, EE+/RG+ compared with EE−/RG−

were measured varied from 1 month [42] to 69 years [5]. No studies included an elderly population. Sample sizes ranged from 140 to 3738. The numbers of cohort, cross-sectional, and case-control studies were 9, 9, and 4, respectively.

Studies were conducted at a variety of locations across Europe, North American, Mexico, Australia, Taiwan, and South Korea. All papers reported the geographic location of their studies, 8 further provided information on ethnicity. Those 8 studies were mainly conducted on Non-Hispanic white/white/Caucasian.

Measurement of Exposures and Outcomes

Genetic Polymorphisms Genotyping methods were reported for all studies. Six of the 22 papers described confirmation of genotyping results by analyzing a duplicated random sample (5%–15%) and all were concordant with the initial results [27, 31, 33, 39, 42, 45].

Environmental Exposures Nineteen of the 22 studies evaluated indoor air pollutants by self-report questionnaires. The remaining 3 studies used more objective measures to quantitate exposure, including blood cotinine level [39], urinary cotinine level [30], and visible mold assessed by a home visit [33]. No studies measured indoor air pollution directly through air sampling (e.g., air quality monitors).

Outcome Assessment Asthma and allergic outcomes were evaluated by self-report questionnaires for most studies, apart from 1 that extracted asthma diagnosis from hospital records [31], and another that undertook independent clinical examination by doctors [26]. All lung function studies performed spirometry, except 1 study in infants that measured $Vmax_{FRC}$ using the rapid thoracoabdominal compression technique during tidal breathing [42].

Assessment of Quality of Included Studies

Based on NOS assessments, 6 studies were low, 11 studies medium, and 5 studies high quality. The main issue identified was failure to adjust for a minimum set of confounders, including age (not applicable for birth cohort studies), gender, socio-economic status (SES), and family history of allergic disease (Fig. 2). Only 5 studies fully adjusted for these variables [27, 28, 36, 38, 39], while 2 did not describe any confounders [30, 43].

Narrative Synthesis of All Identified Studies

Of the 22 included studies, 15 supported the presence of a gene-indoor air pollution interaction on the outcome of allergic disease, lung function, or both [25–29, 32, 34•, 35••, 36, 39–43, 45]. Interestingly, of those 7 papers failing to detect

Table 2 Characteristics and methods of the 8 studies with lung function outcomes

Name of study (acronym)/ type of study/first author/ date published/country	Study numbers and dates	Exposure and age	Outcome age	Outcome measures	Interactions investigated	Association/findings	Conclusions
The Perth Infant Asthma Follow-up (PIAF) Birth Cohort study—women attending local antenatal clinic Murdzoska et al. (2009) Australia	Between 1987 and 1990, DNA available for GSTM1/GSTT1, $n = 179$ GSTP1 $n = 180$	In-utero ever smoking	1, 6, and 12 months.	Maximal flow at functional residual capacity ($Vmax_{FRC}$)	Maternal and infant GSTT1 null/non-null, GSTM1 null/non-null, GSTP1 ile/ile, ile/val, and val/val	*Adjusting length, maternal smoking with GSTT1 non-null ß 37.05 (− 0.06, 74.17).* No association was found on GSTM1; GSTP1 data was not available on infant.	Evidence of GSTT1 interaction ($p = 0.008$)
The Childhood Asthma Management Program (CAMP) Asthma Cohort study (multi-center) Rogers et al. (2009) North American	$N = 511$	In-utero smoking and ETS (postnatal until study enrolment) Both variables retrospectively recorded at study entry—mean age 8.8 years)	8.8 years ± 2.1	FEV1% predicted, FVC % predicted, FEV1/FVC	GSTM1 null and non-null Copy number variants(CNV) Null hemizygotes and homozygotes	With in-utero smoke exposure, children with GSTM1 null have higher FVC% ($p = 0.04$) and lower FEV1/FVC ($p = 0.03$). Postnatal ETS are highly correlated in utero smoking. No number given.	Evidence of GSTM1 interaction ($p < 0.05$)
Swiss Cohort Study on Air Pollution and Lung Diseases in Adults (SAPALDIA) Cohort study Imboden et al. (2007)	Completed Spirometry in 2002. FEV1-4686 FVC-4591 FEF-4528	Ever(> 20 pk/years) and never smokers Ever further classified as persistent and others	Age at baseline 40.8 ± 11.5 years FUP for 11 years	Difference in mean annual change in FEV1, FVC and FEF2575 over 11 years (ml/year)	GSTT1 and GSTM1 (null and non-null), GSTP1 (105 Ile/Ile, Ile/val, val/val); GSTM1T1 (both non-null, either null, both null)	The GSTT1 effect in persistent smokers was modified by pack-years smoked to baseline and follow-up on FEV1 decline.	In male persistent smokers evidence of GSTT1 interaction on FEV1 decline with pack-years smoked at baseline ($p < 0.001$) and follow-up ($p = 0.029$)
Lung Health Study (LHS) Cohort study He et al. (2004) American	High lung function group, $n = 544$ Low lung function group, $n = 554$	Smoking history (pack-year)	35–60 years	High lung function (HL) and low lung (LL) function	GSTM1, GSTT1, null and present GSTP1: recessive, dominant, and Codominant.	GSTT1: *Mild smoker OR 4.28 (1.71, 10.96)* Moderate smoker OR 0.94(0.59, 1.49) Heavy smoker OR 0.91 (0.43. 2.02) No associations were found on GSTM1 and GSTP1.	Evidence of GSTT1 interaction (not with smoking, but pack-years) ($p = 0.038$)
Lung Health Study (LHS) Cohort study He et al. (2002) American	Fastest decline FEV1 $N = 299$ Slowest decline FEV1 $N = 322$	Active smoking during last 5 years	35–60 years	FEV1% predicted	GSTM1, GSTT1, null and wild-type present; GSTP1 AA, AG, GG.	Neither the combination of all GST polymorphisms nor a combination of GSTP1 AA showed a significant interaction with smoking history.	No evidence of interaction (no p value reported)
The Health 2006 Study Cross-sectional study Hersoug et al. (2012) Copenhagen	Final analysis $N = 3471$	ETS, Use of woodstove/candles during wintertime, Smoking status	18–69 years	FEV1% predicted	GSTM1 and GSTT1 with null copies GSTM1 and GSTT1 with 1 or 2 copies	Interaction p value for smoking status*GSTM1 0.281; for ETS*GSTM1 0.567; for wood stove *GSTM1 0.145; candles*GSTM1 0.362; smoking status*GSTT1 0.871; for ETS*GSTT1 0.740; for wood stove *GSTT1 0.761; candles*GSTT1 0.367;	No evidence of interaction (p values ranged from 0.3 to 0.9)
European Community Respiratory Health Survey (ECRHS) Cross-sectional study of Danish participants of ECRHS Malling et al. (2012) Denmark	$N = 1091$ Random sample (728) Symptomatic sample (460) (current asthma symptoms or adult onset asthma)	Current active smoker (I month) in those who have ever smoked for >1 year	22–44 years	FEV1% predicted; $FEV1/height^2$	GSTP1 (ref Ile/Ile) Ile/Ile, Ile/val, val/val, GSTT1, GSTM1 two copies, one copy and null,	Ref GSTM1 two copies. Adjusted FEV1%: With current active smoking, GSTM1 one copies ß − 9.3 (− 16, − 2.3), null ß − 11 (− 18, − 4.0). interaction $p = 0.03$ Interactions were not seen in GSTT1 and GSTP1.	Evidence of GSTM1 interaction ($p = 0.03$)

Table 2 (continued)

Name of study (acronym)/ type of study/first author/ date published/country	Study numbers and dates	Exposure and age	Outcome age	Outcome measures	Interactions investigated	Association/findings	Conclusions
BREATHE study Cross-sectional study of participants with physician diagnosed asthma Palmer et al. (2006) Primary/secondary clinics in 10 practices Scotland	In 2004–2005. $N = 504$	ETS (family smoking in the home environment)	Mean 10 (3–21 years) 2 groups; 3–12 and 13–21	% Predicted PEFR, FEV1, and FVC	GSTM1, GSTT1, null and present and GSTP val/val105 and Ile105/Ile105 or Ile105/val105	In the 13–21 years old, p value of association between GSTM1 genotype and peak expiratory follow rate (PEFR) in ETS exposed group is 0.010, but 0.235 in non-exposed group. (p int 0.003)	Evidence of GSTM1 interaction in older asthmatic children. ($p = 0.003$)

* Interaction

gene-air pollution interactions for allergies or lung function, 2 found interactions instead for outcomes of high total IgE [38]or atopy (positive skin prick test), which are recognized markers of allergy [31, 38]. Seventy-nine percent ($n = 11$) of childhood papers found at least one interaction [25–29, 32, 34•, 35••, 36, 39, 42] compared with only half ($n = 3$) of the papers in adulthood [40, 41, 45]. One paper that spanned both age groups (young children to young adults aged 3–21 years) also found an interaction [43]. All five Taiwanese studies identified genetic susceptibility for environmental factors, while 75% of the European studies and only half of the North American studies found an interaction.

Asthma and Wheeze

Fifteen papers reported results on asthma or wheeze, and nine identified an interaction with one or more of the GST polymorphisms. Four out of eight papers investigating the potentially modifying effect of GSTM1 on the association between indoor air pollution and allergies found that carriers of the null genotype had an increased risk of asthma/wheeze when school-aged children were exposed to markers of increased indoor air pollution [25, 26, 28, 35••]. However, one Taiwanese study found weak conflicting evidence: decreased risk of asthma in children carrying the null GSTM1 genotype and exposed to ETS ($p = 0.0573$) [29]. Another oxidative stress gene Quinone oxidoreductase 1 (NQO1) may be part of a three-way interaction with children carrying the GSTM1 null and NQO1 ser genotype at increased risk of asthma in the exposed group, but not in the unexposed group [29]. Of the three papers reporting on GSTT1, two cross-sectional studies investigating asthma in school-aged children found evidence of an interaction [25, 36]. They found that environmental pollutants including in-utero smoking and incense burning smoke were associated with increased risk of asthma/wheeze if children had GSTT1 null genotypes. The other GSTT1 paper failed to replicate this interaction in adulthood [5]. Of eight papers investigating GSTP1, three found that the GSTP1 val105 allele was associated with an increased risk of wheezing/asthma with in-utero smoking exposure and/or non-exposed children carrying GSTP1 ile105 alleles were at increased risk [27, 32, 34•]. One paper further investigated GSTP1 by using a haplotype-based analysis [34•]. GSTP1 genes were categorized by four haplotype variation tagging SNPs. Consistent with a risk effect of the GSTP1 val 105 variant, the effect of in-utero exposure to maternal smoking on wheezing was largest in children within the h1011 haplotype (105val with no other variants) compared with other haplotypes [34•]. In contrast, there was one study suggesting that GSTP1 ile105 increased the risk of atopy (but not asthma severity) when exposed to ETS [31]. Although a cross-sectional study in Korea did not observe an interaction between ETS, GSTP1, and asthma, when they further stratified by vitamin A intake (low vs high), they found that school children with GSTP1

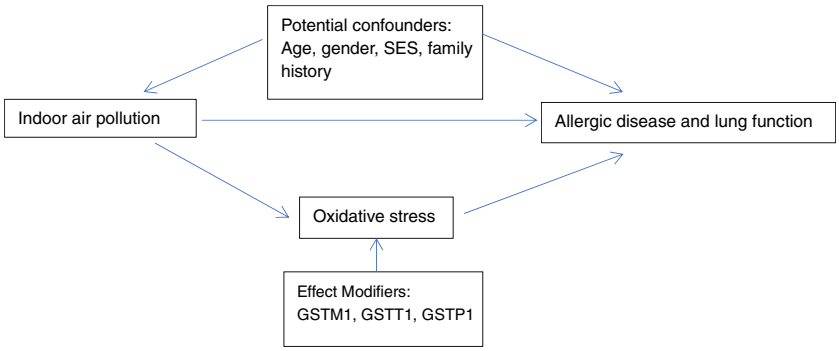

Fig. 2 Casual diagram for GST gene interaction on the association between indoor air pollution and allergic disease and lung function

homozygous ili genotypes exposed to ETS and low vitamin A had an increased risk of asthma [37••]. The remaining three studies showed no evidence of a GSTP1 interaction for all investigated current environmental exposures [30, 31, 33].

Other Allergic Disease

There were two studies investigating rhinitis [35••, 38], and two investigating atopic dermatitis (AD) [35••, 39]. No study investigated the outcome of food allergy. No significant associations were found between allergic rhinitis and GST genotypes in different smoking categories in adulthood [35••, 38]. However, children with GSTM1 null genotypes were found to be more susceptible to AD if they had been exposed to in-utero smoking, while GSTP1 ile105 homogeneity increased the risk of AD in the in-utero unexposed group, but no association was seen in the exposed group [39].

Lung Function

Six of the eight studies that investigated the potential interaction between smoking and GST genes found positive results. All three studies investigating GSTM1 found that carriers of null variants exposed to smoking, had an increased risk of impaired lung function in terms of FEV1% predicted [41, 43], PEFR % predicted [43], FVC% predicted, and FEV1/FVC ratio [26]. These associations were not seen in the non-smoke-exposed group. Two studies formally tested and found statistical evidence for an interaction term [41, 43]. For GSTT1, one study investigated infant lung function, finding that among infants exposed to in-utero smoking, those with null variants had reduced $Vmax_{FRC}$ in the first year compared with non-null infants. No significant associations were seen in the group not exposed to in-utero smoke [42]. Another two adult studies identified significant interactions between GSTT1 genotype and pack-years on lung function in smokers [40, 45]. Two studies which support the presence of interaction by GSTP1 provided evidence that in those exposed to tobacco smoke, having the GSTP1 val105 allele increased the risk of reduced lung function. One found that GSTP1 val105 homozygote individuals demonstrated a reduction in PEFR% predicted in the older ETS exposed group (13–21 years vs 3–12 years) [43]. The second study found that in persistent smokers, those homozygous for GSTP1 val105 had a faster rate of lung function decline in FVC during 11 years' follow-up compared to heterozygotes. However, irrespective of smoking status, no independent effect of GSTP1 was found [45].

Two adult studies did not identify any interaction. The Health 2006 study in Copenhagen found no evidence of GST interaction for a wide range of indoor exposures (active smoking, passive smoking, and use of woodstove or candles during wintertime) and the risk of FEV_1% predicted (interaction p varies from 0.1–0.6) [5]. Another study nested in the Lung Health Study in North America found no significant GST interactions between smoking history and rapid decline in lung function over 5 years, either using single genes or a combination [44].

Discussion

This is the first systematic review to investigate the interaction between indoor air pollution and GST genes on allergic disease and lung function. Our narrative synthesis of 22 studies found evidence that GST polymorphisms interact with indoor air pollution exposures to adversely affect lung health. Carriers of GSTM1 null, GSTT1 null, and GSTP 105val alleles were at greater risk of developing asthma and reduced lung function when exposed to tobacco smoke. Three of the seven papers that did not find an interaction between indoor air pollution, GST, and allergic disease reported interactions on other allergy-related outcomes, in term of IgE [38] or atopy status [31], or after consideration of antioxidant nutrient intake [37••]. Two previous systematic reviews on oxidative stress genes, outdoor air pollution, and lung function outcomes, both suggested the presence of an interaction [16•, 46]. We reviewed GST genes and indoor air pollution, providing further and more current evidence for the presence of an interaction.

Biologic Plausibility

Our findings are supported by a strong biologic plausibility. Oxidative stress is a key component of inflammatory

disorders, and host antioxidant systems are activated in response to an external "oxidant attack" caused by exposure to environmental pollution [47]. ETS has been shown to have detrimental effects on the lung, mostly related to increased oxidative stress [48], as well as suppression of the immune system through modulation of T cell function [49]. Several studies have demonstrated that environmental exposures can influence GST gene activity. A study in rats found that exposure to cigarette smoke five times/week led to an increase in GST activity in both the brain and lungs [50]. Similarly, a human study found that the degree of antioxidant gene expression is directly related to the amount of cigarette smoke exposure [51], suggesting upregulation in response to environmental stimuli. Individuals who lack the protection of certain antioxidant genes may have a lower capacity for antioxidant defense, and be more susceptible to environmental toxins. This may lead to increased risk of asthma or impaired lung function when faced with oxidative exposures. Fourteen studies supported that cigarette smoke-exposed carriers of the GSTM1/T1 null genotypes may have increased risk of allergic disease and impaired lung function.

Evidence for GSTP1 Risk Allele Is Inconsistent

We found that carrying the GSTP1 val105 allele increased the risk of asthma and lung function deficits in individuals exposed to indoor air pollution. One study on asthmatic children found that carriers of GSTP1 val/val genotypes had higher levels of oxidative stress (measured as plasma levels of malondiadehyde) and less antioxidant defense capacity (measured as glutathione levels), compared with other genotypes [47]. Therefore, if ile alleles are replaced by val alleles, the resultant-encoded enzyme can alter the level of antioxidative capacity in the individual and may further contribute to airway inflammation when exposed to oxidative threats [34•, 52]. Conversely, another study indicated that carriers of homozygous GSTP1 ile alleles may have increased risk of atopic dermatitis in those unexposed to in-utero smoking but not in the exposed group [39]. The reason for this contradictory outcome is unclear. There is evidence showing that enzymes with GSTP1 val105 have a greater catalytic effect for diol epoxides of polycyclic aromatic hydrocarbons but less effect with 1-chloro-2,4-dinitrobenzene compared to ile105 [53]. Therefore, we propose that the effect of GSTP1 gene polymorphisms on allergy risk and lung function is likely to vary for different pollutants or in different scenarios.

Reasons for Other Inconsistent Findings

Inconsistent findings from specific exposures may be caused by differing environmental conditions or study methodology. For instance, all 6 papers exploring in-utero smoking suggested that adverse effects were more likely to occur in individuals with a GST risk polymorphism. This suggests a potential critical time window, during which exposure may lead to long-term effects on fetal lung development [6]. Moreover, asthma and other allergic diseases are heterogeneous diseases with complex etiologies. A few studies in our review suggested the presence of complex interactions, including NQO1 [29], other GST genotypes [44, 45], bronchial hyperresponsiveness to methacholine chloride [45], antioxidative vitamins [37••], age [43, 45], and sex [35••, 45]. Asthma and reduced lung function may result from effects of multiple genes and their interactions with environmental and host factors. It is unlikely that a single gene polymorphism is the only mechanism responsible for management of oxidative stress within the lung. It is likely that multiple points of biological redundancy have been developed to protect the airways and prevent adverse respiratory outcomes. The influence of other factors may increase the magnitude of a proposed gene-environment interaction. The Cincinnati Childhood Allergy and Air Pollution Study suggested that GSTP1 genes could modify the negative effect of diesel exhaust particle (DEP) exposure. However, the protective effects of GSTP1 genotypes may be overridden when children are exposed to multiple environmental stresses in early life. In these infants, despite carrying genotypes considered protective for lesser levels and varieties of exposure, adverse outcomes were observed with multiple environmental loads [33]. Interactions were found more commonly in studies on children compared to adults, suggesting that the effect of gene-pollution interaction on respiratory health varies by age. Children's lungs are still developing and therefore may be more susceptible to noxious exposures. Furthermore, immune responses are relatively naïve in children when compared to adults. For both reasons, antioxidant defenses may play a crucial role in overcoming the adverse effects of environmental exposures on childhood respiratory health [54, 55].

Evidence from the Past 5 years

In terms of the most recent evidence, since 2013, there have only been two original research articles published on GST genes and indoor air pollutants [35••, 37••]. One provided evidence that low vitamin A intake in genetically susceptible children (GSTP1 1695 AA) exposed to ETS was associated with an increased risk of asthma [37••]. The authors suggested that low vitamin A diets may increase the level of oxidative stress making genetically susceptible children more likely to be affected by environmental exposures like ETS. This finding suggests that supplemental vitamin A may be a preventive measure for those at high risk. The other recent study provides new evidence on gender differences and risk alleles for the interactive effect of GSTM1 with in-utero smoking on childhood asthma [35••]. This study demonstrated that the GSTM1 null genotype could have a bipolar effect on childhood asthma

at 6 years depending on whether there is in-utero smoke exposure. The GSTM1 null genotype is a protective factor for asthma at age 6 years in girls without in-utero smoke exposure but becomes a risk factor for asthma with in-utero smoke exposure. The associations were not seen for boys. It has been suggested that the relative lack in antioxidant defenses resulting from the GSTM1 null genotype may be compensated for by increased antioxidant production by other members of the GST superfamily [56] or by other antioxidant enzymes. In support of this theory, two studies demonstrated that a protective polymorphism (Ser187) of a second antioxidant enzyme, quinone oxidoreductase 1 (NQO1), provided a protective effect among GSTM1 null subjects [57, 58]. These findings indicate the complexity of the body's buffering system for antioxidant stress and suggest a direction for future studies. Comprehensive studies in this area should consider both a series of antioxidant genes and dietary "antioxidant" intake, when investigating the relationship between environmental exposures such as ETS and lung health outcomes.

Limitations

We were unable to meta-analyze the data due to high heterogeneity. Specific gene-pollutant outcome combinations lacked numbers for meta-analysis. However, the reporting of significant interaction terms from 15 of the 22 studies provides evidence that it is likely this interaction exists. There is potential for negative studies not to be published, which may have led to a bias towards greater magnitude of interaction effects being identified. Some of the estimates were based on unadjusted measures or unexplained adjustment which may increase the differences between study estimates. Finally, most studies were conducted in Asians or Caucasians and thus, our results may only be applicable to these ethnic groups.

Future Directions

Further studies investigating these gene-environment interactions are needed. The ability of individual studies to measure true effects can be improved by larger sample sizes and more objective measures of exposures and outcomes at multiple time points. The majority of the current papers measured exposures and outcomes at one time by self-report. This is problematic as both exposures and outcomes change over time. Moreover, further studies should also consider recruiting participants in specific age ranges especially people over the age of 65 years, because antioxidant capacity is known to decrease with age [59] and the magnitude of gene-environment interaction may also change over time. More publications, with transparent methods, in varied populations in terms of age and ethnicity should be encouraged to obtain a clearer picture of the true magnitude of the relationship.

Conclusions

There is evidence that carriers of *GSTM1* null, *GSTT1* null, and *GSTP1* val genotypes are more susceptible to indoor air pollution exposure, having a higher risk of asthma and lung function deficits, although some findings are conflicting in terms of risk alleles and specific exposures. These interactions may help to explain the inconsistent effects seen when either indoor air pollution or GST genes are studied in isolation for their effects on respiratory health. The recognition of specific genetic risk alleles may allow for targeted preventive measures in susceptible subpopulations to improve respiratory health by avoiding indoor air pollutants or improving their antioxidant defenses through diet or supplemental vitamins.

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Compliance with Ethical Standards

Conflict of Interest All authors declare no support from any organization for the submitted work, no financial relationships with any organizations that might have an interest in the submitted work in the previous 3 years, and no other relationships or activities that could appear to have influenced the submitted work.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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- • Of importance
- •• Of major importance

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SUPPLEMENTARY MATERIAL

DO GLUTATHIONE S-TRANSFERASE GENES MODIFY THE LINK BETWEEN INDOOR AIR POLLUTION AND ASTHMA, ALLERGIES, AND LUNG FUNCTION? A SYSTEMATIC REVIEW.

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Supplemental table 1 Newcastle-ottawa quality assessment scale (NOS) classification

	Cohort Study	Cross-sectional study	Case control study
Full mark	10	7	9
High quality	9-10	6-7	8-9
Medium quality	6-8	5	5-7
Low quality	3-5	4	4
Unsatisfied quality	0-3	0-3	0-3

Supplemental table 2 summery of 49 excluded studies at the full text review stage

49 exclude studies	
40	Exposures did not focus on indoor air pollution; such as outdoor, traffic related or unspecific.
6	Outcomes did not identify allergy or lung function outcomes, instead of sensitization, hyperresponsiveness, atopy, or IgE level.
3	Interacting terms like GSTM2-5, GST-omega

Supplemental document 1

Pubmed searching strategies and terms

(((((((((smok*[Title/Abstract] OR cigarett*[Title/Abstract])) OR (smoke [mesh] or tobacco smoke pollution [mesh]))) OR (((environmental pollution [mesh] or air pollution, indoor [mesh] or ozone [mesh] or particulate matter [mesh] or carbon monoxide [mesh])) OR ("indoor air quality"[Title/Abstract] OR pollut*[Title/Abstract] OR ozone[Title/Abstract] OR "particulate matter"[Title/Abstract] OR particles[Title/Abstract] OR "nitrogen dioxide"[Title/Abstract] OR "carbon monoxide"[Title/Abstract]))) OR (((heating[Title/Abstract] OR cooking[Title/Abstract] OR wood[Title/Abstract] OR heat*[Title/Abstract] OR cook*[Title/Abstract] OR gas[Title/Abstract] OR electri*[Title/Abstract])))) OR (((detergents [mesh] or virus inactivation [mesh])) OR ("chemical exposure"[Title/Abstract] OR "airborne exposure"[Title/Abstract] OR perfume[Title/Abstract] OR cleanser[Title/Abstract])))) AND (((((((spirometry [mesh] or respiratory function test [mesh])) or ("lung function"[Title/Abstract] OR bronchospirometry[Title/Abstract] OR "pulmonary function"[Title/Abstract] OR "respiratory function"[Title/Abstract])))) OR ((((asthma [mesh] or rhinitis, allergic [mesh] or eczema [mesh] or dermatitis [mesh])) OR (asthma[Title/Abstract] OR asthma*[Title/Abstract] OR antiasthma*[Title/Abstract] OR anti-asthma*[Title/Abstract] OR bronchodilator*[Title/Abstract] OR bronchodilator$[Title/Abstract] OR anti-asthma agents[Title/Abstract] OR steroid[Title/Abstract] OR corticosteroid[Title/Abstract] OR allergy[Title/Abstract] OR allerg*[Title/Abstract] OR rhinitis[Title/Abstract] OR eczema[Title/Abstract] OR dermatitis[Title/Abstract] OR hay fever[Title/Abstract] OR

SCORAD[Title/Abstract])))))) AND ((glutathione s transferase [mesh]) OR ("glutathione s transferase"[Title/Abstract] OR "glutathione s-transferase"[Title/Abstract] OR GST*[Title/Abstract]))

Supplemental document 2

Embase searching strategies and terms

[(exp environmental pollution/ or exp indoor air pollution/ or exp ozone/ or exp particulate matter/ or exp carbon monoxide/ or exp indoor air quality/ or pollut*.mp. or exp nitrogen dioxide/) or (exp environmental pollution/ or exp indoor air pollution/ or exp ozone/ or exp particulate matter/ or exp carbon monoxide/ or "indoor air quality".mp. or pollut*.mp. or exp nitrogen dioxide/) or (exp heating/ or exp cooking/ or heat*.mp. or cook*.mp. or wood.mp. or gas.mp. or electri*.mp.) or (exp detergents/ or exp virus inactivation/ or perfume.mp. or chemical exposure.mp. or airborne exposure.mp. or cleanser.mp.)] AND [(exp asthma/ or asthma*.mp. or antiasthma*.mp. or anti-asthma*.mp. or bronchodilator*.mp. or bronchodilator$.mp. or anti-asthma agents.mp. or steroid.mp. or corticosteroid.mp. or allergy.mp. or allerg*.mp. or exp rhinitis/ or exp eczema/ or exp dermatitis/ or exp hay fever/ or SCORAD.mp.) or (exp respiratory function test/ or pulmonary function.mp. or respiratory function.mp. or exp spirometry/ or exp bronchospirometry/)] AND (exp glutathione s transferase/ or glutathione s-transferase.mp. or GST*.mp.)

Chapter 4 STUDY DESIGN AND METHODS

I have used data from two longitudinal cohort studies, the Melbourne Atopy Cohort Study (MACS) and the Tasmanian Longitudinal Health Study (TAHS) to investigate my research questions. MACS is a high-risk birth cohort study, spanning from birth to early adulthood (18 years), and TAHS is a population-based cohort study spanning from childhood (7 years) to the 6th decade (53 years). Both studies have recorded substantial data at multiple time points over the life course, including the genotyping of three key GST genes, providing a great opportunity to address questions concerning gene-environment interactions. In this Chapter, I describe the general study methods for MACS and TAHS. More specific methodology applying to my particular research aims can be found in the results Chapters, such as defined exposure categories, confounders and specific statistical methods.

4.1 Melbourne Atopy Cohort Study (MACS)

4.1.1 Study design and population

MACS is a prospective birth cohort, initially comprising 620 infants born between 1990 and 1994 in Melbourne, Australia. These infants were enrolled whilst *in utero* by recruiting pregnant mothers living in Melbourne through antenatal care clinics at the Mercy hospital for women (East Melbourne), which is a tertiary referral centre for the state of Victoria, Australia. Additionally, a small number of participants were recruited through the rooms of obstetricians affiliated with this hospital. To be eligible for the study, these "*in-utero*" participants required at least one immediate family member (parent and/or sibling) to have a history of asthma, severe food allergy, hay fever or eczema. These infants are also referred to as the "probands". Their family members (1234 parents and 617 siblings) were also recruited into the study for data collection at two different times; baseline and the 18-year follow-up [254].

The initial study began as a randomized controlled trial (RCT) aiming to investigate the relationship between infant milk formula and the subsequent risk of allergy. Formula was randomized and introduced as a substitute only if breastfeeding was unable to be, or was not, initiated, or upon its cessation or partial cessation. Participants were

randomized into four different groups, either: 1) a standard cow's milk-based formula (NAN™, Nestle, Vevey Switzerland) as a control group; 2) a partially hydrolysed formula (NAN™, HA Ibid); or 3) a soy-based formula (ProSobee™, Enfamil, Mead Johnson, USA.). Randomization was performed only at the time when formula was introduced. The trial was retrospectively registered with the Australian and New Zealand Clinical Trial Registry (ACTRN12609000734268). From 2 years of age, the MACS participants have been followed as an observational cohort.

In this thesis, all the analyses were performed based on MACS as an observational birth cohort. Although this cohort was initially recruited for a randomised controlled trial, there are many examples of studies initially conceived as RCTs continuing as prospective observational birth cohorts [255, 256]. As the intervention was randomized, it is unlikely to influence the associations tested in the ongoing observational cohort. Nevertheless, RCT allocation is routinely tested as a potential confounder including in my analyses.

4.1.2 MACS data collection

Parents of the probands completed questionnaires prior to birth, which included questions about their socio-economic status and home environment. After birth, telephone surveys were conducted by a trained research nurse every 4 weeks in the first 15 months, and then at 18 months, and again at 2 years of age. Follow-up surveys were then conducted annually when the children were aged between 3 to 7 years. At the age of 12 years, parents completed written questionnaires and the children were invited to a clinical examination. At the 18 year follow up, probands and their family members were surveyed and invited to attend clinics to undergo testing related to respiratory health and allergic disease. The flowchart of MACS follow-ups and data collection is shown in Figure 4.1.

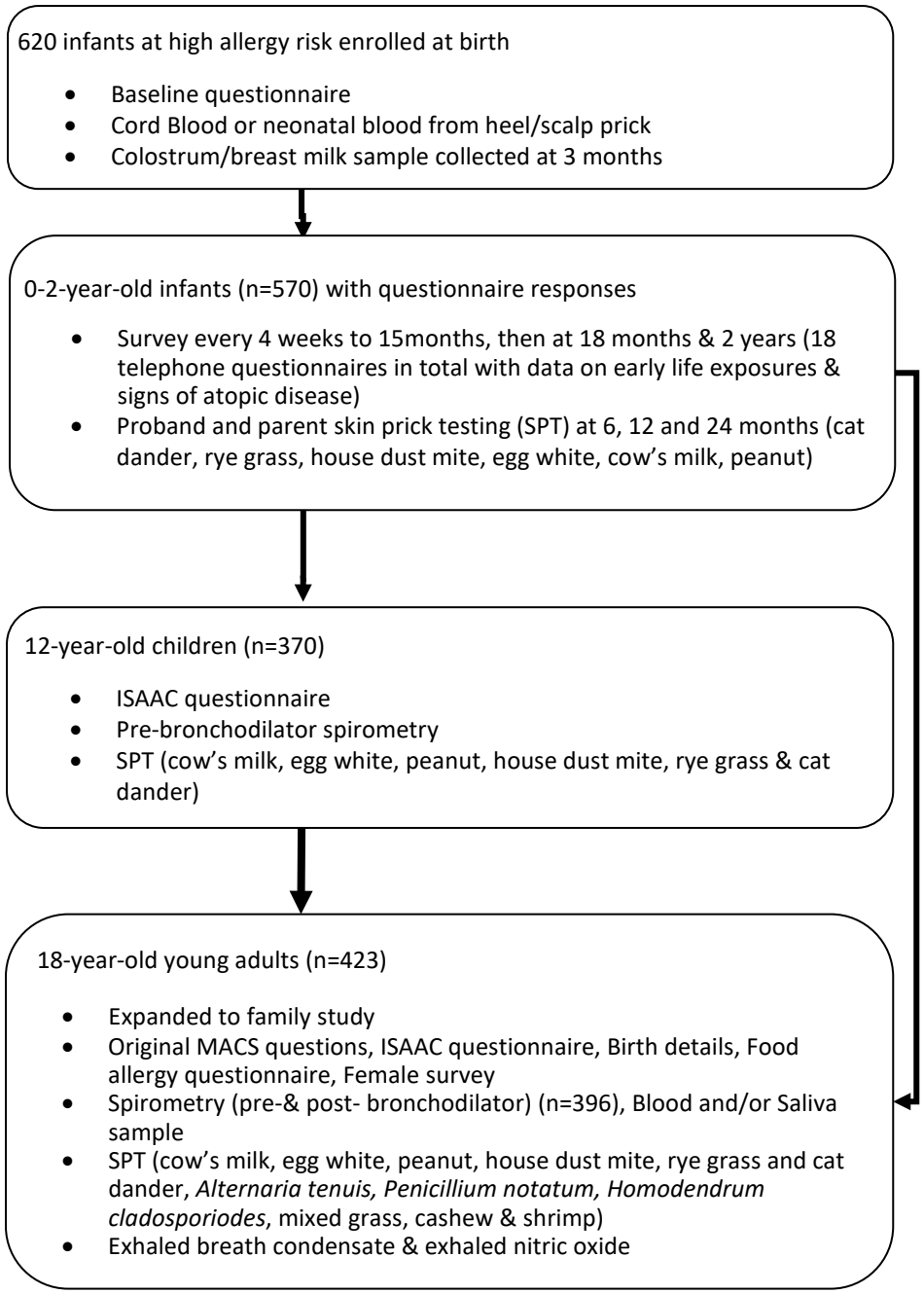


Figure 4.1 Flow chart of participants in MACS

4.1.2.1 Baseline data collection

Baseline information was recorded using an interviewer-administered questionnaire at the initial interview (Appendix 2). The median time of interview was 4 days after the birth of the proband, and the interquartile range was 2 days prior to birth until 26 days after birth [134]. Baseline data concerning family profile and socio-domestic factors included information on parental age, birth place, marital status, parental education, smoking status, occupation and whether the parents were currently receiving social security benefits. Further information on exposures in the home environment was also collected by questionnaires including types of floor covering, domestic pet ownership, types of cooking and heating and exposure to passive smoking.

4.1.2.2 Follow-ups in the first 2 years

Approximately 93% of the MACS participants were followed up during the first 2 years of life. An allergy-trained nurse conducted telephone interviews every 4 weeks from birth to 15 months, and again at 18 months and at 2 years of age. At each interview, 2 main questionnaires were administered, a food reaction questionnaire and an infant illness questionnaire (Appendix 3). The food reaction questionnaire collected detailed information on infant feeding, the timing of introduction of foods, and the occurrence of food reactions. The infant illness questionnaire collected information on the development of allergic diseases and other medical illnesses. Data was collected on many factors including: gastrointestinal symptoms, skin rashes, wheezing, otitis media and allergic reactions to drugs or non-drug substances. The use of any medication including indication for its use, and when and how often it was used was also recorded at these times. Contact with health care professionals and the related reason (in terms of medical conditions) was also documented.

Skin prick tests (SPTs) were conducted at 6 months, 1 year, 2 years, 12 years and 18 years of age according to a standard technique [257]. A trained allergy nurse performed the test by placing a drop of each allergen extract on the infants' backs, then the skin was pricked through this allergen drop using a lancet. Allergens tested included three food allergens: *egg white, cow's milk, and peanut*, and three aeroallergens: *Dermataphagoides Pteronyssinus* (dust mite), *Lolium perenne* (rye grass)

and cat dander [Bayer, Spokane, WA, USA]. At the 18-year follow-up, additional allergens (*Alternaria tenuis, Penicillium notatum, Homodendrum cladosposiodes, mixed grass, cashew and shrimp*) were added on. The SPTs were read at 15-20 minutes using a ruler.

4.1.2.4 Follow-ups from ages 3-7 years

From ages 3-7 years, probands were followed up annually. For each follow-up, the baseline socio-domestic questionnaires were repeated. A new questionnaire (the annual MACS follow-up questionnaire) was added to collect information on childhood exposures and allergic diseases. These data include information on common childhood illnesses and allergies such as asthma, wheeze of any cause, bronchitis, bronchiolitis, croup, eczema, food reactions, drug allergies, non-drug allergies, otitis media, tonsillitis, throat infections, gastroenteritis, conjunctivitis, cold or flus, and physical injuries. For each illness, the symptoms, episodes, frequencies and treatments were documented. Additionally, information on vaccinations, height and weight, day care attendance, kindergarten or school attendance, siblings and number of people living in the house was collected by the same questionnaire.

4.1.2.5 Follow-ups at 12 years of age

At 12 years of age, children were invited to attend a clinical examination and complete a survey (Appendix 4). Of the 620 children enrolled into the cohort at birth, 58% (n=360) participated in the 12-year follow-up at which two objective clinical tests were performed; lung function and SPTs. Lung function was measured by pre-bronchodilator spirometry according to a standard technique and is explained further in the following section on spirometry [258, 259]. The SPT used the same six allergens that were tested in the first 2 years of life comprising *cow's milk, peanut, egg white, house dust mite, rye grass and cat dander*. Positive SPT results were defined as an average wheal diameter of 3mm or greater than the negative control. Two questionnaires were administered by the research nurses on the day of clinical assessments to evaluate the health status of each proband, including 1) the annual MACS follow-up questionnaire that was used at the 3-7-year follow-ups, and 2) a questionnaire on asthma and allergies based on the ISAAC. Answers to both

questionnaires were provided by the parent or guardian of the proband, and for probands who did not attend clinical assessments, their parent or guardian was asked to answer the questionnaires by telephone.

4.1.2.6 Follow-up at 18 years of age

All the probands and their parents and siblings were invited to participate in the 18-year follow-up and 423 (68%) probands, 512 siblings and 691 parents participated. The questionnaires were administered at 18 years to collect information on allergic and respiratory diseases (Appendix 5). In addition to these questionnaires, new questionnaires were administered to collect information on home conditions, household exposures, smoking history, quality of life, education level, occupation and general health status for probands and family members.

Clinical tests including lung function measurements and SPT were performed on probands and their families. Pre-bronchodilator and post-bronchodilator spirometry were conducted. The same six allergens from previous follow-ups for SPTs were tested (*cow's milk, peanut, egg white, house dust mite, rye grass and cat dander),* however an additional six allergens were added; *mixed grass pollen, Alternaria, Cladosporium, Hormodendrum, Penicillium and food allergens*. Positive SPT results were defined as an average wheal diameter of 3mm or greater than the negative control.

4.1.3 Statistical methods

Data from the MACS was explored by using multivariable linear and logistic regression analyses to address the research aims. All analyses were performed using STATA version 14.1 (StataCorp LP, College Station, TX).

Linear regression models were performed to estimate the association between early life exposures and lung function parameters at 12 and 18 years. Logistic regression models were developed to investigate the association between early life exposures and asthma outcomes at 12 and 18 years. Crude models were adjusted for *a priori* confounders, identified through the literature, including age, sex, parental education and parental asthma. Further potential confounders were considered based upon a literature review and on relationships illustrated using a Directed Acyclic Graph (DAG).

To assess the interaction between early life exposures and GST genes for respiratory outcomes in adolescence, a model fitted with an interaction term was compared to a model without the interaction terms by using the likelihood ratio test. Interactions were considered to be present in the event of a p-value<0.1 with the likelihood ratio test, indicating that the compared models were different. Additionally, the results of subgroup strata were presented based on GST polymorphisms of interest.

4.1.4 Ethics

The initial phases of MACS were approved by The Mercy Maternity Hospital Ethics Committee, and the trial was retrospectively registered with the Australian and New Zealand Clinical Trials Registry (ACTRN12609000734268). The 18-year follow-up was approved by the University of Melbourne and the Royal Children's Hospital Ethics Committees (reference 28035). Written informed consent was obtained from all mothers at birth or any parent/guardian for follow-ups and participants over 12 years.

4.2 Tasmanian Longitudinal Health Study (TAHS)

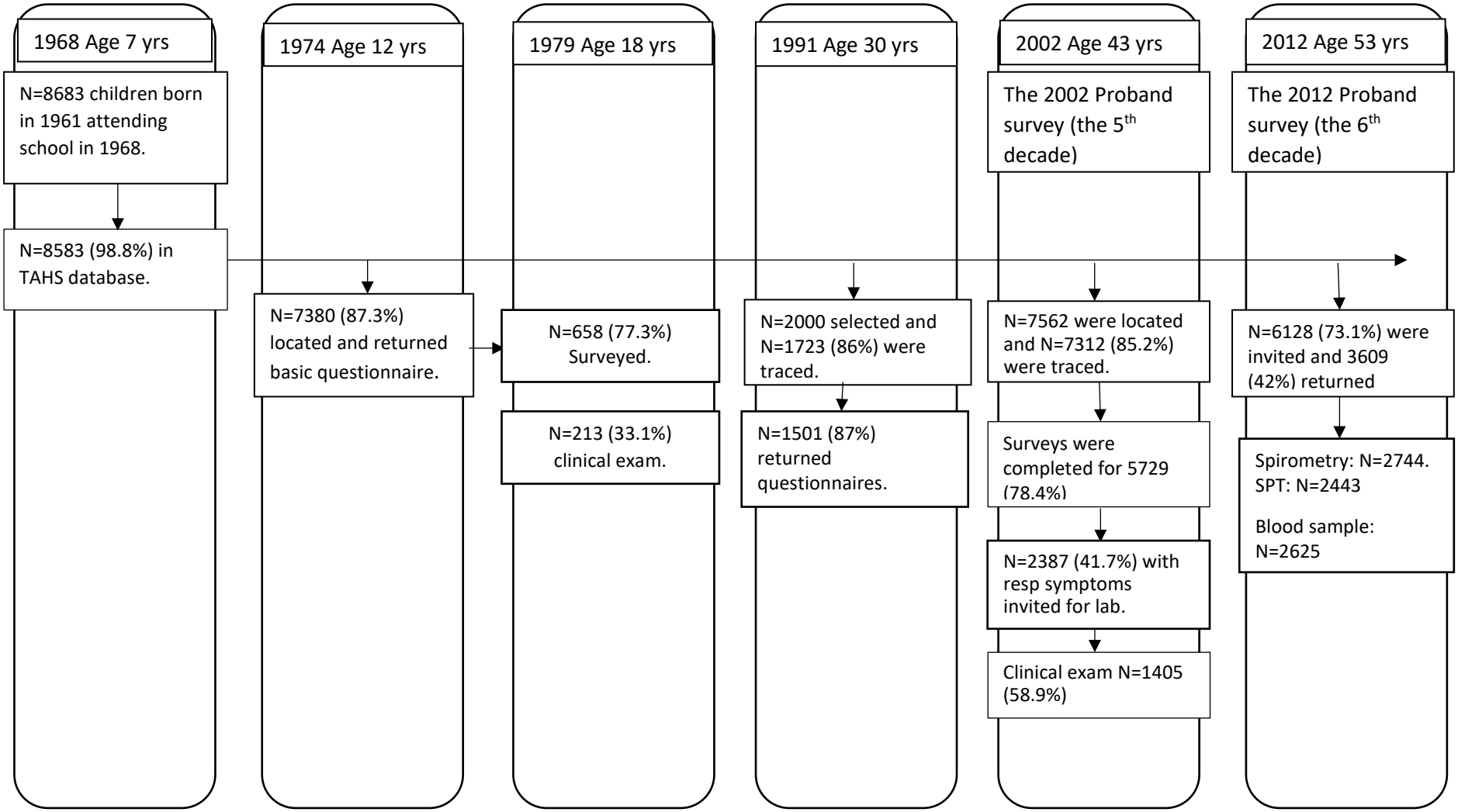
Tasmania is an island state of Australia located 240 kilometres from the mainland of South Australia. The TAHS originally started in Tasmania as the Tasmanian Asthma Survey, and the initial aim was to examine the prevalence and natural history of asthma in childhood. However, this study, in which participants have been followed up to the 6th decade of life, has developed into one of the longest and largest running respiratory health research cohorts in the world. The availability of extensive data over the life course in TAHS provides a unique opportunity to investigate the impact of gene-environment interactions on asthma occurrence and persistence.

4.2.1 Study design and study population

In 1967, the Tasmanian Health Minister granted approval to carry out a survey on all children who were born in 1961 and attending school in Tasmania. The TAHS then started in 1968 as a population-based prospective cohort study and recruited children born in 1961 (aged 7 years) from all public schools in Tasmania, and the private schools

in Tasmania that agreed to participate. A total of 8583 children (98.8% of the 7-year-old population at that time) participated in the baseline survey and were known as "probands". Subsequent follow-up surveys were conducted in 1974, 1979, and 1991 at the ages of 12, 18 and 30 years, respectively. The next proband follow-up study, conducted between 2002-2008, targeted all of the original participants from the baseline study during their 5th decade of life (average 42.8 years). The most recent survey in 2012 obtained health information from probands during their 6th decade of life (average 53 years). The participants who were involved in the two most recent follow-ups (termed the "5th decade" and "6th decade" follow-ups) are the study population of interest in my thesis (Figure 4.2).

Figure 4.2 Flow chart of participants in TAHS



Moreover, information was also collected on the siblings and parents of TAHS probands. The sibling study commenced in 2007 and traced siblings from the original cohort in 1968. Of 17,844 siblings who were sent a postal survey, 71.6% (n=12,073) completed the survey. The parent study started in 2010 and attempted to trace all parents of the original TAHS probands. Of those who were alive and traced to an address, 5,111 (70.3%) responded to a postal survey.

4.2.2 The Chronology of data collection in TAHS.

4.2.2.1 The TAHS 1968 baseline study

The initial study cohort consisted of all Tasmanian school children aged 7 years at the time of the baseline study. Any 7-year-old children who entered Tasmania after June 1968 were not included in the cohort. There were several benefits to selecting children at 7 years including: 1) participants were old enough to be able to cooperate and perform spirometry testing; 2) participants were young enough to be followed-up through the school system; 3) parental recall error of early life diseases and symptoms was reduced by limiting the recall time to a maximum of 7 years.

In 1968, a letter was posted to the parents of each child explaining the aim of the survey and seeking parental consent. There were 8,683 eligible children (born 1961) on the list provided by all public schools, and the private schools in Tasmania that agreed to participate. A completed survey was returned by 98.8% (n=8583) of these children. The baseline questionnaires contained key questions to address proband respiratory health, including asthma, bronchitis, bronchiolitis or bronchiectasis, nasal infection and allergy, and miscellaneous other diseases (Appendix 6). Children who enrolled in the study were also invited to participate in a medical examination that included lung function testing. School medical officers and nurses were involved in the medical examination, and they were given instructions on how to use a Vitalograph spirometer. Spirometers were regularly calibrated by an expert and he/she would document their own ventilatory capacity at regular intervals. Results from spirometers were interpreted by two independent assessors to reduce bias.

School medical officers also performed routine medical examinations focussing on clinical findings pertaining to the upper airways, chest and lungs. Weight and height of the probands were also measured and recorded. Of the 8,583 children who returned completed questionnaires, 8,250 (96.1%) also had complete data on lung function and 331 (3.8%) did not attend the clinical examination.

4.2.2.2 Follow-ups from 1974-1992

Between 1974 to 1992, three proband follow-ups (either major or sub-group) were performed. As I did not use the data from these three follow-ups, the details of the methodology are not described in my thesis.

4.2.2.3 The 5th decade follow-up

This major follow-up occurred between 2002-2008 when the probands were in their 5th decade of life. Since the probands left school their contact details were largely out of date. Therefore, the proband 2002 study aimed to identify the current residential addresses of the 8,583 probands and then follow them up using a mailed questionnaire. Several national databases were used to trace the cohort members. These databases included: the National Death Index (NDI), the Commonwealth electoral roll, the Tasmanian Registry of Births, Deaths and Marriages, the Medicare database and the Australian Twin Registry [260]. After excluding 320 (2.9%) participants who were living overseas, withdrawn or deceased, addresses were identified for 81.5% (6,998) of the baseline proband cohort. Of these traced probands, 81.9% (5,729) provided responses to detailed respiratory questionnaires, representing almost 70% of the total baseline study participants in 1968.

At the 5th decade survey, the mean age was 43 years. Questionnaires at this follow-up were designed to collect information on possible risk factors associated with asthma and allergic diseases up to middle age and focussed on the probands' lifetime symptoms of asthma, wheeze and bronchitis (Appendix 7). This comprehensive questionnaire also collected detailed information on personal smoking history and many current environmental exposures, including passive tobacco smoke exposure, cooking and heating exposures in the home, mould in the home, pets and carpets in the home, as well as personal details, such as height, weight, education level,

occupation, number of children and the health status of these children. For female participants, information was additionally collected on reproductive history including hormonal contraception use.

A subgroup of the probands participating in the 5th decade survey (n=2,387), enriched for cases of asthma or cough reported in childhood or adulthood, were invited to participate in a more detailed laboratory study (Appendix 8). Of 2,387 invited, 58.9% (n=1,405) underwent a full laboratory visit, 14.5% (n=346) only completed a telephone questionnaire for the laboratory visit, and 26.6% (n=636) declined to participate. Clinical testing included SPTs, blood tests and lung function tests. SPTs were performed using a standard technique [261] for eight aero allergens, including *Dermatophag oides pteronyssinus, Cat dander, Cladosporium cladosporioides, Alternaria tenuis, Penicillium mix, Aspergillus fumigatus,* perennial ryegrass, and mixed grasses (Hollister-Stier, EBOS Groups Pty Ltd., Australia) . Blood samples were collected for genotyping using Taq-Man allelic discrimination 5'nuclease assays (Applied Biosystems, Foster City, CA, US) according to the manufacturer's protocol. Pre- and post-BD spirometry were performed at the 5th decade using the EasyOneTM ultrasonic Spirometer (ndd, Medizintechnik, AG, Switzerland), and all lung function tests were conducted according to the ATS and ERS guidelines [77].

4.2.2.4 The 6th decade follow-up

The most recent TAHS follow-up (the 6th decade follow-up) began in 2012 when the probands were aged between 51 and 54 years and aimed to investigate the changes in respiratory health status and lung function with aging and disease. In this follow-up, the entire TAHS cohort who were alive and traceable were invited to contribute information through both respiratory questionnaires (Appendix 9) and clinical testing (which included lung function testing). From a total of 6,128 invited participants, 3,609 (58.9%) returned completed questionnaires, representing 42% of the original 1968 cohort. Spirometry was conducted on 2,744 participants, including 2,659 who attended the clinics and 85 who had home visits. SPTs were performed on 2,443 participants for 12 allergens. A wheal size of at least 3mm greater than the negative control was considered as indicating sensitization. Blood samples were collected on

2,625 participants for objective markers of asthma and other chronic respiratory diseases.

The 6th decade survey collected information on many clinical conditions and exposures, (e.g. asthma symptoms, cough, allergies, infections), lifetime determinants of the various asthma/COPD phenotypes (e.g. growth/obesity, occupational/domestic exposures, smoking, alcohol use, physical activity, diet, reproductive history, menopausal transition), as well as retrospective information on early life determinants (e.g. childhood environment).

4.2.3 Definition of exposures

The 5th and 6th decade surveys in 2002 and 2012 included the same items about the indoor environment, e.g. heating and cooking appliances, mould, carpet, pet ownership, active smoking and passive smoking. These questions were used to assess exposure to indoor air pollution in my analysis are listed as below:

Heating: "what types of heating do you use at home? (Please choose ALL that apply)". Answers recorded one or more of the following types of heating selected. "Gas ducted central heating", "Coal or wood fire", "Gas room heater", "Electric heater (eg. Radiator, fan or Dimplex-type)", other central heating (eg. Electric hydronic, slab floor heating)", "Reverse cycle air conditioning", and "Other";

Cooking: "What kind of stove do you mostly use for cooking?" The participant was required to select only one of the following types: "Gas", "Electric", "Coal, coke or wood", or "Other";

Mould: "Has there ever been mould or mildew on any surface, other than food, in your home?" If the answer to this question was yes, participants would then answer two additional questions to identify the location of mould and whether this mould was current. These questions were: "Which rooms have been affected?" and "Has there ever been mould or mildew on any surface, other than food, in your home in the last 12 months?"

Cat: "Do you keep or own any cats?" If participants answered "yes" to this question, they would need to answer questions about the number of cat, whether cats were allowed indoors, and whether cats were allowed in the bedroom.

Dog: The questionnaires at the 6th decade also asked about dog ownership, which was not asked at the 5th decade.

Carpet: "Is there fitted carpet in the bedroom?" Participants would answer a subsequent question about the age of the carpet if they answered "yes" to this question.

Ever smoking: "In your lifetime, have you smoked at least 100 cigarettes or equal amounts of cigars, pipes or any tobacco product?"

Current smoking: "Do you currently smoke (within the last 4 weeks)?". Participants were required to select 1 of the with following answers: "Not at all", "Yes, daily", "Yes, at least weekly", "Yes, less than weekly". Participants who selected "yes" answers, would need to further indicate the number of cigarettes they smoked per day, per week, and per month.

Passive smoking: "Not counting yourself, how many people in your household currently smoke regularly (most days of the week) inside the house?" Participants would need to indicate the number of people who met the criteria of this question.

4.2.4 Statistical methods

In TAHS, seven different household factor profiles were distinguished using LCA. These profiles were identified as "Wood heating only", "Gas cooking and heater", "Wood heating and smoking", "Electric heater", "Gas heating, cooking and smoking", "Gas cooking and wood heating", and a "Reference" profile characterized by highest probability of membership for participants. Details of the LCA methods can be found in Chapter 7 below. Multivariable logistic regression was then performed to investigate the associations between these identified household air exposure profiles and respiratory outcomes from the 5th to the 6th decade. Interactions between GST genotypes and household risk factors profiles on respiratory outcomes were tested

using the likelihood ratio test, and strata-specific estimates were reported for interaction p-values ≤0.1. All analyses were performed using Stata 14.1 (Stata Corp, College Station, TX, USA).

4.2.5 Ethics

The 5th decade follow-up was approved by the Human Research Ethics Committees at The University of Melbourne and Tasmania (Melbourne University Human Research Ethics Committee approval number 010626X). Written informed consent was obtained from all participants.

The 6th decade follow-up was approved by the Human Research Ethics Committees at The University of Melbourne (1238525), Tasmania (H0012710), New South Wales (HC12417), the Alfred Hospital (367/12), Gold Coast Hospital and Health service (HREC/12/QGC/175) and the Hunter New England Local Health District (HREC/12/HNE423). Written informed consent was provided by all participants.

Chapter 5 INTERACTION OF GLUTATHIONE S-TRANSFERASE M1, T1 AND P1 GENES WITH EARLY LIFE TOBACCO SMOKE EXPOSURE ON LUNG FUNCTION IN ADOLESCENTS

5.1 Chapter introduction

This paper (with data supplement) reports my investigation of the interaction of GST genes on the associations between early life tobacco smoke exposure and asthma and lung function in adolescents. This paper provides evidence that GST genes, involved in the management of oxidative stress in the lungs, modify the associations between early life smoke and lung function.

5.2 Research questions and objectives

5.2.1 Research questions

Is the relationship between early life tobacco smoke exposure and lung health in adolescence modified by GST variants?

5.2.2 Research objectives

To determine if the relationship between early life tobacco smoke exposure and lung health in adolescence is modified by genetic differences in GSTM1 null, GSTT1 null and GSTP1 genotypes.

5.3 Publication

Dai, X, Dharmage, S.C., Bowatte, G., Waidyatillake, N., Perret, J., Hui, J., & …Lodge, C.J (2019). Interaction of Glutathione S-Transferase M1, T1, and P1 Genes with early life tobacco smoke exposure on lung function in adolescents. *Chest*, 155(1), 94-102

CHEST

Interaction of Glutathione S-Transferase M1, T1, and P1 Genes With Early Life Tobacco Smoke Exposure on Lung Function in Adolescents

Check for updates

Xin Dai, MPH; Shyamali C. Dharmage, PhD; Gayan Bowatte, PhD; Nilakshi T. Waidyatillake, PhD; Jennifer L. Perret, PhD; Jennie Hui, PhD; Bircan Erbas, PhD; Michael J. Abramson, PhD; Adrian J. Lowe, PhD; John A. Burgess, PhD; Cecilie Svanes, PhD; and Caroline J. Lodge, PhD

BACKGROUND: Glutathione S-transferase (GST) genes are involved in the management of oxidative stress in the lungs. We aimed to determine whether they modify the associations between early life smoke exposure and adverse lung health outcomes.

METHODS: The Melbourne Atopy Cohort study (a high-risk birth cohort) enrolled 620 children and followed them prospectively from birth. We recorded perinatal tobacco smoke exposure, asthma, and lung function at 12 (59%) and 18 years (66%) and genotyped for *GSTM1*, *GSTT1*, and *GSTP1* (69%).

RESULTS: GST genotypes were found to interact with tobacco smoke exposure on lung function outcomes (P interaction $\leq$ .05). Only among children with *GSTT1* null genotypes was exposure to mother's, father's, or parental tobacco smoke in early life associated with an increased risk of reductions in prebronchodilator (BD) FEV_1 and FVC at both 12 and 18 years. These associations were not seen in children with *GSTT1* present. Similarly, only among children with *GSTM1* null genotypes was exposure to father's or parental smoking associated with reductions in pre- and post-BD FEV_1 and FVC at 18 years. Only among children with Ile/Ile genotypes of *GSTP1* was exposure to mother's smoking associated with increased risk of reduced FEV_1 at 18 years, but this was not the case among children with Val/Val or Ile/Val genotypes.

CONCLUSIONS: Our study provides evidence of interaction between early tobacco smoke exposure and GST genotypes on lung function. Carriers of GST null mutations and *GSTP1* Ile/Ile alleles may be more susceptible when exposed to tobacco smoke in early life. These findings support stronger recommendations to protect all infants from tobacco smoke exposure.

TRIAL REGISTRY: Australian and New Zealand Clinical Trials Registry; No.: ACTRN12609000734268; URL: http://www.anzctr.org.au/ CHEST 2019; 155(1):94-102

KEY WORDS: epidemiology (pulmonary); gene polymorphism; lung function; pediatric asthma; tobacco smoke exposure

ABBREVIATIONS: BD = bronchodilator; GLI = Global Lung Function Initiative; GST = glutathione S-transferase; MACS = Melbourne Atopy Cohort Study

AFFILIATIONS: From the Allergy and Lung Health Unit (Ms Dai and Drs Dharmage, Bowatte, Waidyatillake, Perret, Lowe, Burgess, and Lodge), Centre for Epidemiology and Biostatistics, Melbourne School of Population and Global Health, The University of Melbourne, Melbourne, VIC, Australia; National Institute of Fundamental Studies (Dr Bowatte), Kandy, Sri Lanka; the Centre for Food and Allergy Research (Drs Dharmage and Lowe), Murdoch Children's

Exposure to tobacco smoke in early life has been found to increase the risk of developing asthma and lung function deficits in adolescence and adulthood.[1-5] However, not all children exposed to tobacco smoke in early life develop asthma, which suggests differences in genetic susceptibility.

There is increasing interest in genetic polymorphisms involved in oxidative stress pathways. One of the major enzymatic regulators of oxidative stress in the body is through glutathione S-transferase (GST) enzymes encoded by GST genes. GST enzymes are involved in the detoxification of a variety of foreign chemicals, including several reactive tobacco metabolites and reactive oxygen species.[6-8] Three major GST enzymes are encoded by the *GSTM1*, *GSTT1*, and *GSTP1* genes. Deficiencies in the enzyme activity in *GSTM1* and *GSTT1* are typically caused by deletion of the genes (null genotypes), whereas the most studied *GSTP1* variant contains two polymorphisms: an A→G transition at codon 105, which leads to the ile105val amino acid substitution.[9,10] Substituting Ile^{105} for Val^{105} significantly lowers GST enzyme activity.[11]

However, a 2010 systematic review including 22 studies did not support a substantial direct role of GST genetic polymorphisms in the development of asthma. The authors instead suggested the possibility of a gene-environment interaction influencing the risks of asthma and impaired lung function.[12] However, there are a limited number of longitudinal studies focusing on gene-environment interactions and measuring outcomes at different time periods in older children and adolescents, especially concerning the effect on lung function.

We aimed to investigate the relationship between GST genes and early life tobacco smoke exposure and their interaction on asthma and lung function at 12 and 18 years of age in the Melbourne Atopy Cohort Study (MACS). We have previously found in this cohort that early life tobacco smoke exposure was associated with asthma and lung function impairment at 18 years in girls.[13] We now investigate the potential for GST gene (*GSM1*, *GSTT1*, and *GSTP1*) polymorphisms to modify these associations.

Materials and Methods

Study Population

Between 1990 and 1994, 620 children were recruited (while in utero) into the MACS (Fig 1). All children had at least one first degree family member with allergic disease. Details of the study have been published elsewhere.[14] Data on exposures and respiratory outcomes for these participants were collected from birth to 18 years of age. MACS initially began as a randomized controlled trial of infant formula on weaning,[15] but has since been followed as an observational cohort. The initial phases to the 12-year follow-up of MACS were approved by The Mercy Maternity Hospital Ethics Committee. The 18-year follow-up was approved by the University of Melbourne and the Royal Children's Hospital Ethics Committees (reference No. 28035).

Tobacco Smoke Exposure

Tobacco smoke exposure was collected at the initial interview from mothers (median, 4 days before birth of the child; interquartile range, 26 days prior to birth to 2 days after birth). In this study, mother's or father's smoking in early life was defined by response to the following questions at baseline: Is mother smoking now? and Is father smoking now? Parental smoking in early life was defined as either mother or father smoking or both. Participants completed written questionnaires at 18 years of age.

Asthma

Current asthma at 12 years of age was defined as positive responses by parents to the following question: Any episodes of asthma or treatment with breathing medication within the last 12 months?

Current asthma at 18 years of age was defined by positive responses by the participant to the following question: Have you had any episodes of asthma or used medication for asthma in the last year?

Lung Function

Lung function was measured by spirometry at 12 and 18 years of age, according to standard techniques described by the American Thoracic Society.[16,17] The detailed procedures at both time points have been described elsewhere.[13] SpiroCard spirometers (SpiroCard PC

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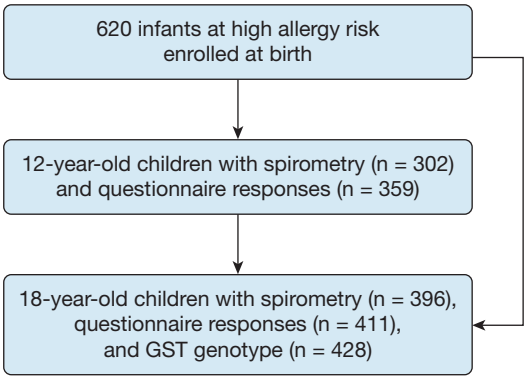

Figure 1 – *Flowchart of participants in the Melbourne Atopy Cohort Study. GST = glutathione S-transferase.*

Spirometer; QRS Diagnostic) were used at 12 years of age. The EasyOne Spirometer system (ndd Medical Technologies) was used for the 18-year follow-up, before and after the inhaled bronchodilator (BD) salbutamol 200 μg via a spacer. Tests were checked for acceptability and reproducibility by the respiratory scientist and one of the authors (C. J. L). FEV_1 and FVC and mid expiratory flow were measured. Both *z* scores (measures of SD from the mean predicted value) and % predicted values were calculated from the Global Lung Function Initiative (GLI) reference values for Caucasians.[18,19]

Genotyping

Blood or saliva was collected at the 18-year follow-up for GST genotyping.[14] Multiplex polymerase chain reaction techniques were used for measurement of the deletion of *GSTM1* and *GSTT1* genotypes. For all experiments, positive primers for beta-globin were included as a positive control. A customized GoldenGate Genotyping Assay (Illumina) was used to genotype the *GSTP1* (rs1695 A→G: ile105val) polymorphism. All assays were performed by scientists unaware of the clinical status of individual subjects. Genotype assignments were based on two consistent experimental results.

Other Variables

Baseline data also collected at the initial interview included parental age, parental education (used as a proxy for socioeconomic status), parental asthma, cooking facilities, and pets at home. Parental higher education was defined as either mother or father or both having a tertiary education at the time of study entry. Parental asthma was defined as either parent ever having a history of asthma. Adolescent smoking was defined by the following question at the 18-year follow-up: Have you smoked at least 100 cigarettes or an equal amount of cigars, pipes, or any other tobacco product?

Statistical Analyses

Multiple linear or logistic regression was used to assess the relationships between early life smoke exposure, GST genotype, adolescent lung function, and asthma outcomes. All ORs from regression models for asthma were adjusted for age, height, parental asthma at birth, and parental education. Results for associations with lung function parameters (FEV_1 and FVC) were expressed as *z* scores (SD and 95% CI) accounting for age, sex, height, and race using GLI reference values.[18,19] Results were also expressed in terms of % predicted values using GLI reference values.

We also considered cooking facilities, adolescent smoking, and pets at home as potential confounders. Variables were included in the multivariable model if they changed the effect on the associations (> 10% change in OR or coefficient).

For *GSTM1* and *GSTT1* genotypes, the reference group consisted of subjects with *GSTM1*/*GSTT1* wild-type alleles, and the exposed (risk) group was those with null genotypes. The referent group for *GSTP1* consisted of subjects having at least one Val allele (ie, Ile/Val, Val/Val). This was compared with the risk group, who were homozygous for Ile/Ile alleles. Strata-specific estimates were reported when an interaction *P* value ≤ .05 was observed. All analyses were conducted using Stata 14 (StataCorp LLC).

Results

MACS recruited slightly more girls (51.1%) than boys (48.9%). Only 38 (6.1%) were exposed to mother's smoking, and 112 (18.2%) were exposed to father's smoking. Overall, 127 (20.5%) were exposed to parental smoking. Spirometry was available for 366 (59%) and 411 (66.3%) at 12 and 18 years, respectively (Fig 1). *GSTM1*/*GSTT1* data were available for 428 participants, and *GSTP1* data were available for 429 participants (Table 1).

Main Effects of Early Life Smoke Exposure and GST Genes on Lung Function and Asthma

There was no association found between early life smoke exposure from parental smoking and lung function (e-Tables 1, 2, % predicted; Table 2, *z* scores) or asthma at age 12 and 18 years (e-Tables 3, 4). We did not find associations between GST null genotypes (*GSTM1* and *GSTT1*) for either lung function or asthma outcomes (e-Tables 5-8). *GSTP1* with Ile/Ile genotype was associated with an increased risk of reduced post-BD FVC at 18 years of age for children with Ile/Ile genotypes compared with those with Ile/Val or Val/Val (*z* score = −0.23; 95% CI, −0.42 to −0.03) (e-Table 6)

Interaction Analyses

Only among those with *GSTT*, *GSTM*, and *GSTP* risk genotypes was exposure to mother's, father's, or parental smoking associated with a reduction in lung function at 18 years of age.

Early Life Tobacco Smoke Exposure, GST Genes, and Lung Function Outcomes at 12 Years of Age: Because we found a significant interaction between *GSTT1* genotype and early life smoke exposure on lung function at 12 years of age (Table 2), we have presented these associations for different strata of GST gene exposure in Table 3.

TABLE 1] Selected Clinical Characteristics of the Study Cohort (N = 620)

Variables	Baseline	12-y Follow-Up	18-y Follow-Up
Sex			
Boy	317 (51.1)	190 (51.9)	208 (50.6)
Girl	303 (48.9)	176 (48.1)	203 (49.4)
Parental higher education			
Yes	417 (72.0)	268 (73.2)	309 (75.2)
No	162 (28.0)	98 (26.8)	102 (24.8)
Parental asthma			
Yes	356 (61.7)	228 (62.5)	253 (61.7)
No	221 (38.3)	137 (37.5)	157 (38.3)
Mother smoking			
Yes	38 (6.1)	16 (4.6)	16 (4.1)
No	582 (93.9)	333 (95.4)	370 (95.9)
Father smoking			
Yes	112 (18.2)	51 (14.6)	49 (12.7)
No	507 (81.8)	298 (85.4)	337 (87.3)
Parental smoking			
Yes	127 (20.5)	58 (16.6)	56 (14.5)
No	493 (79.5)	291 (83.4)	330 (85.5)
Adolescent smoking			
Yes	…	…	38 (9.3)
No	…	…	372 (90.7)
GSTM1			
Present	…	131 (44)	178 (41.6)
Null	…	167 (56)	250 (58.4)
GSTT1			
Present	…	249 (83.6)	353 (82.5)
Null	…	49 (16.4)	75 (17.5)
GSTP1			
Ile/Ile	…	132 (44.1)	184 (42.9)
Val/Ile and Val/Val	…	167 (55.9)	245 (57.1)

Values are No. (%).

Only among those with *GSTT1* null genotypes was exposure to mother's smoking associated with a significant reduction in lung function at 12 years of age (FEV_1: $z = -1.92$; 95% CI, −3.50 to −0.36; FVC: $z = -2.05$; 95% CI, −3.78 to −0.32). That is, *GSTT1* null children exposed to maternal smoking in early life had an increased risk of reduced FEV_1 and FVC by around 2 SDs from the predicted mean at 12 years of age when compared with those with null genotypes not exposed to maternal smoking. This association was not seen in children with *GSTT1* present genotypes. The interaction *P* value confirmed that the effect on lung function was not simply the cumulative effect from both exposures but the result of a synergistic (multiplicative) relationship between tobacco smoke exposure and *GSTT1* status on lung function.

Similarly, we found a significant interaction between *GSTT1* and parental smoking on lung function. Only among children with *GSTT1* null genotypes was exposure to parental smoking associated with an increased risk of reduced FEV_1 at 12 years of age (although the 95% CIs just crossed zero) compared with those with null genotypes but that were not exposed.

Similar analyses and findings for GLI-derived % predicted lung function parameter at 12 years of age are shown in e-Table 9.

TABLE 2] Main Associations Between Early Life Smoke Exposure and Lung Function at 12 and 18 Years of Age and Interaction *P* Values by GST Genes

Pre- or Post-BD	Smoking	Lung Function			Smoking × GST Interaction (*P* Value)		
			Unadjusted	Adjusted	*GSTM1*	*GSTT1*	*GSTP1*
12 y							
Pre-BD	Mother	FEV_1	0.34 (−0.23 to 0.91)	0.48 (−0.13 to 1.08)	.32	< .01[a]	.33
		FVC	1.26 (−0.51 to 0.71)	−0.13 (−0.64 to 0.39)	.79	< .01[a]	.82
	Father	FEV_1	−0.08 (−0.42 to 0.25)	0.26 (−0.38 to 0.90)	.53	.12	.08
		FVC	−0.10 (−0.46 to 0.25)	−0.10 (−0.47 to 0.27)	.73	.17	.27
	Parental	FEV_1	−0.09 (−0.41 to 0.22)	−0.07 (−0.40 to 0.26)	.51	.05	.29
		FVC	−0.16 (−0.49 to 0.18)	−0.11 (−0.46 to 0.23)	.66	.05	.50
18 y							
Pre-BD	Mother	FEV_1	−0.26 (−0.77 to 0.25)	−0.07 (−0.59 to 0.45)	.44	.03[a]	< .01[a]
		FVC	−0.21 (−0.72 to 0.29)	−0.13 (−0.64 to 0.39)	.43	.01[a]	.10
	Father	FEV_1	−0.20 (−0.52 to 0.11)	−0.11 (−0.42 to 0.21)	.15	.12	.60
		FVC	−0.03 (−0.34 to 0.28)	0.02 (−0.29 to 0.33)	.02[a]	.04[a]	.15
	Parental	FEV_1	−0.24 (−0.54 to 0.05)	−0.13 (−0.43 to 0.17)	.21	.09	.61
		FVC	−0.10 (−0.39 to 0.19)	−0.05 (−0.35 to 0.25)	.12	.01[a]	.30
Post-BD	Mother	FEV_1	−0.08 (−0.57 to 0.41)	0.07 (−0.42 to 0.57)	.44	044	.09
		FVC	0.03 (−0.47 to 0.53)	0.10 (−0.42 to 0.61)	.82	.32	.69
	Father	FEV_1	−0.20 (−0.52 to 0.11)	−0.02 (−0.32 to 0.28)	.03[a]	.68	.78
		FVC	−0.03 (−0.34 to 0.28)	0.05 (−0.26 to 0.36)	.02[a]	.89	.28
	Parental	FEV_1	−0.12 (−0.40 to 0.15)	−0.03 (−0.32 to 0.25)	.01[a]	.61	.55
		FVC	−0.03 (−0.31 to 0.25)	0.01 (−0.29 to 0.30)	.04[a]	.57	.41

Values are *z* score (SD, 95% CI) or *P* value. BD = bronchodilator; GST = glutathione S-transferase.
[a]Significant *P* values.

Early Life Tobacco Smoke Exposure, GST Genes, and lung Function Outcomes at 18 Years of Age: We also found some evidence for similar interactions at 18 years of age (Table 3). Only among children with *GSTM1* null genotypes was exposure to father's smoking associated with reductions in pre-BD FVC and post-BD FEV_1 and FVC at 18 years of age when compared with children with GST null not exposed to father's smoking in early life. Although the 95% CIs crossed 0, all the point estimates were reduced by around one-quarter of an SD. Again, the interaction *P* value confirmed that the effect on lung function was multiplicative when compared with either exposure alone. The positive interaction term for *GSTM1* may be partly related to the paradoxical relationship to early life smoke exposure in children with *GSTM1* present. Only among children with *GSTM1* present genotypes was exposure to father's tobacco smoke in early life associated with a significant increase in both pre and post-BD FVC when compared with children not smoke exposed. Significant interactions were also found for *GSTM1* null and father's smoking on pre-BD lung function and for *GSTM1* null and parental smoking on post-BD lung function. Interactions were also demonstrated for children with *GSTT1* null genotypes exposed to early life tobacco smoke on pre-BD lung function (Table 3). An isolated interaction was found for risk alleles of *GSTP1*. Only among children with *GSTP1* Ile/Ile genotypes was exposure to mother's smoking associated with an increased risk of reduced pre-BD FEV_1 compared with those with *GSTP1* Ile/Ile genotypes but not exposed. Similar analyses and findings for GLI-derived % predicted lung function parameters are shown in e-Table 9.

Early Life Tobacco Smoke Exposure, GST Genes, and Asthma/Wheeze at 12 and 18 Years of Age: There were no significant interactions found between early life smoking and GST polymorphisms on asthma or wheeze outcomes at 12 or 18 years of age (e-Tables 3-4).

We were unable to test adolescent smoking as an effect modifier because of the small numbers of adolescents who smoked (n = 38).

TABLE 3] Association Between Early Life Smoke Exposure and Lung Function at 12 and 18 Years of Age by GST Genotype

Pre- or Post-BD			Lung Function		*P* Interaction
12 y					
	Smoking		*GSTT1* Present	*GSTT1* Null	
Pre-BD	Mother	FEV_1	0.88 (0.81 to 1.68)	−1.92 (−3.50 to −0.36)	< .01
		FVC	0.66 (−0.20 to 1.53)	−2.05 (−3.78 to −0.32)	< .01
	Parental	FEV_1	−0.07 (−0.47 to 0.33)	−0.83(−1.75 to 0.09)	.05
		FVC	−0.07 (−0.50 to 0.37)	−0.93 (−1.93 to 0.06)	.05
18 y					
			GSTM1 Present	*GSTM1* Null	
Pre-BD	Father	FVC	0.69 (0.08 to 1.30)	−0.25 (−0.63 to 0.41)	.02
Post-BD	Father	FEV_1	0.47 (−0.15 to 1.10)	−0.26 (−0.62 to 0.09)	.03
		FVC	0.61 (0.01 to 1.21)	−0.22 (−0.60 to 0.16)	.02
	Parental	FEV_1	0.45 (−0.07 to 0.96)	−0.28 (−0.62 to 0.06)	.01
		FVC	0.40 (−0.10 to 0.91)	−0.24 (−0.61 to 0.12)	.04
			GSTT1 Present	*GSTT1* Null	
Pre-BD	Mother	FEV_1	0.30 (−0.35 to 0.95)	−0.76 (−2.00 to 0.48)	.03
		FVC	0.17 (−0.49 to 0.83)	−0.96 (−2.05 to 0.13)	.01
	Father	FVC	0.14 (−0.22 to 0.50)	−0.45 (−1.19 to 0.30)	.04
	Parental	FVC	0.12 (−0.22 to 0.46)	−0.71 (−1.43 to 0.01)	.01
			GSTP1 Ile/Val or Val/Val	*GSTP1* Ile/Ile	
Pre-BD	Mother	FEV_1	0.54 (−0.21 to 1.28)	−1.07 (−1.87 to −0.28)	< .01

Values are *z* score (SD, 95% CI) or as otherwise indicated. See Table 2 legend for expansion of abbreviations.

Discussion

In this study, we found evidence that individuals with *GSTM1* and *GSTT1* null genotypes were more susceptible to reduced FEV_1 and FVC at 18 years when exposed in early life to second-hand smoke. Similar associations were found for lung function at 12 years of age for early life smoke exposure and *GSTT1*. Although the trend was similar for asthma outcomes, interactions between the effects of GST genes and parental smoking were not significant.

The relevance of GST deficiency for lung function impairment and respiratory symptoms in children exposed to oxidative inhalants has been suggested by previous epidemiologic studies.[20-22] The Perth Infant Asthma Follow-up cohort investigated infant lung function at 12 months and found evidence for an interaction between smoke exposure in utero and *GSTT1* genes.[20] Kabesch et al[21] conducted a cross-sectional study of schoolchildren and found evidence suggesting increased susceptibility for asthma/wheeze in children with *GSTM1*/*GSTT1* null; however, the interactions were not significant. Our findings in a prospective birth cohort strengthen this growing body of evidence on this association up to late adolescence.

These findings are supported by strong biologic plausibility. Exposure to early life tobacco smoke, especially before 2 years of age, can damage the rapidly growing alveolar cells and stimulate inflammatory responses that may lead to development of impaired lung function and asthma in later life.[1,2,23] GST genes generate enzymes that detoxify and catalyze the conjugation of glutathione with oxidant substrates, including tobacco-derived substances. Through this mechanism, they may reduce inflammation and lung injury when exposed to oxidative stress.[24] Therefore, this may lead to impaired lung function when an individual has a GST gene deficiency (null genotype) or a reduction in enzymatic activity associated with genetic polymorphisms.

We found few differential effects on lung function for exposure to mother's and father's smoking in relation to *GSTM1* and *GSTT1*. However, effects related to mother's or father's smoking may differ because mothers tend to spend greater amounts of time with young children and mothers may also smoke during pregnancy. This

exposure in utero is thought to have different effects on the child than second-hand smoke exposure after birth. Although there was a suggestion that mothers smoking had a greater effect on lung function for children with *GSTT1* null genotypes and fathers smoking for *GSTM1* null genotypes, we were unable to draw definitive conclusions. The slight differences we observed may simply be because of lack of power associated with the small numbers of smoking mothers.

We did not find significant associations with asthma. Nicotine exposure in utero/early life may cause a global reduction in both FEV_1 and FVC rather than preferentially affecting FEV_1 as is the usual scenario for people with asthma.[25] Another possible reason could be that asthma is a dichotomous outcome, whereas lung function is a continuous outcome, leading to less power to detect an effect with asthma. A cross-sectional study investigating interactive effect between GST genes and passive smoking also found little effect on childhood asthma for *GSTM1*/*GSTT1* null groups although their sample size was $>$ 3,000.[21]

We found that among children with *GSTM1* present, those exposed to father's smoking in early life had an increased risk of improved lung function (FVC) compared with those unexposed. This result was inconsistent and unexpected. It is possible that this finding may be related to the small numbers of children exposed to both tobacco smoke and genotype. This contrary finding may also be because of compensation by other antioxidant genes or networks which are more successful at detoxifying substances other than tobacco smoke. There is evidence from two studies that a polymorphism (Ser187) of an alternative antioxidant enzyme, quinone oxidoreductase 1, provides a protective effect among *GSTM1* null subjects.[26,27] An alternative hypothesis is that tobacco smoke exposure in early life may induce upregulation of GST enzymes through DNA methylation changes. These improved oxidation defenses may then protect the lung from oxidative threats in the environment (other than tobacco smoke) that are also related to lung function. There is no conclusive support of this hypothesis in the literature.

Studies examining the *GSTP1* 105 variant reported that associations vary depending on the specific risk alleles of *GSTP1*.[28-30] Similar to our findings, some research exploring second-hand smoke has found no evidence of *GSTP1* interaction on asthma outcomes,[31-33] but studies investigating lung function were limited. We found little evidence of interaction for *GSTP1* genes with early life smoking and lung function impairment or asthma. One explanation may be because of the small numbers limiting our power to find associations. A further reason might be because the effect of *GSTP1* genes on allergy risk and lung function varies for different pollutants. For instance, enzymes encoded by Val genotypes have a sevenfold greater catalytic efficiency for polycyclic aromatic hydrocarbon diol epoxides but a threefold lower efficiency for 1-chloro-2,4-dinitrobenzene than those with Ile genotypes.[34,35] Careful measurement and definitions of pollutant exposures may be necessary before these relationships become clear.

Strengths and Limitations

An important strength of this study is the prospective birth cohort design. Early life smoking exposure was determined prospectively perinatally. Objective measurements of respiratory function were collected at 12 and 18 years of age. All these factors lessen any information bias, improving the validity of our findings.

Although our study collected early life tobacco smoke exposure data, these data were limited by the lack of information on the duration of smoking exposure, its intensity, and extent of exposure throughout life. In terms of personal smoking, very few of the adolescent participants had commenced smoking, making it unlikely for this to have materially impacted on the results. Moreover, MACS may have been underpowered to investigate the role of early life smoking exposure, particularly on asthma outcomes. We did not investigate other antioxidant genes/enzymes. There is a network of genes that interact with each other and form complex pathways that regulate oxidative stress and determine an individual's antioxidant capacity, including GST, quinone oxidoreductase 1, tumor necrosis factor, and Toll-like receptors.[36] Unfortunately, the limited size of this study precluded our ability to explore associations with other genes.

Additionally, as with all long-running cohorts, there was potential for attrition bias of our findings because data were obtained from only 66% of the children at 18 years of age. Furthermore, multiple testing may have generated spurious associations. We did not perform correction for multiple comparisons because our associations were based on pre-established hypotheses,

and the pattern of associations was consistent throughout the analyses. Finally, the high-risk nature of MACS may limit the generalizability of our results, and our findings should be validated in an independent cohort. The prevalence and degree of our observed lung function effects in our cohort would be higher than in a general population. However, it is likely that the interaction we found for high-risk children would also be present for high-risk individuals in a general population.

Conclusions

We found evidence that the impact of early life tobacco smoke exposure on reducing adolescent lung function was modified by GST gene polymorphisms, particularly *GSTT1*. These interactions may help to explain the inconsistent effects seen when either tobacco smoke exposure or GST genotypes were investigated alone for their effects on respiratory health.

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Additional information: The e-Tables can be found in the Supplemental Materials section of the online article.

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CHEST Online Supplement

Interaction of Glutathione S-Transferase M1, T1, and P1 Genes With Early Life Tobacco Smoke Exposure on Lung Function in Adolescents

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e-Table 1. Adjusted %predicted values (derived from GLI reference values) for the main effect of early life smoking on lung function at 12 years.

		Lung function
Mother smoking	FEV_1	5.27 (-1.79, 12.33)
	FVC	3.11 (-4.38, 10.59)
Father smoking	FEV_1	-1.46 (-5.52, 2.60)
	FVC	-1.22 (-5.52, 3.08)
Parental smoking	FEV_1	-0.88 (-4.68, 2.92)
	FVC	-1.33 (-5.35, 2.69)

*Mother smoking and father smoking was mutually adjusted for each other and parental education, parental smoking adjusted for parental education only.

e-Table 2. Adjusted %predicted values (derived from GLI reference values) for the main effect of early life smoking on lung function at 18 years.

			Lung function
Pre-BD	Mother smoking	FEV_1	-0.57 (-6.86, 5.71)
		FVC	-1.54 (-7.79, 4.70)
	Father smoking	FEV_1	-1.25 (-5.06, 2.56)
		FVC	0.48 (-3.31, 4.27)
	Parental smoking	FEV_1	-1.58 (-5.11, 1.96)
		FVC	-0.53 (-4.05, 2.98)
Post-BD	Mother smoking	FEV_1	0.97 (-4.95, 6.89)
		FVC	1.01 (-5.16, 7.18)
	Father smoking	FEV_1	-0.32 (-3.87, 3.23)
		FVC	0.49 (-3.21, 4.19)
	Parental smoking	FEV_1	-0.37 (-3.67, 2.93)
		FVC	0.09 (-3.35, 3.52)

*Mother smoking and father smoking was mutually adjusted for each other and parental education, parental smoking adjusted for parental education only.

e-Table 3. Adjusted Odds Ratios (ORs) for associations between early life smoking and asthma/wheeze at 12 years

Exposure	Outcome	OR (95% CI)	Smoking * GST Interaction (p value)		
			GSTM1	GSTT1	GSTP1
Mother smoking	Current asthma	1.23 (0.28, 5.37)	0.72	-	0.55
	Current wheeze	1.48 (0.42, 5.26)	0.74	0.81	-
Father smoking	Current asthma	1.53 (0.68, 3.74)	0.63	-	0.96
	Current wheeze	1.39 (0.65, 3.00)	0.83	0.12	0.85
Parental smoking	Current asthma	1.19 (0.53, 2.68)	0.64	-	0.44
	Current wheeze	1.55 (0.75, 3.22)	0.72	0.10	0.40

*All mother smoking results adjusted for age, height, gender, parent asthma and father smoking; all father smoking results adjusted for age, height, gender, parent asthma and mother smoking; parental smoking results adjusted for age, height, gender and parent asthma.

e-Table 4. Adjusted ORs for associations between early life smoking and asthma/wheeze at 18 years and interaction p values for GST genes

Exposure	Outcome	OR(95%CI)	Smoking * GST Interaction (p value)		
			GSTM1	GSTT1	GSTP1
Mother smoking	Current asthma	1.03 (0.32, 3.29)	0.27	0.53	0.30
	Current wheeze	1.38 (0.48, 3.96)	0.26	0.95	0.24
Father smoking	Current asthma	1.39 (0.67, 2.87)	0.63	0.35	0.36
	Current wheeze	1.33 (0.68, 2.62)	0.47	0.47	0.47
Parental smoking	Current asthma	1.27 (0.65, 2.50)	0.62	0.85	0.39
	Current wheeze	1.26 (0.68, 2.35)	0.15	0.82	0.31

All mother smoking results adjusted for age, height, gender, parent asthma and father smoking; all father smoking results adjusted for age, height, gender, parent asthma and mother smoking; parental smoking results adjusted for age, height, gender and parent asthma.

e-Table 5. Associations between GST genes and lung function (z score) at 12 years

	FEV_1	**FVC**
GSTM1 null	0.15 (-0.11, 0.40)	0.27 (-0.01, 0.54)
GSTT1 null	-0.11 (-0.45, 0.22)	0.07 (-0.29, 0.43)
GSTP1 ile/ile	0.04 (-0.22, 0.29)	-0.03 (-0.31, 0.24)

e-Table 6. Associations between GST genes and lung function (z score) at 18 years

	Pre-bronchodilator		**Post-bronchodilator**	
	FEV_1	FVC	FEV_1	FVC
GSTM1 null	0.06 (-0.15, 0.27)	0.15 (-0.05, 0.35)	0.13 (-0.07, 0.33)	0.15 (-0.05, 0.35)
GSTT1 null	-0.09 (-0.36, 0.19)	0.01 (-0.26, 0.27)	-0.05 (-0.31, 0.21)	0.06 (-0.21, 0.32)
GSTP1 ile/ile	-0.08 (-0.29, 0.13)	-0.20 (-0.40, 0)	-0.13 (-0.32, 0.07)	-0.23 (-0.42, -0.03)

e-Table 7. Associations between GST genes and asthma/wheeze at 12 years

	Current asthma	**Current wheeze**
GSTM1 null	1.04 (0.60, 1.81)	1.33 (0.80, 2.21)
GSTT1 null	0.86 (0.40, 1.82)	1.36 (0.71, 2.60)
GSTP1 ile/ile	1.44 (0.61, 3.43)	1.48 (0.70, 3.14)

e-Table 8. Associations between GST genes and asthma/wheeze at 18 years

	Current asthma	**Ever asthma**	**Current wheeze**
GSTM1 null	1.26 (0.80, 1.99)	1.48 (0.98, 2.22)	1.50 (0.99, 2.27)
GSTT1 null	0.78 (0.42, 1.42)	1.08 (0.64, 1.81)	0.77 (0.45, 1.32)
GSTP1 ile/ile	1.00 (0.53, 1.89)	1.33 (0.75, 2.39)	1.20 (0.67, 2.17)

e-Table 9. Associations between early life smoking and lung function (%predicted from GLI reference values) by GST genotype at 12 & 18 years.

Pre or post BD			Lung function (%predicted, 95%CI)				Interaction p
			Unadjusted	Adjusted	Unadjusted	Adjusted	
			12 years				
	Smoking		**GSTT1 present**		**GSTT1 null**		
Pre	Mother	FEV_1	8.94 (0.17, 17.70)	10.18 (0.88, 19.48)	-25.80 (--41.94, -9.66)	-23.45 (-41.97, -4.94)	<0.01
		FVC	6.09 (-3.38, 15.57)	7.72 (-2.33, 17.76)	-25.85 (-43.27, -8.43)	-23.45, -43.59, -3.31)	<0.01
	Parental	FEV_1	-0.77 (-5.33, 3.80)	-0.83 (-5.53, 3.86)	-11.35 (-21.93, -0.76)	-9.90 (-20.79, 0.99)	0.05
		FVC	-1.08 (-5.99, 3.83)	-0.81, -5.86, 4.24)	-12.16 (-23.43, -0.88)	-10.68 (-22.29, 0.93)	0.05
			18 years				
			GSTM1 present		**GSTM1 null**		
Pre	Father	FVC	4.23 (-2.18, 10.64)	8.25 (1.03, 15.46)	-3.38 (-7.81, 1.05)	-2.96 (-7.48, 1.57)	0.02
Post	Father	FEV_1	4.85 (-1.71, 11.10)	5.58 (-1.70, 12.86)	-3.84 (-7.89, 0.20)	-3.06 (-7.17, 1.04)	0.03
		FVC	5.95 (-0.44, 12.34)	7.21 (0.06, 14.37)	-2.88 (-7.23, 1.47)	-2.62 (-7.07, 1.83)	0.02
	Parental	FEV_1	4.32 (-1.70, 10.34)	5.25 (-0.81, 11.30)	-4.29 (-8.10, -0.48)	-3.32 (-7.28, 0.64)	0.01
		FVC	4.38 (-1.51, 10.27)	4.79 (-1.18, 10.77)	-3.11 (-7.22, 0.99)	-2.85 (-7.14, 1.45)	0.04
			GSTT1 present		**GSTT1 null**		
Pre	Mother	FEV_1	1.24 (-6.21, 8.70)	3.53 (-4.11, 11.16)	-12.51 (-25.34, 0.31)	-9.41 (-24.14, 5.31)	0.03
		FVC	1.91 (-5.58, 9.40)	2.06 (-5.70, 9.83)	-14.17 (-25.40, -2.94)	-11.13 (-24.00, 1.73)	0.01
	Father	FVC	1.40 (-2.68, 5.48)	1.62 (-2.62, 5.86)	-7.70 (-16.30, 0.89)	-5.15 (-13.90, 3.61)	0.04
	Parental	FVC	0.95 (-2.91, 4.81)	1.40 (-2.57, 5.37)	-9.49 (-17.25, -1.73)	-8.26 (-16.71, 0.19)	0.01
			GSTP1 ile/val or val/val		**GSTP1 ile/ile**		
Pre	Mother	FEV_1	2.87 (-5.54, 11.27)	6.32 (-2.45, 15.09)	-15.19 (-24.17, -6.21	-13.23 (-22.65, -3.81)	<0.01

Mother smoking and father smoking was mutually adjusted for each other and parental education, parental smoking adjusted for parental education only. GSTP ile/ile is the risk genotype

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Chapter 6 EARLY LIFE PARACETAMOL EXPOSURE, GLUTATHIONE S-TRANSFERASE GENES AND DEVELOPMENT OF ADOLESCENT ASTHMA

6.1 Chapter introduction

This paper (with data supplement) reports my investigation of the potential interaction of GST genes on the associations between paracetamol exposure before 2 years of age and asthma and lung function in adolescents. This paper provides evidence that paracetamol use during infancy may be linked to risk of asthma and lower lung function for children with particular genetic profiles. This manuscript is currently submitted for publication to the *Journal of Allergy and Clinical Immunology*.

6.2 Research questions and objectives

6.2.1 Research questions

Does paracetamol use during early life influence lung health in adolescence, and is this relationship modified by GST gene variants?

6.2.2 Research objectives

1. To investigate the relationship between paracetamol use during early life and asthma and lung function in adolescents;
2. To determine whether these relationships were modified by variants of GST genes.

6.3 Manuscript

Dai, X., Lodge, C.J. Associations between acetaminophen exposure up to 2 years and lung function at 18 years are modified by Glutathione s-Transferase genes in a prospective birth cohort (Submitted for publication to *Journal of Allergy and Clinical Immunology on 14/08/2019*).

*In this manuscript, paracetamol was written as “acetaminophen”, per the journal’s preference in US.

Word counts:2920

Early life acetaminophen exposure, Glutathione s-Transferase genes and development of adolescent asthma

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Key messages: This work contributes to emerging evidence that early life acetaminophen may be a potential contributor to asthma causation and reduced lung function in young adults, particularly in genetically susceptible individuals.

Declaration of all sources of funding: The first 6 years of the MACS was funded (study formula and staff) by Nestec Ltd, a subsidiary of Nestle Australia. The 12-year follow-up was funded by a project grant from the Asthma Foundation of Victoria. The National Health and Medical Research Council of Australia (NHMRC) funded the 18 year (APP454856). Xin Dai, and Dr. Caroline Lodge are funded by NHMRC.

Keywords: Asthma, Oxidative stress, Drug induced lung disease, Asthma genetics, Asthma epidemiology, Respiratory measurement.

Abbreviations:

Avon Longitudinal Study of Parents and Children (ALSPAC)

Bronchodilator (BD)

Forced Expiratory Volume in 1 second (FEV_1)

Forced Vital Capacity (FVC)

Global Lung Function Initiative (GLI)

Glutathione S-Transferase (GST)

Lower respiratory tract infections/symptoms (LRTI)

Melbourne Atopy Cohort Study (MACS)

Mid-Expiratory Flow (MEF)

N-acetyl-p-benzo-quinone imine (NAPQI)

Otitis media (OM)

Upper respiratory tract infections/symptoms (URTI)

Abstract

Background

While the impact of early life acetaminophen on asthma risk is still not clear, potential interactions with antioxidative Glutathione S-Transferase (GST) genes, and possible impact on lung function, have never been investigated.

Objective

We aimed to investigate associations between early life acetaminophen use, adolescent asthma and lung function and to assess potential interactions by GST polymorphisms.

Methods

Acetaminophen use was recorded 18 times up to 2 years in 620 children enrolled in the Melbourne Atopy Cohort Study. Participants were genotyped for GST polymorphisms (GSTM1, GSTT1, GSTP1). Asthma and lung function were measured at 12 and 18 years. Regression models assessed associations and interactions.

Results

Doubling of days of acetaminophen use was associated with reduced pre-bronchodilator (BD) Forced Expiratory Volume in 1 second/Forced Vital Capacity (FEV1/FVC) (β-coefficient=-0.10 [95%CI -0·19, -0·02]) and Mid-Expiratory Flow (MEF) (-0.09 [-0·18, -0·01]) at 18 years, but this association was not found when restricted for non-respiratory reasons, suggesting confounding by indication. However, in children with GSTM1 null and GSTT1 present, increasing acetaminophen use for non-respiratory reasons was associated with reduced FEV1 and MEF at 18 years (p interaction <0·05). Increased acetaminophen use was associated with asthma at 18 years for children with GSTP1 Ile/Ile (OR=1·66, 1·07, 2·57), but not other GSTP1 genotypes.

Conclusions

These novel findings need to be investigated for consistency in other studies but suggest that children carrying risk genotypes may be susceptible to respiratory consequences from acetaminophen use.

Clinical Trial Registration

The Australian and New Zealand Clinical Trials Registry (ACTRN12609000734268).

Word count: 247

Introduction

Acetaminophen is a commonly used analgesic and antipyretic in young children. Evidence suggests that exposure to acetaminophen in early life may increase the incidence of asthma [1, 2], but finding are not consistent [3, 4]. This evidence is based on observational studies which have reported that exposure to acetaminophen during pregnancy [1, 3, 5], the postnatal period up to 6 months or during first year of life [1, 2, 6, 7], or in childhood [6 7] is associated with an increased risk the subsequent asthma. The hypothesized mechanism for this link is through suppression of glutathione levels. The acetaminophen metabolite N-acetyl-p-benzo-quinone imine (NAPQI) is known to bind with glutathione, depleting the amount available to detoxify reactive oxygen species (ROS) [1]. However, the true nature of this association is not clear due to the potential for confounding by indication, as acetaminophen is commonly used to treat respiratory tract infections, a known risk factor for childhood asthma.

It is plausible that the effects of early life acetaminophen exposure on respiratory outcomes might be modified by the Glutathione S-transferase (GST) superfamily of enzymes. The GST family is believed to be important for the prevention of lung damage through detoxification of ROS generated from inflammation, infection and/or exposure to airborne toxins[8]. Three major GST enzymes are encoded by the GSTM1, GSTT1 and GSTP1 genes. Deletion of GSTM1 and GSTT1 (recessive model), and GSTP1 with homozygous Ile genotypes (dominant model) are all associated with reduced GST enzyme activity, which may put individuals carrying these genotypes at increased risk of respiratory morbidity when exposed to oxidative stress [9, 10]. The possible interactions between GST polymorphisms and acetaminophen use during the first 2 years of life for asthma and lung function outcomes have not been investigated.

The objective of this study was to assess the association between acetaminophen use up to the age of 2 years and GST genes with asthma and lung function at 12 and 18 years in the Melbourne Atopy Cohort Study (MACS). In an earlier analysis using this same cohort, we found no association between acetaminophen use between birth and 2 years of age and the risk of asthma at 7 years after adjustment for respiratory infection, or when acetaminophen use was restricted to non-respiratory indications [11]. The present analysis extends our work by investigating potential modification by GST polymorphisms and by following participants up to 18 years with outcomes of asthma and lung function.

Methods

Study population

A total of 620 pregnant women were recruited into the MACS between 1990 and 1994 in Melbourne, Australia[12]. Infants had at least one first degree family member with self-reported asthma, eczema, hay fever or severe food allergy. MACS initially began as a randomized controlled trial of infant formulae[13], but has since been followed as an observational cohort. Ethics approvals are detailed in eMethods.

Acetaminophen use

An allergy-trained research nurse conducted telephone interviews every 4 weeks in the first 15 months, at 18 months, and at 2 years of age. Each survey documented illness occurrence and medication use since the last interview. The number of episodes, days of use, and reason for the administration of acetaminophen were recorded at each telephone interview. The total days of acetaminophen use before 2 years of age for each child (regardless of dose or frequency per day) was recorded as the main exposure. In line with our previous analyses [11], we classified indications for acetaminophen use into four categories including for: lower respiratory tract infections/symptoms (LRTI), upper respiratory tract infections/symptoms (URTI), otitis media (OM) and other reasons (non-respiratory tract infection reasons) (eTable 1).

Asthma & Lung function

Definitions of current asthma and wheeze at 12 and 18 years are provided in the eMethods. Lung function testing was performed at both 12 and 18 years, following standard techniques described by the American Thoracic Society [14, 15]. Only pre-bronchodilator (BD) spirometry was measured at 12-years, while both pre- and post-BD spirometry were measured at 18 years.

Genotyping methods and definitions of other variables are detailed in the eMethods.

Statistical analyses

Complete case analysis was performed in this study. Multiple linear and logistic regression were used to assess the relationship between total days of acetaminophen use in the first two years of life, GST genotypes, adolescent lung function and asthma. The distribution of acetaminophen was found to be left skewed (median: 17, mean: 19, (IQR): 10-27]). Therefore, we applied a log (base 2) transformation with an offset of 1 added for days of acetaminophen exposure. This enabled interpretation of the estimates from the regression models as the effect per doubling of days of acetaminophen use. After transformation, the median $\log_2$ acetaminophen days of exposure was 4 (IQR: 3-5).

For asthma outcomes, the models were adjusted for age, sex, maternal asthma, paternal asthma and parental education, plus frequency of all forms of respiratory infection including LRTI, URTI and OM. The raw values of lung function were transformed to the resultant β coefficients described differences in the lung function z-scores, accounting for age, gender and height using Global Lung Function Initiative (GLI) equations[16, 17], and were adjusted for parental education, maternal asthma, paternal asthma and respiratory infections.

The following variables were tested as potential confounders: birth weight at 4 weeks, gestational age, breast feeding, childcare in first 12 months, the presence of older siblings, other infections in the first 2 years, randomized group allocation from the original RCT, and parental smoking. These variables were included in the final model if they changed the associations by more than 10%.

To determine whether the indication for use of acetaminophen affected the associations, we examined the association between total days of acetaminophen use for non-respiratory indications. Potential GST interactions were assessed using likelihood ratio tests. Analyses were carried out initially on the complete dataset and then ORs and coefficients were calculated for each GST stratum. Interactions were considered significant if interaction p values were <0.1. All analyses were conducted using Stata® ver 14 (2013 StataCorp, College Station, TX).

Results

Of the 620 infants enrolled, 575 (92·7%) were followed to age 2 years. There were 365 at 12 and 413 at 18 years with spirometry data available (Figure 1). Approximately half of the participants were male (table 1). Almost all infants (97%) received at least 1 day of acetaminophen between birth and 2 years of age, with most being exposed for non-respiratory reasons (93·4%) or for URTIs (88·7%) (eTable 2). Total days of acetaminophen use was not associated with loss to follow-up at 18 years (p=0·5). The proportion of participants with current asthma was similar at 12 and 18 years (23.7% (n=85) and 23.5% (n=97) respectively). Between 12 and 18 years remission of asthma (8.1%) was higher than incidence of asthma (6.4%).

Associations between days of acetaminophen use and asthma and lung function at 12 and 18 years of age

Any indication for acetaminophen use

There was no evidence of an association between total days of acetaminophen use and current asthma (OR 1·05, 95%CI 0·83, 1·31) but a marginal association was found for wheeze at 18 years (OR 1·20, 95%CI 0·97, 1·48) (Table 2). Total days of

acetaminophen use were not associated with either asthma or wheeze at 12 years (etable 3)

However, total days of acetaminophen use were associated with reduced pre-BD Forced Expiratory Volume in 1 second/Force Vital Capacity (FEV1/FVC) at 18 years (β-coefficient= -0·10; 95%CI -0·19, -0·01) (Table 2). Trends were also seen for modestly reduced pre-BD FEV1, and Mid-Expiratory Flow (MEF) at 18 years. Similarly, we found associations with decreased FEV1/FVC at 12 years and marginally elevated FVC (eTable 3).

Acetaminophen use for non-respiratory indications

We found little evidence of an association between acetaminophen use for non-respiratory reasons and the risk of asthma outcomes or impaired lung function. However, all lung function parameter point estimates were negative with increasing acetaminophen use and there was a modest association with reduced pre-BD FEV1/FVC (p=0·07) (Table 2).

Interaction analysis between acetaminophen use and GST genes and asthma and lung function at 12 and 18 years of age

Any indication for acetaminophen use

Subjects with GSTP1 Ile/Ile genotypes were more likely to report asthma at 18 years if they had more days of acetaminophen exposure between birth and 2 years of age (OR 1·66; 95%CI 1·07, 2·57; interaction p=0·02) (Table 3). There was similar but weaker interaction between acetaminophen use and GSTP1 on current wheeze at 18 years (interaction p=0·07). Evidence of interactions between acetaminophen use and GSTP1 on asthma outcomes can be seen in Figure 2. There was no evidence that GSTM1/T1 polymorphisms modified the association between increased acetaminophen use and asthma outcomes (eTables 4 and 5).

There was little evidence that GSTM1/T1/P1 polymorphisms modified the associations between acetaminophen use and lung function at 12 and 18 years (eTable4,5 and 6)

Acetaminophen use for non-respiratory indications

There was evidence of interactions between acetaminophen use for non-respiratory indications and GSTM1 genotypes for FEV1 and MEF at 18 years (Table 4). Increasing exposure to acetaminophen for non-respiratory indications in children with GSTM1 null genotypes was associated with reduced pre- and post-BD lung function, while this was not found for children with GSTM1 present (Figure 3) Similar trends were also found for lung function at 12 years (eTable 7).

There was also some evidence for a paradoxical finding on pre-BD lung function (FEV1/FVC, MEF), where increasing acetaminophen use for non-respiratory reasons was associated with reduced lung function in those with GSTT1 present, but not in those with GSTT1 null (Table 5). Similar trends were also found for lung function at 12 years but not for asthma outcomes (eTable 8). We found no evidence of interaction for GSTP1 genotypes and acetaminophen use for non-respiratory indications at 12 or 18 years (eTable 9).

We also performed a sensitivity analysis, including a wider range of confounders into our analysis. As the measured associations did not appreciably change when these variables were included, and their inclusion resulted in restriction of the available sample numbers for analyses, we did not include these variables in the final models as confounders. The sensitivity analysis is presented in eTables 10-19.

Discussion

This prospective cohort study is the first to assess gene x environment interactions between early post-natal life acetaminophen exposures and GST polymorphisms on associations with asthma and lung function up to early adult life. We found that increasing total days of acetaminophen use for any indication between birth and 2 years of age was associated with reduced FEV1/FVC ratio at 18 years. When limited to non-respiratory indications these associations were attenuated. However, we found evidence that GST genotype may modify the association between acetaminophen use and the risk of asthma and lower lung function. Total doubling days of acetaminophen use was associated with increased risk of asthma for people with Ile/Ile genotypes and individuals with *GSTM1* null and *GSTT1 present* genotypes were at risk of lower lung function by the age of 18 years when given acetaminophen for non-respiratory indications, compared to those with *GSTM1* present/*GSTT1* null genotypes.

In a previous analysis of this cohort we found little evidence of a main association between GSTM1/T1 null genotypes for either lung function or asthma outcomes, except that we found some evidence that carriers of GSTP1 with Ile/Ile genotypes were at an increased risk of reduced post-BD FVC at 18 years compared to those with Ile/Val or Val/Val [18]. However, in the current analysis we found some evidence of an interaction between acetaminophen use from birth to 2 years of age and GST genotype on the risks of asthma and reduced lung function up to 18 years of age. These findings are biologically plausible. Glutathione, an important antioxidant, can offer some defence against ROS resulting from environmental stimuli [9]. Acetaminophen reduces the availability of glutathione and therefore reduces one mechanism for managing oxidative stress [19]. The lungs, including airways, alveoli and blood vessels, grow rapidly before 2 years of age. Impairment

of antioxidant defences may render individuals susceptible to environmental exposures during this critical window for lung development. Acetaminophen exposure may cause structural lung damage when there is a deficit in the lung's ability to detoxify the oxidant substrates [20, 21]. This may particularly be the case that when an exposed individual has GSTM1/T null and/or GSTP1 with Ile/Ile genotypes, as reduced enzyme activity in genetically affected individuals may lead to increased toxicity of acetaminophen metabolites and inability to compete for glutathione.

There are limited studies investigating the interaction between infant acetaminophen use and GST genes on respiratory outcomes. One cross-sectional study investigated interactions between acetaminophen use and asthma outcomes in preschool children (aged 3 to 7 years) and suggested similar findings; the adverse effect of acetaminophen use during the 1st year of life on current asthma was only seen among those with GSTP1 Ile/Ile and Ile/Val genotypes, not for those with Val/Val genotypes [22]. In contrast, The Avon Longitudinal Study of Parents and Children (ALSPAC) in the UK did not identify an interaction between infant acetaminophen exposure and GST genes on childhood asthma at 7 years. This study collected exposure on acetaminophen intake for 6 months only, so the total days would be fewer compared to our data, and asthma outcomes were assessed at 7 years, so this study was potentially investigating different asthma phenotypes than in our current analysis [4]. With our more comprehensive methodology, we found evidence that GSTM1, T1 and P1 modified the association between acetaminophen use and lung function and asthma at 12 and 18 years. Our study is the first to assess these interactions in young adults.

Our findings for GSTT1 were unexpected. We hypothesized that acetaminophen use may be associated with reduced lung function in GSTT1 null individuals, but instead found that increasing acetaminophen use for non-respiratory indications in those with GSTT1 present was associated with reduced lung function (FEV1/FVC and MEF) but found no association in those with GSTT1 null. Interestingly, Shaheen et al. reported similar paradoxical associations in the ALSPAC [4], where late gestation exposure to acetaminophen was associated with higher risk of asthma at 7 years in children whose mothers carried a GSTT1 present genotype compared to mothers with a null genotype. The reason for these apparently paradoxical associations remains unclear and these results need to be replicated and the mechanisms explored.

To account for the potential influence of early life respiratory infections, our study and other observational studies [11, 23], investigated the effect of acetaminophen use for non-respiratory reasons. Like these studies, before taking GST polymorphisms

into account we did not find an overall association between acetaminophen use for non-respiratory reasons and increased risk of adverse respiratory outcomes. We also considered that parents who frequently gave acetaminophen to their children for respiratory infection might use more acetaminophen for non-respiratory reasons, therefore our associations were also adjusted for frequency of respiratory infections. However, we observed that acetaminophen use for non-respiratory indications was associated with increased risk of impaired lung function in young adults with susceptible genotypes.

Our study is a prospective birth cohort, with frequent assessment of acetaminophen use and recorded indications for its use before 2 years of age along with objective measurements of respiratory function at 12 and 18 years. All these factors limited the potential for recall bias for acetaminophen exposure in the early years and reduced the potential for measurement error.

However, we did not collect data on acetaminophen use by mothers whilst their children were in utero. Prenatal acetaminophen use is believed to be associated with asthma in children [24], and acetaminophen use during pregnancy and early post-natal life may be highly correlated. As such, it is difficult to determine whether post-natal acetaminophen use is only related to lung outcomes through its potential association with pregnancy use by mothers.

As with all long-running cohorts, there is the possibility of bias due to attrition; data were obtained from 66% of the children at the 18-year follow-up. We have previously shown that there were no apparent differences between children lost to follow-up and those who continued to participate for many parameters including gender, family history of allergic diseases, and a range of residential characteristics [12]. Losses to follow-up resulted in smaller numbers of participants at 12 and 18 years and limited our power to detect robust stratum-specific associations. As we did not perform correction for multiple comparisons, multiple testing may have generated spurious associations, and some interactions may arise through chance. However, it should be noted that the associations were based on pre-established hypotheses, and we did not focus on particular estimated effects in isolation. All point estimates for associations between children with risk alleles for GSTM1 and GSTP1 and lung function and asthma were in the same direction when exposed to more acetaminophen between birth and 2 years of age. Additionally, as high-risk children with a family history of allergic disease were recruited into the MACS, these findings should be extrapolated to the general population with care. Finally, we did not investigate some alleles with low frequencies, for instance GSTP1 Val114Ala, but it is an avenue for further research.

Conclusions

In summary, we found some evidence that acetaminophen use in the first two years of life was associated with reduced lung function in adolescents. There was evidence that this association was confounded by respiratory tract infection, with the models limited to non-respiratory acetaminophen use finding no robust evidence of reduced lung function. However, we found evidence that GST genotypes, determinants of antioxidant function, may confer increased risk of reduced lung function from acetaminophen exposure in early life. We observed that increasing acetaminophen use was associated with increased risk of adverse respiratory health in adolescents with GSTM1 null and GSTP1 Ile/Ile polymorphisms for acetaminophen used for non-respiratory indications and total acetaminophen use respectively. These findings need to be investigated and corroborated by other studies before translation into clinical practice.

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Table 1 Demographic and clinical characteristics of study population (n=620)

	Initial interview	**2 years' follow-up**	**12 years' follow up**	**18 years' follow up**
	N (%)	Mean (SD) or N (%)	Mean (SD) or N (%)	Mean (SD) or N (%)
Total participants	620	575	365	429
Sex				
Boy	317 (51.1%)	280 (48.7%)	189 (51.8%)	212 (49.4%)
Girl	303 (48.9%)	295 (51.3%)	176 (48.2%)	217 (50.6%)
Parental completion of a tertiary degree				
Yes	443 (71.4%)	417 (72.5%)	268 (73.2%)	324 (75.5%)
No	177 (28.6%)	158 (27.5%)	98 (26.8%)	105 (24.5%)
Parental asthma				
Yes	379 (61.2%)	348 (60.7%)	227 (62.5%)	259 (60.7%)
No	239 (38.8%)	225 (39.3%)	136 (37.5%)	168 (39.3%)
Age (years)			11.50 (1.87)	17.98 (1.33)
Height (cm)			149.5 (13.0)	172.5 (9.3)
Current asthma			85 (23.7%)	97 (23.5%)
GSTM1			299	428
Present			128 (44%)	178 (41.6%)
Null			171 (56%)	250 (58.4%)
GSTT1			299	428
Present			248 (83.6%)	353 (82.5%)
Null			51 (16.4%)	75 (17.5%)
GSTP1			299	429
Ile/Ile			132 (44.1%)	184 (42.9%)
Val/Ile and Val/val			167 (55.9%)	245 (57.1%)

Variable "parental asthma" has 2 missing data of total participants at each follow up, respectively; Variable "current asthma" has 18 missing data of total participants at 12 years, 28 missing of 18 years' follow up; GSTM1/T1/P1 have 66 missing data of total participants at 12 years' follow-up, GSTM1/T1 have 1 missing data of 18 year's follow up.

There was no significant difference between baseline data and data for each follow-up, and data who were missing.

Table 2 Association between days of acetaminophen use before 2 years and respiratory outcomes at 18 years.

		Total days of acetaminophen use		days of acetaminophen use for non-respiratory infection use	
Asthma		ORs		ORs	
	Current asthma	1.05 (0.83, 1.31)	0.62	0.96 (0.78, 1.17)	0.70
	Current wheeze	1.20 (0.97, 1.48)	0.10	1.08 (0.88, 1.31)	0.46
Lung function		Coefficients†	P	Coefficients	P
Pre-BD	FEV_1	-0.07 (-0.16, 0.01)	0.11	-0.06 (-0.14, 0.03)	0.19
	FVC	-0.01 (-0.09, 0.08)	0.82	-0.01 (-0.10, 0.07)	0.80
	FEV_1/FVC	-0.10 (-0.19, -0.01)	0.02	-0.08 (-0.17, 0.01)	0.07
	MEF	-0.09 (-0.18, 0)	0.06	-0.07 (-0.16, 0.02)	0.11
Post-BD	FEV_1	-0.05 (-0.13, 0.03)	0.22	-0.06 (-0.14, 0.02)	0.16
	FVC	-0.02 (-0.10, 0.06)	0.66	-0.03 (-0.12, 0.05)	0.42
	FEV_1/FVC	-0.06 (-0.14, 0.03)	0.19	-0.05 (-0.13, 0.03)	0.24
	MEF	-0.04 (-0.13, 0.04)	0.34	-0.06 (-0.14, 0.03)	0.18

*Lung function outcomes were adjusted for parent education maternal asthma and paternal asthma plus frequency of respiratory infections, including URTI, LRTI and OM. with asthma/wheeze outcomes additionally also adjusted for age and sex of the participant.

†The results for lung function are the β-coefficients from linear regression, representing by z-score.

Table 3 Associations between total days of acetaminophen use and asthma/wheeze at 12 and 18 years stratified by GSTP1 genotypes.

	GSTP1 Ile/val or val/val		**GSTP1 ile/ile**		**Interaction P**
	OR	P	OR	P	
Asthma at 12 years	1.07 (0.71, 1.60)	0.75	1.47 (0.88, 2.47)	0.14	0.20
Wheeze at 12 years	1.05 (0.76, 1.46)	0.77	1.43 (0.95, 2.20)	0.10	0.13
Asthma at 18 years	0.84 (0.64, 1.09)	0.19	**1.66 (1.07, 2.57)**	**0.02**	**0.02**
Wheeze at 18 years	1.01 (0.78, 1.31)	0.93	**1.74 (1.14, 2.64)**	**0.01**	0.07

*ORs were adjusted for age and sex of the participant, parent education, maternal asthma, paternal asthma and frequency of respiratory infection (URTI, LRTI and OM).

Table 4 Associations between days of acetaminophen use for non-respiratory reasons and lung function (z-score) at 18 years stratified by GSTM1 genotypes.

		GSTM1 present		**GSTM1 null**		**Interaction P**
		coefficients†	p	coefficients	p	
Pre-BD	FEV_1	0.06 (-0.09, 0.21)	0.43	**-0.14 (-0.25, -0.03)**	**0.02**	**0.01**
	FVC	0.09 (-0.05, 0.23)	0.22	-0.07 (-0.18, 0.04)	0.22	0.05
	FEV_1/FVC	0.05 (-0.21, 0.10)	0.49	**-0.11 (-0.22, 0)**	**0.05**	0.42
	MEF	0.02 (-0.14, 0.18)	0.80	**-0.14 (-0.25, -0.03)**	**0.01**	**0.04**
Post-BD	FEV_1	0.05 (-0.09, 0.19)	0.45	**-0.12 (-0.22, -0.02)**	**0.03**	**0.01**
	FVC	0.06 (-0.08, 0.19)	0.41	-0.08 (-0.19, 0.03)	0.17	0.06
	FEV_1/FVC	0.02 (-0.15, 0.12)	0.82	-0.07 (-0.18, 0.03)	0.19	0.38
	MEF	0.04 (-0.11, 0.19)	0.57	**-0.12 (-0.22, -0.02)**	**0.02**	**0.02**

*Coefficients were adjusted for parent education, maternal asthma, paternal asthma and frequency of respiratory infections (URTI, LRTI, and OM).

†The results for lung function are the β-coefficients from linear regression, representing by z-score.

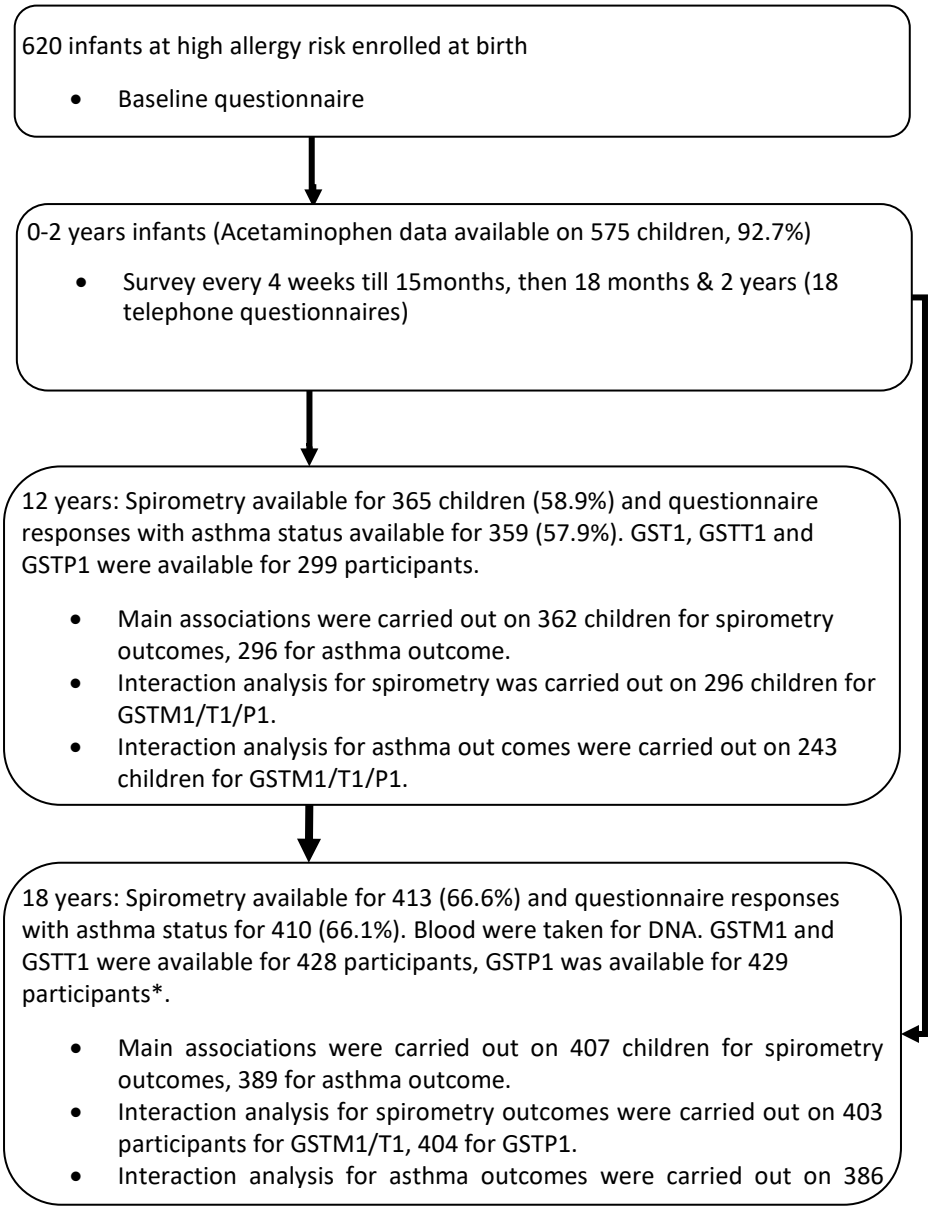
Table 5 Associations between days of acetaminophen use for non-respiratory reasons and lung function (z-score) at 18 years stratified by GSTT1 genotypes.

		GSTT1 present		GSTT1 null		Interaction p
		Coefficients†	**p**	**Coefficients**	**p**	
Pre-BD	FEV_1	-0.08 (-0.17, 0.01)	0.08	0.09 (-0.22, 0.41)	0.55	0.46
	FVC	-0.02 (-0.11, 0.07)	0.73	0.05 (-0.24, 0.33)	0.75	0.87
	FEV_1/FVC	**-0.11 (-0.21, -0.02)**	**0.02**	0.11 (-0.19, 0.42)	0.47	0.13
	MEF	**-0.11 (-0.21, -0.02)**	**0.02**	0.23 (-0.09, 0.55)	0.16	**0.04**
Post-BD	FEV_1	-0.04 (-0.13, 0.05)	0.36	-0.17 (-0.42, 0.09)	0.19	0.31
	FVC	-0.01 (-0.10, 0.08)	0.80	-0.19 (-0.45, 0.07)	0.15	0.15
	FEV_1/FVC	-0.06 (-0.15, 0.03)	0.19	0.04 (-0.22, 0.30)	0.78	0.40
	MEF	-0.07 (-0.16, 0.02)	0.14	0.06 (-0.20, 0.32)	0.66	0.45

*Coefficients were adjusted for parent education, maternal asthma, paternal asthma and frequency of respiratory infections (URTI, LRTI, and OM).

†The results for lung function are the β-coefficients from linear regression, representing by z-score.

Figure 1. Flow chart of participants in MACS



*There were 21 participants with missing information on asthma.

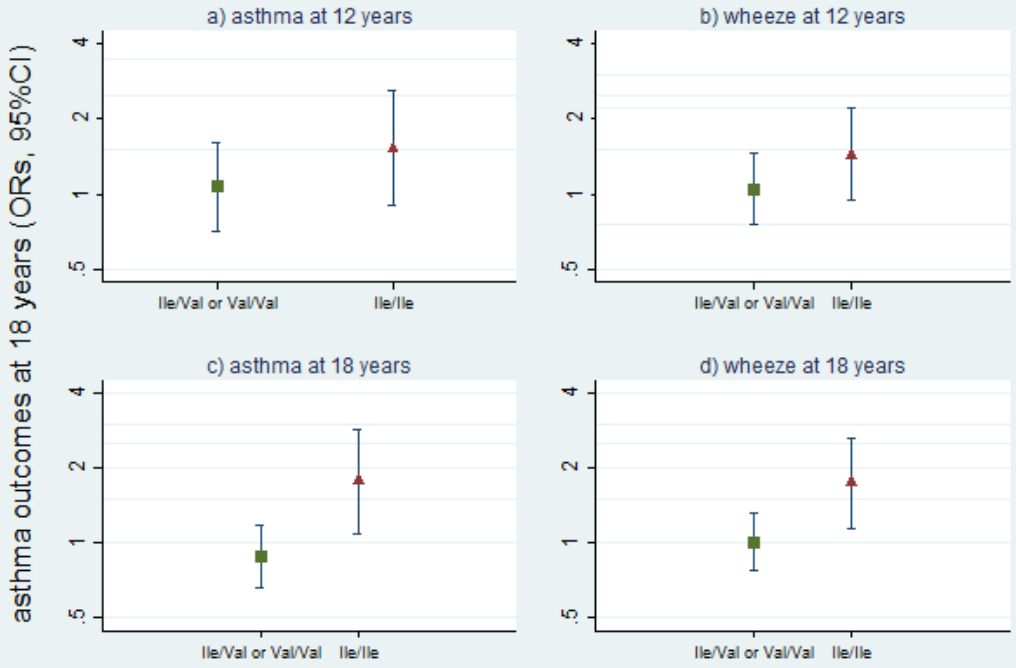


Figure 2. Interaction between early life acetaminophen use and GSTP1

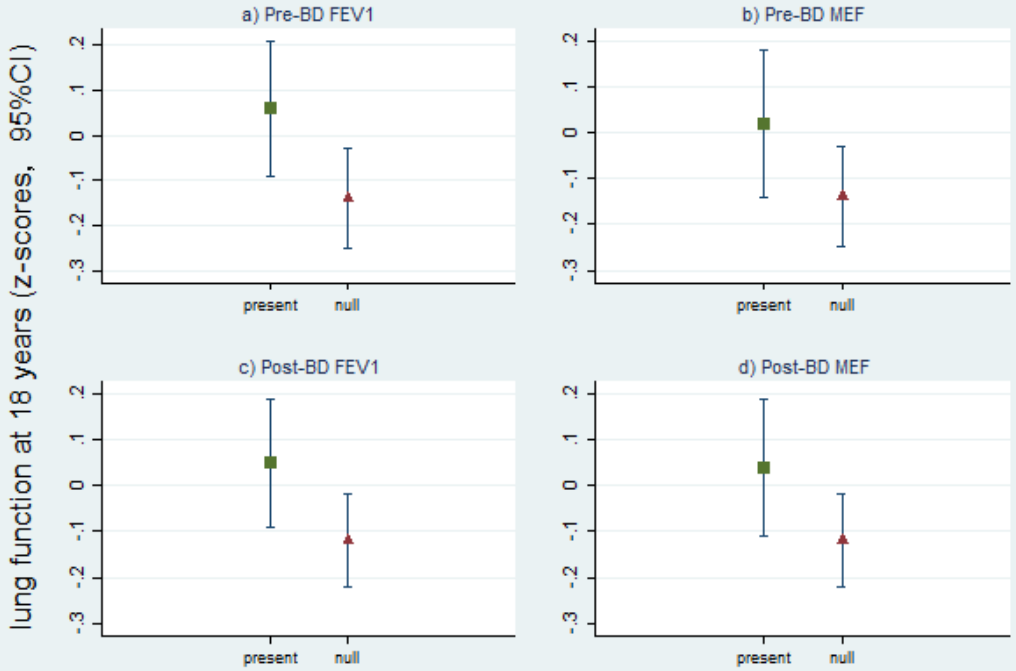


Figure 3. Interaction between early life acetaminophen use for non-respiratory reasons and GSTM1

eMethods:

The initial trial arm

The initial study began as a randomized controlled trial (RCT) aiming to investigate the relationship between infant milk formula and the subsequent risk of allergy. Formula was randomized and introduced as a substitute only if breastfeeding was unable to be or was not initiated, or on its cessation, or partial cessation. Participants were randomized into four different groups, either: 1) a standard cow's milk based formula (NANTM, Nestle, Vevey Switzerland) as a control group; 2) a partially hydrolysed formula (NANTM, HA Ibid); or 3) a soy based formulas (ProSobeeTM, Enfamil, Mead Johnson, USA.). This randomization was performed only at the time when formula was introduced. We added the trial arm in our analyses as a potential confounder, however it did not materially change our results, indicating that the randomized intervention did not confound the relationship between acetaminophen use and lung outcomes.

Ethics

The Mercy Maternity Hospital Ethics Committee approved the recruitment and follow-up of the cohort until 12-years. Consent forms were completed by all parents. The 18-year follow-up was approved by the University of Melbourne and the Royal Children's Hospital Ethics Committees. Informed consent was provided by all participants.

Current asthma

Questionnaire responses by parents were used to define children's asthma status at 12 years and 18 years of age. Current asthma at 12 years and 18 years was defined as any episodes of asthma and/or treatment with any asthma medication within the last 12 months. Current wheeze at 12 and 18 years was defined by question "has your child (12 years) / have you (18 years) ever had wheezing or whistling in the chest in the past 12 months?"

Lung function

SpiroCard™ [PC Spirometer, QRS Diagnostic. Plymouth, MN, USA] devices were used at 12 years, at which time only pre-bronchodilator (BD) spirometry was measured. EasyOne Spirometers [NDD Medical Inc, Andover MA] were used for 18-year follow-up, before and after the inhaled BDsalbutamol via spacer.

Genotyping

Blood or saliva samples were collected from 69.2% of total participants at the 18-year follow-up and DNA extracted (n=429). Deletion of GSTM1 and GSTT1 genotypes were detected by a previously described multiplex Polymerase Chain Reaction (PCR) technique[25], and for all experiments positive

primers for beta-globin were included as a positive control. For GSTM1 and GSTT1, individuals were categorised by recessive model as either GSTM1/T1 null (homozygous for GSTM1/T1*0 allele) or GSTM1/T1 present (at least one GSTM1/T1 alleles). A customised illuminaGoldenGate Genotyping Assay (www.illumina.com) was used to genotype the GSTP1 (rs1695 A→G: Ile105Val) polymorphism. Because GSTP1 with any Val alleles were suggested with a possible protective effect and no clear evidence of different effects between GSTP1 with Ile/Ile and Ile/Val alleles on asthma phenotypes [8], we combined those two genotypes in dominant genetic model for subsequent analyses.

Other variables

Baseline data collected at the initial interview (birth) included parental age, parental education (used as a proxy for socio-economic status) and parental asthma status. Parental education was defined as either mother or father or both having tertiary education at the time of study entry. Parental asthma was defined as either father or mother reporting ever having a history of asthma at baseline. The number of episodes of LRTI, URTI and OM requiring medical attention, regardless of treatment, were classified as 0, 1-2, or $\geqslant$3 in the first two years of life. Parental smoking was defined by either father or mother reporting smoking at baseline; maternal age was made into a binary variable with the cut point at 31 years; the presence of siblings was defined by a positive answer to any older siblings at baseline; breastfeeding was defined by weeks of total breastfeeding; other infection was defined by gastroenteritis, conjunctivitis or other infections which was not due to respiratory reasons before 2 years of age. At the one-year survey, childcare in the first 12 months of the child's life was defined by a positive answer to question "Did your child attend childcare in the past 12 months?"

Randomized intervention was classified by three groups based on formula introduced at the baseline. The initial study began as a randomized controlled trial and aim to test the relationship between infant milk formula and the risk of allergy. Formula was introduced as a substitute only if breastfeeding was unable to start, or on its cessation, or partial cessation. Participants were assigned into four different groups, either: 1) a standard cow's milk based formula (NANTM, Nestle, Vevey Switzerland) as a control group; 2) a partially hydrolysed formula (NANTM, HA Ibid); or 3) a soy based formulas (ProSobeeTM, Enfamil, Mead Johnson, USA.); 4) exclusive breastfeeding.

eTable 1 Classification of indicators for exposure to acetaminophen

Category of acetaminophen indication	Description of indicators
Lower respiratory tract infection	Episodes of acetaminophen exposures due to lower respiratory tract infection, asthma, wheeze of any cause, bronchitis, bronchiolitis, or croup, regardless of any other concomitant reasons for administration
Upper respiratory tract infection/symptoms	Episodes of acetaminophen exposures due to upper respiratory tract infection, tonsillitis, throat infection, regardless of any other concomitant reasons.
Otitis media	Otitis media
Others (Non-respiratory tract infection reasons)	Episodes of acetaminophen exposures due to eczema or food reaction, regardless of any other concomitant reasons in category above, gastroenteritis, conjunctivitis, fever without associated evidence of respiratory tract infection, immunisations, or physical injury.

eTable 2 Acetaminophen use up to 2 years of age for stated indications (N=575).

	N (%) children exposed	Episode of exposure (Median (IQR*))	Cumulative days of exposure (Median (IQR))
Any indication	556 (96.7%)	7 (4-9)	17 (10-27)
LRI	104 (18.1)	1 (1-1)	4 (2-5)
URI	510 (88.7%)	3 (2-5)	9 (5-16)
OM	274 (47.7%)	2 (1-2)	5 (3-5)
Non-respiratory reasons	537 (93.4%)	3 (2-5)	7 (4-11)

*IQR=interquartile range

eTable 3 Association between total days of acetaminophen use before 2 years and respiratory outcome at 12 years.

Asthma	**ORs**	**P values**
Current asthma	1.04 (0.80, 1.37)	0.75
Current wheeze	1.03 (0.82, 1.28)	0.81
Lung function	Coefficients	P values
FEV_1	0.02 (-0.08, 0.13)	0.65
FVC	0.10 (-0.01, 0.21)	0.07
FEV_1/FVC	**-0.16 (-0.28, -0.04)**	**0.01**
MEF	-0.05 (-0.21, 0.10)	0.50

*Standard models were adjusted for parent education and parent asthma, with asthma/wheeze outcomes additionally also adjusted for age and sex of the participant.

Fully adjusted models were additionally adjusted for frequency of respiratory infections, including URTI, LRTI and OM.

eTable 4 associations between total days of acetaminophen use and respiratory outcomes at 12 and 18 years stratified by GSTM1 genotypes.

		GSTM1 present		GSTM1 null		Interaction P values
		OR	P values	OR	P values	
Asthma at 12 years		1.43 (0.83, 2.49)	0.20	1.03 (0.71, 1.50)	0.87	0.67
Wheeze at 12 years		1.01 (0.63, 1.63)	0.95	1.16 (0.86, 1.57)	0.32	0.70
Asthma at 18 years		1.48 (0.92, 2.40)	0.11	0.99 (0.77, 1.27)	0.54	0.99
Wheeze at 18 years		1.47 (0.93, 2.31)	0.10	1.15 (0.89, 1.48)	0.30	0.99
12 years' lung function						
		Coefficients	P values	Coefficients	P values	
FEV_1		0.02 (-0.17, 0.22)	0.80	-0.01 (-0.16, 0.13)	0.86	0.86
FVC		0.07 (-0.12, 0.27)	0.46	0.08 (-0.08, 0.24)	0.33	0.80
FEV_1/FVC		-0.09 (-0.31, 0.14)	0.44	**-0.21 (-0.38, -0.04)**	**0.01**	0.28
MEF		0.16 (-0.14, 0.46)	0.29	-0.15 (-0.36, 0.07)	0.18	0.09
18 years' lung function						
Pre-BD	FEV_1	0.07 (-0.82, 1.96)	0.94	-0.12 **(**-0.22**, -0.10)**	**0.03**	0.14
	FVC	1.22 (-0.58, 3.02)	0.18	-0.66 (-1.90, 0.57)	0.29	0.11
	FEV_1/FVC	-0.14 (-0.31, 0.03)	0.10	-0.10 (-0.21, 0)	0.06	0.93
	MEF	-0.07 (-0.24, 0.09)	0.36	-0.11 (-0.21, 0)	0.05	0.60
Post-BD	FEV_1	0.06 (-0.09, 0.21)	0.41	**-0.11 (-0.20, -0.01)**	**0.03**	**0.03**
	FVC	0.10 (-0.04, 0.25)	0.15	-0.07 (-0.18, 0.03)	0.18	0.05
	FEV_1/FVC	-0.07 (-0.22, 0.08)	0.37	-0.06 (-0.16, 0.04)	0.27	0.78
	MEF	0 (-0.16, 0.17)	0.96	-0.07 (-0.17, 0.02)	0.14	0.22

*ORs were adjusted for age and sex of the participant, parent education, parent asthma and frequency of respiratory infection (URTI, LRTI and OM);

*Coefficients were adjusted for parent education, parent asthma and frequency of respiratory infections (URTI, LRTI, and OM).

eTable 5 associations between total days of acetaminophen use and respiratory outcomes at 12 and 18 years stratified by GSTT1 genotypes.

		GSTT1 present		GSTT1 null		Interaction P values
		OR	P values	OR	P values	
Asthma at 12 years		1.18 (0.85, 1.64)	0.33	0.32 (0.05, 2.27)	0.26	0.37
Wheeze at 12 years		1.19 (0.91, 1.54)	0.20	0.31 (0.07, 1.33)	0.12	0.10
Asthma at 18 years		1.06 (0.84, 1.35)	0.60	0.84 (0.45, 1.55)	0.58	0.41
Wheeze at 18 years		1.21 (0.95, 1.54)	0.12	0.93 (0.51, 1.70)	0.82	0.55
12 years' lung function						
		Coefficients	P values	Coefficients	P values	
FEV_1		-0.02 (-0.14, 0.10)	0.77	0.13 (-0.26, 0.53)	0.50	0.04
FVC		0.08 (-0.05, 0.21)	0.21	0.14 (-0.30, 0.58)	0.53	0.36
FEV_1/FVC		**-0.22 (-0.36, -0.08)**	**<0.01**	0.04 (-0.41, 0.49)	0.86	0.07
MEF		-0.07 (-0.26, 0.11)	0.45	0.04 (-0.53, 0.61)	0.88	0.61
18 years' lung function						
Pre-BD	FEV_1	-0.07 (-0.16, 0.02)	0.13	-0.04 (-0.34, 0.26)	0.79	0.98
	FVC	0.01 (-0.08, 0.10)	0.90	-0.02 (-0.29, 0.25)	0.87	0.40
	FEV_1/FVC	**-0.13 (-0.22, -0.03)**	**0.01**	-0.03 (-0.30, 0.29)	0.97	0.14
	MEF	**-0.11 (-0.21, -0.02)**	**0.02**	0.07 (-0.24, 0.38)	0.64	0.22
Post-BD	FEV_1	-0.03 (-0.12, 0.06)	0.54	-0.15 (-0.38, 0.08)	0.20	0.34
	FVC	0.01 (-0.08, 0.10)	0.81	-0.14 (-0.38, 0.10)	0.24	0.15
	FEV_1/FVC	-0.07 (-0.16, 0.02)	0.14	0 (-0.24, 0.24)	1.00	0.27
	MEF	-0.05 (-0.14, 0.04)	0.31	0.03 (-0.21, 0.27)	0.82	0.58

*ORs were adjusted for age and sex of the participant, parent education, parent asthma and frequency of respiratory infection (URTI, LRTI and OM);

*Coefficients were adjusted for parent education, parent asthma and frequency of respiratory infections (URTI, LRTI, and OM).

eTable 6 associations between total days of acetaminophen use and lung function (z-score) at 12 and 18 years stratified by GSTP1 genotypes

		GSTP1 Ile/val or val/val		GSTP1 ile/ile		Interaction P values
12 years' lung function						
		Coefficients	P values	Coefficients	P values	
FEV_1		0.01 (-0.15, 0.16)	0.93	0.02 (-0.16, 0.20)	0.84	0.90
FVC		0.11 (-0.05, 0.26)	0.17	0.08 (-0.13, 0.29)	0.51	0.59
FEV_1/FVC		-0.19 (-0.36, -0.02)	0.03	-0.18 (-0.40, 0.05)	0.12	0.66
MEF		-0.04 (-0.24, 0.17)	0.71	-0.11 (-0.42, 0.20)	0.49	0.98
18 years' lung function						
Pre-BD	FEV_1	-0.08 (-0.20, 0.04)	0.21	-0.08 (-0.21, 0.05)	0.23	0.91
	FVC	-0.02 (-0.13, 0.10)	0.79	-0.03 (-0.17, 0.11)	0.79	0.89
	FEV_1/FVC	-0.09 (-0.21, 0.02)	0.11	-0.12 (-0.27, 0.03)	0.13	0.50
	MEF	-0.09 (-0.21, 0.03)	0.13	-0.09 (-0.24, 0.06)	0.25	0.97
Post-BD	FEV_1	-0.07 (-0.18, 0.04)	0.23	-0.03 (-0.17, 0.11)	0.67	0.88
	FVC	-0.03 (-0.14, 0.08)	0.54	-0.01 (-0.15, 0.12)	0.84	0.71
	FEV_1/FVC	-0.05 (-0.15, 0.06)	0.40	-0.04 (-0.18, 0.10)	0.53	0.59
	MEF	-0.06 (-0.16, 0.05)	0.31	0 (-0.14, 0.15)	0.98	0.77

*Coefficients were adjusted for parent education, parent asthma and frequency of respiratory infections (URTI, LRTI, and OM).

eTable 7 associations between days of acetaminophen use for non-respiratory infection reasons and respiratory outcomes at 12 and 18 years stratified by GSTM1 genotypes.

	GSTM1 present		**GSTM1 null**		**Interaction P**
	OR	P	OR	P	
Asthma at 12 years	1.10 (0.66, 1.83)	0.71	1.26 (0.87, 1.83)	0.22	0.45
Wheeze at 12 years	0.90 (0.59, 1.37)	0.62	1.06 (0.78, 1.44)	0.69	0.33
Asthma at 18 years	1.25 (0.83, 1.86)	0.29	0.89 (0.69, 1.15)	0.39	0.35
Wheeze at 18 years	1.26 (0.86, 1.84)	0.23	1.03 (0.79, 1.32)	0.84	0.44
12 years' lung function					
	Coefficients	P values	Coefficients	P values	
FEV_1	0.18 (0, 0.35)	0.05	-0.09 (-0.23, 0.06)	0.23	**0.01**
FVC	0.18 (-0.01, 0.36)	0.06	-0.03 (-0.19, 0.13)	0.68	**0.03**
FEV_1/FVC	0.01 (-0.20, 0.23)	0.90	-0.14 (-0.31, 0.04)	0.12	0.38
MEF	0.09 (-0.19, 0.37)	0.52	-0.14 (-0.36, 0.08)	0.21	0.24

*ORs were adjusted for age and sex of the participant, parent education, parent asthma and frequency of respiratory infection (URTI, LRTI and OM);
*Coefficients were adjusted for parent education, parent asthma and frequency of respiratory infections (URTI, LRTI, and OM).

eTable 8 associations between days of acetaminophen use for non-respiratory infection reasons and respiratory outcomes at 12 and 18 years stratified by GSTT1 genotypes.

	GSTT1 present		**GSTT1 null**		**Interaction P values**
	OR	P values	OR	P values	
Asthma at 12 years	1.28 (0.95, 1.77)	0.10	0.17 (0.02, 1.04)	0.10	0.17
Wheeze at 12 years	1.07 (0.82, 1.38)	0.62	0.51 (0.19, 1.35)	0.18	0.28
Asthma at 18 years	0.98 (0.79, 1.22)	0.85	0.89 (0.48, 1.63)	0.70	0.74
Wheeze at 18 years	1.05 (0.84, 1.30)	0.69	1.02 (0.57, 1.83)	0.94	0.87
12 years' lung function					
	Coefficients	P values	Coefficients	P values	
FEV_1	-0.03 (-0.15, 0.09)	0.61	0.26 (-0.11, 0.63)	0.27	0.10
FVC	0.03 (-0.09, 0.16)	0.56	0.13 (-0.29, 0.56)	0.80	0.80
FEV_1/FVC	**-0.14 (-0.28, 0)**	**0.05**	0.32 (-0.09, 0.74)	0.13	**0.01**
MEF	-0.11 (-0.29, 0.07)	0.24	0.26 (-0.28, 0.80)	0.26	0.12

*ORs were adjusted for age and sex of the participant, parent education, parent asthma and frequency of respiratory infection (URTI, LRTI and OM);
*Coefficients were adjusted for parent education, parent asthma and frequency of respiratory infections (URTI, LRTI, and OM).

eTable 9 associations between days of acetaminophen use for non-respiratory infection reasons and respiratory outcomes at 12 and 18 years stratified by GSTP1 genotypes.

		GSTP1 Ile/val or val/val		GSTP1 ile/ile		Interaction P
		OR	P values	OR	P values	
Asthma at 12 years		1.29 (0.86, 1.93)	0.22	1.15 (0.71, 1.88)	0.57	0.98
Wheeze at 12 years		0.94 (0.69, 1.27)	0.68	1.20 (0.77, 1.88)	0.43	0.41
Asthma at 18 years		0.87 (0.67, 1.14)	0.31	1.18 (0.84, 1.65)	0.35	0.22
Wheeze at 18 years		0.93 (0.72, 1.20)	0.56	1.35 (0.96, 1.90)	0.09	0.15
12 years' lung function						
		Coefficients	P values	Coefficients	P values	
FEV_1		0.04 (-0.11, 0.18)	0.64	-0.02 (-0.21, 0.17)	0.83	0.57
FVC		0.08 (-0.07, 0.23)	0.28	0.01 (-0.21, 0.23)	0.94	0.46
FEV_1/FVC		-0.09 (-0.25, 0.08)	0.31	-0.09 (-0.33, 0.14)	0.44	0.97
MEF		0 (-0.19, 0.20)	0.98	-0.25 (-0.57, 0.07)	0.13	0.37
18 years' lung function						
Pre-BD	FEV_1	-0.05 (-0.18, 0.07)	0.38	-0.08 (-0.21, 0.05)	0.24	0.81
	FVC	0 (-0.10, 0.12)	0.87	-0.07 (-0.21, 0.07)	0.32	0.43
	FEV_1/FVC	-0.10 (-0.22, 0.01)	0.07	-0.05 (-0.20, 0.10)	0.54	0.60
	MEF	-0.09 (-0.21, 0.02)	0.12	-0.05 (-0.20, 0.10)	0.53	0.61
Post-BD	FEV_1	-0.02 (-0.12, 0.09)	0.67	-0.11 (-0.25, 0.03)	0.11	0.33
	FVC	0.01 (-0.10, 0.12)	0.90	-0.11 (-0.24, 0.03)	0.12	0.24
	FEV_1/FVC	-0.04 (-0.15, 0.06)	0.43	-0.03 (-0.17, 0.11)	0.66	0.92
	MEF	-0.05 (-0.16, 0.06)	0.37	-0.05 (-0.19, 0.10)	0.52	0.84

*ORs were adjusted for age and sex of the participant, parent education, parent asthma and frequency of respiratory infection (URTI, LRTI and OM);
*Coefficients were adjusted for parent education, parent asthma and frequency of respiratory infections (URTI, LRTI, and OM).

eTable 10 Association between total days of acetaminophen use before 2 years and respiratory outcomes at 18 years.

		Total days of acetaminophen use		days of acetaminophen use for non-respiratory infection use	
Asthma		ORs	p	ORs	P
	Current asthma	1.12 (0.87, 1.43)	0.38	0.97 (0.73, 1.29)	0.84
	Current wheeze	1.40 (1.08, 1.82)	0.01	0.99 (0.79, 1.25)	0.96
Lung function		Coefficients	P	Coefficients*	P
Pre-BD	FEV_1	-0.07 (-0.16, 0.03)	0.15	-0.04 (-0.13, 0.06)	0.48
	FVC	0.01 (-0.09, 0.10)	0.85	-0.02 (-0.07, 0.12)	0.63
	FEV_1/FVC	-0.12 (-0.22, -0.02)	0.02	-0.10 (-0.20, 0)	0.06
	MEF	-0.10 (-0.19, 0)	0.05	-0.07 (-0.17, 0.03)	0.19
Post-BD	FEV_1	-0.06 (-1.45, 0.04)	0.24	-0.06 (-0.15, 0.04)	0.25
	FVC	0 (-0.10, 0.09)	0.96	-0.01 (-0.11, 0.09)	0.86
	FEV_1/FVC	-0.90 (-0.18, 0.01)	0.07	-0.09 (-0.18, 0.01)	0.07
	MEF	-0.06 (-0.15, 0.03)	0.19	-0.08 (-0.17, 0.02)	0.10

*Standard models were adjusted for parent education, parent asthma, birth weight at 4 weeks, breastfeeding, childcare in first 12 months, older sibling, other infections, parental education, parental smoking, RCT formula with asthma/wheeze outcomes additionally also adjusted for age and sex of the participant.
Fully adjusted models were additionally adjusted for frequency of respiratory infections, including URTI, LRTI and OM.

eTable 11 associations between total days of acetaminophen use and asthma/wheeze at 12 and 18 years stratified by GSTP1 genotypes.

	GSTP1 Ile/val or val/val		GSTP1 ile/ile		Interaction P
	OR	P	OR	P	
Asthma at 12 years	1.30 (0.78, 2.17)	0.31	**3.00 (1.20, 7.49)**	**0.02**	0.10
Wheeze at 12 years	1.12 (0.76, 1.66)	0.57	1.61 (0.93, 2.77)	0.09	0.13
Asthma at 18 years	0.88 (0.64, 1.21)	0.43	**1.77 (1.09, 2.88)**	**0.02**	**<0.01**
Wheeze at 18 years	1.01 (0.79, 1.50)	0.60	**2.21 (1.33, 3.68)**	**<0.01**	**0.01**

*ORs were adjusted for age and sex of the participant, parent education, parent asthma, birth weight at 4 weeks, breastfeeding, childcare in first 12 months, older sibling, other infections, parental education, parental smoking, RCT formula and frequency of respiratory infection (URTI, LRTI and OM).

eTable 12 Associations between days of acetaminophen use for non-respiratory reasons and lung function (z-score) at 18 years stratified by GSTM1 genotypes.

		GSTM1 present		**GSTM1 null**		**Interaction P**
		coefficients	p	coefficients	p	
Pre-BD	FEV_1	0.05 (-0.12, 0.22)	0.54	-0.10 (-0.23, 0.03)	0.12	**0.05**
	FVC	0.13 (-0.03, 0.29)	0.12	-0.04 (-0.18, 0.09)	0.53	0.06
	FEV_1/FVC	-0.14 (-0.32, 0.05)	0.15	-0.10 (-0.23, 0.04)	0.16	0.95
	MEF	-0.03 (-0.20, 0.15)	0.77	-0.11 (-0.24, 0.02)	0.09	0.23
Post-BD	FEV_1	0.05 (-0.11, 0.20)	0.53	**-0.13 (-0.25, 0.??)**	**0.04**	**0.02**
	FVC	0.08 (-0.07, 0.23)	0.31	-0.06 (-0.20, 0.07)	0.36	0.06
	FEV_1/FVC	-0.06 (-0.22, 0.09)	0.42	-0.11 (-0.24, 0.02)	0.10	0.58
	MEF	-0.01 (-0.17, 0.16)	0.93	**-0.13 (-0.25, -0.02)**	**0.02**	**0.05**

*Coefficients were adjusted for parent education, parent asthma, birth weight at 4 weeks, breastfeeding, childcare in first 12 months, older sibling, other infections, parental education, parental smoking, RCT formula and frequency of respiratory infections (URTI, LRTI, and OM).

eTable 13 Association between total days of acetaminophen use before 2 years and respiratory outcome at 12 years.

	Fully adjusted Model	
Asthma	ORs	P
Current asthma	1.14 (0.83, 1.56)	0.41
Current wheeze	1.11 (0.87, 1.40)	0.41
Lung function	Coefficients	P values
FEV_1	0.02 (-0.09, 0.13)	0.71
FVC	0.10 (-0.01, 0.21)	0.08
FEV_1/FVC	**-0.16 (-0.29, -0.04)**	**0.01**
MEF	-0.05 (-0.21, 0.12)	0.58

*Standard models were adjusted for parent education, parent asthma birth weight at 4 weeks, breastfeeding, childcare in first 12 months, older sibling, other infections, parental education, parental smoking, RCT formula, with asthma/wheeze outcomes additionally also adjusted for age and sex of the participant, and frequency of respiratory infections, including URTI, LRTI and OM.

eTable 14 associations between total days of acetaminophen use and respiratory outcomes at 12 and 18 years stratified by GSTM1 genotypes.

		GSTM1 present		GSTM1 null		Interaction P values
		OR	P	OR	P	
Asthma at 12 years		1.62 (0.49, 5.36)	0.43	1.06 (0.69, 1.62)	0.80	0.26
Wheeze at 12 years		1.16 (0.59, 2.28)	0.66	1.21 (0.88, 1.66)	0.25	1.00
Asthma at 18 years		1.41 (0.80, 2.50)	0.23	1.08 (0.80, 1.45)	0.61	0.87
Wheeze at 18 years		1.53 (0.90, 2.61)	0.12	**1.41 (1.02, 1.95)**	**0.04**	0.63
12 years' lung function						
		Coefficients	P	Coefficients	P	
FEV_1		0 (-0.20, 0.21)	0.97	-0.01 (-0.15, 0.14)	0.92	0.94
FVC		0.04 (-0.17, 0.26)	0.69	0.09 (-0.07, 0.25)	0.27	0.55
FEV_1/FVC		-0.05 (-0.29, 0.20)	0.71	**-0.21 (-0.39, -0.04)**	**0.02**	0.19
MEF		0.14 (-0.18, 0.46)	0.38	-0.11 (-0.33, 0.10)	0.30	0.07
18 years' lung function						
Pre-BD	FEV_1	0.01 (-0.17, 0.18)	0.95	-0.11 (-0.22, 0.01)	0.06	0.28
	FVC	0.13 (-0.04, 0.31)	0.14	-0.04 (-0.16, 0.08)	0.50	0.14
	FEV_1/FVC	**-0.19 (-0.39, 0)**	**0.05**	-0.11 (-0.22, 0.01)	0.08	0.64
	MEF	-0.10 (-0.29, 0.09)	0.30	-0.10 (-0.22, 0.01)	0.07	0.87
Post-BD	FEV_1	0.07 (-0.10, 0.23)	0.41	**-0.11 (-0.22, 0)**	**0.04**	**0.08**
	FVC	0.13 (-0.03, 0.29)	0.11	-0.06 (-0.19, 0.06)	0.32	**0.07**
	FEV_1/FVC	-0.09 (-0.26, 0.08)	0.28	-0.08 (-0.20, 0.03)	0.15	0.94
	MEF	-0.01 (-0.19, 0.16)	0.87	-0.09 (-0.19, 0.02)	0.09	0.29

*ORs were adjusted for age and sex of the participant, parent education, parent asthma, birth weight at 4 weeks, breastfeeding, childcare in first 12 months, older sibling, other infections, parental education, parental smoking, RCT formula and frequency of respiratory infection (URTI, LRTI and OM);
*Coefficients were adjusted for parent education, parent asthma, birth weight at 4 weeks, breastfeeding, childcare in first 12 months, older sibling, other infections, parental education, parental smoking, RCT formula and frequency of respiratory infections (URTI, LRTI, and OM).

eTable 15 associations between total days of acetaminophen use and respiratory outcomes at 12 and 18 years stratified by GSTT1 genotypes.

		GSTT1 present		GSTT1 null		Interaction P values
		OR	P	OR	P	
Asthma at 12 years		1.37 (0.93, 2.03)	0.11	-	-	0.48
Wheeze at 12 years		1.30 (0.98, 1.73)	0.70	-	-	0.14
Asthma at 18 years		1.12 (0.86, 1.47)	0.41	0.35 (0.03, 4.78)	0.43	0.58
Wheeze at 18 years		1.41 (1.05, 1.88)	0.02	1.75 (0.67, 4.54)	0.25	0.66
12 years' lung function						
		Coefficients	P	Coefficients	P	
FEV_1		-0.01 (-0.14, 0.11)	0.83	0.15 (-0.24, 0.55)	0.43	**0.02**
FVC		0.09 (-0.05, 0.22)	0.20	0.13 (-0.37, 0.63)	0.60	0.20
FEV_1/FVC		**-0.21 (-0.36, -0.06)**	**<0.01**	0.07 (-0.56, 0.69)	0.82	0.17
MEF		-0.04 (-0.22, 0.15)	0.70	-0.33 (-0.98, 0.33)	0.32	0.67
18 years' lung function						
Pre-BD	FEV_1	-0.06 (-0.16, 0.04)	0.23	-0.08 (-0.40, 0.23)	0.60	0.67
	FVC	0.02 (-0.08, 0.12)	0.69	-0.06 (-0.36, 0.24)	0.69	0.40
	FEV_1/FVC	**-0.13 (-0.24, -0.03)**	**0.01**	-0.04 (-0.38, 0.31)	0.84	0.31
	MEF	**-0.11 (-0.21, -0.01)**	**0.03**	0.05 (-0.32, 0.42)	0.79	0.33
Post-BD	FEV_1	-0.04 (-0.14, 0.06)	0.74	-0.16 (-0.43, 0.11)	0.23	0.41
	FVC	0.01 (-0.09, 0.12)	0.79	-0.12 (-0.41, 0.17)	0.39	0.31
	FEV_1/FVC	-0.09 (-0.19, 0.01)	0.09	-0.05 (-0.34, 0.24)	0.74	0.46
	MEF	-0.07 (-0.17, 0.04)	0.20	0 (-0.28, 0.29)	0.99	0.59

*ORs were adjusted for age and sex of the participant, parent education, parent asthma, birth weight at 4 weeks, breastfeeding, childcare in first 12 months, older sibling, other infections, parental education, parental smoking, RCT formula and frequency of respiratory infection (URTI, LRTI and OM);
*Coefficients were adjusted for parent education, parent asthma, birth weight at 4 weeks, breastfeeding, childcare in first 12 months, older sibling, other infections, parental education, parental smoking, RCT formula and frequency of respiratory infections (URTI, LRTI, and OM).

eTable 16 associations between days of acetaminophen use for non-respiratory infection reasons and respiratory outcomes at 12 and 18 years stratified by GSTM1 genotypes.

	GSTM1 present		**GSTM1 null**		**Interaction P**
	OR	P	OR	P	
Asthma at 12 years	0.49 (0.17, 1.42)	0.19	1.13 (0.73, 1.74)	0.59	0.94
Wheeze at 12 years	1.03 (0.59, 1.80)	0.91	1.11 (0.79, 1.56)	0.56	0.67
Asthma at 18 years	1.06 (0.66, 1.71)	0.80	0.92 (0.68, 1.26)	0.62	0.60
Wheeze at 18 years	1.15 (0.75, 1.77)	0.52	1.21 (0.88, 1.65)	0.23	0.99
12 years' lung function					
	Coefficients	P	Coefficients	P	
FEV_1	0.18 (-0.02, 0.37)	0.07	-0.05 (-0.21, 0.10)	0.51	**0.02**
FVC	0.15 (-0.05, 0.35)	0.14	-0.01 (-0.18, 0.17)	0.94	**0.09**
FEV_1/FVC	0.09 (-0.15, 0.32)	0.47	-0.11 (-0.30, 0.08)	0.25	0.26
MEF	0.14 (-0.17, 0.44)	0.38	-0.07 (-0.30, 0.16)	0.55	0.27

*ORs were adjusted for age and sex of the participant, parent education, parent asthma, birth weight at 4 weeks, breastfeeding, childcare in first 12 months, older sibling, other infections, parental education, parental smoking, RCT formula and frequency of respiratory infection (URTI, LRTI and OM);
*Coefficients were adjusted for parent education, parent asthma, birth weight at 4 weeks, breastfeeding, childcare in first 12 months, older sibling, other infections, parental education, parental smoking, RCT formula and frequency of respiratory infections (URTI, LRTI, and OM).

eTable 17 associations between days of acetaminophen use for non-respiratory infection reasons and respiratory outcomes at 12 and 18 years stratified by GSTT1 genotypes.

		GSTT1 present		GSTT1 null		Interaction P values
		OR	P	OR	P	
Asthma at 12 years		1.40 (0.95, 1.98)	0.09	-	-	0.14
Wheeze at 12 years		1.23 (0.91, 1.64)	0.17	-	-.	0.05
Asthma at 18 years		0.96 (0.74, 1.24)	0.81	-	-	0.48
Wheeze at 18 years		1.13 (0.87, 1.46)	0.38	1.19 (0.49, 2.91)	0.70	0.94
12 years' lung function						
		Coefficients	P	Coefficients	P	
FEV_1		0.01 (-0.12, 0.14)	0.88	0.30 (-0.05, 0.66)	0.09	0.16
FVC		0.06 (-0.08, 0.19)	0.41	0.09 (-0.38, 0.56)	0.69	0.91
FEV1/FVC		**-0.11 (-0.26, 0.04)**	**0.17**	0.46 (-0.10, 1.02)	0.10	**0.02**
MEF		-0.04 (-0.23, 0.15)	0.65	0.20 (-0.43, 0.83)	0.51	0.20
18 years' lung function						
Pre-BD	FEV_1	-0.05 (-0.15, 0.05)	0.33	0.09 (-0.25, 0.42)	0.60	0.87
	FVC	0.02 (-0.08, 0.13)	0.67	-0.01 (-0.33, 0.32)	0.75	0.64
	FEV_1/FVC	**-0.13 (-0.24, -0.01)**	**0.03**	0.16 (-0.20, 0.53)	0.36	0.20
	MEF	**-0.11 (-0.21, 0)**	**0.05**	0.27 (-0.11, 0.65)	0.16	**0.07**
Post-BD	FEV_1	-0.04 (-0.14, 0.06)	0.45	-0.20 (-0.50, 0.10)	0.19	0.31
	FVC	0.01 (-0.10, 0.12)	0.85	-0.24 (-0.55, 0.08)	0.14	0.10
	FEV_1/FVC	-0.09 (-0.20, 0.01)	0.08	0.08 (-0.25, 0.50)	0.63	0.39
	MEF	-0.09 (-0.19, 0.01)	0.08	0.10 (-0.22, 0.41)	0.54	0.41

*ORs were adjusted for age and sex of the participant, parent education, parent asthma, birth weight at 4 weeks, breastfeeding, childcare in first 12 months, older sibling, other infections, parental education, parental smoking, RCT formula and frequency of respiratory infection (URTI, LRTI and OM);
*Coefficients were adjusted for parent education, parent asthma, birth weight at 4 weeks, breastfeeding, childcare in first 12 months, older sibling, other infections, parental education, parental smoking, RCT formula and frequency of respiratory infections (URTI, LRTI, and OM).

eTable 18 associations between days of acetaminophen use for non-respiratory infection reasons and respiratory outcomes at 12 and 18 years stratified by GSTP1 genotypes.

		GSTP1 Ile/val or val/val		GSTP1 ile/ile		Interaction P
		OR	P	OR	P	
Asthma at 12 years		1.29 (0.78, 2.13)	0.32	1.47 (0.77, 2.80)	0.24	0.53
Wheeze at 12 years		1.01 (0.70, 1.45)	0.97	1.39 (0.80, 2.41)	0.25	0.20
Asthma at 18 years		0.76 (0.55, 1.06)	0.10	1.21 (0.79, 1.87)	0.38	**0.02**
Wheeze at 18 years		0.93 (0.68, 1.26)	0.46	**1.75 (1.03, 2.41)**	**0.04**	**0.03**
12 years' lung function						
		Coefficients	P	Coefficients	P	
FEV_1		0.10 (-0.06, 0.26)	0.23	-0.08 (-0.28, 0.12)	0.43	0.25
FVC		0.14 (-0.03, 0.30)	0.10	-0.06 (-0.30, 0.17)	0.59	0.17
FEV_1/FVC		-0.07 (-0.25, 0.11)	0.46	-0.06 (-0.32, 0.21)	0.67	0.47
MEF		0.03 (-0.18, 0.24)	0.77	-0.19 (-0.55, 0.17)	0.29	0.49
18 years' lung function						
Pre-BD	FEV_1	-0.05 (-0.18, 0.09)	0.51	-0.07 (-0.23, 0.09)	0.36	0.97
	FVC	0.06 (-0.70, 0.19)	0.38	-0.12 (-0.30, 0.05)	0.18	0.13
	FEV_1/FVC	**-0.16 (-0.29, -0.03)**	**0.02**	0.05 (-0.15, 0.23)	0.71	0.20
	MEF	-0.12 (-0.25, 0.01)	0.12	0.03 (-0.15, 0.20)	0.76	0.24
Post-BD	FEV_1	-0.02 (-0.15, 0.10)	0.37	-0.15 (-0.31, 0.01)	0.07	0.41
	FVC	0.04 (-0.90, 0.18)	0.53	-0.16 (-0.33, 0.??)	0.05	0.18
	FEV_1/FVC	-0.12 (-0.24, 0)	0.06	0 (-0.18, 0.17)	0.99	0.45
	MEF	-0.09 (-0.21, 0.03)	0.13	-0.03 (-0.20, 0.15)	0.75	0.59

*ORs were adjusted for age and sex of the participant, parent education, parent asthma, birth weight at 4 weeks, breastfeeding, childcare in first 12 months, older sibling, other infections, parental education, parental smoking, RCT formula and frequency of respiratory infection (URTI, LRTI and OM);
*Coefficients were adjusted for parent education, parent asthma, birth weight at 4 weeks, breastfeeding, childcare in first 12 months, older sibling, other infections, parental education, parental smoking, RCT formula and frequency of respiratory infections (URTI, LRTI, and OM).

eTable 19 associations between total days of acetaminophen use and lung function (z-score) at 12 and 18 years stratified by GSTP1 genotypes

		GSTP1 Ile/val or val/val		GSTP1 ile/ile		Interaction P values
12 years' lung function						
		Coefficients	P	Coefficients	P	
FEV_1		0.03 (-0.15, 0.20)	0.77	-0.01 (-0.19, 0.17)	0.92	0.80
FVC		0.14 (-0.04, 0.31)	0.12	0.06 (-0.16, 0.28)	0.60	0.43
FEV1/FVC		-0.21 (-0.39, -0.02)	0.03	-0.18 (-0.43, 0.06)	0.14	0.53
MEF		-0.06 (-0.28, 0.16)	0.58	-0.08 (-0.41, 0.26)	0.65	0.74
18 years' lung function						
Pre-BD	FEV_1	-0.12 (-0.25, 0.01)	0.08	-0.06 (-0.21, 0.08)	0.93	0.51
	FVC	0.01 (-0.12, 0.14)	0.92	-0.05 (-0.21, 0.11)	0.51	0.84
	FEV_1/FVC	**-0.18 (-0.31, -0.05)**	**0.01**	-0.06 (-0.23, 0.12)	0.52	0.81
	MEF	**-0.16 (-0.29, -0.03)**	**0.02**	-0.03 (-0.19, 0.13)	0.25	0.43
Post-BD	FEV_1	-0.12 (-0.24, 0.01)	0.07	-0.05 (-0.20, 0.11)	0.55	0.38
	FVC	-0.03 (-0.16, 0.11)	0.72	-0.04 (-0.20, 0.11)	0.58	0.67
	FEV_1/FVC	-0.14 (-0.26, -0.02)	0.03	-0.02 (-0.18, 0.14)	0.80	0.63
	MEF	-0.13 (-0.25, -0.01)	0.03	0.01 (-0.15, 0.18)	0.86	0.30

*Coefficients were adjusted for parent education, parent asthma, birth weight at 4 weeks, breastfeeding, childcare in first 12 months, older sibling, other infections, parental education, parental smoking, RCT formula and frequency of respiratory infections (URTI, LRTI, and OM).

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Chapter 7 EXPOSURE TO HOUSEHOLD AIR POLLUTION OVER 10 YEARS IS RELATED TO ASTHMA AND LUNG FUNCTION DECLINE

7.1 Chapter introduction

This paper (with data supplement) reports my investigation of the association between 10-year household air pollution (HAP) exposure and the risk of asthma and lung function decline in middle-aged adults. I also investigated the potential interaction of GST genes on the associations between household air pollution and asthma and lung function. This paper provides evidence that long-term HAP exposures was linked to risk of asthma and greater lung function decline in middle-age, particularly for susceptible participants with specific genetic polymorphisms. This manuscript is in revision following peer review by *the American Journal of respiratory and critical care medicine*.

7.2 Research questions and objectives

7.2.1 Research questions

Are common HAP exposures related to asthma risk and greater lung function decline in middle-aged adults, in terms of cooking and heating facilities, tobacco smoke, and mould. Are those relationships modified by GST gene variants?

7.2.2 Research objectives

1. To identify distinct longitudinal patterns of HAP exposure;
2. To determine whether these patterns were associated with risks of asthma and/or accelerated lung function decline between ages 43 and 53 years;
3. To determine whether these associations were modified by GST genotypes.

7.3 Manuscript

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Exposure to household air pollution over 10 years is related to asthma and lung function decline

Short running head: household air pollution, GST and lung health

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Abstract 249 word count

Rationale: Currently, guidelines on obstructive lung disease contain few recommendations on Household air pollution (HAP), although it is known to cause airway oxidative stress, which is associated with obstructive lung disease The associations may be modified by Glutathione S-Transferase (GST) gene polymorphisms.

Objective: We aimed to identify longitudinal HAP profiles associated with asthma risk and lung function decline in middle-aged adults and determine whether these associations were modified by GST gene variants.

Methods: We used repeated measurement on HAP exposures (heating, cooking, mould, smoking) and asthma collected by the Tasmanian Longitudinal Health Study (TAHS) at mean ages 43 and 53 years (n=3314). A sub group of this cohort also had data on lung function (n=897) and GST genes (GSTM1, GSTT1 and GSTP1) (n=928). Latent class analysis was used to characterize exposure longitudinal patterns. Regression models assessed assocations and interactions.

Measurements and Main Results: We identified seven longitudinal HAP profiles. “wood heating”,“all gas”, “wood heating/smoking” and “wood heating/gas cooking” were associated with persistent asthma and greater lung function decline by age 53 years. Participants with GSTP1 Ile/Ile and GSTM1 null genotypes were at a higher risk of asthma or greater lung function decline when exposed compared to other genotypes.

Conclusions: Longitudinal exposures to wood heating, gas cooking and heating, and tobacco smoke over 10 years increased the risks of persistent asthma and lung function decline, with evidence of interaction by GST genes. These new findings inform the development of more comprehensive asthma and COPD guidelines that include specific HAP exposures.

Key words: asthma, respiratory function tests, indoor air pollution, glutathione transferase, cohort studies

Word count: 3490

Introduction

Asthma is the most common chronic respiratory disease across the population, and is especially prevalent in Australia. Persistent asthma and excess lung function decline are risk factors for chronic obstructive pulmonary disease (COPD)(1). Household air pollution (HAP) sources implicated in asthma causation and persistence include exposure to tobacco smoke, heating, cooking emissions and surface mould (2). Apart from tobacco smoke and biomass fuel as risk factors for COPD and asthma-COPD overlap (3) current asthma and COPD guidelines rarely mention specific sources of HAP as modifiable risk factors (4, 5). This is not surprising given the inconsistent evidence on associations between these exposures and respiratory health (6-13). There is a need to provide high quality evidence to incorporate strategies addressing HAP into clinical and public health guidelines for the management and prevention of obstructive lung diseases.

There are several reasons for the inconsistent evidence concerning HAP and respiratory health. Previous studies have usually investigated single HAP exposures without considering their interplay. It is important to accurately identify patterns of HAP as home characteristics cluster together, and the effect of certain exposures may be accentuated or reduced by others. The relationship between HAP patterns overtime and respiratory health has not previously been reported. Another important inconsistency between studies is whether or not they have taken venting

of HAP into account. Ventilation and flues could modify the the impact of HAP (14, 15), or may be an independent beneficial factor for respiratory health (16, 17).

Finally, inconsistency of the reported associations may also reflect that many previous studies have not taken gene-environment interactions into account. Glutathione S-Transferase (GST) genes regulate the response to oxidative stress, that is induced by HAP. GST polymorphisms have been identified as modifiers of the effects of outdoor air pollution on respiratory health (18, 19). Studies suggest that carriers of GSTM1 null, GSTT1 null and GSTP1 with homozygous Ile genotypes may be more susceptible to the adverse effects of outdoor air pollution (18, 20). Such interactions have been explored less in studies investigating HAP exposures and asthma and lung function.

Using the 5th and 6th decade follow-ups from the Tasmanian Longitudinal Health Study (TAHS), we aimed to determine the relationship between longitudinal exposure to a range of common HAPs, and their potential interaction with GST polymorphisms, on respiratory health in middle-aged adults. Specifically, we aimed to: 1) identify distinct longitudinal patterns of HAP exposure; 2) determine whether these patterns were associated with risks of asthma and/or accelerated lung function decline between ages 43 and 53 years; and 3) determine whether these associations were modified by GST genotypes.

Methods

Study design and data collection

TAHS is a population-based prospective cohort study, which commenced in 1968 including 8583 children at the age of 7 years (98.8% of the entire Tasmanian population at that age) (21). We have recently followed participants into their 5th and 6th decades (Beginning in 2002 and 2012, respectively) when they were aged on average, 43 years (Standard Deviation [SD]: 0.82) and 53 years (SD: 0.93) (Figure 1) . A total of 5729 (78.4%) participants completed 5th decade, and 3609 (41.9%) completed 6th decade questionnaires. Of these, 3314 participated at both follow-ups with complete information, and 897 had spirometry at both follow-ups, with and without inhaled bronchodilator (Figure 2). Additionally, 1215 subjects contributed genetic information at the 5th decade follow-up. Of these, 930 also completed the 6th decade questionnaire. (Figure 2).

Household air pollution exposures

The 5th and 6th decade questionnaires included the same items for determining HAP exposure in the last 12 months. The details of specific questions from the questionnaires concerning HAP exposures are in the Supplement.

Asthma

Current asthma was defined by a participants' positive response to "Have you ever had asthma" and also to having any episode of asthma in the past 12 months or any asthma medication taken within the past 3 months (5th decade) or 12 months (6th decade). At the 6th decade we defined new-onset or persistent asthma depending on previous status. The details of definitions are in the Supplement.

Lung function decline

The procedures for lung function testing is detailed in the supplement. Forced expiratory volume (FEV_1) and forced vital capacity (FVC) were recorded as the best of three manoeuvres. Z-scores for lung function indices were calculated from the Global Lung Initiative (GLI) reference equations for Caucasians (22, 23). Lung function decline was determined by reductions in pre- or post-bronchodilator (BD) values between the 5th and 6th decade follow-ups, derived from reductions in z-scores, for FEV_1, FVC and FEV_1/FVC. The amount of decline in exposure profiles was compared to the baseline reference group (low exposed) to investigate evidence of excess lung function decline.

Other variables

Atopy was defined as an average Skin Prick Test (SPT) wheal diameter of 3mm or greater for 1 or more of the allergens, compared to the negative control tested at the 6th decade. Details of atopy, occupation and definitions of fixed airway obstruction (COPD) used for our sensitivity analysis are in the Supplement.

Genotyping of GST genes

DNA was isolated from whole blood samples provided at the 6th decade follow-up. GSTM1 and GSTT1 genotype were classified as either present or null genotypes. Individuals were categorised by GSTP1 genotypes as Val/Val, Ile/Ile or Val/Ile. More details of GST genotyping are in the Supplement.

Statistical analysis

Identification of HAP profiles

The following HAP exposures were included in the Latent Class Analysis (LCA): heating method (wood or coal fire, gas heaters, electric heater and reverse-cycle air conditioning); cooking method (gas or electric); visible mould, and; smoking (combined passive and/or active). Participants were assigned to the class for which they had the highest probability of membership. The details of LCA statistical methods are in the Supplement. We also performed a standard multivariable regression analysis, to compare the results with those found from the LCA model.

The associations between LCA profiles and risk of asthma/lung function decline was determined using multivariable regression. Potential confounders, chosen with reference to the literature and causal modelling theory, were included in the final model if they changed the ORs or coefficients for the associations by more than 10%. For asthma, we adjusted for age, sex, occupation, exhaust fan and atopy at the 6th decade. For lung function decline, we adjusted for change of age, change of BMI, sex, exhaust fan and occupation at the 6th decade.

Two-way interactions between LCA defined exposure classes: and a) GST polymorphisms and b) atopy were assessed using likelihood ratio tests. The variable for atopy was initially tested as an effect modifier. There was no evidence to support interaction, so atopy was included as a potential confounder. P-values ≤0.1 were considered as evidence of interaction. If interactions were found, then stratified

analyses were presented. Stata® 14.2 (2015 Stata Corp, College Station, TX) was used for all analyses.

Results

The study sample included 1684 women (50.8%) and 1630 men (49.2%). Characteristics of those who participated in the 5th and 6th decade follow-ups are presented in Table 1. The proportion of people reporting current asthma increased from 11.3% in the 5th to 15.7% in the 6th decade. By the 6th decade there were 283 (8.6%) participants with new-onset asthma and 139 (4.2%) participants with remitted asthma. A comparison of 5th decade characteristics for the remaining participants with those lost to follow-up is presented in Table S1. Those who participated in both follow-ups were more likely to have been non-smokers, used gas ducted central heating or reverse cycle air-conditioning gas cooking, and to have reported visible mould than those lost to follow-up.

Latent class exposure profiles

We explored LCA models comprising between 3 and 8 classes. A 7-class model (AIC=3259, BIC=3540, log-likelihood=20640) was found to be the best fit (table S2). The 7 latent classes found were labelled “least exposed”, “wood heating”, “all gas”, “wood heating/smoking”, “all electric”, “wood & gas heating/gas cooking/smoking” and “wood heating/gas cooking”(Figure 3). The prevalence and characteristics of household profiles identified by LCA are shown in Table 2. The “least exposed” profile was chosen as the reference group. The prevalence of mould exposure was

similar for all profiles at both time points and therefore exposure to mould was removed from the model.

Main associations of HAP profiles with asthma

Several profiles were associated with increased risk of persistent asthma, compared with the reference profile (Table 3). The highest risk of persistent asthma was seen for the "all gas" (OR: 3.40, 95%CI 1.58, 7.32), followed by the "wood heating/gas cooking" (OR: 3.17, 95%CI 1.34, 7.47), "wood heating/smoking" (OR: 3.40, 95%CI 1.58, 7.32) and "wood heating" (OR: 2.04, 95%CI 1.04, 3.98). The profiles of "wood & gas heating/gas cooking/ smoking" and the "all electric" profile did not demonstrate evidence for this clinical association.

The profiles of "wood & gas heating/gas cooking/smoking" and "wood heating/smoking" were associated with new-onset asthma (OR: 2.34, 95%CI 0.99, 5.53; and 1.82, 95%CI 0.98, 3.38, respectively). In contrast, adults in the "wood heating/gas cooking" profile had a moderately decreased risk of new-onset asthma (OR 0.39, 95%CI 0.14, 1.06). We further analysed participant choices in this profile, finding that people who developed new-onset asthma were more likely to stop using wood heating and switching to other methods (37.0%), compared with those who never had asthma (33.2%). Similarly, people with new-onset asthma had a higher rate of change away from gas cooking to other cooking methods (44.4%), compared with those who did not develop asthma (23.1%).

It was possible that some of the "asthma" derived from self-report may have been fixed airway obstruction, and therefore meeting a definition of COPD. As a sensitivity analysis, we repeated the investigation after exclusion of those with fixed airway obstruction at the 5th and 6th decades (n=181) (supplement S3). Associations found were largely unchanged and similar to those using self-reported asthma outcomes. One difference was that the "wood heating/smoking" exposure profile was no longer associated with asthma outcomes, and further, the highest proportion of participants with COPD were found in this profile (13/45, 28.9%) (Table S3).

Main associations of HAP profiles with lung function decline

The profiles of "wood heating", "all gas", "wood heating/smoking", and "wood heating/gas cooking" were associated with increased lung function decline for FEV_1 and/or FVC. There was no evidence of association with decline in FEV_1/FVC (Table 4). Participants who were exposed to the "Wood heating/smoking" profile had the most lung function decline for pre-BD FEV_1 (β-coef representing change in z score: -0.38, 95%CI -0.65, -0.11) and FVC (-0.53 95%CI -0.81, -0.25) over 10 years follow-up, compared with reference. Post-BD lung function reduction followed a similar pattern to pre-BD (Table 4).

Individual household exposures

Logistic regression to assess associations between individual household exposures and asthma and lung function decline showed a number of similarities with the LCA

profile analysis. Ducted gas heating and smoking were associated with increased risk of asthma and increased lung function decline along with gas room heating(Tables S4 and S5). However, we found that consistent use of coal or wood for heating (at both times) (OR 0.63, 95%CI 0.39, 1.00) was associated with a reduced risk of new-onset asthma. The numbers of participants with COPD by the 6th decade were too small to allow a meaningful analysis of association with HAP exposures. In a limited analysis we only observed that active smoking was associated with development of COPD by the 6th decade (P<0.001).

Interactions by GST genotype/atopy

We used current asthma at the 6th decade for the GST genotype interaction analyses. Due to limited power, we were unable to stratify this analysis by persistent and new-onset asthma. There was no evidence of main associations between GST genes and asthma or lung function decline (Tables S6 and S7). However, we found significant evidence of interactions between HAP profiles, GSTP1 genotypes, and asthma. Thus, the profile "All gas" was associated with an increased risk of current asthma for participants with GSTP1 Ile/Ile genotype (OR=9.68), but this association was not seen for participants with Ile/Val and Val/Val genotypes (interaction p= 0.03) (Table 5). The profile "Wood heating/smoking" was also associated with a similarly increased risk of current asthma at the 6th decade for participants with the GSTP1 Ile/Ile genotype (interaction p = 0.02). We did not observe any interactions for GSTM1 or GSTT1 genotypes (Table S8).

We found significant interactions between HAP profiles and GST genotypes on lung function decline (Tables S9-11). Participants with GSTM1 null or GSTP1 with Ile/Ile genotypes were more likely to have greater lung function decline compared with other genotypes, when exposed to some HAP profiles (Table 6). The profile "Wood & gas heating/gas cooking/smoking" was associated with greater FEV_1/FVC decline for people with GSTM1 null genotypes, compared with those with GSTM1 present genotypes (interaction p value<0.01). The "wood heating/gas cooking" profile was associated with greater post-BD FEV_1 and FVC decline for those with GSTP1 Ile/Ile genotype (interaction p= 0.04 and <0.01, respectively). We also found some moderate evidence for increased post-BD FEV_1/FVC for those with GSTP1 Ile/Ile genotype when exposued to "all gas" and "wood heating/gas cooking" profiles (interaction p= 0.06 and 0.08,respectively). We did not observe evidence of interaction for GSTT1 genotypes.

We found no evidence of interaction by atopy.

Discussion

Using comprehensive data from TAHS participants studied for a decade during middle-age, we have identified seven HAP profiles of which four were associated with asthma, and four with lung function decline. Self-reported exclusive electric heating/cooking was the only profile not associated with these outcomes. Of the various combinations of cumulative wood/gas/smoking exposures most were associated with asthma and accelerated lung function decline. Notably, those with

GSTM1 null and GSTP1 Ile/Ile genotype were more predisposed to worsening lung function.

Risk reduction, including reducing the risk of persistent asthma and future adverse outcomes such as COPD, is an important goal of asthma management. Risk factors for accelerated decline in lung function and strategies related to reducing the impact of these risk factors are poorly identified in current guidelines (3-5). While GINA specifies HAP as a modifiable risk factor, there is no information on what sources of HAP are important. Including a list of potentially harmful exposures will help health care practitioners to advise their patients accordingly. Our study provides evidence supporting potential asthma prevention strategies, which may inform asthma guidelines and prevention of future COPD burden related to accelerated lung function decline.

The exposure profiles “wood heating”, “all gas”, “wood heating/smoking” and wood heating/gas cooking” were associated with increased risk of persistent asthma and accelerated FEV_1 and FVC decline over ten years’ follow-up. These findings are consistent with results from previous studies for a range of household risk factors and adverse respiratory health (24-29). Inefficient combustion of solid fuels (including wood) generates a complex mixture of carbon-based particles and gases that cause health effects in humans (30). Gas appliances also produce complex mixtures of airborne chemicals including volatile organic compounds, sulphur dioxide, water vapour, particulate matter (PM), carbon dioxide and oxides of

nitrogen (10). Cigarette smoke contains a mixture of up to 4000 individual chemical constituents that induce oxidative stress in the airways (31, 32). HAP may induce an inflammatory response through effects of ROS on alveolar epithelial cells (33). In this way HAP may be involved in the pathogenesis, and long term prognosis, of obstructive respiratory diseases and lung function deficits (30-32).

Our reference profile "reverse-cycle air conditioning, electric cooking and a non-smoking environment" was associated with the lowest risk of asthma and lung function decline. This finding is consistent with limited previous data on the beneficial effect with the use of non-polluting home heater (air-conditioning, flued gas, wood pellet burner) on respiratory health (34, 35). These findings may be explained by lack of generation of particulate matter by air conditioning and electric cooking. They may also be due to the associated air filtration in reverse cycle air conditioning removing airborne PM. There is some evidence that methods incorporating air filters can reduce household PM by an average of 50% (36). Absence of smoking could also reduce the risk of airway inflammation and enhance the responsiveness of asthma treatment (37).

Three multiple exposure profiles ("wood heating/smoking", "wood & gas heating/gas cooking/smoking" and "wood heating/gas cooking") were associated with increased respiratory health risk. The measures of association were higher for the dual exposure profile of "Wood heating/smoking" than the sum of the individual values for wood and smoking obtained from our logistic regression analysis,

suggesting there may be a synergistic effect which is not captured when investigating these exposures singly. More severe lung function decline was also seen for this profile when compared with the individual exposures. This observation was consistent with the findings of a previous cross-sectional study in Brazil. This study reported that adults exposed to both smoking and wood burning were at a far higher risk than those with single exposure to household air pollution in relation to asthma severity and reduced lung function (38).

There is some evidence that wood heating and gas cooking have adverse effects on respiratory health (30), but paradoxically, we found a moderately negative association between this combined exposure profile and new-onset asthma (p=0.06). We hypothesize that the mechanism may be similar to a "healthy survivor effect" (39), in that people in this HAP profile who developed new-onset asthma during the follow-up period were more likely to change their heating/cooking exposures than people who remained asthma free (as mentioned in results). These changes may have resulted in apparent reductions in asthma risk for those remaining in this profile. Even if a risk were occurring in the exposed group, it may be "hidden" from epidemiological measures of association due to this reversed-causation.

We found some evidence that the effects of HAP profiles were modified by GST gene polymorphisms. GSTM1 null genotypes were associated with increased lung function decline for FEV_1 and FVC. We found stronger evidence of an interaction

for GSTP1: Ile/Ile polymorphisms increased risk of both asthma and lung function decline for specific HAP profiles. Although the relationship between household pollutants and lung function did not differ between GSTT1 polymorphisms, the possibility of modification by GSTT1 was not excluded as there were few participants with both the GSTT1 null genotype and particular HAP profiles (eg "wood heating/smoking" (n=10)).

Strengths & Limitations

Identification of longitudinal HAP profiles using LCA is novel. Household exposures tend to cluster into patterns, so LCA, which considers multiple exposures together may be more useful in exposure risk profiling than considering the exposures individually. Additionally, HAP profiles can more comprehensively capture the complexities of HAP and may help identify a "lower exposed" category.

As TAHS is a large whole-of-population birth-cohort study, it would have been difficult and expensive to directly measure household air quality to capture differences in toxicity and concentrations of the pollutants generated by indoor air pollution sources, especially over time. Moreoever, there were 27.1% of participants living elsewhere in Australia by the 5th decade follow-up, making household air quality measurement impossible. As we measured exposures and outcomes across the same time period, we were unable to conclude the relationships were temporally related. Similarly, we were unable to account fully for participant-initiated lifestyle changes in exposures across the 10-year period,

possibly due to respiratory symptoms, and this may introduce a degree of misclassification, but any such bias would most likely be towards the null. Also, sample sizes were small for considering interactions for GST genotypes, reducing the power to find statistically significant differences between GST polymorphisms. Finally, we noted that some exposures were associated with loss to follow up eg. Smokers were more likely to be lost to follow-up, and this may have reduced the power to detect associations.

We recognise that asthma symptoms defined from questionnaires in this age group may represent fixed airway obstruction or COPD. A third of patients with persistent asthma may develop COPD when they are older than 40 years of age (40), some of these participants may have COPD without asthma, and the relationships with HAP profiles may differ for asthma and COPD. We therefore performed a sensitivity analysis after removal of participants with COPD. We found most COPD cases in the "Wood heating/smoking" profile. This finding is consistent with other studies that have found evidence that smoking history and biomass exposure are key risk factors for COPD (41, 42). HAP profiles may be associated with development of COPD, but we were unable to robustly investigate this relationship in the TAHS due to the small numbers with COPD.

conclusion

We identified longitudinal HAP profiles associated with the risk of obstructive lung diseases and greater lung function decline in middle-age. The high risk profiles

included “Wood heating”, “All gas”, “Wood heating/smoking”, “Wood & gas heating/gas cooking/smoking” and “Wood heating/gas cooking”. Our findings also support a synergistic effect of multiple exposures on respiratory health compared with single exposures. We found some evidence of individual susceptibility to HAP for carriers of GST risk genotypes, as exposed participants with GSTP1 with Ile/Ile genotypes, and GSTM1 null genotypes had greater risks of persistent asthma and increased lung function decline. Our findings provide further evidence of the importance of clean household air, particularly for those who are at high individual risk, and have the potential to inform evidence-based strategies for asthma and COPD guidelines.

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Table 1 Characteristics of study sample

	Participants at both follow-ups (N=3314)	
	5th decade follow-up (average age 43 years)	6th decade follow-up (average age 53 years)
Age (SD)	42.7 (0.82)	53.0 (0.93)
Men (%)	1630 (49.2)	1630 (49.2)
Tertiary education (%)	1790 (53.9)	1872 (59.4)
GSTM1 null (%)	500 (53.8)	500 (53.8)
GSTT1 null (%)	158 (17.0)	158 (17.0)
GSTP1 Ile/Ile alleles (%)	372 (40.1)	372 (40.1)
Current asthma (%)	376 (11.3)	520 (15.7)
Ducted gas central heating (%)	215 (6.5)	261 (7.9)
Wood or coal fire (%)	1461 (44.1)	1216 (36.7)
Gas room heater (%)	386(11.6)	314 (9.7)
Electric heater (%)	1279 (38.6)	990 (30.6)
Reverse cycle air conditioning (%)	715 (21.6)	1578 (47.6)
Gas cooking (%)	783 (23.6)	927 (28.0)
Electric cooking (%)	2507 (75.6)	2276 (68.7)
Mould in last 12 months (%)	1250 (37.7)	1135 (34.9)
Active smoking (%)	793 (24.0)	545 (16.6)
Passive smoking (%)	391 (10.5)	261 (8.0)

Table 2 Prevalence and characteristics of Household Air Pollution (HAP) profiles identified by Latent Class Analysis (LCA).

Series number	Profile name	N (Prevalence)	Description
1	Least exposed	453 (13.7%)	Highest probability of reverse cycle air conditioning, electric cooking, and lowest probability of smoking and other risk factors.
2	Wood heating	952 (28.7%)	Highest probability of coal or wood fire for heating at both surveys, but probability of other risk factors was similar to “least exposed”.
3	All gas	538 (16.2%)	Higher probability of gas used for heating and cooking at both surveys, the probability of other risk factors was similar to “least exposed”.
4	Wood heating/smoking	446 (13.4%)	Highest probability of current smoking at both surveys, moderate probability of coal or wood fire for heating at both surveys. The probability of other risk factors was similar to “least exposed”.
5	All electric	450 (13.6%)	Highest probability of electric heating, lowest probability of gas cooking indicating higher probability of electric cooking. The probability of other risk factors was similar to “least exposed”.
6	Wood & gas heating/gas cooking/smoking	157 (4.7%)	Highest probability of current smoking at both surveys, moderate probability of gas cooking and wood heating. The probability of other risk factors was similar to “least exposed”.
7	Wood heating/gas cooking	323 (9.7%)	Higher probability of wood heating and moderate probability of gas cooking at both surveys. The probability of other risk factors was similar to “least exposed”.

Table 3 Associations between longitudinal indoor exposure profiles and asthma outcomes by the 6th decade

HAP exposure profiles	N of exposed	New Onset asthma			Persistent asthma		
		N	ORs+	P	N	ORs+	P
Least exposed (Reference*)	453	29	1		26	1	
Wood heating	952	50	0.91 (0.56, 1.64)	0.76	91	**2.04 (1.04, 3.98)**	**0.04**
All gas	538	37	1.15 (0.61, 2.16)	0.67	56	**3.40 (1.58, 7.32)**	**<0.01**
Wood heating and smoking	446	45	1.82 (0.98, 3.38)	0.06	48	**2.83 (1.27, 6.35)**	**0.01**
All electric	450	38	1.59 (0.86, 2.93)	0.14	34	1.46 (0.68, 3.16)	0.33
Wood & gas heating/gas cooking/smoking	157	13	**2.34 (0.99, 5.53)**	**0.05**	17	1.35 (0.45, 3.99)	0.59
Wood heating/gas cooking	323	10	0.39 (0.14, 1.06)	0.06	28	**3.17 (1.34, 7.47)**	**0.01**

*Reference group included people used electric cooking, air conditioning for heating, and no smoking

+ All ORs were adjusted for age, sex, atopy, exhaust fan use and occupation at the 6th decade.

Baseline outcome for new onset asthma was participants who had never reported asthma in previous surveys, baseline outcome for persistent asthma was participants who had remitted asthma at the 6th decade.

Table 4 the associations* between HAP profiles and lung function decline (Beta coefficients from linear regression, representing change in z scores) between the two follow-ups.

HAP exposure profiles	**Pre-bronchodilator**			**Post-bronchodilator**		
	FEV_1	FVC	FEV_1/FVC	FEV_1	FVC	FEV_1/FVC
Least exposed (reference)	0	0	0	0	0	0
Wood heating	-0.08 (-0.27, 0.12)	-0.19 (-0.39, 0.02)	0.16 (-0.08, 0.39)	**-0.22 (-0.42, -0.02)**	-0.19 (-0.39, 0.02)	-0.01 (-0.23, 0.20)
All gas	-0.19 (-0.42, 0.03)	**-0.34 (-0.58, -0.11)**	0.19 (-0.07, 0.46)	**-0.24 (-0.47, -0.01)**	**-0.33 (-0.56, -0.09)**	0.14 (-0.12, 0.39)
Wood heating and smoking	**-0.38 (-0.65, -0.11)**	**-0.53 (-0.81, -0.25)**	0.17 (-0.15, 0.49)	**-0.30 (-0.57, -0.02)**	-0.26 (-0.54, 0.02)	0 (-0.30, 0.30)
All electric	-0.02 (-0.25, 0.22)	-0.09 (-0.33, 0.15)	0.10 (-0.18, 0.37)	-0.04 (-0.28, 0.19)	-0.06 (-0.30, 0.18)	0 (-0.25, 0.26)
Wood & gas heating/gas cooking/smoking	-0.28 (-0.69, 0.12)	-0.37 (-0.79, 0.06)	0.06 (-0.42, 0.53)	-0.33 (-0.77, 0.11)	-0.38 (-0.83, 0.07)	0.12 (-0.35, 0.60)
Wood heating/gas cooking	-0.16 (-0.41, 0.09)	**-0.26 (-0.52, 0)**	0.14 (-0.15, 0.43)	-0.23 (-0.48, 0.03)	**-0.27 (-0.53, -0.01)**	0.07 (-0.21, 0.35)

*Adjusted for sex, change of age, change of BMI, exhaust fan use and occupation at the 6th decade.

Table 5 Associations between longitudinal HAP profiles and current asthma at the 6th decade stratified by GSTP1.

HAP exposure profiles	**Current asthma, OR (95%CI)**		
	GSTP1 Ile/Val and Val/Val	GSTP1 Ile/Ile	Interaction p values
Least exposed (Reference*)	1	1	
Wood heating	1.45 (0.60, 3.48)	3.94 (0.75, 20.9)	0.17
All gas	1.25 (0.40, 3.92)	**9.68 (1.54, 60.7)**	**0.03**
Wood heating and smoking	1.30 (0.45, 3.77)	**11.02 (1.27, 95.9)**	**0.02**
Electric heating	1.65 (0.63, 4.31)	2.08 (0.16, 27.04)	0.96
Wood & gas heating/gas cooking/smoking	2.30 (0.48, 11.10)	-	0.66
Wood heating/gas cooking	1.39 (0.42, 4.62)	4.14 (0.38, 44.54)	0.66

*Adjusted for age, sex, occupation, atopy at the 6th decade, exhaust fan and asthma status at the 5th decade.

Table 6 Associations between HAP profiles and lung function decline by GST genotype (β coefficients representing change in z scores)

			Change of lung function		P interaction
			GSTM1 present	**GSTM1 null**	
Wood heating and smoking	Post-BD	FEV_1/FVC	-0.39 (-0.82, 0.03)	0.26 (-0.32, 0.84)	0.03
Wood & gas heating/gas cooking/smoking	Pre-BD	FEV_1/FVC†	**0.87 (0.21, 1.53)**	-0.51 (-1.31, 0.28)	<0.01
			GSTT1 present	**GSTT1 null**	
Wood heating/smoking	Pre-BD	FEV_1	-0.21 (-0.58, 0.16)	-0.97 (-2.38, 0.44)	0.08
		FVC	-0.28 (-0.64, 0.08)	-1.99 (-7.41, 3.44)	<0.01
		FEV_1/FVC	0.03 (-0.39, 0.44)	1.60 (-6.46, 9.66)	<0.01
	Post-BD	FVC	-0.09 (-0.42, 0.25)	-0.53 (-6.10, 5.04)	0.02
		FEV_1/FVC	-0.10 (-0.44, 0.24)	0.31 (-5.89, 6.12)	0.02
			GSTP1 Ile/Val and Val/Val	**GSTP1 Ile/Ile**	
All gas	Post-BD	FVC†	-0.22 (-0.52, 0.07)	**-0.70 (-1.19, -0.21)**	0.08
		FEV_1/FVC	0.06 (-0.23, 0.34)	**0.74 (0.18, 1.31)**	0.06
All electric	Post-BD	FEV_1	0.16 (-0.11, 0.43)	0.13 (-0.42, 0.67)	0.05
		FVC	0.26 (-0.03, 0.55)	-0.15 (-0.81, 0.51)	<0.01
		FEV_1/FVC	-0.22 (-0.58, 0.13)	0.44 (-0.42, 1.31)	0.06
Wood & gas heating/gas cooking/smoking	Post-BD	FEV_1/FVC	-0.19 (--0.80, 0.43)	0.71 (-0.42, 1.83)	0.08
Wood heating/gas cooking	Pre-BD	FEV_1†	-0.01 (-0.34, 0.33)	-0.44 (-0.91, 0.03)	0.10
	Post-BD	FEV_1†	0 (-0.32, 0.33)	**-0.46 (-0.87, -0.05)**	0.04
		FVC†	0.02 (-0.32, 0.35)	**-0.75 (-1.17, -0.33)**	<0.01
		FEV_1/FVC	-0.03 (-0.33, 0.28)	**0.54 (0.07, 1.01)**	0.08

*Adjusted for sex, change of age, change of BMI, exhaust fan and occupation at the 6th decade.

†Significant interactions (Criteria for interaction: 1. Interaction p<0.1; 2. P value for stratified association<0.05)

Figure 1 Flowchart for TAHS

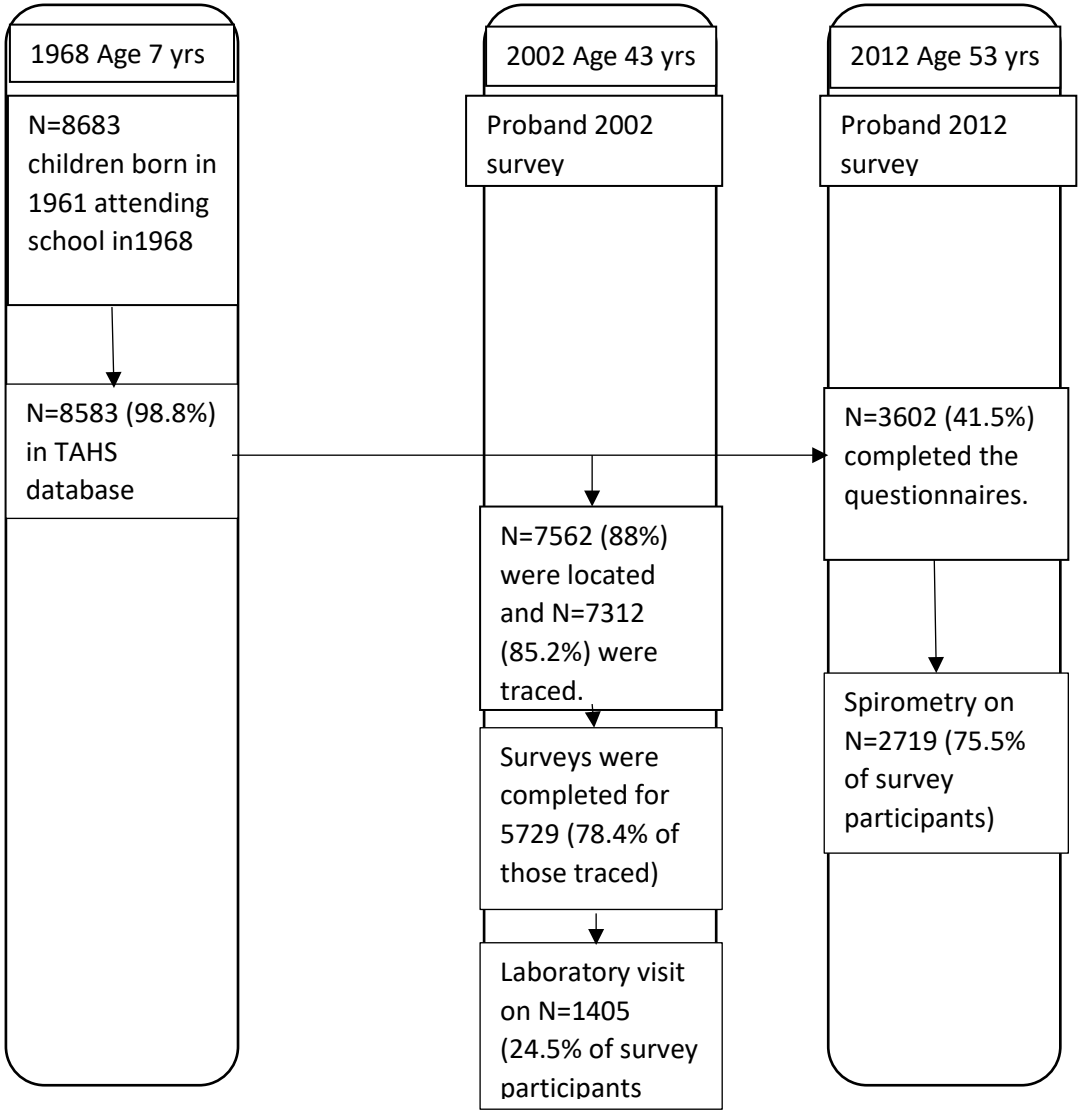


Figure 2 Flowchart for TAHS proband in the 5th to the 6th decade stratified by asthma status

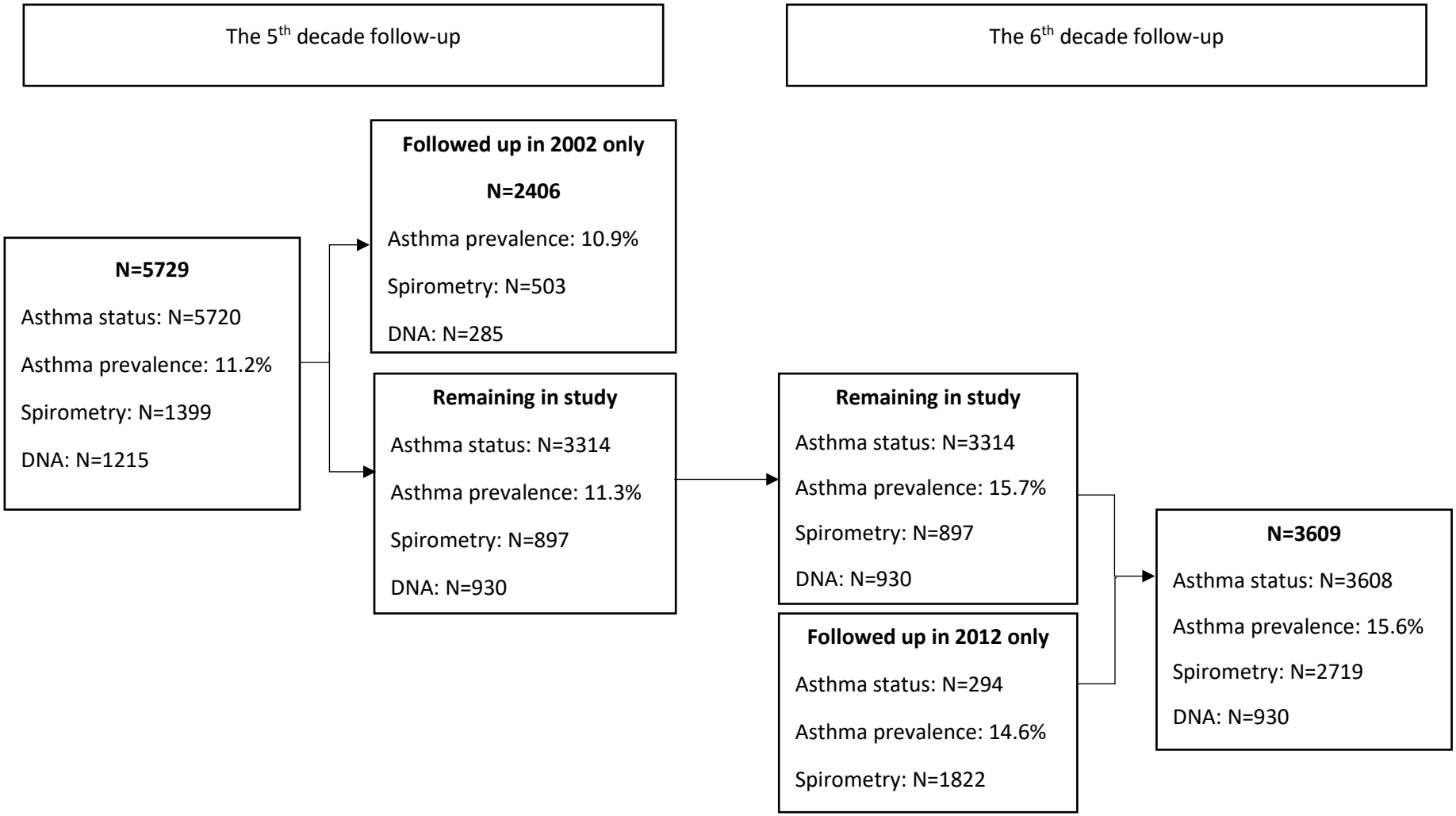
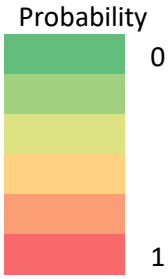


Figure 3 Heatmap for household risk profiles identified by LCA

	Reference	Wood heating	Gas cooking and heater	Wood heating and smoking	Electric heater	Gas heating, cooking and smoking	Gas cooking and wood heating
Gas cooking†2002	0.097967	0.052981	0.731927	0.010941	0.033405	0.685639	0.542972
Gas cooking2012	0.175787	0.000724	0.733367	0.100769	0.058942	0.601905	0.977605
Gas heater*2002	0.044326	0.010293	0.690142	0.019700	0.033084	0.382615	0.037042
Gas heater2012	0.050936	0.032974	0.582789	0.018023	0.018226	0.311650	0.108389
Elect. heater2002	0.195569	0.061132	0.108247	0.284174	0.633246	0.084451	0.107085
Elect. heater2012	0.001012	0.104458	0.089589	0.231450	0.630050	0.101571	0.071864
Wood heater2002	0.193059	0.847641	0.002129	0.548447	0.010398	0.380418	0.817756
Wood heater2012	0.008782	0.718974	0.088526	0.484094	0.009973	0.295981	0.692834
Other heater2002	0.068329	0.031250	0.103524	0.039782	0.167030	0.064648	0.000113
Other heater2012	0.051987	0.021274	0.060906	0.035623	0.124002	0.091955	0.036795
current smoke2002	0.098923	0.059717	0.083414	0.952337	0.090313	0.938638	0.058855
current smoke2012	0.031173	0.016896	0.021117	0.801040	0.018780	0.809814	0.011036
Passmoke2002	0.095545	0.081242	0.029824	0.372942	0.047056	0.372113	0.037856
Passmoke2012	0.073546	0.047937	0.019196	0.238957	0.031736	0.240849	0.039507
Mould2002	0.291937	0.387128	0.371624	0.352833	0.483483	0.298111	0.410020
Mould2012	0.235971	0.396789	0.292658	0.349097	0.455920	0.261450	0.360703



*Gas heater combined gas heater and ducted gas heating.

†For cooking choices, over 98% of participants used gas or electric cooking at both surveys. The choices were mutually exclusive with participants indicating which form of energy was predominantly used. We modelled with gas cooking as the exposure group and electric as reference, thus only gas appears in heat map.

Supplement

Section 1- Variables from questionnaires

Heating exposures were defined by selection of one or more of the following categories: “Gas ducted central heating”, “Coal or wood fire”, “Gas room heater”, “Electric heater (eg. Radiator, fan or Dimplex-type)”, “other central heating (eg. Electric hydronic, slab floor heating)”, “Reverse cycle air conditioning”, and “Other”;

Cooking exposures were defined by the question “What kind of stove do you mostly use for cooking”: “Gas”, “Electric”, “Coal, coke or wood”, or “Other”;

Mould exposure was defined from the question “Has there been mould or mildew on any surface, other than food, in your home in the last 12 months?”;

Active current smoking was identified by any smoking within the last 4 weeks; passive smoking was identified by any currently active smoking inside the house but not counting yourself.

Exposure status was categorized as consistent exposure and no exposure. Consistent exposure to any category was defined by a positive answer for that category on both the 5th and 6th decade questionnaires. No exposure was defined as a negative answer at both time points.

New onset asthma: Participants were determined to have new-onset asthma if they had never reported asthma previously, but reported current asthma at the 6th decade.

Persistent asthma: Participants were determined to have persistent asthma if they had reported asthma during the study up until the 5th decade and also reported current asthma at the 6th decade.

Lung function

Lung function at the 5th and 6th decade was measured with an EasyOne ultrasonic spirometer (NDD Medizintechnik AG, Zürich, Switzerland). Participants were asked not to smoke for 4-6 hours prior to testing. Spirometry was repeated 10 minutes after 200 μg of salbutamol was administered via a spacer. Forced expiratory volume (FEV_1) and Forced vital capacity (FVC) were recorded as the best of three manoeuvres.

Other variables

As a proxy for **socioeconomic status (SES),** occupation recorded at the 6th decade and coded according to the International Standard Classification of Occupations (ISCO-88) four-digit classification

Atopy was defined as an average Skin Prick Tests (SPTs) wheal diameter of 3mm or greater for 1 or more of the allergens tested at the 6th decade survey. Skin Prick Tests (SPTs) were performed at the TAHS 6th decade of follow-up by using a standard technique (ref). In total, 10 allergens were included in the testing: *house dust mite, cat, hormodendrum, alternaria, penicillium, aspergillus, rye grass, mixed grasses, egg white, peanut, shellfish and cow's milk.* Histamine was used as a positive control in SPTs. Saline was used as the negative control .Trained research scientists performed all the SPTs and a wheal size of ⩾3 mm or more than the negative control was regarded as an indication of atopy.

COPD was defined as post-BD FEV1/FVC< lower limit of normal (LLN) derived from Global Lung Initiative (GLI) reference equations for Caucasian ethnicity, plus at least one of the key indicators (shortness of breath at rest or after exercise, chronic cough or chronic sputum production; a history of smoking ⩾10 packyears; occupational exposure to vapour/gas/dust/fumes; a family history of COPD) (3).

Genotypes of GSTM1 null and GSTT1 null were detected using a multiplex polymerase chain reaction (PCR) technique as described previously(43) , and for all experiments positive primers for β-globin were included as a positive PCR control. A customised GoldenGate genotyping assay (Illumina, San Diego, CA, USA) was used to genotype the GSTP1 (resulting in Ile105Val at codon 105) polymorphism.

LCA methods: The final number of latent classes was determined by model fit criteria comparing models with increasing numbers of classes starting from a 2-class model (Table S3). All model fit parameters were considered in choosing the final model (although precedence was given to the Bayesian information criterion (BIC)), along with plausibility of the classes found and mean class membership.

Table S1 Characteristic at the 5th decade for participants followed to the 6th decade and those not followed

Participants at 5th decade (N=5720)		
	Participants followed–up to the 6th decade	**Participants lost to follow up**
N	3314	2406
Age	42.7 (41-44)	43.0 (42-44)
Men	1630 (49.2)	1318 (54.8)
Tertiary education	1790 (53.9)	975 (40.6)
GSTM1 null	481 (53.7)	165 (57.7)
GSTT1 null	150 (16.8)	40 (14.0)
GSTP1 Ile/Ile alleles	352 (39.4)	116 (40.3)
Current asthma	376 (11.3)	262 (10.9)
***Ducted gas central heating**	215 (6.5)	124 (5.2)
Wood or coal fire	1461 (44.1)	1012 (42.1)
Gas room heater	386(11.6)	246 (10.2)
Electric heater	1279 (38.6)	908 (37.7)
***Reverse cycle air conditioning**	715 (21.6)	426 (17.7)
***Gas cooking**	783 (23.6)	487 (20.2)
***Electric cooking**	2507 (75.6)	1889 (78.5)
***Mould in last 12 months**	1250 (37.7)	735 (30.8)
***Active smoking**	793 (24.0)	908 (38.0)
***Passive smoking**	391 (10.5)	447 (19.3)

*factors significantly differed between those lost and not lost to follow-up. Participants who attended both follow-ups were more likely to: use gas ducted central heating ($p=0.04$), reverse cycle air-conditioning ($p<0.001$), gas cooking ($p=0.01$), and report visible mould ($p<0.000$); and less likely to: use electric cooking ($p=0.004$), be smokers ($p<0.000$) or be exposed to passive smoking ($p<0.000$).

Table S2 Model fit characteristics for 3-8 class models.

No of class	Fit criteria					Class size							
	Entropy	BIC	AIC	Adjusted BIC	Log-likelihood	1	2	3	4	5	6	7	8
3	0.81	4714	4408	4555	-21357	1944	578	797					
4	0.78	4208	3799	3995	-21035	644	1246	867	562				
5	0.79	4077	3564	3810	-20901	524	1275	394	537	589			
6	0.82	4005	3388	3684	-20795	441	975	866	337	163	537		
7	0.8	**3969**	3259	**3540**	**-20640**	453	952	538	446	450	157	323	
8	0.79	3980	3145	3605	-20714	532	887	435	518	324	282	224	117

Table S3 Associations between longitudinal indoor exposure profiles and asthma outcomes at the 6th decade (excluding COPD).

Grouped environment exposures	N of exposed	New Onset asthma			Persistent asthma		
		N	ORs^{+}	P	N	ORs^{+}	P
Least exposed (Reference*)	440	29	1		20	1	
Wood heating	919	47	0.80 (0.45, 1.42)	0.45	77	1.90 (0.94, 3.83)	0.07
All gas	527	36	0.99 (0.53, 1.85)	0.99	49	**2.92 (1.32, 6.48)**	**0.01**
Wood heating and smoking	375	32	1.37 (0.71, 2.63)	0.35	29	1.73 (0.66, 4.57)	0.27
All electric	436	35	1.31(0.71, 2.40)	0.38	31	1.40 (0.62, 3.12)	0.42
Wood & gas heating/gas cooking/smoking	142	11	2.11 (0.87, 5.12)	0.10	12	0.62 (0.15, 2.50)	0.50
Wood heating/gas cooking	314	7	**0.21 (0.06, 0.73)**	**0.01**	26	**3.15 (1.30, 7.65)**	**0.01**

*Reference group included people used electric cooking, air conditioning for heating and no smoking

Table S4 Adjusted ORs* for indoor environmental exposures in the 5th to the 6th decade for participants with and without asthma at the 5th decade and asthma outcomes at the 6th decade

Consistent household exposures	**N of exposed**	**New onset asthma**		**Persistent asthma**	
		ORs	P	ORs	P
Ducted gas central heating	140	0.89 (0.33, 2.37)	0.81	**5.84 (1.73, 19.78)**	**0.01**
Never (ref)	2983	1		1	
Wood or coal fire	967	**0.63 (0.39, 1.00)**	**0.05**	1.13 (0.70, 1.80)	0.62
Never (ref)	4306	1		1	
Gas room heater	166	1.36 (0.68, 2.72)	0.39	0.99 (0.38, 2.56)	0.99
Never (ref)	2787	1		1	
Electric heater	590	1.30 (0.80, 2.13)	0.29	0.84 (0.49, 1.45)	0.54
Never (ref)	1636	1		1	
Reverse cycle air conditioning	553	1.32 (0.77, 2.27)	0.31	1.33 (0.71, 2.48)	0.37
Never (ref)	1578	1		1	
Gas cooking	547	0.61 (0.36, 1.03)	0.07	1.07 (0.71, 1.61)	0.74
Never (ref)	2106	**1**		1	
Electric cooking	2069	1.50 (0.90, 2.49)	0.12	0.92 (0.62, 1.38)	0.70
Never (ref)	575	1		1	
Mould exposure	619	1.02 (0.65, 1.60)	0.93	1.01 (0.62, 1.66)	0.94
Never	1512	1		1	
Active smoking	477	**2.57 (1.57, 4.21)**	**<0.01**	1.09 (0.56, 2.10)	0.80
Never	1450	1		1	
Passive smoking	117	1.40 (0.58, 3.36)	0.45	0.77 (0.23,2.52)	0.66
Never	2694	1		1	

*All exposures were adjusted for age, sex, atopy and occupation, heating appliances were adjusted for other heating appliance use, cooking appliances were adjusted for vented fan use over the stove, active smoking was adjusted for passive smoking at home; passive smoking was adjusted for active smoking.

Table S5 Adjusted coefficients[+] for consistent household exposures and lung function decline between the 5th and 6th decade follow-ups.

Consistent household exposures	Pre-Bronchodilator			Post-bronchodilator		
	FEV1	FVC	FEV1/FVC	FEV1	FVC	FEV1/FVC
Ducted gas central heating	**-0.39 (-0.78, -0.01)**	**-0.52 (-0.93, -0.11)**	0.12 (-0.33, 0.56)	-0.37 (-0.76, 0.03)	**-0.63 (-1.03, -0.23)**	0.39 (-0.03, 0.81)
Never (ref)	0	0	0	0	0	0
Wood or coal fire	-0.20 (-0.44, 0.03)	-0.01 (-0.18, 0.17)	0.05 (-0.13, 0.22)	-0.12 (-0.35, 0.11)	-0.04 (-0.20, 0.12)	-0.05 (-0.22, 0.13)
Never (ref)	0	0	0	0	0	0
Gas room heater	-0.20 (-0.52, 0.11)	**-0.39 (-0.72, -0.06)**	0.33 (-0.02, 0.69)	-0.28 (--0.60, 0.04)	-0.31 (-0.63, 0.01)	0.09 (-0.25, 0.42)
Never (ref)	0	0	0	0	0	0
Electric heater	-0.10 (-0.28, 0.08)	-0.09 (-0.27, 0.09)	-0.10 (-0.20, 0.18)	-0.15 (-0.33, 0.03)	-0.14 (-0.33, 0.06)	0.01 (-0.19, 0.20)
Never (ref)	0	0	0	0	0	0
Reverse cycle air conditioning	0.07 (-0.11, 0.24)	0.04 (-0.15, 0.22)	0.05 (-0.16, 0.26)	0.04 (-0.14, 0.22)	-0.03 (-0.24, 0.18)	0.16 (-0.06, 0.37)
Never (ref)	0	0	0	0	0	0
Gas cooking	0 (-0.18, 0.17)	-0.07 (-0.26, 0.13)	0.07 (-0.13, 0.28)	0.01 (-0.17, 0.19)	-0.08 (-0.26, 0.11)	0.10 (-0.10, 0.30)
Never (ref)	0	0	0	0	0	0
Electric cooking	0.04 (-0.13, 0.22)	0.08 (-0.10, 0.27)	-0.03 (-0.23, 0.18)	0.05 (-0.14, 0.23)	0.15 (-0.04, 0.33)	-0.11 (-0.31, 0.08)
Never (ref)	0	0	0	0	0	0
Mould exposure	-0.03 (-0.21, 0.14)	-0.10 (-0.28, 0.08)	0.06 (-0.14, 0.27)	0.14 (-0.32, 0.04)	0.14 (-0.33, 0.04)	0.04 (-0.15, 0.22)
Never	0	0	0	0	0	0
Active smoking	**-0.35 (-0.59, -0.10)**	**-0.34 (-0.61, -0.07)**	-0.08 (-0.37, 0.22)	**-0.25 (-0.50, 0)**	-0.11 (-0.37, 0.14)	-0.13 (-0.43, 0.16)
Never	0	0	0	0	0	0
Passive smoking	-0.60 (-1.14, -0.06)	-0.13 (-0.72, 0.45)	**-0.84 (-1.45, -0.23)**	**-0.78 (-1.33, -0.23)**	-0.54 (-1.13, 0.05)	-0.36 (-0.90, 0.19)
Never	0	0	0	0	0	0

*All associations were adjusted for sex, change of age, change of BMI and occupation, heating appliances were adjusted for other heating appliance use, cooking appliances were adjusted for exhaust fan use, active smoking was adjusted for passive smoking at home; passive smoking was adjusted for active smoking.

Table S6 the associations between GST genes and lung function decline (beta coef for change in z-score) between the 5th and 6th decades.

	Pre-Bronchodilator			Post-bronchodilator		
	FEV1	FVC	FEV1/FVC	FEV1	FVC	FEV1/FVC
GSTM1	0 (-0.13, 0.13)	-0.02 (-0.16, 0.12)	0.04 (-0.11, 0.19)	0.08 (-0.06, 0.21)	0.09 (-0.04, 0.23)	-0.04 (-0.19, 0.10)
GSTT1	0.14 (-0.05, 0.32)	0.13 (-0.07, 0.33)	0.04 (-0.18, 0.25)	0.07 (-0.12, 0.26)	0.16 (-0.04, 0.35)	-0.03 (-0.24, 0.17)
GSTP1	0 (-0.13, 0.13)	-0.04 (-0.18, 0.10)	0.03 (-0.12, 0.19)	0.06 (-0.08, 0.20)	0.05 (-0.10, 0.19)	0.01 (-0.14, 0.16)

*Coefficients were adjusted for sex, change of age, change of BMI and occupation at 6th decade.

Table S7 the associations between GST genes and current asthma/wheeze outcomes at the 6th decade

	Current asthma		Current wheeze	
	OR	P	OR	P
GSTM1	0.90 (0.66, 1.23)	0.49	0.88 (0.65, 1.18)	0.39
GSTT1	0.71 (0.46, 1.11)	0.14	0.91 (0.61, 1.36)	0.66
GSTP1	0.87 (0.63, 1.20)	0.41	1.82 (0.60, 1.11)	0.19

*ORs were adjusted for sex, age, and occupation at 6th decade survey.

Table S8 Interaction P values on associations between longitudinal HAP profiles and current asthma at the 6th decade.

Grouped environmental exposures	Current asthma		
	GSTM1	GSTT1	GSTP1
Wood heating only	0.82	0.75	0.17
Gas cooking and heater	1.00	0.63	**0.01**
Wood heating and smoking	0.72	0.67	**0.08**
Electric heater	0.80	0.67	0.96
Gas heating, cooking and smoking	0.29	0.74	0.65
Gas cooking and wood heating	0.53	0.93	0.66

*Adjusted for age, sex, occupation and atopy at the 6th decade, exhaust fan and asthma status at the 5th decade.

Table S9 Interaction p values for associations between longitudinal HAP profiles and lung function decline for GSTM1.

Grouped environment exposures	**Pre-bronchodilator**			**Post-bronchodilator**		
	FEV1	FVC	FEV1/FVC	FEV1	FVC	FEV1/FVC
Least exposed (reference)	1	1	1	1	1	1
Wood heating	0.92	0.85	0.91	0.63	0.99	0.47
All gas	0.46	0.39	0.49	0.87	0.26	0.15
Wood heating and smoking	0.80	0.44	0.43	0.23	0.87	**0.03**
All electric	0.49	1.00	0.41	0.65	0.29	0.36
Wood & gas heating/gas cooking/smoking	0.17	0.69	**<0.01**	0.89	0.88	0.85
Wood heating/gas cooking	0.81	0.72	0.76	0.30	0.37	0.76

*Adjusted for sex, change of age, change of BMI, exhaust fan and occupation at the 6th decade.

Table S10 Interaction p values for associations between HAP profiles and lung function decline for GSTT1.

Grouped environment exposures	**Pre-bronchodilator**			**Post-bronchodilator**		
	FEV1	FVC	FEV1/FVC	FEV1	FVC	FEV1/FVC
Least exposed (reference)	1	1	1	1	1	1
Wood heating	0.48	0.85	0.85	0.30	0.55	0.61
All gas	0.29	0.29	0.70	0.24	0.14	0.54
Wood heating and smoking	**0.03**	**<0.01**	**<0.01**	0.57	**0.05**	**0.01**
All electric	0.40	0.30	0.68	0.26	0.53	0.72
Wood & gas heating/gas cooking/smoking	0.34	0.80	0.13	0.55	0.80	0.64
Wood heating/gas cooking	0.20	0.27	0.70	0.35	0.36	0.56

*Adjusted for sex, change of age, change of BMI, exhaust fan and occupation at the 6th decade.

Table S11 Interaction p values for associations between HAP profiles and lung function decline for GSTP1.

Grouped environment exposures	**Pre-bronchodilator**			**Post-bronchodilator**		
	FEV1	FVC	FEV1/FVC	FEV1	FVC	FEV1/FVC
Least exposed (reference)	1	1	1	1	1	1
Wood heating	0.25	0.18	0.72	0.86	0.38	0.30
All gas	0.36	0.34	0.95	0.50	**0.08**	**0.06**
Wood heating and smoking	0.34	0.44	0.70	0.56	0.58	0.94
All electric	0.22	0.14	0.74	**0.05**	**<0.01**	**0.06**
Wood & gas heating/gas cooking/smoking	0.44	0.98	0.16	0.41	0.90	**0.08**
Wood heating/gas cooking	**0.10**	0.21	0.38	**0.04**	**<0.01**	**0.08**

*Adjusted for sex, change of age, change of BMI, exhaust fan and occupation at the 6th decade.

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Chapter 8 OVERALL DISCUSSION, IMPLICATIONS AND CONCLUSION

The findings from each research question have been discussed in detail within each individual chapter. In this discussion chapter, I will:

1. Summarize the findings from my four research aims.
2. Bring together the findings from my four research aims and discuss aspects that were not published due to the word limits for published manuscripts.
3. Discuss the overall strengths and limitations concerning study design, exposure and outcome data, confounding and interaction issues.
4. Explain how my research has addressed key knowledge gaps.
5. Discuss the implications of my research and make recommendations.

8.1 Thesis summary

Asthma is responsible for a significant health and economic burden in Australia. It affects people of all ages and is associated with an increased risk of COPD in later life. Household exposures are thought to be important determinants, but there are very few longitudinal studies focusing on common household exposures, particularly those that influence indoor air quality, and their relationships with asthma and lung function in adolescence and adulthood. Moreover, the potential influence of the interactions between household exposures and antioxidant genes on respiratory health is still a novel area of research. There is a critical need for studies that can provide robust evidence concerning common household exposures, their interactions with genetic determinants and potential influence on respiratory health. This knowledge would inform preventive strategies and guidelines, targeting people who are at increased risk.

My doctoral research examined the influence of common household exposures on the risk of asthma and impaired lung function and investigated whether this relationship was modified by GST genes. I utilized two prospective cohort studies, MACS and TAHS, to identify potential risk factors for lung health in susceptible populations.

The summary of my findings can be seen in Table 8.1.

Table 8.1 Summary of findings from the four projects in my research program

	Research type	Oxidative exposure	Respiratory outcome	Outcome age	Main association	Interaction found	Risk alleles
1	Systematic review	Indoor air pollution	Asthma, allergies and lung function	Childhood and adulthood	N/A- Not the subject of the review.	Yes, the majority of studies investigating whether GST genes modify the link between indoor air pollution and asthma, lung function and allergies supported the presence of a gene-environment interaction	GSTM1 null, GSTT1 null and GSTP1 105 Val alleles
2	Data analysis	Early life smoking	Asthma and lung function	18 years	Yes, girls were more susceptible (Previously published -Masters research program).	Yes, on lung function only. (New PhD research)	GSTM1 null, GSTT1 null and GSTP1 Ile/Ile alleles
3	Data analysis	Early life paracetamol use	Asthma and lung function	18 years	Yes, but was confounded by early life RTIs.	Yes, on both asthma and lung function outcomes.	GSTM1 null, GSTT1 present and GSTP1 Ile/Ile alleles
4	Data analysis	Household air pollution	Asthma, COPD and lung function	Middle-aged adulthood	Yes.	Yes, on asthma, COPD and lung function outcomes	GSTM1 null and GSTP1 Ile/Ile alleles

The specific research aims and key findings of my doctoral work were:

Research Aim 1 (Chapter 3)**:** To systematically synthesise the current evidence for gene-environment interactions on the risk of asthma, reduced lung function and allergies, focusing on indoor air pollution and one of the most commonly explored antioxidant genes – GST.

Key findings: My review showed that the majority of studies investigating whether GST genes modify the link between indoor air pollution and asthma, lung function and allergies, supported the presence of a gene-environment interaction. Overall, there was evidence that carriers of GSTM/T1 null and GSTP1 Val genotypes were more susceptible to indoor air pollution exposure, having a higher risk of asthma and lung function deficits. However, findings differed in terms of specific risk alleles and exposure types. The major gaps in the literature identified from this review were the imprecision of pollution exposure assessments and the lack of evidence on age-specific effects. Prospectively collected evidence from longitudinal studies is important as collection of data on indoor pollution and asthma status is unaffected by recall bias. Further studies are needed to provide thoroughly and objectively measured exposure and outcome data, in order to improve the ability to determine the true effects.

Research Aim 2 (Chapter 5)**:** To investigate whether the relationships between early life parental smoking exposure and risk of asthma and lung function deficits in adolescence were modified by GST polymorphisms.

Key findings: My analysis of data from the MACS birth cohort study found evidence of an interaction between early life tobacco smoke exposure and GST genotypes in relation to an adverse impact on lung function in adolescence and early adulthood. Participants with GSTM1/T1 null mutations and GSTP1 Ile/Ile alleles may be at a higher risk for reduced lung function during adolescence and early adulthood if they are exposed to tobacco smoke in early life. However, I did not find significant associations with asthma. A possible reason could be tobacco smoke in utero/early life may cause

a parallel reduction in both FEV_1 and FVC, rather than preferentially affecting FEV_1 to indicate obstructive airway diseases measured using the FEV_1/FVC ratio.

Research Aim 3 (Chapter 6): To Investigate whether early life paracetamol exposure was associated with increased risk of asthma and impaired lung function in adolescence and whether GST genes modified the associations between paracetamol exposure and respiratory health.

Key findings: I found some evidence that early life paracetamol use may increase the risk of asthma and reduced lung function. However, when limited to non-respiratory indications for paracetamol use, these associations were attenuated. There was evidence that children with GSTP1 Ile/Ile genotypes were at higher risk of developing asthma by the age of 18 years if they were exposed to more paracetamol in early life compared to children with other GSTP1 genotypes. A sensitivity analysis limited to paracetamol use for non-respiratory reasons showed evidence that children with GSTM1 null genotypes were at greater risk of reduced lung function at 18 years of age if they had been exposed to more paracetamol during early life compared to GSTM1 null children with less paracetamol exposure. I also found some unexpected results that increasing paracetamol use for non-respiratory indications in GSTT1 present individuals was associated with reduced lung function (FEV_1/FVC and MEF) but found no association in those with GSTT1 null. The reason behind these findings is not clear, so these results need to be replicated and the mechanisms explored. It is possible that multiple testing may have generated spurious associations.

Research Aim 4 (Chapter 7): To investigate whether long-term contact with a range of common household exposures like heating, cooking facilities, tobacco smoke, and mould were associated with the risk of asthma and greater lung function decline, and whether GST polymorphisms modify these relationships.

Key findings: I found that exposure to wood heating, gas facilities, and tobacco smoke over a 10-year period increased the risk of asthma and lung function reduction in middle-aged adults. There was evidence of an interaction with GST genes, that is, participants with GSTM1 null, GSTT1 null and GSTP1 Ile/Ile genotypes were more susceptible to these household exposures and demonstrated an increased risk of

current asthma and greater lung function reduction over the 10-year follow-up, when compared to similarly exposed participants with other genotypes.

In this thesis discussion chapter, I bring together the overall strengths and limitations of my doctoral work and further discuss the potential for these findings to advance knowledge in the field of household exposures and asthma and lung function outcomes.

8.2 Overall discussion of findings

In the subsequent sections, 8.2.1 through to 8.2.4, I present a summary discussion of my results with reference to the four aims of my thesis, their sub-objectives, and any unexpected inconsistencies. Additionally, I provide further discussion that was not included in the published/accepted papers in Chapter 3, 5 and 6 due to the journals' word limit requirements.

8.2.1 Evidence of GST and indoor air pollution interactions on respiratory health from my systematic review

My systematic review provided some evidence that GST genes modify the link between indoor air pollution and asthma, allergies, and lung function. There was evidence that GST gene interactions were more consistent for studies conducted on children suggesting that the effect of gene-pollution interactions on respiratory health may vary by age. There are many differences between children and adults regarding respiratory system pathophysiology and immune responses. Children are relatively naïve with respect to many body systems and may be more susceptible to noxious exposures. Consequently, oxidative capacity may be more important for children compared to adults. Furthermore, the exposures documented in childhood studies usually occurred during early life. The lung, including airways, alveoli and blood vessels grow rapidly up to 2 years of age and continue to expand until early adulthood. Exposures during this critical period have been found to detrimentally affect respiratory health in later life. My systematic review also supports the notion that there are critical time windows for adverse exposures for lung growth in early life.

Though GSTs play an important role in regulating oxidative stress, particularly for children, the most recent article in my review suggested that supplemental nutrition intake, like vitamin A, could enhance antioxidant capacity and possibly prevent respiratory harm in susceptible children with risk GST genotypes exposed to ETS. In future, clinicians could consider antioxidant supplements as additions to personalised therapy for children found to have a high-risk genotype on genetic screening.

A limitation of my systematic review was the inability to pool the evidence from the included studies, leading to a lack of statistical evidence for a pooled effect from a meta-analysis. I was not able to statistically synthesize all the evidence using meta-analyses for possible interactions due to high heterogeneity and different methodology between studies. As mentioned above, there may be additional interactions by exposure time, outcome time and nutrition on the associations between indoor air pollution, GSTs and asthma.

However, a limited meta-analysis was performed for 3 studies (n=6,405) investigating the exposure to tobacco smoke and the GSTM1 deletion polymorphism and the risk of wheeze. The power to detect gene-pollution interactions was limited in my meta-analysis due to the marked differences in exposure and outcome measures, and specific gene-pollutant outcome combinations lacked numbers for meta-analysis. In addition, the statistical method used to measure interactions in meta-analyses is not perfect, as the assumption is no correlation between gene and environmental factors. Therefore, I derived Standard Errors (SE) for the interactions by combining SEs from strata. The SE is likely to be underestimated by using this method so the true interactions may be weaker than those found. For these reasons, I did not include this limited meta-analysis in my published paper; however, I included this meta-analysis as a supplement (unpublished) in Chapter 3. Along with the narrative synthesis of all included studies, the overall evidence from my systematic review supports the presence of a gene-indoor air pollution interaction on the outcomes of allergic disease and lung function.

Findings from the systematic review differed in terms of the specific risk allele and exposure. This was particularly the case for GSTP1 risk alleles where the evidence was

contradictory and inconsistent. Though my findings showed a pattern that carrying the GSTP1 105Val genotype had a negative effect on the occurrence of allergies and lung health when exposed to environmental risks, there are also contradictory findings in the literature. Thus, it was difficult to draw a firm conclusion on GSTP1 risk alleles. One of the reasons for this lack of consistency could be that this polymorphism was investigated using Single Nucleotide Polymorphisms (SNPs), not haplotypes. GSTP1 has a more complex genetic variation than GSTM1 and GSTT1, and has recognized haplotypes, in terms of 4 alleles: GSTP1*A (Ile105-Ala114), GSTP1*B (Val105-Ala114), GSTP1*C (Val105-Val114) and GSTP1*D (Ile105-Val114). The included papers generally focussed on the Ile105-Val105 substitution because the minor Ala114Val allele has a lower frequency and many of the studies had small numbers so were unable to address GSTP1 using these 4 distinct haplotypes. My analysis of GSTP1 interaction in this thesis did not take into account the effect of the Ala114Val allele, like many other studies. However, Ala114Val may interact with Ile105Val alleles to produce heterogeneity in different scenarios [262]. Future studies investigating more comprehensive genetic variation and haplotypes of the GSTP1 genes would improve our understanding of the role of GSTP1 on respiratory health.

8.2.2 Associations between early life smoking, GST genotypes (M1, T1, P1) and respiratory health up to 18 years in the MACS

My second analysis found evidence of interactions between early life tobacco smoke exposure and GST genotypes on lung function. The overall findings of this investigation in the MACS are consistent with my findings, but there were a few unexpected discoveries. Firstly, I did not find significant associations for gene-environment interactions and asthma outcomes. Although I expected to find an increased risk of asthma in those exposed to tobacco smoke with risk genotypes, interactions were seen for lung function only. I further hypothesized that early life smoke exposure may affect lung capacity rather than airflow obstruction, as consistent with the lung function findings. My study found that, for those exposed and with risk genotypes, both FEV_1 and FVC were reduced rather than a preferential reduction in FEV_1, as would be expected with asthmatic airways. Therefore, the FEV_1/FVC ratio was not reduced. FVC is thought to reflect the total lung capacity, while FEV_1 and FEV_1/FVC are

considered to be measures of airway obstruction in the larger airways, and reduction of these measurements is commonly found in people with asthma. My findings suggest that smoke exposure in early life may influence the development of all lung structures (lung parenchyma and airways) and cause reduction in lung capacity.

Another unexpected result is the borderline association between exposure to father's smoking and increased lung function in the group of people with GSTM1 pre`sent (Table 5.E9). The present allele of GSTM1 results in partial or complete lack of GSTM1 enzymatic activity, thus it should not affect the individual's ability to respond to the oxidative threat of early life tobacco smoke exposure. It is not clear why lung function was found to be better among individuals with GSTM1 present in those exposed to fathers' smoking compared to those not exposed. This finding may be due to compensation by other antioxidant genes or networks which have greater capacity to detoxify substances and are associated with GSTM1 present carriage.

In my systematic review, I found that people carrying the GSTP1 Val105 allele were at increased risk of asthma and reduced lung function when exposed to indoor air pollution. However, I found different associations in my MACS investigation. Participants with GSTP1 Ile/Ile genotypes were more likely to have lower lung function when exposed to early life maternal smoking compared to those who were not exposed, and I found no evidence of an association for people with other GSTP1 genotypes. Though there were some inconsistent results, most papers from my systematic review indicated that individuals with GSTP1 with Val genotypes were more susceptible to indoor air pollution exposures. The reason for these conflicting findings is unclear, although sampling variation could be a possible reason. This phenomenon has been found in several studies. A systematic review proposed that the effect of GSTP1 genes on asthma is likely to vary for different asthma phenotype outcomes. In one study, the Val allele showed a protective effect for bronchial hyper-responsiveness (BHR) and asthma but was associated with increased risk of wheezing [205]. Another study concluded that the GSTP1 Ala114Val polymorphism influences the activity of the GSTP1 Ile105Val substitution. They found evidence that, individuals having a Val in the 105 position along with an Ala allele in the 114 position exhibited a

stronger catalytic efficiency compared to individuals with the Val allele in the 105 position but lacking the Ala allele in the 114 position [262]. MACS genotyped a selection of 110 SNPs. Candidate genes were selected for characterization based upon having at least 10% prevalence for minor or wild type alleles and were also required to have some published evidence of association with at least one of the respiratory or allergic disease outcomes in which we were interested. In terms of GSTP1 genes with Ala114Val, these were not characterized because they have a minor type allele frequency of 8.9% [263]) and at the time of candidate gene selection, limited evidence was available to support an association with respiratory outcomes. Thus, MACS did not genotype the GSTP1 Val114Ala allele, limiting the characterization to GSTM1 null, GSTT1 null and GSTP1 Val105Ile alleles. One future direction for this study is to characterize further genes related to oxidative stress, to better understand how they may interact with each other and explore GSTP1 interactions on respiratory health.

8.2.3 Associations between early life paracetamol use, GST genes and respiratory health up to 18 years in the MACS

My third analysis was also performed in the MACS and the major findings are consistent with my hypothesis that the adverse effects of early life paracetamol on respiratory health were modified by GST genes. However, I also found some unexpected results as described below.

When investigating the main association between paracetamol and lung health, I adjusted for early life respiratory tract infection (RTI), including lower respiratory tract infection (LRTI), upper respiratory tract infection (URTI) and otitis media (OM). These were categorized for the number of each type of infection as 0, 1 and 2, in order to investigate a dose-response relationship. I did not see a dose-response relationship, likely due to small sample sizes. I expected to find that early life RTIs were negative confounders. Interestingly, my study showed that associations between paracetamol use and reduced lung function at 12 and 18 years (FEV_1/FVC and MEF) were somewhat strengthened after adjusting for all forms of early life RTIs, indicating that RTIs were acting as positive confounders. I performed a further analysis on the association between early life RTIs and lung health. As I expected, early life LRTI was still associated

with a reduced ratio of FEV_1/FVC (p=0.05), but early life OM was associated with increased pre-BD FEV_1/FVC in adolescents (p=0.08), behaving as a positive confounder. The reason for this difference by type of RTI is not clear. I hypothesize that exposure to mild early life URTIs may be beneficial in terms of their ability to enhance immune system function by providing immune modulatory stimuli during early life. Through this mechanism, mucosal lung surfaces may develop an enhanced ability to manage encounters or inflammation from environmental stimuli, subsequently avoiding damage to the developing airway and lung tissue [264, 265]. This hypothesis is consistent with my findings of improved lung function following mild OM in early life. As data in the MACS were collected prospectively from frequent interviews every 4 weeks before the age of 2 years, it was more likely that milder episodes of RTI would be captured compared to other studies which had fewer surveys at longer intervals.

I also performed sensitivity analyses for paracetamol use for non-respiratory indications, in order to remove the potential influence of early life respiratory infections. I did not find an association between paracetamol use for non-respiratory infection reasons and increased risk of adverse respiratory outcomes when the analysis was not stratified by GST gene status, which indicates that RTIs during early life are a potentially important confounder on these associations.

The results in my MACS analysis concerning GSTM1 and GSTP1 are consistent with my hypothesis. However, I found some evidence that was not consistent concerning GSTT1. Participants with GSTT1 present genotypes were more prone to reduced lung function with increasing days of paracetamol exposure. These unexpected findings possibly due to the prevalence of the GSTT1 risk allele (17.5%) being the lowest of all three GST genes tested, thus providing limited power to robustly investigate this association. However, my findings were consistent with another similar study on the association between prenatal paracetamol exposure, GSTT1 polymorphisms in mothers and the risk of asthma in their offspring [176]. In that study, a higher risk of offspring asthma was associated with maternal late gestational exposure to paracetamol when the maternal GSTT1 genotype was present, compared to the offspring of similarly exposed mothers with null GSTT1 genotypes. Overall, the reason

for these findings is not clear. My results need to be investigated in other studies, and further research is needed to explore the biological mechanisms underlying the association between paracetamol and respiratory health in order to confirm the potential modifying effect of GSTT1.

8.2.4 Associations between household exposures, GST genes and respiratory health in the TAHS

My fourth investigation used TAHS data to investigate household exposures and respiratory health while considering possible GST interactions in middle-aged adults. This research used a different statistical methodology - LCA, to identify household air pollution patterns. It is the first study to use LCA on household air pollution in a middle-aged population. Various combinations of cumulative wood/gas/smoking exposures had significant associations with the risk of asthma, fixed flow airway obstruction and greater lung function decline. I also found that GST gene polymorphisms interacted with household air pollution to influence respiratory health in middle-age.

In this study, I changed my focus from the effect of single environmental factors to that of multiple exposure profiles. Multiple exposures characterized in profiles appear to have a stronger impact on obstructive airway diseases and lung function decline outcomes, even when considering the additive combination of two exposures from separate multivariable regression models. Moreover, people with COPD were most commonly linked to the "Wood heating/smoking" profile, compared with other household air pollution exposure profiles, indicating wood heating and smoking may have a stronger effect on fixed flow airway obstruction. This finding indicates that multiple exposures may interact with each other and lead to greater lung damage, and also has an important public health message, that common household air pollution may be associated with worse lung health outcomes for smokers.

Household mould was included in the LCA model but did not show a difference in exposure prevalence between profiles, and therefore, exposure to mould was not discussed in Chapter 7. Although there is evidence that mould IgE production and airway hyperresponsiveness [266], indicating that asthma morbidity among adults may be related to household mould [145, 267]; in multivariable analyses I did not find

significant associations between consistent exposure to mould and asthma or lung function decline outcomes (all p values >0.2). The TAHS data were previously used to perform a cross-sectional analysis, when the participants were aged 43 years (The 5th decade follow-up), that found some evidence that the presence of visible household mould was significantly associated with current asthma and wheeze [268]. In my study, I did not find similar associations between longitudinal exposure to mould and respiratory outcomes. Indirect measurements of mould, ass in this study, may reduce the observed associations with mould exposures. In my analysis, exposure to mould was collected from two surveys ten years apart. However, I have not captured any preventive or corrective measures undertaken by participants to control visible mould in their houses, nor the changing mould status at times between these two follow-ups.

My previous studies in MACS provided evidence that gene-environment interactions may be more important during childhood and very early life, such as *in-utero* exposures like smoking and paracetamol. However, my current study in the TAHS confirms the presence of interactions between GST genes and household air pollutants for middle-aged adults. Conversely, previous population-based studies were less likely to provided evidence on GST-pollution interactions in adulthood. A population-based, cross-sectional study in Copenhagen did not find any significant interactions between GSTM1/T1 genotypes and indoor air pollution exposure and respiratory symptoms in adulthood [16]. Another population-based Swiss cohort study (mean age 41.6 years) did not identify any significant interactions between second-hand smoke exposure and GST genotypes on the risk of allergic rhinitis [243]. In contrast, my study found significant evidence of an interaction with GSTP1 genotypes on risk of asthma (interaction p-value=0.01). Notably, my work on lung function is novel, as it is the first study to identify these interactions for lung function decline outcomes. To date there have been no population-based studies investigating the relationship between indoor air pollution, GST genes and lung function. There were no significant strata-specific results for GSTT1 in my study, although GSTT1 null participants exposed to household air pollution had lower lung function, as illustrated by the point estimates for the Beta coefficients. It is possible that GSTT1 also modifies the association between household

air pollution exposures and lung function decline but that my results were limited by small numbers of exposed participants with GSTT1 null genotypes.

Previous studies investigating the association between environmental risk factors and asthma suggested considering atopy either as an effect modifier or a confounder. Several studies have found evidence for a role of atopy as an effect modifier for the association between air pollution and asthma or respiratory health. A cross-sectional study in Toronto found that outdoor air pollution was associated with lifetime asthma and wheeze in school aged children, and that these associations were stronger in atopic participants, with no or weaker effects found for non-atopic participants (no interaction P value provided) [269]. There is some evidence that air pollution may act as an adjuvant for atopy status, and then lead to the development of airway inflammation and bronchial hyperresponsiveness [269]. A systematic review and meta-regression found that the association between second-hand tobacco smoke and asthma in childhood was reduced if the effect was not controlled for atopy. The findings suggested that atopy was acting as a confounding variable and lack of inclusion would bias estimated associations toward the null [270]. In my fourth analysis, I initially tested atopy as an effect modifier, but there was no evidence to support this hypothesis. I then adjusted for atopy as a confounder for the associations between HAP and asthma outcomes in the final model. I found my results strengthened after controlling atopy, a finding that is consistent with the results from the meta-regression mentioned above [270].

8.3 Strengths and limitations

My findings should be interpreted in the context of strengths and limitations relating to methodological and analytical considerations as below. I bring together the overall strengths and limitations for various elements of my studies including specific issues concerning research design, exposure and outcome measurements, as well as confounding and interaction (Table 8.2).

Table 8.2 Summary of the strengths and limitations of my studies

	Strength	Potential limitations
Research design	Long-term longitudinal design	Attrition bias due to loss to follow up
Exposure measurement	Longitudinal assessment at multiple time points Validated exposure measurements	Non-differential misclassification due to prospective self-report questionnaires
Outcome measurement	Validated lung function measurement along with asthma outcomes	Self-report questionnaires for asthma outcomes
Confounding	Novel data to consider early life respiratory tract infection as confounder	Residual confounding from unknown factors
Interaction	Three genes tested	Results are unable to be compared due to heterogeneity between studies.

8.3.1 Research design

My doctoral work was nested within two longitudinal studies: the MACS and the TAHS. Both studies are prospective cohort studies, investigating exposures with the potential to be determinants of respiratory health. MACS, a birth cohort study of children with a family history of allergic disease, was used to investigate the associations between early life exposures and asthma and lung function in adolescence. TAHS, a long-term follow-up study of a general population-based cohort, was used to investigate the associations between common household exposures and asthma in middle-aged adults. A major strength common to all my studies is data across long follow-up periods, as MACS has followed participants from birth to 18 years of age, and TAHS has followed participants from the age of 7 years to the age of 53 years.

The MACS is amongst the world's leading studies in the field of allergic disease. One of the major strengths of the MACS is that it is a birth cohort study with frequent follow-ups. After birth, telephone interviews were conducted every 4 weeks in the first 15 months, then at 18 months, 2 years of age, then annually until 7 years of age, and again at 12 years and 18 years. At these follow-ups, substantial amounts of data concerning many different exposures and outcomes along with biological specimens were collected. Individual genotypes were characterized from specimens at 18 years. This wealth of data provided a great opportunity for me to address questions

concerning gene environment interactions. The prospective collection of data on early-life exposures at frequent intervals substantially reduces the issue of recall bias and provides excellent data that can be used to establish temporality between exposures and outcomes, strengthening evidence for causal associations. In the MACS, this is especially true for data collected in the first 2 years of life, before research questions were even considered for the outcomes I am investigating in my thesis and certainly before there were any concerns about the use of paracetamol. For instance, in MACS, early life paracetamol exposures were collected from frequent interviews every 4 weeks before the age of 2 years. This reduces recall bias when compared to studies where parents answered these questions retrospectively when their children were much older and may have developed asthma. The method of data collection in MACS also ensured that parents were more likely to record all paracetamol use and indications for this use, even mild respiratory infections, compared to other studies with fewer surveys at longer intervals. Parents were also less likely to be biased in their reporting with respect to respiratory outcomes which had not yet occurred.

A major strength of the TAHS is its large population-based sample. TAHS recruited almost all (99%) of eligible school children in Tasmania aged 7 years in 1968 (n=8,683), with modest loss to follow-up over the next 50 years. This large sample size is unique and provides adequate power for investigating associations between longitudinal environmental exposures and asthma outcomes as well as gene-environment interaction analyses. Secondly, the TAHS also has multiple follow-ups throughout life, allowing us to observe and document the change in household environmental exposure profiles and asthma status over time. This is especially important for my analyses as asthma outcomes may be differentially influenced by environmental profiles over time. Thirdly, the two most recent follow-ups collected information on plausible confounders and modifiers for the associations between these exposures and respiratory health. Skin prick tests, an objective indicator of atopy measured at 53 years, is one of these important confounders. Finally, TAHS recorded household exposures that may influence asthma and poor lung function risks. Several of these recorded household exposures have not been thoroughly explored in previous studies, such as reverse cycle air conditioning and electric cooking. My TAHS analysis provides

further insight into respiratory risks in adulthood from household exposures and may influence the choice of heating and cooking methods in susceptible middle-aged populations.

One major limitation in both MACS and TAHS is the potential for attrition bias. As with all long-running cohorts, there is the possibility of bias from selective loss to follow-up with increasing length of studies. Attrition bias in cohort studies may arise from differences in population characteristics between the baseline cohort and the subsequent population in the follow-ups. If there are significant differences between people who leave the study and those who remain, attrition bias can be introduced. However, this depends upon whether the loss to follow-up is related to both the exposure and outcomes of interest. Complete follow-up of all baseline participants with no attrition is the best way to prevent against possible bias, but this is rarely achieved in practice [271]. In the MACS, data were obtained from 58% of the children at the 12-year follow-up and 66% at the 18-year follow-up. In the TAHS, 7,562 (88%) of the baseline participants were located, though 250 (2.2%) were deceased. The overall tracing (n=7,312, 85.2%) and responses rate to surveys (5,729, 78.4%) were high in 2002 (5th decade). In 2012, contact details were available for 71% (n=6128) of the original cohort (mean age 53 years) from the original cohort. Of those invited, 3,609 (58.9%) participated. My analyses in this thesis mainly focused on the 5th decade and 6th decade follow-up. I found that participants with some risk exposures (e.g. smoking) were more likely to be lost to follow-up between 5th decade and 6th decade. Therefore, my LCA pattern may underestimate the effect of household air exposure profiles as some exposed participants were missing. However, as the statistical power was great enough to detect significant associations, the observed associations are likely to be valid.

In TAHS, as both exposures and outcomes were ascertained at the same time or exposures were ascertained after asthma disease status was known, it is possible that disease status may have influenced responses. For instance, people who had asthma may have been more likely to attribute this to factors in their household environment, claiming exposure to more risk factors compared than people with similar exposures

but no asthma. This bias may have increased the strength of my observed associations. However, the lung function outcomes are an objective measure and findings were consistent for both asthma and lung function. It is possible that this bias may still exist in my analysis however it is probably unlikely to affect directions of association and conclusions.

Another limitation was small sample sizes, particularly for my sub-strata analyses for interactions affecting the power to detect associations. Furthermore, in both cohorts, participation rates in the clinical studies were lower than survey responses as participants were usually invited after completing the written questionnaires. This further restricted the power to find significant results relating to lung function outcomes. In both MACS and TAHS, evidence was not found to support some biologically plausible associations. For example, analyses concerning susceptible strata of GST polymorphisms. This lack of evidence for some associations may be because there is no association or may also be due to small numbers limiting the power to detect stratum-specific associations. The issues regarding loss to follow up and small sample sizes were discussed in more detail in Chapter 5, 6, and 7.

8.3.2 Exposure assessment

Generally, both MACS and TAHS have conducted robust longitudinal assessments of exposures which may be risk factors for the respiratory outcomes of interest. This exposure information was collected through interviews or self-administered questionnaires which have many strengths including their non-invasive nature, cost-effectiveness and feasibility in larger studies, and ability to obtain large response numbers within short periods of time. Moreover, both studies also have strengths in terms of the unique timing of their exposure data collection. In MACS, extensive exposure data were collected at baseline (median, 4 days after birth of the child), which is particularly important for identifying early influences on lung growth, but few studies have recorded data from this critical time window. For this reason, the effect of exposure to smoke during this period on childhood and adolescent asthma and lung function has remained unclear. In TAHS, household exposures were prospectively collected in the 5th and the 6th decade of life, enabling the identification of a range of

long-term household exposures that may be important for respiratory health in middle-age. Previous studies have mainly focused on exposures during childhood, and my analysis of the TAHS data is the first to assess the relationship between longitudinal household indoor air exposures and asthma and lung function outcomes in middle-aged adults.

Compared to other studies, the MACS has collected unique data on exposure to early life paracetamol that minimizes the possibility of recall bias. In the Norwegian Mother and Child Cohort study (Moba), authors assessed early life paracetamol exposure before 6 months by using one question posed to parents when their children were 6 months old: “whether infants used paracetamol“ [99]; The Avon Longitudinal Study of Parents and Children (ALSPAC) identified early life paracetamol exposure by asking mothers, when their infants were aged 6 months, how often they had given their infants paracetamol since birth, and the possible answers provided much less detailed information than is available from the MACS. The ALSPAC responses included: (“Never”, “Once”, “More than once”) [176]. In these studies, parents who reported more illnesses for their children may also think that they used more paracetamol. Paracetamol exposure may, therefore, be overestimated in those studies for children with recurrent infections, leading to differential misclassification. MACS had frequent assessment of paracetamol use including indications for use of paracetamol before the age of 2 years, limiting the potential for recall bias for paracetamol exposure.

However, there are also some limitations of using interviews or self-administered questionnaires, as in MACS and TAHS, with respect to assessment of exposures. This method of exposure assessment may lead to non-differential misclassification between non-exposed participants and exposed participants. For instance, in TAHS, when confronted with a list of ten different heating/cooking types (multiple choices allowed), people may select many facilities listed even though the frequency or duration of use may not be reflected in their response. As the amount of exposure is not reflected in this question, those with little or infrequent exposure would still be classified as exposed to a similar amount as people who predominantly use these forms of heating/cooking. In this scenario, the estimates of association may

underestimate the true effects. In order to reduce exposure misclassifications and subsequent introduction of bias, accurately assessing indoor air pollution by using objective air pollution measurements would be ideal when investigating the associations between these exposures and health outcomes. However, this is usually not economically or technically feasible in long-term cohort studies, particularly for large studies conducted at the general population level.

There were also, in some instances, too few and/or too simplistic questions to fully characterise some exposures. In MACS, I identified whether mothers and/or fathers were smoking at baseline using the question "Is smoking now". Although the MACS baseline survey in had a question on the amount and frequency of smoking, the small sample size of smoking parents limited the power to further investigate a dose-response relationship.

Another limitation is that both cohorts were unable to specifically define the timing of exposures. In MACS, from the data collected I was unable to separate *in-utero* exposure from early post-natal environmental tobacco smoke exposures. Therefore, the observed associations may be the effects from exposures at both times and may also be related to pre-conception smoking. In TAHS, I identified longitudinal exposures from two surveys conducted ten years apart. Using two discrete time points may not capture important changes in exposure status at times between these two follow-ups. However, LCA modelling indicated a high probability of consistent exposure to the same household factors over the ten years follow-up.

8.3.3 Outcome assessment

The definitions of asthma in my thesis were based on participant reports from both TAHS and MACS. Similar to the issues related to the collection of exposure data via interview or self-administered questionnaires, there may be some non-differential misclassification related to the collection of outcome information. However, in both MACS and TAHS, validated questionnaires were used to define asthma outcomes, and this would have reduced potential non-differential misclassification [272]. Since all participants in the cohorts used the same questionnaires, any information bias arising is most likely to be non-differential misclassification. Such misclassification in

outcomes could be minimised by collecting additional data with physiological tests, and for this reason my thesis did not use interviewer- or self-reported outcomes alone. In particular, lung function measured using spirometry is an objective measure of lung health, which provides more specific measurements to assess the potentially adverse effects of exposures in MACS and TAHS.

In TAHS, I explored several different definitions of asthma including new-onset asthma and persistent asthma. I used prospective data on asthma reported from the two most recent follow-ups and by parents at baseline (age 7 years). Asthma has an episodic nature that may persist or remit at any time of life. The parent responses concerning asthma status at the age of 7 are more likely to be correct than the answers for proband's self-reported asthma status in childhood, collected retrospectively when they are in their 40s or 50s and may not recall asthma from many years ago. This highlights the benefits of prospective data collection for asthma at repeated time points in the TAHS, to minimize misclassification of outcomes.

8.3.4 Confounding issues

The selection of confounders in my thesis was based on causal modelling theory with the use of DAGs. This method models potential relationships and derives a minimum set of confounders required to provide the most accurate association between exposures and outcomes whilst accounting for all relevant confounders. In contrast to other similar studies, there were two key sets of confounders I used in my thesis that strengthened my ability to suggest the causality relationships between household exposures and respiratory outcomes:

1. Confounding by RTIs on the associations between early life paracetamol use and lung health, and
2. Reducing over-adjustments by closing backdoor paths with minimum set of confounders.

Whether there is an association between paracetamol use and asthma is still not clear. A major concern is whether observed associations have been confounded by indication, ie. giving paracetamol for RTIs. A previous review found that adjusting for

early life RTIs may reduce the association between paracetamol exposure and subsequent childhood asthma [13], thus it is important to correctly assess the role of early life RTIs in this relationship. However, not all studies have considered the confounding effect of early life RTIs, and even for studies that have adjusted for RTIs, crude measurements of RTIs have been used. Therefore, their findings may not reflect the true associations between paracetamol and respiratory health. There are several notable examples. The ALSPAC study and the Czech European Longitudinal study of Pregnancy and Childhood (ELSPAC) did not adjust for early life RTIs when investigating the association between infant paracetamol exposure and subsequent childhood asthma [165, 176]. The Moba study included early life RTIs as a confounder[99], but their method of data collection for this variable allows for a high possibility of recall bias. A Korean study only adjusted for a history of bronchiolitis when modelling the relationship between paracetamol use before 1 year and asthma, but bronchiolitis does not account for all types of RTIs [173]. MACS comprehensively collected data on all types of early life RTIs, in terms of LRTI and URTI), and OM. Thirdly, each type of RTI was categorized into three groups: 0, 1-2 and ≥3 times (before 2 years of age). The analytical models also adjusted for LRTIs, URTIs and OM in separate groups. This methodology allowed me to improve control over potential confounding, as there may be dose-response relationships between RTIs and subsequent lung health.

In the TAHS, I aimed to investigate the relationship between household exposures and asthma outcomes in middle-age. A total of ten different exposures were collected in my study, covering multiple elements of the household environment. Most previous studies have been designed to identify these relationships via multivariable regression analyses, to explore the unique effect of each environmental feature after adjusting for the others. Using this methodology, I would have needed to adjust for more than 10 potential confounders and the results may be overfitted if the number of confounders is large. Overfitting generally occurs when a model is excessively complex, such as having too many variables [273]. Addition of unnecessary “noise variables” may result in inaccurate associations through the introduction of open backdoor paths and bias or collinearity. Future studies will be unable to identify similar associations by using same set of confounders. Therefore, I used LCA to determine classes of

environmental exposure patterns which reflect unmeasured (latent) beliefs and behaviours in this cohort, rather than attempting to isolate the contribution of each environmental characteristic by using more traditional methods. For addressing confounding, I aimed to include all confounders from the DAG and found that including all potential confounders reduced the sample size due to missing data in some confounders. So, I created a model with a set of confounders that also minimized missing data, to avoid a large reduction in sample size. This model using confounders with little missing values did not appreciably change the associations compared with the model including all potential confounders. In the LCA analysis, I was able to limit the variables to be included in each regression to less than 5 as the LCA classes I determined already took into account the potential impact of the combined presence or absence of a variety of household exposures. Therefore, the findings from TAHS are less likely to be influenced by overfitting or noise variables compared to the findings from other studies.

Although I adjusted for a number of possible confounding variables in both the MACS and TAHS analyses, it is possible that some important known and unknown confounders may not have been included in the regression models. As discussed in the literature review (Chapter 2), residual confounding may be a reason for inconsistent results between published studies. In the TAHS analysis, I found that exposure to indoor wood fires for heating was associated with an increased risk of asthma outcomes. However, this analysis was unable to be adjusted for a potentially important confounder-rurality, as these data were not available in TAHS. In a retrospective cohort study in Germany, there was no evidence for an association between wood stove use and asthma in childhood, and the authors indicated that residential environment, especially the farm environment, was an important variable for deciphering the relationship between wood heating and allergic diseases [158].

8.3.5 Analysis for genetic interactions

In this thesis, I selected three GST genes and their polymorphisms based on their biologically plausible roles in influencing the risk of asthma. The GSTs selected were mainly based on the importance of their role in antioxidative functions in relation to

environmental exposures. When investigating the effects of environmental exposures, my consideration of genetic interaction is a major strength in my studies. Though I hypothesized that all three GST risk polymorphisms were associated with increased risk of asthma and poor lung function, when individuals carrying these risk traits were exposed to oxidative stress inducing exposures, the observed effects of gene-environment interactions were not consistent and relatively small. I believe the lack of strong evidence for an interaction may be because antioxidant capacity is determined by a complex combination of factors, rather than individual polymorphisms in candidate genes.

In MACS, all three GST polymorphisms were found to modify the associations between early life smoking and lung health in adolescents. In TAHS, I found evidence for an interaction only for GSTM1 and GSTP1 and household exposures on the risk of asthma and lung function deficits in adulthood. I cannot fully explain the inconsistent relationships for GSTs in different populations, and the issue of inconsistencies is rarely discussed in the literature. However, I have proposed several possible reasons for these inconsistencies in Chapters 5, 6, and 7, such as different antioxidant function between children and adults, different detoxification effects between GST polymorphisms, and the different methodologies used for assessment of exposures and outcomes. Nevertheless, it is important to note that such inconsistent results are common in the literature regarding the interactive effects of GST variants on respiratory health.

8.4 Contribution to current knowledge gaps

My thesis has made several novel and important contributions to the scientific literature. These include new knowledge on the interactions between common household environmental exposures (including indoor air and pharmaceutical exposures) and antioxidant GST genes on the relationships with asthma and lung function. The key contributions of my research are:

- Evidence from my systematic review that carriers of GSTM1/T1 null and GSTP1 Val/Val genotypes were more susceptible to household exposures leading to a higher risk of asthma and lung function deficits.

- Early life tobacco smoke exposures may have a greater impact on adverse respiratory health in adolescents with susceptible GST genes.
- Early life paracetamol exposures may be associated with adverse respiratory health in adolescents, particularly for those with susceptible GST genes.
- Many common household air pollution exposures from heating/cooking may be associated with subsequent adverse respiratory health in middle-age, particularly for those with susceptible GST genes.
- Oxidative stress may play an important role in the occurrence of asthma as both GSTs and household exposures are involved in pathogenesis of oxidative stress.

8.5 Implications and recommendations

8.5.1 Establishing an "Asthma-friendly" home environment

My research found that oxidative household exposures were associated with long-term risk of asthma, fixed airflow obstruction and lung function deficits. This has the potential to be translated into important public messages to avoid or minimise household exposures that lead to oxidative stress. My study suggests reverse cycle air conditioning and electric cooking are the heating and cooking methods with the lowest risk of adverse respiratory outcomes. Clinicians need to consider patients' home environments when assessing their risk of persistent asthma and developing management plans for patients with existing asthma. From my work, suggestions could be made concerning "Asthma-friendly" home environments. "Asthma-friendly" home environments are those that endeavour to keep the indoor air as clean as possible.

A key component of an "asthma-friendly" home environment is a smoke-free zone. Indoor air should be free of tobacco smoke that contains irritants and chemicals known to be harmful for respiratory health. Public health messages should continue to advocate for protection from tobacco smoke exposure for all people. A survey from the Australian Bureau of Statistics indicated that over 2.8 million (16.1%) people aged over the age of 18 years reported smoking daily, and nearly half (48.9%) of all adults had ever smoked (Data from 2011-12). This is particularly disturbing in the case of pregnant women who expose their children in utero and usually continue to expose

them in infancy. A 2010 report found that 14 percent of women smoked during pregnancy in Australia. The early years of life are a critical time window during which indoor environmental exposures can significantly increase the risk of disease in later life.

Furthermore, identification of gene-environment interactions from my work provides information on at-risk groups for normal household exposures on respiratory outcomes. With genetic screening, clinicians could advise parents of infants and young children or other high-risk individuals on ways to modify their home for an "asthma-friendly" home environment.

8.5.2 Is it safe to use paracetamol during early life?

In my study, I found evidence that paracetamol use in early life may increase the risk of asthma and reduced lung function in late adolescence/early adulthood. I also found that some children appear to be more susceptible to these risks. Children who had risk genotypes of GST genes, had a greater risk of asthma and reduced lung function by 18 years of age if given more paracetamol in infancy. At present, although what I have found is important, it is not a robust finding and has not yet been investigated in other studies. There is also not enough evidence to imply a causal relationship. This finding needs to be confirmed in other populations including non-atopic populations before this evidence could be translated to practice. Furthermore, even if this is a true effect, more work needs to be done to understand the amount of paracetamol which may put children at risk and also how great that risk may be. So, at this stage I have not found enough evidence to impact guidelines on paracetamol use in very young children.

Paracetamol is safe to treat fever and mild to moderate pain when the indication and dose are appropriate. There is, at this stage, no advantage for children or parents knowing whether they carry a GST risk genotype, nor is there enough evidence to support advising a change of guidelines concerning paracetamol administration to children. However, fever is a common symptom of childhood illness. The infectious process which leads to fever may be harmful but fever itself is part of a normal immune response and may not be harmful [274]. There is increasing evidence that routine fever

reduction is unnecessary [275]. Moreover, inadvertently giving too much paracetamol may cause hepatotoxicity in children, especially if repeated supratherapeutic doses are given [274]. Parents are advised to use fever and pain medications only when necessary and as guided by medical practitioners.

8.5.3 What does gene-environment interaction mean for the public?

Genetic screening identifies the differences in chromosomes and genes that may influence susceptibility to particular diseases. So far, genetic screening is not part of the usual approach to the clinical management of asthma. However, in my doctoral work, I have found that it may be important for identifying people who are more vulnerable to household, and other oxidative, exposures. As the gene-environment interactions I observed are likely to occur via an oxidative stress mechanism, leading to impaired lung function and increased risk of asthma, people who know they are at high risk may choose to enhance their antioxidative capacity through the use of standard, readily available supplements to reduce or eliminate these adverse effects from household pollution. However, a few studies from my systematic review suggested the presence of complex interactions in human antioxidative networks. Thus, it is unlikely that a single gene polymorphism is responsible for regulating all oxidative stress in the body, and range of nutritional compounds may help to prevent asthma and allergic disease through improving antioxidant defences. A Korean study provided evidence that low vitamin A intake was associated with an increased risk of asthma for participants with genetic risk alleles who were exposed to ETS. This finding suggests that supplemental vitamin A may be able to be taken as a preventive measure for those at high risk. Apart from vitamin A, many other nutritional supplements may help to boost antioxidant defences, including vitamin C, vitamin E, vitamin D, zinc, selenium and more. Many randomized control trials have already found the association between supplemental nutrition and risk of pulmonary diseases. For instance, the most recent meta-analysis of randomized control trials on the effect of supplemental vitamin D suggested that vitamin D could reduce frequency and severity of asthma exacerbations and improve lung function in patients with air flow limitation [276].

8.6 Future directions for research

Firstly, heterogeneity and data quality are some of the major challenges in understanding evidence from observational studies. The more common and traditional approaches of assessing exposures and outcomes using interviewer- or self-administered questionnaires are unable to measure actual levels of exposure to various exposures of interest and may also not be detailed enough to determine different asthma phenotypes. The nature of self-administered questionnaires is also prone to bias. Though indoor air monitoring may not be feasible in large epidemiological studies, a well-designed questionnaire along with the collection of specific objective biomarkers (e.g. urinary cotinine as a proxy for tobacco smoke exposure) or clinical investigations may improve the degree of accuracy in exposure and outcome measurement.

Secondly, further studies should consider more factors that may interact with environmental exposures in the oxidative stress pathway including multiple antioxidant genes, nutrition, food and physical activity, rather than GST genes alone. Body systems, including those for the management of oxidative stress, usually have a large degree of biological redundancy and work together to protect the airways from adverse respiratory outcomes. Within the PhD timeline, I did not extend my work into these areas. However, it could be a valuable direction for further studies.

Thirdly, oxidative stress plays a critical role in the pathogenesis of age-related lung diseases [277], thus the magnitude of gene-environment interactions may also change with aging. However, current research has only focussed on narrow periods during childhood and adulthood. More publications with populations of varying ages should be encouraged to obtain a clearer picture of asthma aetiology via this oxidative stress mechanism across the lifespan. Finally, the possible mechanisms underlying the associations I uncovered between paracetamol exposure and respiratory health are not completely clear. Further exploring these mechanisms will help us to understand whether the relationship between paracetamol and asthma may be causal, and hence, determine the need to revise our clinical and therapeutic strategies for paracetamol use as it pertains to the risks for asthma and lung function deficits.

8.7 Conclusions

At the beginning of my doctoral work, I systematically synthesised the evidence for GST interactions in the associations between indoor air pollution and asthma, allergies and lung function. Subsequently, using the data from MACS and TAHS, I critically investigated the associations between oxidative household exposures and asthma and lung function outcomes, and potential effect modification by Glutathione S-Transferase gene polymorphisms in Australian adolescents and adults. In summary, I have demonstrated the following:

- I found significant GST interactions in the associations between early life passive smoking and reduced lung function in adolescents. Carriers of GST null genotypes and GSTP1 with Ile/Ile alleles were more susceptible when exposed to tobacco smoke in early life (Chapter 5).
- I found some evidence that paracetamol use in infancy may have an adverse effect on respiratory health for children with risk genotypes. Children who had GSTM1 null genotypes and GSTP1 Ile/Ile alleles had a greater risk of asthma and reduced lung function by 18 years of age if given more paracetamol before 2 years of age (Chapter 6).
- I found that several household exposures were associated with increased risk of asthma and excess lung function decline in middle-age. These exposures included gas cooking and heating, wood fires for heating and tobacco smoke. Mixed environmental exposures had stronger effects on risk of asthma compared to the effects from individual factors alone. There was evidence showing that participants with GSTM1 null and GSTP1 Ile/Ile alleles were more susceptible to household exposures on asthma and lung function decline outcomes (Chapter 7).

In conclusion, my doctoral work has filled significant gaps in our understanding of the relationship between household exposures and respiratory health. The findings from my work provide evidence for the presence of gene-environment interactions and highlight the potential biological mechanisms relating to oxidative stress in the development and progression of chronic respiratory diseases. Many household air

pollution exposures, such as smoking, wood or coal fires, and gas may have health effects for susceptible populations. My findings on the adverse effect of paracetamol use during early life need to be further investigated in future studies. The overarching goal of future research is to raise public awareness about common, and potentially harmful, household exposures and integrate strategies to improve household indoor air pollution. These strategies will benefit our children, individuals and the community.

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Newcastle Ottawa Scale for Case Control Studies – GST interaction Systematic Review

Reference:		
Date:		
Reviewer:		
Selection	Score	Notes
1) Is the case definition adequate? a) Yes, with independent validation* b) Yes, e.g. record linkage or based on self-reports, describe if "other" c) No description		
2) Representativeness of the cases a) consecutive or obviously representative of cases* b) Potential for selection biases or not stated		
3) Selection of the controls a) Community controls* b) Hospital controls c) No description		
4) Definition of controls a) No history of disease (endpoint)* b) No description of source		
Comparability		
1) Comparability of cases and controls cohorts on the basis of the design or analysis a) Study controls for a minimum set of confounders including age, gender, SES, and family history of allergic disease [important factor]* b) Study controls for a minimum set of confounders and other confounders [second important factor]*		
Exposure		
1) Ascertainment of exposure a) Secure record* b) Structured interview where blind to case/control status* c) Interview not blinded to case/control status d) Written self-report or medical record only e) No description		
2) Same method of ascertainment for cases and controls a) Yes* b) No		
3) Non-response rate a) Same rate for both groups* b) Non respondents described c) Rate different and no designation		
Total score (Max = 9)		

Reference:		
Date:		
Reviewer:		
Selection	Score	Notes
1) Representativeness of the exposed cohort a) truly representative of the average _____ (describe) in the community * b) somewhat representative of the average _____ in the community * c) selected group of users d) no description of the derivation of the cohort		
2) Selection of the non-exposed cohort a) drawn from the same community as the exposed cohort * b) drawn from a different source c) no description of the derivation of the non-exposed cohort		
3) Ascertainment of exposure a) secure record (e.g. surgical records) * b) structured interview * c) written self-report d) no description		
4) Demonstration that outcome of interest was not present at start of study a) yes * b) no		
Comparability		
1) Comparability of cohorts on the basis of the design or analysis a) study controls for a minimum set of confounders including age (not for birth cohort), gender, SES, and family history of allergic disease * b) Study controls for any additional factor * (This criteria could be modified to indicate specific control for a second important factor.		
Outcome		
1) Assessment of the outcome a) Independent blind assessment. ** b) Record linkage. ** c) Self report. * d) No description.		
2) Was follow-up long enough for outcomes to occur a) yes (select an adequate follow up period for outcome of interest) * b) no		
3) Adequacy of follow up of cohorts		
a) complete follow up - all subjects accounted for * b) subjects lost to follow up unlikely to introduce bias - small number lost - > ____ % (select an adequate %) follow up, or description provided of those lost) * c) follow up rate < ____% (select an adequate %) and no description of those lost d) no statement		
Total score (Max = 10)		

Reference:		
Date:		
Reviewer:		
Selection	Score	Notes
1) Representativeness of the exposed cohort a) truly representative of the average _____ (describe) in the community * b) somewhat representative of the average _____ in the community * c) selected group of users d) no description of the derivation of the cohort		
2) Selection of the non-exposed cohort a) drawn from the same community as the exposed cohort * b) drawn from a different source c) no description of the derivation of the non-exposed cohort		
3) Ascertainment of exposure a) secure record (e.g. surgical records) * b) structured interview * c) written self-report d) no description		
Comparability		
1) Comparability of cohorts on the basis of the design or analysis a) study controls for a minimum set of confounders including age, gender, SES, and family history of allergic disease * b) Study controls for any additional factor * (This criteria could be modified to indicate specific control for a second important factor.)		
Outcome		
1) Assessment of the outcome a) Independent blind assessment. ** b) Record linkage. ** c) Self report. * d) No description.		
Total score (Max = 7)		

Score classification
Very good = 6-7
Good = 5
Satisfactory = 4
Unsatisfactory = 0-3

QUESTIONNAIRE FAMILY DATA/PERSONAL WITH ANTE NATAL PROFILE

SURVEY NUMBER:.

FAMILY NAME (MRS):

ADDRESS:

TELEPHONE:

DATE OF QUESTIONNAIRE:

E.D.C.

SOCIAL BACKGROUND:

MOTHER'S NAME:

FATHER'S NAME:

MOTHER'S AGE:

FATHER'S AGE:

COUNTRY OF BIRTH:
1. Australia/New Zealand
2. England/North America
3. Europe
4. Asia
5. Other (specify)

COUNTRY OF BIRTH:

MARITAL STATUS:
1. Married. 2. Single
3. Separated. 3. Divorced
4. De facto

MARITAL STATUS:

EDUCATION:
State number of years of formal education:

e.g. Completed Year 11 = 11

University/Technical
3 year course = 15

(For part-time course, write full-time equivalent).

EDUCATION:

OCCUPATION:

OCCUPATION:

CURRENT RESIDENCE:
1. Owner occupied house
2. Owner occupied flat
3. Rented house
4. Rented flat
5. Housing Commission flat (which floor?....)
6. Housing Commission walk-up
7. Other - specify....

ARE YOU IN RECEIPT OF A SOCIAL SECURITY BENEFIT PENSION:

1 = YES 2 = NO

ARE YOU IN RECEIPT OF A SOCIAL SECURITY BENEFIT PENSION:

1 = YES 2 = NO

FAMILY HISTORY

Write in 1 = Yes 2 = No 9 = Don't know

SIBLINGS:- (NUMBERED FROM OLDEST (1) TO YOUNGEST)

1 = FEMALE 2 = MALE

1	2	3	4	5	6

1. Natural child to both parents.
2. Natural child to mother only.
3. Natural child to father only.
4. Adopted.
5. Other.

Suffers from now, or has in past had -

1.	MILK ALLERGY					
2.	EGG ALLERGY					
3.	OTHER FOOD ALLERGY (Specify... .)					
4.	ECZEMA					
5.	ASTHMA/WHEEZY BRONCHITIS					
6.	HAYFEVER					
7.	INFANTILE COLIC					
8.	DRUG ALLERGY (Specify.....)					
9.	OTHER ILLNESS (Specify.....)					

PARENTAL HISTORY:

Suffers from now, or has in past had -	MOTHER	FATHER
1. MILK ALLERGY	☐	☐
2. EGG ALLERGY	☐	☐
3. OTHER FOOD ALLERGY (Specify.....)	☐	☐
4. ECZEMA	☐	☐
5. ASTHMA	☐	☐
6. HAYFEVER	☐	☐
7. DRUG ALLERGY (Specify.	☐	☐
8. OTHER ILLNESS (Specify...	☐	☐

SMOKING HISTORY

1. is smoking now?
2. not smoking but has in past 3/12
3. not smoking now but has in past 6/12
4. has smoked > 6/12 ago
5. has never smoked.

INSERT CORRECT ANSWER ☐ ☐

Intensity of smoking:

i) Currently smokes:

1. no cigarettes
2. smokes 1 - 4 cigs./day
3. smokes 5 - 10 cigs./day
4. smokes 11 - 20 cigs./day
5. smokes > 21 cigs./day

☐ ☐

ii) At peak of smoking in last 6/12 smoked

1. no cigarettes
2. 1 - 4 cigs./day
3. 5 - 10 cigs./day
4. 11 - 20 cigs./day
5. > 21 cigs./day

INSERT CORRECT ANSWER ☐ ☐

Appendix 3: MACS infant follow-up questionnaire

APPENDIX 3: Questionnaire Infant Illness, Physical Examination, Diet Chart, and Socio-domestic

QUESTIONAIRE

INFANT ILLNESS, PHYSICAL EXAMINATION,

DIET CHART, AND SOCIO-DOMESTIC.

FAMILY NAME (MRS.)

ADDRESS.

TELEPHONE:

NAME OF CHILD

SEX:

CHILD NUMBER IN STUDY

QUESTIONNAIRE NUMBER

DATE OF QUESTIONAIRE:

AGE OF CHILD IN WEEKS

INFANT ILLNESS

1. TEMPERAMENT

In the last 3 nights between 7pm. and 12 midnight how many hours of irritable, colicky or distressed behavior has baby shown?

ASSESSOR: Write in average number of hours per night; i.e. 1,2,3, 6 etc.

2. GASTROINTESTINAL SYMPTOMS

a) In the last three days has there been any vomiting > 1 desertspoon i.e. 15 ml? How many times per day?

ASSESSOR : Write in number of episodes of vomiting.

b) In the last three days on average, how many bowel actions per day?

ASSESSOR: Write in number of bowel actions.

c) How many bouts of illness with vomit and/or diarrhoea have occured in the last 4 weeks?

ASSESSOR: Write in number of episodes.

3. SKIN SYMPTOMS

a) In the last 4 weeks has baby shown persistent skin rash for more than a week?

1 = Yes 2 = No 3 = Don't know.

ASSESSOR: Write in number.

If rash persistent > 1 week what is distibution?

1. Scalp
2. Face
3. Nappy Area
4. Trunk
5. Legs
6. Arms

ASSESSOR: 1 = Yes 2 = No
(If no rashes at all put 2 for each).

In the past 4 weeks how many days of Topical treatment were given?

i) Cortisone(steroid) application

ii) Non-cortisone applications

ASSESSOR: If no treatment = 0 , otherwise write in days of treatment required.

4. RESPIRATORY SYMPTOMS

In the last 4 weeks record the total number of days of:

a) blocked, snuffly or runny nose.

b) cough and/ or chest rattle and /or wheeze.

ASSESSOR : Write in days.

5. OTHER ILLNESSES IN THE LAST 4 WEEKS:

In the last 4 weeks record total number of days with, or episodes of:-

a) High temperature (fever) with illness.
Specify illness if known, e.g. Measles .....

b) Hives.
If cause known, specify.....

c) Otitis Media
i.e. Middle ear infection diagnosed by Doctor.

d) Hospital admission.
Specify illness if known.....
Operation .....

e) Episodes of allergic reaction not due to drugs.
Specify cause, if known .....

f) Episodes of allergic reaction to drugs.
Specify if known .....

ASSESSOR: Write in days of fever, or episodes of illness as indicated; 0 if no illness.

Has there been any visits to the Doctors, or any other allied Health Workers?

ASSESSOR: Write in who was visited and reason why. .
0 if no visits.

6. USE OF MEDICATIONS

In the last 4 weeks, specify the number of days baby has received:

i) Antibiotics .....
Specify type.

ii) Cough Mixtures

iii) Asthma medication
e.g. Ventolin, Nuelin, Intal, Steroids.
Specify .....

iv) Anti - allergy drugs.
i.e. Antihistamines

v) Nasal sprays or drops.

vi) Other
Specify .....

ASSESSOR : Write in days of treatment required with each.
Write in 0 if none given.

7. IMMUNIZATIONS

In the last 4 weeks specify any immunizations.

1 Triple Antigen
2. CDT
3. Sabin
4. MMR vacc.
5. Other

ASSESSOR: 1 = Yes 2 = None given 3 = Not sure

PHYSICAL EXAMINATION

1. Height = Percentile

2. Weight = Percentile

Is nose blocked, runny or snuffly?

1 = Yes 2 = No 3 = Don't know.

Is cough, chest rattle present.

1 = Yes 2 = No 3 = Don't know

5. Is rash evident on:

1. Scalp
2. Nappy area
3. Face
4. Trunk
5. Arms/legs

ASSESSOR: 1 = Yes 2 = No 3 = Don't know

Appendix 4: MACS 12 year follow-up questionnaire

INTERNATIONAL SURVEY OF BREATHING, NOSE AND SKIN PROBLEMS IN CHILDREN

QUESTIONNAIRE FOR PARENTS OF 6 – 7 YEAR OLDS

Office Use Only [1] [ ][ ] [ ][ ] [ ][ ][ ][ ] [ ][ ] [ ][ ][ ]

INSTRUCTIONS FOR COMPLETING THE QUESTIONNAIRE

On this sheet are questions about your child's name, school and birthdate. Please write your answers to these questions in the spaces provided.

All other questions require you to tick your answer in a box. If you make a mistake, put a cross in the box and tick the correct answer. Tick only one option unless otherwise instructed.

Examples of how to mark the questionnaire:

To answer "yes": Yes ☑ No ☐

To answer "no": Yes ☐ No ☑

Today's Date: ____ / ____ / ____ (Day / Month / Year)

Child's Name: ____ (First Name) ____ (Last Name)

Child's School: ____

Child's Date of Birth: ____ / ____ / ____ (Day / Month / Year)

Child's Age: ____ Years

(Tick all your answers for the rest of the questionnaire)

Is your child a: ☐ Male or ☐ Female

In which country was your child born? ☐ Australia ☐ Other → ____ Please Specify ☐ Office Use

In which country was the child's Mother born? ☐ Australia ☐ Other → ____ Please Specify ☐ Office Use

1. Has your child ever had wheezing or whistling in the chest at any time in the past?

☐ Yes ☐ No

IF YOU HAVE ANSWERED "NO" PLEASE GO TO QUESTION 6

2. Has your child ever had wheezing or whistling in the chest in the past 12 months?

☐ Yes ☐ No

IF YOU HAVE ANSWERED "NO" PLEASE GO TO QUESTION 6

3. How many attacks of wheezing has your child had in the past 12 months?

☐ None ☐ 1 to 3 ☐ 4 to 12 ☐ More than 12

4. In the past 12 months, how often, on average, has your child's sleep been disturbed due to wheezing?

☐ Never woken with wheezing ☐ Less than one night per week ☐ One or more nights per week

5. In the past 12 months, has wheezing ever been severe enough to limit your child's speech to only one or two words at a time between breaths?

☐ Yes ☐ No

6. Has your child ever had asthma?

☐ Yes ☐ No

7. In the past 12 months, has your child had a dry cough at night, apart from a cough associated with a cold or chest infection?

☐ Yes ☐ No

8. In the past 12 months, has your child's chest sounded wheezy during or after exercise?

☐ Yes ☐ No

IF YOU HAVE ANSWERED "NO" PLEASE GO TO QUESTION 13

9. In the past 12 months, has asthma limited the amount of sport or physical activity your child does?

☐ Yes ☐ No

10. Do you sometimes try and stop your child from taking part in sports or physical education because your child has asthma? ☐ Yes ☐ No

11. Do the teachers at your child's school sometimes exclude your child from taking part in physical education or sport because your child has asthma? ☐ Yes ☐ No ☐ Do Not Know

12. Before your child does any exercise that may make him/her wheezy, do you give him/her any medicine? ☐ Yes ☐ No

a) If you answered "yes", what medicine do you give your child before he/she exercises?

Name of Medication	Office Use

IF YOU ANSWERED "YES" TO QUESTION 2 (HAS HAD WHEEZE IN THE LAST 12 MONTHS) THEN PLEASE ANSWER QUESTIONS 13 TO 17, OTHERWISE GO TO QUESTION 18

13. In the past 12 months, has your child taken any medication (medicines/pills/puffers) for wheezing or asthma? ☐ Yes ☐ No

IF YOU ANSWERED YES, THEN PLEASE NAME THE MEDICATIONS:

Name of Medication	When medication is taken (please circle one or both)	Office Use
	When wheezy / regularly*	
	When wheezy / regularly*	
	When wheezy / regularly*	
	When wheezy / regularly*	

(* every day for at least 2 months in the year)

14. Do you have a written plan which tells you how to look after your child's asthma? ☐ Yes ☐ No

15. In the past 12 months, how many visits has your child made to the doctor (family doctor, general practitioner or specialist) for his/her wheezing or asthma?

a). For a wheezy episode? ☐ None ☐ 1 to 3 ☐ 4 to 12 ☐ More than 12

b). For a regular "check-up" for asthma? ☐ None ☐ 1 to 3 ☐ 4 to 12 ☐ More than 12

16. In the past 12 months, how many visits has your child made to a hospital Casualty or Emergency Department because of his/her wheezing or asthma? ☐ None ☐ 1 ☐ 2 ☐ 3 or More

17. In the past 12 months, how many times has your child been admitted to hospital because of his/her wheezing or asthma? ☐ None ☐ 1 ☐ 2 ☐ 3 or More

THE FOLLOWING QUESTIONS ARE ABOUT PROBLEMS WHICH OCCUR WHEN YOUR CHILD **DOES NOT** HAVE A COLD OR THE FLU

18. Has your child ever had a problem with sneezing or a runny or blocked nose when he/she DID NOT have a cold or the flu?

☐ Yes ☐ No

IF YOU HAVE ANSWERED "NO" PLEASE GO TO QUESTION 23

19. In the past 12 months, has your child had a problem with sneezing or a runny or blocked nose when he/she DID NOT have a cold or the flu?

☐ Yes ☐ No

IF YOU HAVE ANSWERED "NO" PLEASE GO TO QUESTION 23

20. In the past 12 months, has this nose problem been accompanied by itchy-watery eyes?

☐ Yes ☐ No

21. In which of the past 12 months did this nose problem occur? (Please tick any which apply)

☐ January ☐ February ☐ March ☐ April

☐ May ☐ June ☐ July ☐ August

☐ September ☐ October ☐ November ☐ December

22. In the past 12 months, how much did this nose problem interfere with your child's daily activities?

☐ Not at all ☐ A little ☐ A moderate amount ☐ A lot

23. Has your child ever had hayfever?

☐ Yes ☐ No

24. Has your child ever had an itchy rash which was coming and going for at least 6 months?

☐ Yes ☐ No

IF YOU HAVE ANSWERED "NO" PLEASE GO TO QUESTION 30

25. Has your child had this itchy rash at any time in the past 12 months?

☐ Yes ☐ No

IF YOU HAVE ANSWERED "NO" PLEASE GO TO QUESTION 30

26. Has this rash at any time affected any of the following places?

- The folds of the elbows
- Behind the knees
- In front of the ankles
- Under the buttocks
- Around the neck, ears or eyes?

☐ Yes ☐ No

27. At what age did this itchy rash first occur?

☐ Under 2 Years ☐ Age 2-4 Years ☐ Age 5 or More

28. Has this rash cleared completely at any time during the past 12 months?

☐ Yes ☐ No

29. In the past 12 months, how often, on average, has your child been kept awake at night by this itchy rash?

☐ Never in the past 12 months ☐ Less than one night per week ☐ One or more nights per week

30. Has your child ever had eczema?

☐ Yes ☐ No

The Royal Children's Hospital, Melbourne

THE UNIVERSITY OF MELBOURNE

SURVEY 1: TO BE COMPLETED BY ALL PARTICIPANTS WHO ARE 14 YEARS AND OLDER

Date survey completed

_____

Day/month/year

First name _____

Last name _____

ID _____

(Or affix sticker here) _____

Respiratory health

1. Have you ever had wheezing or whistling in the chest at any time in the past? (Wheezing means a whistling sound, however high or low pitched and however faint).

☐ **No**→ go to Q3

☐ **Yes**→ continue

1.1 At what age did you first have wheezing or whistling in the chest?

_____ Years

1.2 At what age did you last have wheezing or whistling in the chest?

_____ Years

2. Have you had wheezing or whistling in the chest in the last 12 months?

☐ **No**→ go to Q3

☐ **Yes**→ continue

2.1 How many attacks of wheezing have you had in the last 12 months?

_____ attacks

2.2 In the last 12 months, how often, on average, has your sleep been disturbed due to wheezing?

☐ Never woken with wheezing

☐ Less than one night per week

☐ One or more nights per week

2.3 In the last 12 months, has wheezing ever been severe enough to limit your speech to only one or two words between each breath?

☐ **No**

☐ **Yes**

3. Have you ever had asthma?

☐ **No**→ go to Q4

☐ **Yes**→ continue

3.1 Was this asthma confirmed by a doctor?

☐ **No**

☐ **Yes**

3.2 How many episodes of asthma have you had in the past 12 months?

_____ episodes or ☐ continuous

3.3 Do you have a written plan which tells you how to look after your asthma?

☐ **No**

☐ **Yes**

4. In the past 12 months, have you seen a doctor (family doctor, general practitioner or specialist), attended a hospital casualty or emergency department or been admitted to hospital for your **wheezing** OR **asthma**?

☐ **No**→ go to Q5

☐ **Yes**→ continue

4.1 In the past 12 months, how many visits have you made to the doctor (family doctor, general practitioner or specialist) for your wheezing or asthma?

a) for a wheezy episode: _____ visits

b) for a regular "check-up for asthma: _____ visits

4.2 In the past 12 months, how many visits have you made to hospital Casualty or Emergency Department because of wheezing or asthma (but not subsequently admitted to hospital)?

_____ visits

4.3 In the past 12 months, how many times have you been admitted to hospital because of wheezing or asthma?

_____ times

5. In the last 12 months, has your chest sounded wheezy during or after exercise?

☐ **No**

☐ **Yes**

6. Before you do any exercise that may make you wheezy, do you take any medications?

☐ **No**

☐ **Yes**

7. Have you taken any medicines including inhalers or tablets for asthma or wheezy breathing, in the last 12 months?

☐ **No**

☐ **Yes**

7.1 If yes, please list these medications

_____

_____

_____

8. Have you, had **cough with phlegm** on most days for at least three months and for two years in a row?

☐ **No**

☐ **Yes**

9. In the past 12 months, have you had any episodes of cough?

☐ **No**→ go to Q10

☐ **Yes**→ continue

9.1 In the past 12 months, how long was the longest episode of cough?

☐ less than one week

☐ one week

☐ two weeks

☐ three or four weeks

☐ more than four weeks

9.2 In the past 12 months, how many episodes of cough have you had?

_____ episodes or ☐ continuous

9.3 In the last 12 months, have you had a dry cough at night, apart from a cough associated with a cold or chest infection?

☐ **No**

☐ **Yes**

9.4 In the past 12 months, have you taken any treatments for cough?

☐ **No**

☐ **Yes**

9.5 If Yes, please list these medications.

_____

_____

_____

Nasal symptoms

THE FOLLOWING QUESTIONS ARE ABOUT PROBLEMS WHICH OCCUR WHEN YOU **DO NOT** HAVE A COLD OR THE FLU

10. 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?

☐ **No**→ go to Q11

☐ **Yes**→ continue

10.1 At what age did you first have a problem with sneezing, or a runny, or a blocked nose?

_____ Years

10.2 At what age did you last have a problem with sneezing, or a runny, or a blocked nose?

_____ Years

10.3 In the past 12 months, have you had a problem with sneezing, or a runny, or a blocked nose when you **DID NOT** have a cold or the flu?

☐ **No**→ go to Q11

☐ **Yes**→ continue

10.4 In the past 12 months, has this nose problem been accompanied by itchy-watery eyes?

☐ **No**

☐ **Yes**

10.5 In which of the past 12 months did this nose problem occur? (please tick any which apply)

☐ No particular time of year

☐ January	☐ May	☐ September
☐ February	☐ June	☐ October
☐ March	☐ July	☐ November
☐ April	☐ August	☐ December

10.6 In the past 12 months, how much did this nose problem interfere with your daily activities?

- ☐ Not at all
- ☐ A little
- ☐ A moderate amount
- ☐ A lot

11. Have you ever had hay fever?

- ☐ **No**→ go to Q12
- ☐ **Yes**→ continue

11.1 How many episodes of hay fever have you had in the past 12 months?

_____ episodes or ☐ continuous

12. Have you taken any medicines including nasal inhalers or tablets for hay-fever or problem with sneezing, or a runny, or a blocked nose in the last 12 months?

- ☐ **No**
- ☐ **Yes**

12.1 If yes, please list these medications

_____

_____

_____

13. Do you ever get a runny or stuffy nose or start to sneeze when you are near:

	No	**Yes**
cats	☐	☐
dogs	☐	☐
horses	☐	☐
feathers (including pillows, quilts or duvets)	☐	☐
a dusty part of the house	☐	☐
pollen, grass, trees or flowers	☐	☐
other please list _____ _____	☐	☐

Skin conditions

14. Have you ever had an itchy rash which was coming and going for at least 6 months?

- ☐ **No**→ go to Q15
- ☐ **Yes**→ continue

14.1 At what age did this itchy rash first occur?

_____ years

14.2 At what age did you last have this rash?

_____ years

14.3 Have you had this itchy rash at any time in the last 12 months?

- ☐ **No**→ go to Q15
- ☐ **Yes**→ continue

14.4 Has this itchy rash at any time affected any of the following places: (tick all that apply)

- ☐ The folds of the elbows
- ☐ Behind the knees
- ☐ In front of the ankles
- ☐ Under the buttocks
- ☐ Around the neck, ears or eyes
- ☐ Trunk (chest, stomach or back)
- ☐ Other. Please list: _____

14.5 Has this rash cleared completely at any time during the last 12 months?

- ☐ **No**
- ☐ **Yes**

14.6 In the past 12 months, how often, on average, have you been kept awake by this itchy rash?

- ☐ Never in the last 12 months
- ☐ Less than one night per week
- ☐ One or more nights per week

15. Have you ever had eczema?

- ☐ **No**→ go to Q18
- ☐ **Yes**→ continue

15.1 How many episodes of eczema have you had in the past 12 months?

_____ episodes or ☐ continuous

If no episodes of eczema, please go to question 18

Severity of eczema – only complete if you have had eczema IN THE LAST 12 MONTHS

16. Please circle the number under the column that describes the skin on each part of your body. **Please circle any and all that apply.**

	Clear	Dry	Scaly	Redness	Cracks/ openings	Oozing
Head (face, ears scalp)	0	1	2	3	4	5
Neck	0	1	2	3	4	5
Trunk (body, not including limbs)	0	1	2	3	4	5
Arms	0	1	2	3	4	5
Hands	0	1	2	3	4	5
Legs	0	1	2	3	4	5
Feet	0	1	2	3	4	5

17. How itchy has your skin been in the following places? **Please circle only one**

	None at all	A little bit	Somewhat bothered	Quite bothered	Very bothered	Extremely bothered/losing sleep
Head (face, ears scalp)	0	1	2	3	4	5
Neck	0	1	2	3	4	5
Trunk (body, not including limbs)	0	1	2	3	4	5
Arms	0	1	2	3	4	5
Hands	0	1	2	3	4	5
Legs	0	1	2	3	4	5
Feet	0	1	2	3	4	5

18. Have you used any medicines including creams and ointments for rash or eczema during the last 12 months?

☐ **No**

☐ **Yes**

18.1 If yes, please list these medications

_____

_____

_____

_____

Food and other allergies

19. Do you have an allergy to any of the following? (tick all that apply to you)

	No	**Yes**
Latex	☐	☐
Medicines please list	☐	☐
Other please list	☐	☐

_____

_____

_____

_____

_____

_____

20. Have you ever had any food allergies?

☐ Yes → please continue

☐ No → go to Q21

☐ Not sure → please continue

		Food 1 (eg egg)	Food 2	Food 3
		_____	_____	_____
20.1 Who diagnosed this food allergy? (tick all that apply)	Self-diagnosis	☐	☐	☐
	Doctor diagnosis	☐	☐	☐
	Other health care professional: Please list	☐ _____	☐ _____	☐ _____
20.2 Have you ever had a skin prick test (SPT) or a food challenge at an allergy clinic (challenge) to confirm this food allergy? (tick all that apply)		SPT ☐ challenge ☐	SPT ☐ challenge ☐	SPT ☐ challenge ☐
20.2.1 What were the results of the SPT for this food?		+ve ☐ -ve ☐ unsure ☐	+ve ☐ -ve ☐ unsure ☐	+ve ☐ -ve ☐ unsure ☐
20.2.2 What were the results of food challenge for this food?		+ve ☐ -ve ☐ unsure ☐	+ve ☐ -ve ☐ unsure ☐	+ve ☐ -ve ☐ unsure ☐
20.3 Have you been prescribed with an epipen for this food allergy?		☐ **No** ☐ **Yes**	☐ **No** ☐ **Yes**	☐ **No** ☐ **Yes**
20.4 At what age were you **first diagnosed** with this food allergy?		___ years	___ years	___ years
20.5 At what age did you **last consume this food**?		___ years or ☐ never	___ years or ☐ never	___ years or ☐ never
20.6 At what age did you **last react to this food?**		___ years or ☐ never	___ years or ☐ never	___ years or ☐ never
20.7 What symptoms have you experienced when you have reacted to this food? Write "never", if you have never reacted to this food. Common symptoms of food include: hives/urticaria/wheals, (small raised itchy areas likened to mosquito bites), itchy skin or rash, swelling of the lips, red swollen eyes, vomiting, diarrhoea, wheezing, coughing, collapse.		_____ _____ _____ _____	_____ _____ _____ _____	_____ _____ _____ _____

Eczema and rash

WE REALISE THE FOLLOWING QUESTIONS ARE SIMILAR TO THOSE YOU HAVE ALREADY ANSWERED, BUT THE WORDING IS SLIGHTLY DIFFERENT

21. IN THE LAST YEAR, have you had an ITCHY skin condition - by itchy we mean scratching or rubbing the skin?

☐ **No**→ go to Q22

☐ **Yes**→ continue

21.1 How old were you when this skin condition began? (please tick one box only)

☐ Under 2

☐ 2 to 5

☐ 5 to 10

☐ Over 10

21.2 Has this skin condition ever affected the skin creases in the past - by skin creases we mean fronts of elbows, behind the knees, fronts of ankles, around the neck, or around the eyes?

☐ **No**

☐ **Yes**

22. Have you ever suffered from asthma - by asthma we mean bouts of wheezing or whistling in the chest?

☐ **No**

☐ **Yes**

23. Have you ever suffered from hay fever - by hay fever we mean bouts of sneezing with a runny nose or itchy eyes in the summer?

☐ **No**

☐ **Yes**

24. In the last year, have you suffered from a dry skin in general?

☐ **No**

☐ **Yes**

General questions

25. What is your highest level of education?

☐ Primary School

☐ High School

☐ Trade / Apprenticeship (eg Hairdresser, electrician, plumber etc)

☐ Certificate or Diploma (eg. Child care, technician etc)

☐ University degree (eg. Bachelor)

☐ Postgraduate university degree (eg. Graduate Diploma, Masters, PhD)

26. If you are currently working, what is your job?

_____

26.1 On average, how many hours per day do you sit at a desk during working hours?

☐ none, or ____ hours per day.

26.2 What industry would you like to work in?

_____

27. Have you smoked at least 100 cigarettes or equal amounts of cigars, pipes or any other tobacco product?

☐ **No**→ go to Q28

☐ **Yes**→ continue

27.1 How old were you when you started and stopped smoking, and how much did you smoke during these times?

Many people start and stop smoking, and smoke different amounts during these times. Please use a separate line for each period that you have smoked.

Age started	Age stopped (current age if you still smoke)	Average cigarettes smoked
___ years	___ years	___ per day
___ years	___ years	___ per day
___ years	___ years	___ per day

Quality of life

28. In general, would you say your health is:

☐ Excellent

☐ Very good

☐ Good

☐ Fair

☐ Poor

29. The following questions are about activities you might do during a typical day. Does your health *now* limit you in these activities? If so, how much?

a) **Moderate activities,** such as moving a table, pushing a vacuum cleaner, bowling, or playing golf

☐ Yes, limited a lot

☐ Yes, limited a little

☐ No, not limited at all

b) Climbing **several** flights of stairs

☐ Yes, limited a lot

☐ Yes, limited a little

☐ No, not limited at all

30. During the *past 4 weeks*, have you had any of the following problems with your work or other regular daily activities as a result of your physical health?

a) **Accomplished less** than you would like

☐ **No**

☐ **Yes**

b) Were limited in the **kind** of work or other activities

☐ **No**

☐ **Yes**

31. During the *past 4 weeks*, have you had any of the following problems with your work or other regular daily activities *as a result of any emotional problems* (such as feeling depressed or anxious)?

a) **Accomplished less** than you would like

☐ **No**

☐ **Yes**

b) Didn't do work or other activities as **carefully** as usual

☐ **No**

☐ **Yes**

32. During the *past 4 weeks*, how much did pain interfere with your normal work (including both work outside the home and housework)?

☐ Not at all

☐ A little bit

☐ Moderately

☐ Quite a bit

☐ Extremely

These questions are about how you feel and how things have been with you during the past 4 weeks. For each question, please give the one answer that comes closest to the way you have been feeling.

		All of the time	Most of the time	A good bit of the time	Some of the time	A little of the time	None of the time
33. How much of the time during the *past 4 weeks*...							
33.1	Have you felt calm and peaceful?	☐	☐	☐	☐	☐	☐
33.2	Did you have a lot of energy?	☐	☐	☐	☐	☐	☐
33.3	Have you felt downhearted and blue?	☐	☐	☐	☐	☐	☐

	All of the time	Most of the time	Some of the time	A little of the time	None of the time
34. During the *past 4 weeks*, how much of the time has your physical health or emotional problems interfered with your social activities (like visiting friends, relatives, etc.)?	☐	☐	☐	☐	☐

35. If you have any current pain, disability, or illness what is the main cause for this?

_____

36. The following questions are about your feelings **in the past 4 weeks:**

About how often did you feel	All of the time	Most of the time	Some of the time	A little of the time	None of the time
36.1 Nervous?	☐	☐	☐	☐	☐
36.2 Hopeless?	☐	☐	☐	☐	☐
36.3 Restless or fidgety?	☐	☐	☐	☐	☐
36.4 That everything was an effort?	☐	☐	☐	☐	☐
36.5 So sad that nothing could cheer you up?	☐	☐	☐	☐	☐
36.6 Worthless?	☐	☐	☐	☐	☐

General health and activity

37. Has a doctor ever told you that you have or have had this condition? (please tick all that apply).

	No	Yes
Diabetes Mellitus?	☐	☐
Heart Disease?	☐	☐
High Blood Pressure?	☐	☐
Chronic Bronchitis?	☐	☐
Emphysema?	☐	☐
Chronic obstructive lung disease or chronic obstructive airways disease (COPD, COAD)?	☐	☐
Other medical condition/s	☐	☐

Please list: _____

38. During a normal week, how many hours of each type of exercise do you perform **per week**?

38.1 **moderate exercise** (for example walking)

☐ none, or _____ hours per week

38.2 **strenuous** physical exertion (for example running or lifting weights)

☐ none, or _____ hours per week

39. During a normal week, how many hours of each type of activity do you perform **per day**?

39.1 watch television

☐ none, or _____ hours per day

39.2 use a computer or games console for non-work related reasons (such as games, research or study)

☐ none, or _____ hours per day

40. In the past 12 months, how often, on average, have you taken paracetamol (eg Panadol), ibuprofen (eg Nurofen), or aspirin? (please tick all that apply)

Medicine	Never	Once per year	Monthly	Weekly
Paracetamol	☐	☐	☐	☐
Ibuprofen	☐	☐	☐	☐
Aspirin	☐	☐	☐	☐

40.1 Did you use each of these medicines for treatment of **migraine**?

Medicine	Yes	No
Paracetamol	☐	☐
Ibuprofen	☐	☐
Aspirin	☐	☐

Supplemental questions for females

Please continue if you are a female. Please skip to question 46 if you are a male.

41. Have you ever had a menstrual period?

☐ **No** → Go to question 46

☐ **Yes** → Please continue

42. At what age did you first have your first menstrual period?

_____ (for example "12 years")

43. What was the date of the first day of your last menstrual period?
If you have reached menopause, please list the year of your last menstrual period

_____ (date)
(For example 14 July 2008)

The "pill" (oral contraceptive pill) and hormone replacement therapy (HRT) are used for a number of reasons, including treatment for period pain and "hot flushes", and for birth control.

44. Have you ever used the "pill" or HRT?

☐ **No** → Go to question 45

☐ **Yes** → Please continue

44.1 Between what ages did you use the "pill" or HRT, and what type?

Ages (eg 18 to 25)	"Pill"	HRT	Type (brand name)	OR can't remember
___ to ___	☐	☐	_____	☐
___ to ___	☐	☐	_____	☐
___ to ___	☐	☐	_____	☐

45. Do you generally have regular menstrual periods? Regular menstrual periods are between 21 to 35 days apart, and do not vary by more than 4 days between cycles.

- If you have reached menopause, were your menstrual periods generally regular?
- If you take oral contraception or HRT, were your menstrual periods generally regular when you **were not taking** these medicines?

☐ **No**

☐ **Yes**

Attitudes and beliefs about asthma

Questions 46 to 52 should only be completed by participants **who have had symptoms of asthma in the last 12 months.** If you do not have current asthma, please skip to question 53.

INSTRUCTIONS: Below there is a list of statements. At the end of each statement, please tick ONE of the boxes to show how much you agree/disagree with the statement

	Strongly disagree	Tend to disagree	Tend to agree	Strongly agree
46. Think first about how it feels to be an asthmatic:				
a) I feel different from other people because I am an asthmatic	☐	☐	☐	☐
b) Being an asthmatic often makes me feel angry	☐	☐	☐	☐
c) Being an asthmatic often makes me feel depressed	☐	☐	☐	☐
d) I feel somehow to blame for being an asthmatic	☐	☐	☐	☐
e) I became an asthmatic because of emotional upset	☐	☐	☐	☐
47. Consider the effect on your relationship to others:				
a) I avoid letting other people know I am an asthmatic	☐	☐	☐	☐
b) The people who are closest to me seem overly protective of me because of my asthma	☐	☐	☐	☐
c) I am worried that my asthma may interfere with the lives of the people closest to me	☐	☐	☐	☐
48. Now think about the severity of your asthma:				
a) My asthma is severe	☐	☐	☐	☐
b) Looking toward the future, I feel certain that my asthma will get better	☐	☐	☐	☐
c) I worry about the long-term effects of asthma on my health	☐	☐	☐	☐
d) Asthma has made me physically less attractive	☐	☐	☐	☐
e) I worry that I might die from asthma	☐	☐	☐	☐
49. Consider the effect of asthma on your activities:				
a) I can't enjoy a full life because of my asthma	☐	☐	☐	☐
b) I do all the things I want to, regardless of their effect on my asthma	☐	☐	☐	☐
c) I know what things start my asthma attacks	☐	☐	☐	☐
d) I don't do anything about my asthma until it gets bad	☐	☐	☐	☐

INSTRUCTIONS: Below there is a list of statements. At the end of each statement, please tick ONE of the boxes to show how much you agree/disagree with the statement

	Strongly disagree	Tend to disagree	Tend to agree	Strongly agree
50. Think now about your asthma attacks:				
a) I have confidence in my ability to cope with an asthma attack	☐	☐	☐	☐
b) I know when an asthma attack is beginning	☐	☐	☐	☐
c) I can prevent asthma attacks	☐	☐	☐	☐
d) Even when I feel well, I worry about getting an attack of asthma	☐	☐	☐	☐
e) At the first sign of an asthma attack I feel panicky and frightened	☐	☐	☐	☐
51. Now consider your asthma medication:				
a) I worry that my asthma medication may have unwanted effects on my health	☐	☐	☐	☐
b) I find it easy to remember to take my asthma medication	☐	☐	☐	☐
c) It embarrasses me to use asthma inhalers or other asthma medication in public	☐	☐	☐	☐
d) It's a good idea to increase or decrease asthma medication without consulting the doctor	☐	☐	☐	☐
52. Think about the doctor who looks after your asthma:				
a) My doctor has helped to make my asthma better	☐	☐	☐	☐
b) I have confidence in my doctor's management of my asthma	☐	☐	☐	☐
c) I avoid troubling the doctor about my asthma	☐	☐	☐	☐
d) 1 wish that my doctor talked more to me about my asthma	☐	☐	☐	☐
e) My doctor tells me everything I want to know about my asthma	☐	☐	☐	☐

Home Conditions

THESE QUESTIONS ARE TO BE COMPLETED BY ONE PERSON PER HOUSEHOLD.

Please tick all that are appropriate.

All family members live together ☐ → **only the mother OR father answers these questions**
Mother and father live separately ☐ → **both the mother AND father answers these questions**
If you have moved away from family home ☐ → **you also have to answer these questions**

53. What is the address of your home?

_____

_____

_____

54. Of your immediate family, who lives with you and how many days per week do they normally live in this home?

Name	Days per week
_____	_____
_____	_____
_____	_____
_____	_____
_____	_____
_____	_____
_____	_____
_____	_____

55. How long have you lived in this home?

_____ years

56. In total how many people live in this house?

_____ people

57. Which types of heating and cooling do you use at home? ***(tick all that apply)***

- ☐ Gas ducted central heating
- ☐ Coal or wood fire
- ☐ Gas room heater
- ☐ Electric heater (eg. Radiator, fan or dimplex-type)
- ☐ Other central heating (eg Electric, hydronic, slab floor)
- ☐ Reverse cycle air-conditioning
- ☐ Evaporative cooler
- ☐ Other
- ☐ No Heating

58. What kinds of stove do you **mostly** use for **cooking**? ***(tick one only)***

- ☐ Gas
- ☐ Coal, coke or wood
- ☐ Electric
- ☐ Other

59. Do you have an exhaust fan over the stove?

- ☐ No → go to Q60
- ☐ Yes → continue

59.1 When cooking how often do you use the fan? ***(tick one only)***

- ☐ All the time
- ☐ Some of the time
- ☐ None of the time

59.2 Does the fan take the fumes out of the house?

- ☐ No
- ☐ Yes
- ☐ Don't Know

60. Has there **ever** been mould or mildew on any surfaces, other than food, in your home?

- ☐ No → go to Q61
- ☐ Yes → continue

60.1 Which rooms have been affected? ***(tick all that apply)***

- ☐ Bathrooms
- ☐ The child's bedroom
- ☐ Other bedrooms
- ☐ Living areas
- ☐ Kitchen
- ☐ Any other area/s

60.2 Has there been mould or mildew on any surface, other than food, in your home **in the last 12 months**?

- ☐ No
- ☐ Yes

61. Which rooms have wall to wall carpet as the floor covering ***(tick all that apply)***

	No	Yes
Bedrooms	☐	☐
Living areas	☐	☐
Kitchen	☐	☐
Any other area/s	☐	☐

62. Do you usually sleep with the window open?

- ☐ No
- ☐ Yes

63. How often is the bedroom aired? (ie windows open for more than two hours)

- ☐ Most days (5 or more times per week)
- ☐ 2-4 times a week
- ☐ Once a week
- ☐ Less often than once a week

64. How often do you open windows in your house?

- ☐ Daily
- ☐ 2-4 times per week
- ☐ Weekly or less often
- ☐ Never

65. Do the windows you open have fly screens?

- ☐ Yes
- ☐ No
- ☐ Not all

66. What type of backyard do you have?

- ☐ Grass
- ☐ Dirt
- ☐ Concrete
- ☐ Paving
- ☐ Grass and combination of the above
- ☐ No backyard

67. How many people in your household currently smoke regularly (most days of the week) outside the house?

_____ people

68. How many cigarettes are smoked on average per day outside your home?

_____ per day

69. How many people in your household currently smoke regularly (most days of the week) inside the house?

_____ people

70. How many cigarettes are smoked on average per day inside your home?

_____ per day

71. In the last 12 months, did you normally have any animals living with you?

- ☐ **No** → Thanks! That completes the survey.
- ☐ **Yes** → please continue

If yes, which type(s) of animal do you have?

	How many of these animals live with you?	Do they live inside your home	
		Inside	Outside
☐ Dog	____ dog/s	☐	☐
☐ Cat	____ cat/s	☐	☐
☐ Guinea pig	____ guinea pig/s	☐	☐
☐ Rabbit	____ rabbit/s	☐	☐
☐ Birds	____ bird/s	☐	☐
☐ Other (please list below)	____ animal/s	☐	☐

_____

TAS. 10/P.1

TASMANIAN ASTHMA SURVEY

CHILD'S MEDICAL HISTORY — CONFIDENTIAL

THIS SECTION IS FOR OFFICE USE ONLY

Code

1 Survey Number

CHILD'S SURNAME CALLARY

CHILD'S CHRISTIAN NAME Bernadette

SCHOOL HEALTH NUMBER HCK 092
(not to be coded)

Sex F

145 ~~155~~ ~~145~~ School Name ~~BURNIE~~ Montello 155

108 ~~111~~ ~~108~~ Child's School Number 111

TO BE FILLED IN BY PARENT, GUARDIAN OR RELATIVE

Important

(a) Before completing this form, please indicate your relationship to the child named in the panel above by putting a cross in the appropriate square below:

MOTHER	FATHER	STEP-PARENT OR ADOPTED PARENT OR GUARDIAN	OTHER RELATION	NO RELATION
☒ 1	☐ 2	☐ 3	☐ 4	☐ 5

(b) Has the child named above got a twin, or any brothers or sisters born in the same year (that is 1961)? (put a cross in the appropriate square) YES ☐ NO ☒

If the answer is YES, please give Christian names and the name of the school the brother or sister attends:

Name.....

School.....

(c) Please state the number of living full brothers and sisters in the family (excluding any born in 1961). Nil

(d) If you do not read English well and have difficulty understanding the questions for this reason, please put a cross here ☐

(e) Please name the local doctor or hospital usually attended by this child for any ailment.
Burnie Medical Centre

HOW TO ANSWER THE QUESTIONS:

In most cases, unless otherwise indicated, you are asked to answer YES or NO by putting a cross ☒ in the appropriate square. Sometimes, if the answer is not simply YES or NO, there are more than two squares provided, but you should still put a cross in only one of the several squares. Only three questions (relating to months of the year) may need a cross in more than one square, but these exceptions are specifically mentioned.

Please make sure that **ALL** questions are answered; please do not omit one, as this might mean we can not use the other information you will have given us. We understand that the answers to some questions can only be approximate, just as we understand that one's memory cannot always be perfectly accurate. If you wish to add more information or more details, please do so on a separate sheet of paper. Do **NOT** write extra notes on the form, and always answer the question with a cross in the most appropriate square, even if you add extra notes on a separate piece of paper.

Note that there are really four separate forms — the first deals with the health of the child named in the panel above, the second deals with the health of the mother, the third with the health of the father, and the fourth with the health of brothers and sisters. All these are equally important in helping us to understand the importance of the whole family in respiratory and allergic illnesses.

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1. (a) Where was he/she born?

Tasmania	☒ 1
Other Australian State	☐ 2
United Kingdom, New Zealand, South Africa, Canada, United States of America	☐ 3
Other Overseas country (Please Name.....)	☐ 4

(b) Date of birth 22-7-61

2. Did he/she have infantile (baby) eczema? — YES ☐ NO ☒

3. Has he/she ever had eczema in the creases (bends) of elbows, wrists, or knees? — ☐ ☒

4. How was he/she fed in the first three months of life?
   - Breast only ☐ 1
   - Bottle only ☒ 2
   - Breast and bottle ☐ 3

5. Does he/she have a stutter or stammer? — YES ☐ NO ☒

6. Has he/she a habit of biting the fingernails? — ☐ ☒

7. During the first three years of life did you have to take the child to the doctor because of difficulties with feeding?
   - Often ☒ 1
   - Occasionally ☐ 2
   - Never ☐ 3

8. For how much time in the last twelve months has the child been confined to the house because of chest illnesses?
   - Not at all ☐ 1
   - One to seven days ☐ 2
   - Eight to thirty days ☒ 3
   - More than a month ☐ 4

9. Has he/she had the tonsils removed? — YES ☒ NO ☐

10. Has he/she had **more** than two sore throats or attacks of tonsilitis in the past twelve months? — ☐ ☒

11. Have you ever been told by a doctor that he/she had pneumonia or pleurisy?
   - No, Never ☐ 1
   - Yes, Once or twice ☐ 2
   - Yes, More than twice ☒ 3

12. Have you been told by a doctor that he/she is allergic to any foods or medicines? — YES ☒ NO ☐

13. Does he/she get hives?
   - Never ☐ 1
   - Once or twice a year ☐ 2
   - More than twice a year ☒ 3

14. Has he/she **at any time** in his/her life suffered from attacks of asthma or of wheezy breathing? — YES ☐ NO ☒
   (**Note:** Please regard "asthma" and "wheezy breathing" as being much the same thing for this survey; we do not ask you to try to tell the difference)

The following questions (Nos. 15 to 21) relate to the details of these illnesses, and need to be answered if the answer to question 14 was YES. If the answer to question 14 was NO, omit these questions, and go on to question 21.

15. How long is it since the last attack?
   - Less than a month ago 1 ☐
   - Over one but less than three months ago 2 ☐
   - Over three but less than six months ago 3 ☐
   - Over six but less than twelve months ago 4 ☐
   - Over one year but less than two years ago 5 ☐
   - Over two years ago 6 ☐

16. On the average (as near as you can say), how often do these attacks tend to occur over the last two years or so?
   - About once in twenty-four hours 1 ☐
   - About once a week 2 ☐
   - About once a fortnight 3 ☐
   - About once a month 4 ☐

17. On the average usually last (with

18. At what age did

19. Since the attacks altogether

20. If the attacks tend lar time of the y the appropriate s FOUR of the twe leave all squares
JAN. ☐ FEB. ☐ MCH. ☐ APRI ☐

21. Has he/she **at an** chitis or attacks c or "rattly" cough)
(**Note:** Please reg in the che same thin the differe

The following questior to be answered if the was NO, omit these qu

22. How long is it sin

23. On the average (i tend to occur over

NO ☒

NO ☒

NO ☒

NO ☐

NO ☐

NO ☒

About once every three months 5 ☐

About once every six months 6 ☐

About once a year (or less often) 7 ☐

No attacks at all in the last two years 8 ☐

17. On the average (as near as you can say), how long do these attacks usually last (with usual treatment)?

Less than twelve hours 1 ☐

A day or so 2 ☐

A week or so 3 ☐

A month or so 4 ☐

"Continuous" (never free of asthma or wheezing for more than a day or two) 5 ☐

18. At what age did these attacks begin?

Under 1 year 1 ☐

Over 1 and under 2 years 2 ☐

Over 2 and under 3 years 3 ☐

Over 3 and under 4 years 4 ☐

Over 4 and under 5 years 5 ☐

Over 5 and under 6 years 6 ☐

Over 6 and under 7 years 7 ☐

Over 7 years 8 ☐

19. Since the attacks began, approximately how many has he/she had altogether

One attack only 1 ☐

Two to five attacks 2 ☐

Six to ten attacks 3 ☐

Eleven to twenty attacks 4 ☐

Over twenty attacks 5 ☐

20. If the attacks tend to be more frequent or more severe at any particular time of the year, indicate the "bad" months by putting a cross in the appropriate square(s) (but DO NOT put a cross against more than FOUR of the twelve months). If no month is worse than the others, leave all squares blank.

JAN.	FEB.	MCH.	APRIL	MAY	JUNE	JULY	AUG.	SEPT.	OCT.	NOV.	DEC.
☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

→ 21. Has he/she **at any time** in his/her life suffered from attacks of bronchitis or attacks of cough with sputum (phlegm) in the chest ("loose" or "rattly" cough)?

(**Note:** Please regard "bronchitis" and "cough with sputum (phlegm) in the chest," and "loose or rattly cough" as being much the same thing for this survey; we do not ask you to try to tell the difference).

YES ☒ NO ☐

The following questions (Nos. 22-27) relate to the details of these illnesses, and need to be answered if the answer to question 21 was YES. If the answer to question 21 was NO, omit these questions and go on to question 28.

22. How long is it since the last attack?

Less than a month ago 1 ☐

Over one but less than three months ago 2 ☐

Over three but less than six months ago 3 ☒

Over six but less than twelve months ago 4 ☐

Over one year but less than two years ago 5 ☐

Over two years ago 6 ☐

23. On the average (as near as you can say) how often do these attacks tend to occur over the last two years or so?

About once in twenty-four hours 1 ☐

About once a week 2 ☐

About once a fortnight 3 ☐

About once a month 4 ☐

About once every three months 5 ☒

About once every six months 6 ☐

About once a year (or less often) 7 ☐

No attacks at all in the last two years 8 ☐

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24. On the average (as near as you can say), how long do these attacks usually last (with usual treatment)?

Less than twelve hours	☐ 1
A day or so	☐ 2
A week or so	☒ 3
A month or so	☐ 4
"Continuous" (never free of loose cough for more than a day or two)	☐ 5

25. At what age did these attacks begin?

Under one year	☒ 1
Over 1 and under 2 years	☐ 2
Over 2 and under 3 years	☐ 3
Over 3 and under 4 years	☐ 4
Over 4 and under 5 years	☐ 5
Over 5 and under 6 years	☐ 6
Over 6 and under 7 years	☐ 7
Over 7 years	☐ 8

26. Since the attacks began, approximately how many has he/she had altogether?

One attack only	☐ 1
Two to five attacks	☐ 2
Six to ten attacks	☐ 3
Eleven to twenty attacks	☐ 4
Over twenty attacks	☒ 5

27. If the attacks tend to be more frequent or more severe at any particular time of the year, indicate the "bad" months by putting a cross in the appropriate square(s) (but DO NOT put a cross against more than FOUR of the twelve months). If no month is worse than the others, leave all squares blank.

JAN.	FEB.	MCH.	APRIL	MAY	JUNE	JULY	AUG.	SEPT.	OCT.	NOV.	DEC.
☒	☐	☐	☐	☐	☒	☒	☐	☐	☐	☐	☒

28. Does he/she get attacks of "hay fever" (that is, sneezing, running or blocked nose, sometimes with itchy eyes or nose)? YES ☒ NO ☐

If the answer to question 28 is NO, omit question 29.

29. If these hay fever attacks tend to be more frequent or more severe at any particular time of the year, indicate the "bad" months by putting a cross in the appropriate square(s) (but DO NOT put a cross against more than FOUR of the twelve months). If no month is worse than another, leave all squares blank.

JAN.	FEB.	MCH.	APRIL	MAY	JUNE	JULY	AUG.	SEPT.	OCT.	NOV.	DEC.
☒	☒	☒	☐	☐	☐	☐	☒	☒	☒	☐	☐

30. Is he/she prone to "colds in the head" (that is, more than two or three colds a year)? YES ☐ NO ☒

If the answer to question 30 is NO, omit question 31.

31. If these head colds tend to be more frequent or more severe at any particular time of the year, indicate the "bad" months by putting a cross in the appropriate square(s) (but DO NOT put a cross against more than FOUR of the twelve months). If no month is worse than another, leave all the squares blank.

JAN.	FEB.	MCH.	APRIL	MAY	JUNE	JULY	AUG.	SEPT.	OCT.	NOV.	DEC.
☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

32. Has he/she ever had a chest X-ray? YES ☒ NO ☐

If the answer is YES:

Where was the X-ray taken? Royal Hobart Hospital
(Name of hospital, doctor or clinic)

What year was it taken? 19 66

Was it normal, as far as you know? YES ☒ NO ☐

33. Has he/she ever attended a chest clinic as a contact of a person with tuberculosis? YES ☐ NO ☒

Please check your answers to all the questions: Thank you.

MERCURY 67-4866

TAS. 20/1

TASMANIAN ASTHMA SURVEY

HEALTH OF MOTHER — CONFIDENTIAL

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THIS SECTION FOR OFFICE USE ONLY

Code

1 Survey Number

CHILD'S SURNAME CALLARY

CHILD'S CHRISTIAN NAME Bernadette

SCHOOL HEALTH NUMBER HCK 092
(not to be coded)

Sex F

145 School Name BURNIE

108 Child's School Number

COD

Important

(a) Before completing this form, please indicate your relationship to the child named in the panel above by putting a cross in the appropriate square below:

MOTHER	FATHER	STEP-PARENT OR ADOPTED PARENT OR GUARDIAN	OTHER RELATION	NO RELATION
☒	☐	☐	☐	☐
1	2	3	4	5

(b) This form seeks information about the actual mother of the child named above so that we can study the possible importance of heredity. Naturally, we would prefer that she filled it in, but if the mother is not available, information would still be welcomed on any of the questions below, **provided** you can be reasonably definite from your own knowledge of the mother's health. If you feel you cannot give any useful or reliable information please put a cross here ☐ and pass on to the next form.

MOTHER'S SURNAME AND CHRISTIAN NAME Anne Marie Callary AGE 35

	YES 1	NO 2
1. Do you go out to work (part or full-time)?	☒	☐
2. Have you **ever** had asthma or attacks of wheezing like asthma?	☐	☒
3. Do you suffer from chronic bronchitis, or from more than one attack of bronchitis every three years or so?	☐	☒
4. Have you **ever** suffered from "hay fever"?	☒	☐
5. Do you suffer frequently from "colds in the head" (that is, more than three colds a year)?	☒	☐
6. Have you ever been told by a doctor that you have a chest complaint called "emphysema"? (If you have never heard of emphysema, record your answer as NO).	☐	☒

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		1 YES	2 NO
7.	Do you usually have a cough either first thing in the morning or some time during the day? ("Usually" means on three or four days in the week, or for three or four months of the year)	☒	☐
8.	Do you usually clear or bring up some phlegm (sputum) from your chest in the mornings or during the day? ("Usually" means on three or four days of the week, or for three or four months of the year)	☒	☐
9.	Do you smoke every day (or six days out of seven)?	☒	☐

If your answer to question 9 is NO, omit question 10 and go on to question 11.

10. How much do you smoke?

More than 20 cigarettes a day?	☐ 1
Six to 20 cigarettes a day?	☒ 2
Less than 6 cigarettes a day?	☐ 3

11. Are you troubled by shortness of breath when hurrying on the level or walking up a slight rise so that you cannot keep up with other people of your own age? (NOTE: If you are physically handicapped, so that you cannot take walking exercise, put a cross in the square for "not applicable").

Yes	☒ 1
No	☐ 2
Not applicable	☐ 3

12. Are you able to keep up when walking on the flat with other people of your own age? (NOTE: If you are physically handicapped so that you cannot take walking exercise, put a cross in the square for "not applicable").

Yes	☒ 1
No	☐ 2
Not applicable	☐ 3

13. Has any brother or sister of this child died of a chest illness? ☐ ☒

If the answer is NO, omit the remaining questions.

If the answer is YES, please state:

	1		2	
Year of deceased child's birth				
Year of death				
Sex				
Illness causing death				
	YES	NO	YES	NO
Did the child ever suffer from asthma?	☐	☐	☐	☐
Did the child suffer from bronchitis on more than one occasion?	☐	☐	☐	☐

Please check your answers to all the questions: Thank you.

MERCURY 67-4865

OWN FATHER DECEASED – HAS STEP FATHER

TAS. 30/P.1

TASMANIAN ASTHMA SURVEY

HEALTH OF FATHER — CONFIDENTIAL

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THIS SECTION FOR OFFICE USE ONLY

Code

1 Survey Number

CHILD'S SURNAME GALLAGHER

CHILD'S CHRISTIAN NAME Bernadette

SCHOOL HEALTH NUMBER HCK 092
(not to be coded)

Sex F

145 School Name BURRIS

108 Child's School Number

Important

(a) Before completing this form, please indicate your relationship to the child named in the panel above by putting a cross in the appropriate square below:

MOTHER	FATHER	STEP-PARENT OR ADOPTED PARENT OR GUARDIAN	OTHER RELATION	NO RELATION
☐	☐	☐	☐	☐

(b) This form seeks information about the actual father of the child named above so that we can study the possible importance of heredity. Naturally, we would prefer that he filled it in. If the father is not available, information would still be welcomed on any of the questions below, **provided** you can be reasonably definite from your own knowledge of the father's health. If you feel you cannot give any useful or reliable information, please put a cross here ☒ and pass on to the next form.

FATHER'S SURNAME AND CHRISTIAN NAME.....AGE.....

		YES	NO
1.	Please indicate your occupation.....		
2.	Have you **ever** had asthma or attacks of wheezing like asthma?	☐	☐
3.	Do you suffer from chronic bronchitis, or from more than one attack of bronchitis every three years or so?	☐	☐
4.	Have you **ever** suffered from "hay fever"?	☐	☐
5.	Do you suffer frequently from "colds in the head" (that is, more than three colds a year)?	☐	☐
6.	Have you ever been told by a doctor that you have a chest complaint called "emphysema"? (If you have never heard of emphysema, record your answer as NO).	☐	☐

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	YES	NO
7. Do you usually have a cough either first thing in the morning or some time during the day? ("Usually" means on three or four days in the week, or for three or four months of the year)	☐	☐
8. Do you usually clear or bring up some phlegm (sputum) from your chest in the mornings or some time during the day? ("Usually" means on three or four days in the week or for three or four months of the year).	☐	☐
9. Do you smoke every day (or six days out of seven)? **If your answer to question 9 is NO, omit questions 10 and 11, and go on to question 12.**	☐	☐
10. Do you smoke		
(a) cigarettes?	☐	☐
(b) a pipe?	☐	☐
(c) cigars?	☐	☐

Note: If you smoke a pipe and cigarettes on most days, answer YES to (a) and (b), and NO to (c). If you mostly smoke cigarettes and only **rarely or occasionally** smoke a pipe or cigars, answer YES to (a) and NO to (b) and (c). If you mostly smoke a pipe and only **rarely or occasionally** smoke cigarettes, answer NO to (a) and (c), and YES to (b)

11. If you smoke cigarettes, how many cigarettes do you smoke each day?

More than 20 cigarettes a day	☐
Six to 20 cigarettes a day	☐
Less than 6 cigarettes a day	☐

12. Are you troubled by shortness of breath when hurrying on the level or walking up a slight rise so that you cannot keep up with other people of your own age? (Note: If you are physically handicapped, so that you cannot take walking exercise, put a cross in the square for "not applicable").

Yes	☐
No	☐
Not applicable	☐

13. Are you able to keep up when walking **on the flat** with other people of your own age? (Note: If you are physically handicapped, so that you cannot take walking exercise, put a cross in the square for "not applicable".)

Yes	☐
No	☐
Not applicable	☐

Please check your answers to all the questions: Thank you.

MERCURY 67-4866

TASMANIAN ASTHMA SURVEY

HEALTH OF BROTHERS and SISTERS—CONFIDENTIAL

Office use only

THIS SECTION IS FOR OFFICE USE ONLY

Code

.....Survey Number

CHILD'S SURNAME.....

CHILD'S CHRISTIAN NAME.....

SCHOOL HEALTH NUMBER.....
(not to be coded)

..... Sex.....

..... School Name.....

..... Child's School Number

Important

(a) Before completing this form, please indicate your relationship to the child named in the panel above by putting a cross in the appropriate square below:

MOTHER	FATHER	STEP-PARENT OR GUARDIAN OR ADOPTED PARENT	OTHER RELATION	NO RELATION
☐	☐	☐	☐	☐

(b) This form seeks information about the brothers and sisters of the child named in the panel above. Use a separate set of questions for each brother or sister.

If the child has any half-brothers or half-sisters (step-brothers or step-sisters), please put a cross here ☐

However, **only full brothers and sisters** of the child need to be included in the detailed form below. If you do not know enough about the child's full brothers and sisters to fill in the form completely, please give at least their names, sex and approximate year of birth in the spaces provided.

SURNAME AND CHRISTIAN NAME.....SEX.....

DATE OF BIRTH.....

	YES	NO
1. Has he/she **ever** had asthma or attacks of wheezing like asthma?	☐	☐
2. Has he/she **ever** been diagnosed by a doctor as suffering from chronic bronchitis or from recurrent bronchitis (more than three or four attacks of bronchitis)	☐	☐
3. Has he/she been diagnosed by a doctor as having a chest condition called "bronchiectasis"? (If you have not heard of bronchiectasis, record the answer as NO).	☐	☐
4. Has he/she been confined to the house, or to bed, for a total of more than seven days in the last year because of asthma, bronchitis, coughs and colds?	☐	☐
5. Does he/she suffer from "hay-fever"?	☐	☐
6. Does he/she suffer frequently from "colds in the head" (that is, more than three colds a year)?	☐	☐
7. Did he/she have infantile (baby) eczema?	☐	☐
8. Has he/she **ever** had eczema in the creases (bends) of elbows, knees or wrists?	☐	☐
9. Has he/she had tonsils removed?	☐	☐

Please check your answers to all the questions: Thank you.

[OVER

Office use only

SURNAME AND CHRISTIAN NAME.....SEX.....

DATE OF BIRTH.....

	YES	NO
1. Has he/she **ever** had asthma or attacks of wheezing like asthma?	☐	☐
2. Has he/she **ever** been diagnosed by a doctor as suffering from chronic bronchitis or from recurrent bronchitis (more than three or four attacks of bronchitis)	☐	☐
3. Has he/she been diagnosed by a doctor as having a chest condition called "bronchiectasis"? (If you have not heard of bronchiectasis, record the answer as NO).	☐	☐
4. Has he/she been confined to the house, or to bed, for a total of more than seven days in the last year because of asthma, bronchitis, coughs and colds?	☐	☐
5. Does he/she suffer from "hay-fever"?	☐	☐
6. Does he/she suffer frequently from "colds in the head" (that is, more than three colds a year)?	☐	☐
7. Did he/she have infantile (baby) eczema?	☐	☐
8. Has he/she **ever** had eczema in the creases (bends) of elbows, knees or wrists?	☐	☐
Has he/she had tonsils removed?	☐	☐

Please check your answers to all the questions: Thank you.

SURNAME AND CHRISTIAN NAME.....SEX.....

DATE OF BIRTH.....

	YES	NO
1. Has he/she **ever** had asthma or attacks of wheezing like asthma?	☐	☐
2. Has he/she **ever** been diagnosed by a doctor as suffering from chronic bronchitis or from recurrent bronchitis (more than three or four attacks of bronchitis)	☐	☐
3. Has he/she been diagnosed by a doctor as having a chest condition called "bronchiectasis"? (If you have not heard of bronchiectasis, record the answer as NO).	☐	☐
4. Has he/she been confined to the house, or to bed, for a total of more than seven days in the last year because of asthma, bronchitis, coughs and colds?	☐	☐
5. Does he/she suffer from "hay-fever"?	☐	☐
6. Does he/she suffer frequently from "colds in the head" (that is, more than three colds a year)?	☐	☐
7. Did he/she have infantile (baby) eczema?	☐	☐
8 Has he/she **ever** had eczema in the creases (bends) of elbows, knees or wrists?	☐	☐
9. Has he/she had tonsils removed?	☐	☐

Please check your answers to all the questions: Thank you.

MERCURY 66-868

TASMANIAN ASTHMA SURVEY

SCHOOL TEACHER'S ASSESSMENT — CONFIDENTIAL

Office use only

THIS SECTION FOR OFFICE USE ONLY

Code

1 ..... Survey Number

CHILD'S SURNAME ..... CALLARY .....

CHILD'S CHRISTIAN NAME ..... Bernadette .....

SCHOOL HEALTH NUMBER ..... HCK 092 .....
(not to be coded)

..... Sex ..... F .....

155 ..... School Name ..... Montello .....

111 ..... Child's School Number

Please answer each question by putting a cross in the appropriate square. From your own observations and from any other reliable information available to you:

	YES	NO	
1. Is this child more liable to "head colds," "hay fever" or a "snuffly nose" than most members of the class?	1 ☐	2 ☒	0
2. Is this child more liable to bronchitis or a loose ("chesty") cough than most members of the class?	☐	☒	
3. Does the child suffer from asthma or wheezing?			0
Yes — More or less constantly	☐		1
Yes — Frequently (more than once a month)	☐		2
Yes — Occasionally (once a month or less often)	☐		3
No	☒		4
4. Is the child's physical activity restricted by shortness of breath, asthma or wheezing?			
Never	☒		1
At times only	☐		2
More or less constantly	☐		3
5. If your knowledge of the child is insufficient to allow an answer to these questions, please put a cross here ☐			0 / 1 Insufficient knowledge

MERCURY 68-771

TAS. 60/P.1

TASMANIAN ASTHMA SURVEY

CLINICAL DATA — CONFIDENTIAL

THIS SECTION FOR OFFICE USE ONLY

Code
1

.....Survey Number

CALLARY
CHILD'S SURNAME.....Bernadette.....
CHILD'S CHRISTIAN NAME.....HCK 092.....
SCHOOL HEALTH NUMBER.....
(not to be coded)
F

155 Sex.....Montello

111 School Name.....

..... Child's School Number

Office use only

Date of Examination 27 - 6 - 68

Medical Officer's Number 3

Height (ins.) 4 3 3/4

Weight (lbs.) 43

Scales Calibration ☑ 28 lbs. = ...

56 lbs. = ...

	YES	NO
Flexural Eczema (knees, elbows and wrists)	☐	☒
Generalised Eczema	☐	☒

Nasal Discharge

None	☒
Present Mucoid	☐
Present Coloured	☐

Nasal Obstruction

	YES	NO
Right	☒	☐
Left	☐	☒

Post Nasal Discharge ☐ ☒

Chest Deformity

	SEVERE	MILD	ABSENT
1. Kyphosis			X
2. Scoliosis			X
3. Funnel			X
4. Pigeon			X
5. "Asthmatic"			X

OVER

Office use only

Cough

Dry	☒
Loose	☐
Would not cough	☐

	YES	NO
Audible Wheezing	☐	☒

Auscultation

Normal	☒	1
Rhonchi unilateral	☐	2
bilateral	☐	3
Rales unilateral	☐	4
bilateral	☐	5

Ventilatory Function

Date of Test 27-6-68 ~~a.m.~~/p.m.

Room Temperature 19 °C

Spirometer Number 1619

Co-operation

Satisfactory	☐
Doubtful	☐
Poor	☒

	YES	NO
Spirometer Calibration checked	☒	☐

If Ventilatory Test not performed, state reason.....

.....

.....

Attach Spirogram

RESULTS (Office use only) INCOMPLETE SPIROGRAM

F.E.V. 0.5 (ml) 1190

F.E.V. 1.0 (ml) —

V.C. (ml) —

Ratio F.E.V./V.C.

M.E.F.R. (ml/sec.) —

MERCURY 68-771

TASMANIAN ASTHMA SURVEY

MEDICAL DIAGNOSES—CONFIDENTIAL

THIS SECTION FOR OFFICE USE ONLY

Code

..... Survey Number

CHILD'S SURNAME.....

CHILD'S CHRISTIAN NAME.....

SCHOOL HEALTH NUMBER.....
(not to be coded)

..... Sex.....

..... School Name.....

..... Child's School Number

Office use only

Section A. **CHILD'S MEDICAL DIAGNOSIS**

Tuberculin Test — POS. ☐ NEG. ☐ NOT KNOWN ☐

Parental evidence for

	Definite	Doubtful	Absent
Infantile Eczema			
Flexural Eczema			
U.R.T. abnormality			
"Asthma"			
"Bronchitis"			

	YES	NO
Tonsils Removed	☐	☐
School Teacher evidence for		
U.R.T. abnormality	☐	☐
L.R.T. abnormality	☐	☐
Medical Evidence for		
Eczema	☐	☐

	Definite	Doubtful	Absent
U.R.T. Abnormality			
L.R.T. Abnormality			
"Asthma"			
"Bronchitis"			

Office use only

Overall assessment of child

	Definite	Doubtful	Absent
Infantile Eczema			
Flexural Eczema			X
U.R.T. Abnormality	X		
L.R.T. Abnormality	X		
"Asthma"			
"Bronchitis"			

Section B. **PARENTS MEDICAL DIAGNOSES**

	Definite	Doubtful	Absent
Mother—Evidence of U.R.T. Abnormality	X		
Evidence of L.R.T. Abnormality			X
Father—Evidence of U.R.T. Abnormality			
Evidence of L.R.T. Abnormality			

Section C. **SIBLINGS MEDICAL DIAGNOSES**

	Definite	Doubtful	Absent	Total
No. with evidence of (a) Eczema				
(b) U.R.T. Abnormality				
(c) L.R.T. Abnormality				

Results of Spirometry (not to be coded on this form)

F.E.V. 0.5 (ml)	
F.E.V. 1.0 (ml)	
V.C. (ml)	
Ratio F.E.V./V.C.	
M.E.F.R. (ml/sec.)	

TASMANIAN ASTHMA SURVEY

FAMILY RECORD—FOR OFFICE USE ONLY

Code

1 Survey Number

CHILD'S SURNAME CALLARY

CHILD'S CHRISTIAN NAME Bernadette

SCHOOL HEALTH NUMBER HCK 092
(not to be coded)

Sex F

155 School Name Montello

111 Child's School Number

	YES	NO
Mother available	☐	☐
Information recorded	☐	☐
Father available	☐	☐
Information recorded	☐	☐

SIBLINGS BORN IN 1961

NAME	SCHOOL No.	TAS./10 COMPLETED YES	NO
		☐	☐
		☐	☐

Siblings excluding those born in 1961

Number.....

Number recorded.....

Assistance required to fill in forms

Considerable	☐ 1
A little	☐ 2
None	☐ 3
Unco-operative in spite of personal interview	☐ 1
Language barrier insuperable	☐ 1

Child unable to participate owing to:

1. Absence from school	☐ 1
2. Geographical isolation	☐ 2
3. Physical handicap	☐ 3
4. Mental deficiency	☐ 4

MERCURY 68-771

UNIVERSITY OF TASMANIA

MONASH University

WINDOW

THE UNIVERSITY OF MELBOURNE

The Fourth Follow-up Study of the 1968 Tasmanian Asthma Survey

We would like to invite you to take part in a postal survey for a follow-up study of the 1968 Tasmanian Asthma Survey. In 1968, as part of a statewide survey of all 7 year old children, your parents filled in a questionnaire about your health. They also completed surveys for your brothers and sisters as well as for themselves. With 45,900 Tasmanians involved, this research is the largest asthma study in the world.

This current follow-up study will look at the factors that affect respiratory health in adults. We have found your address on the federal electoral rolls *(allowed under sections 91(4A)(e) and 91(2A)(c) of the Commonwealth Electoral Act 1918 and Regulation 10 of the Electoral and Referendum Regulations)* or recently from your brothers or sisters.

We would be grateful if you could help us by completing this survey booklet and returning it in the reply paid envelope provided. We'd like as many of you to complete the survey booklet as possible, **even if you do not have any asthma or any respiratory problems**. This is so we can compare information from people with and without respiratory illnesses to identify the causes of these conditions. This survey booklet contains:

- **Consent Form** which you need to sign if you agree to fill in this survey booklet
- **Respiratory Health Questionnaire**
- **Family Contact Details Form**

We are asking for the details of your family so we can also survey them with a similar questionnaire in the future. Providing their details does not commit them to be involved in this study. You may wish to discuss this with them to make sure they are happy for you to pass on their information. A lot of time has gone by since the original study and if this letter happens to bring up memories of upsetting events that have occurred in your family, we sincerely apologise.

If you have questions about this research or need help in completing the survey, please contact Cathryn Wharton on **1800 110 711** (free call Australia wide) or email: inq-tas@unimelb.edu.au
Alternatively, you could contact the chief investigator Dr Shyamali Dharmage on (03) 8344 0737.

Your participation in this survey is completely voluntary. If you do not wish to take part you are not obliged to, but please let us know by ringing 1800 110 711 (free call) so we do not send you a reminder letter. All information you provide is strictly confidential. Your Consent Form (page 2) and Family Contact Details Form (pages 11 and 12) which have your identifying information will be removed from this booklet and stored separately from your questionnaire. If you have a complaint about this project or concerns about your rights as a research participant, you can contact the Executive Officer, Human Research Ethics, The University of Melbourne on (03) 8344 4071.

Over the years this study has made many great contributions to the understanding of respiratory illnesses. Thank you for your help with this research so far and for taking the time to consider our request.

Sincere regards,

Dr Shyamali Dharmage, Senior Lecturer
Chief Investigator of the T.A.S.

Cathryn Wharton, Senior Research Assistant
Project Coordinator of the T.A.S.

THE UNIVERSITY OF MELBOURNE

Fourth Follow-up Study of the 1968 Tasmanian Asthma Survey

CONSENT FORM

If you agree to fill in this survey booklet we need you to sign below. Signing this form simply means that you are willing to take part in this postal survey and provide information in this booklet. It does not remove any of your legal rights as a research participant.

Please do not fold this booklet or remove any of the pages.
Before you post this booklet please make sure you have:

1. signed this Consent Form
2. completed the Respiratory Health Questionnaire (pages 3 to 10)
3. filled in the Family Contact Details Form (pages 11 and 12)

Post the completed booklet in the reply paid envelope provided or mail to:

Tasmanian Asthma Study
Department of Public Health
The University of Melbourne
Reply Paid 75370
CARLTON VIC 3053

If you have any questions please contact Cathryn Wharton on **1800 110 711.**

Participant Name: _____ ID number: _____

*If the above name is **not correct** please record your current full name below.*

Current name: _____

I agree to complete this survey booklet that is part of a research study titled **"The fourth follow-up study of the 1968 Tasmanian Asthma Survey"**, which is being conducted by Dr Shyamali Dharmage of The University of Melbourne.

I have been informed that the general purpose of this study is to identify the causes of respiratory health problems in adults. I know that my involvement in this research is voluntary and I am free to withdraw at any time. I also understand that my personal information will be kept confidential and my identity will not be revealed in publications of results.

I would like to receive a report on the findings of the study *(please shade in the oval)* ○ Yes

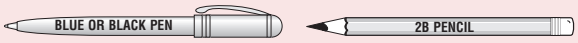
Participant signature: _____ **Date:** _____

RESPIRATORY HEALTH QUESTIONNAIRE
TASMANIAN ASTHMA STUDY 1968 - 2003/04

Mail-out 1

Please read these instructions & examples before you fill in the questionnaire.

- Please do not fold or bend this booklet.



- Please use a blue or black pen or 2B pencil.
- Please mark your responses like this: ○ ● ○ ○
- Ovals must be completely shaded in.
- Cross out any mistakes and shade in the correct oval, e.g: ○ ⊗ ○ ● ○
- If you need help to answer any of the questions please contact us on 1800 110 711 (free call).
- Unless indicated, shade in only one oval

  Example: To indicate "female"

  Ⓐ Male
  ● Female

- Where a number is required, write the number in the boxes and shade in the ovals.

  Example: To indicate "2"

Write number AND shade in ovals

Today's Date:

Day		Month	Year
		○ JAN	○ 2003
		○ FEB	○ 2004
0	0	○ MAR	○ 2005
1	1	○ APR	○ 2006
2	2	○ MAY	○ 2007
3	3	○ JUN	
	4	○ JUL	
	5	○ AUG	
	6	○ SEP	
	7	○ OCT	
	8	○ NOV	
	9	○ DEC	

Your date of birth:

If this is **NOT CORRECT**, please record your date of birth below:

Day		Month	Year		
		○ JAN	19		
		○ FEB			
0	0	○ MAR		0	0
1	1	○ APR		1	1
2	2	○ MAY		2	2
3	3	○ JUN		3	3
	4	○ JUL		4	4
	5	○ AUG		5	5
	6	○ SEP		6	6
	7	○ OCT		7	7
	8	○ NOV		8	8
	9	○ DEC		9	9

Write number AND shade in ovals

HEALTH QUESTIONS

1. Have you ever been allergic to any of the following?
   SHADE ALL THAT APPLY

   ○ Foods
   ○ Medicine
   ○ Dust
   ○ Animals
   ○ Pollen, grass, trees or flowers

2. Have you ever had eczema or any kind of skin allergy?

   ○ No
   ○ Yes

3. Have you ever had hay fever (that is sneezing, running or blocked nose *when you **do not** have a cold or the flu*)?

   ○ No
   ○ Yes

4. Have you had treatment for heart trouble in the past 10 years?

   ○ No
   ○ Yes

BARCODE & ID NUMBER TO BE PRINTED HERE

5. Are you disabled from walking by a condition other than heart or lung disease?

- ○ No
- ○ Yes

6. Are you troubled by shortness of breath when hurrying on level ground or walking up a slight hill?

- ○ No → go to question **7**
- ○ Yes → continue

6.1 Do you have to walk slower than people of your age because of breathlessness?

- ○ No
- ○ Yes

6.2 Do you have to stop for breath when walking at your own pace on level ground?

- ○ No
- ○ Yes

6.3 Do you ever get short of breath when resting?

- ○ No
- ○ Yes

7. Do you **usually** cough when you **do not** have a cold?

- ○ No → go to question **8**
- ○ Yes → continue

7.1 Are there months in which you cough on most days?

- ○ No → go to question **8**
- ○ Yes → continue

7.1.1 Do you cough on most days for at least three months of each year?

- ○ No
- ○ Yes

7.1.2 For how many years have you had this cough?

- ○ Less than 2 years
- ○ 2 - 5 years
- ○ More than 5 years

8. Do you **usually** have phlegm in your chest when you **do not** have a cold?

- ○ No → go to question **9**
- ○ Yes → continue

8.1 Are there months in which you have phlegm in your chest on most days?

- ○ No → go to question **9**
- ○ Yes → continue

8.1.1 Do you bring up this phlegm on most days for at least three months each year?

- ○ No
- ○ Yes

8.1.2 For how many years have you had this phlegm?

- ○ Less than 2 years
- ○ 2 - 5 years
- ○ More than 5 years

9. Have you, **at any time in your life**, suffered from **cough with phlegm** in the chest (with or without a cold)?

- ○ No → go to question **10**
- ○ Yes → continue

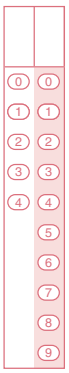
9.1 Have you had cough with phlegm on most days for at least three months of each year for two successive years?

- ○ No
- ○ Yes

10. Have you, **at any time in your life**, suffered from attacks of asthma or wheezy breathing? (Regard asthma and wheezy breathing as being much the same for this question.)

- ○ No → go to question **13**
- ○ Yes → continue

10.1 At what age did these attacks begin? *(age in years)*



Write number AND shade in ovals

10.2 How long ago did you have the last attack?

- ○ Within the last year
- ○ Over 1 year but less than 2 years ago
- ○ Over 2 years ago

11. Are you:

- ○ Male → go to question **13**
- ○ Female → continue

11.1 Have you ever noticed that your asthma changes with your monthly menstrual period?
SHADE ONE ONLY

- ○ No, my asthma does not change when I have my period
- ○ Yes, my asthma gets worse when I have my period
- ○ Yes, my asthma gets better when I have my period
- ○ I don't have periods
- ○ Don't know

12. Have you been pregnant since you first had asthma?

- ○ No → go to question **13**
- ○ Yes → continue

12.1 What happened to your asthma during your pregnancies?
SHADE ONE ONLY

- ○ Improved
- ○ Got worse
- ○ Stayed the same
- ○ Not the same for all pregnancies
- ○ Don't know

→ go to question **13**

Please answer A, B and C for questions 13 to 16.

	A. Has a doctor ever told you that you have or had this condition?	**B.** How old were you when you were told you had this condition? *(age in years)* Write number AND shade in ovals (Eg. 2 years: 02)	**C.** Have you taken any medicine (including inhalers or tablets) for this condition in the **last three months**?
13. Asthma?	○ No → go to question **14** ○ Yes → continue		○ No ○ Yes
14. Chronic bronchitis?	○ No → go to question **15** ○ Yes → continue		○ No ○ Yes
15. Emphysema?	○ No → go to question **16** ○ Yes → continue		○ No ○ Yes
16. Chronic obstructive pulmonary disease or chronic obstructive airways disease?	○ No → go to question **17** ○ Yes → continue		○ No ○ Yes

17. Have you had wheezing or whistling in your chest **in the last 12 months**? (wheezing means a whistling sound, however high or low pitched and however faint.)

- ○ No → go to question **18**
- ○ Yes → continue

17.1 Have you been at all breathless when the wheezing noise was present?

- ○ No
- ○ Yes

17.2 Have you had this wheezing or whistling when you **did not** have a cold?

- ○ No
- ○ Yes

18. Have you, at any time **in the last 12 months**, woken up with a feeling of tightness in your chest?

- ○ No
- ○ Yes

19. Have you, at any time **in the last 12 months**, been woken at night by an attack of shortness of breath?

- ○ No
- ○ Yes

YOURSELF, WORK AND HOME

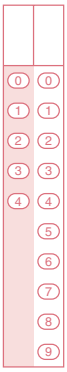
Now, we are asking for some information about yourself, your work and home to look at the effect of these things on people's respiratory health.

20. In your lifetime, have you smoked at least 100 cigarettes or equal amounts of cigars, pipes, or any other tobacco product?

- ○ No → go to question **21**
- ○ Yes → continue

20.1 How old were you when you started smoking?
(age in years)

Write number AND shade in ovals



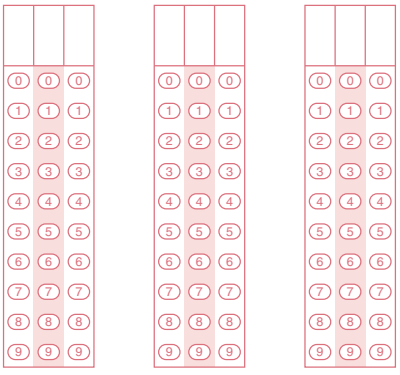
20.2 Do you currently smoke (within the last 4 weeks)?

- ○ Not at all → go to question **20.3**
- ○ Yes, daily → continue
- ○ Yes, at least weekly → continue
- ○ Yes, less than weekly → continue

20.2.1 On average, how much do you **currently** smoke (total number of cigarettes or equivalent product)? (Provide the average number per day **OR** per week **OR** per month)

per day **OR** per week **OR** per month

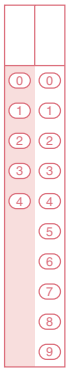
Write number AND shade in ovals



→ go to question **21**

20.3 How old were you when you stopped smoking?
(age in years)

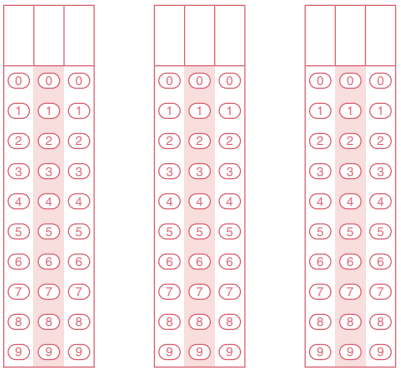
Write number AND shade in ovals



20.3.1 On average, during periods when you smoked, how much **did** you smoke (total number of cigarettes or equivalent product)? (Provide the average number per day **OR** per week **OR** per month)

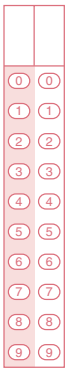
per day **OR** per week **OR** per month

Write number AND shade in ovals



21. Not counting yourself, how many people in your household currently smoke regularly (most days of the week) inside the house?

Write number AND shade in ovals



22. Do people smoke regularly (most days of the week) in the room where you work (not counting yourself)?

- ○ No
- ○ Yes

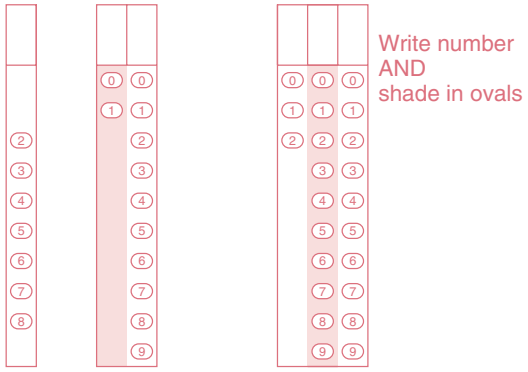
23. On average, how many hours per day are you exposed to other people's tobacco smoke (work and home)?

Write number AND shade in ovals

24. What is your height without shoes? (answer either in feet and inches **OR** in centimetres.)

Feet and Inches **OR** Centimetres

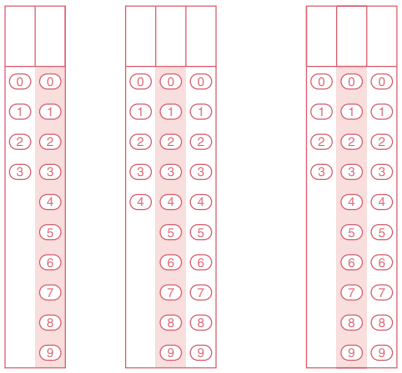
Write number AND shade in ovals



25. What is your weight? (answer either in stone and pounds **OR** in pounds **OR** in kilograms)?

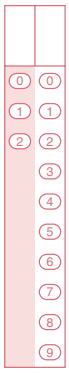
Stone and Pounds **OR** Kilograms

Write number AND shade in ovals



26. How many of your own (biological) children have you had?

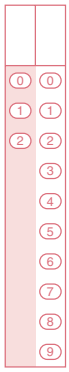
Write number AND shade in ovals



If you have not had children → go to question **27**
If you have had children ⟶ continue

26.1 How many of your own (biological) children have ever suffered from attacks of asthma or wheezy breathing?

Write number AND shade in ovals



27. What is the highest educational or vocational qualification that you have completed?

SHADE ONE ONLY

- ○ Grade 1 to 6
- ○ Grade 7 to 9
- ○ Grade 10 or 11
- ○ Grade 12 or equivalent (eg. Higher School Certificate)
- ○ Trade / Apprenticeship (eg. Hairdresser, electrician, plumber etc)
- ○ Certificate or Diploma (eg. Child care, technician etc)
- ○ University degree (eg. Bachelor)
- ○ Higher University Degree (eg. Graduate Diploma, Masters, PhD)

28. Are you currently employed or self-employed?
SHADE ONE ONLY

- ○ Yes
- ○ No, I am currently studying.
- ○ No, unemployed for less than 6 months and actively seeking work
- ○ No, unemployed for more than 6 months and actively seeking work
- ○ No, I am not working due to health, family or other reasons
- ○ No, retired
- ○ No, other

29. What is your main occupation?
SHADE ONE ONLY

- ○ Manager or administrator (eg. magistrate, general manager, school principal, director of nursing, farm manager)
- ○ Professional (eg. scientist, doctor, nurse, allied health professional, teacher, artist)
- ○ Associate professional (eg. technician, manager, youth worker, police officer)
- ○ Tradesperson or related worker (eg. hairdresser, gardener, florist)
- ○ Advanced clerical or service worker (secretary,flight attendant, law clerk, personal assistant)
- ○ Intermediate clerical, sales or service worker (eg. typist, data entry operator, receptionist, child care worker, nursing assistant, hospitality worker)
- ○ Intermediate production or transport worker (eg. machine operator, bus driver, sewing machinist)
- ○ Elementary clerical, sales or service worker (eg. filing/mail clerk, parking inspector, sales assistant, telemarketer, housekeeper)
- ○ Labourer or related worker (cleaner, factory worker, farm hand, kitchen hand)
- ○ House person

30. Which types of heating do you use at home?
SHADE ALL THAT APPLY

- ○ Gas ducted central heating
- ○ Coal or wood fire
- ○ Gas room heater
- ○ Electric heater (eg. radiator, fan or Dimplex-type)
- ○ Other central heating (eg. electric, hydronic, slab floor heating)
- ○ Reverse cycle air-conditioning
- ○ Other

31. What kind of stove do you **mostly** use for **cooking**?
SHADE ONE ONLY

- ○ Gas
- ○ Electric
- ○ Coal, coke or wood
- ○ Other

32. Do you have an exhaust fan over the stove?

- ○ No → go to question **33**
- ○ Yes → continue

32.1 When cooking how often do you use the fan?

- ○ All of the time
- ○ Some of the time
- ○ None of the time

32.2 Does the fan take the fumes outside the house?

- ○ No
- ○ Yes
- ○ Don't know

33. Has there ever been mould or mildew on any surfaces, other than food, in your home?

- ○ No → go to instructions on bottom of page
- ○ Yes → continue

33.1 Which rooms have been affected?
SHADE ALL THAT APPLY

- ○ Bathrooms
- ○ Your bedroom
- ○ Other bedrooms
- ○ Living areas
- ○ Kitchen
- ○ Any other area/s

33.2 Has there been mould or mildew on any surface, other than food, in your home **in the last 12 months**?

- ○ No
- ○ Yes

If you are male, please make sure you have signed the Consent Form (page 2) and completed the Family Contact Details Form (pages 11 & 12). Please return this whole booklet in the reply paid envelope provided. Thank you for your time and contribution to the T.A.S.

If you are female, please go to the next page.

SUPPLEMENT FOR WOMEN

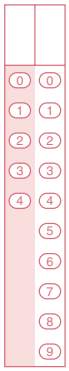
We are looking at the reasons why more women have asthma than men, especially since it is more common in boys than in girls. This is why we are asking the following questions. Thank you for your help.

34. Have you ever had a menstrual period?

- ○ No → go to question **35**
- ○ Yes → continue

34.1 What was the age of your first period? *(age in years)*

Write number AND shade in ovals

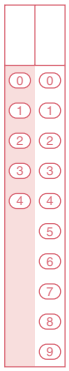


35. Have you **ever** used birth control pills or other hormonal contraceptives (implants or injections)?

- ○ No → go to question **36**
- ○ Yes → continue

35.1 At what age did you first use birth control pills or other hormonal contraceptives? *(age in years)*

Write number AND shade in ovals



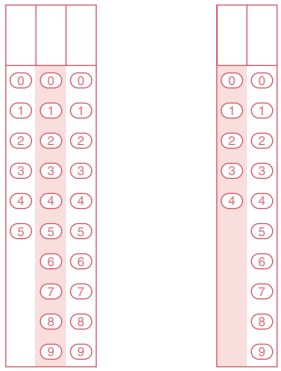
35.2 Are you currently taking birth control pills or other hormonal contraceptives?

- ○ No
- ○ Yes

35.3 Over your whole lifetime, in total how many months or years have you taken birth control pills or other hormonal contraceptives? (answer in months **OR** years.)

Write number AND shade in ovals

Months **OR** Years



36. Are you currently pregnant?

- ○ No
- ○ Yes
- ○ Don't know

37. Have you ever been pregnant in the past?

- ○ No → go to instructions below
- ○ Yes → go to question **38** (next page)

If No, please make sure that you have signed the Consent Form (page 2) and completed the Family Contact Details Form (pages 11 and 12). Please return this whole booklet in the reply paid envelope provided.
Thank you for your time and contribution to the T.A.S.

38. For each of your past pregnancies (including live births, miscarriages, abortions or still births), please fill in the following information going down the page. If more than 8 pregnancies, please record the details on a separate piece of paper.

	1st pregnancy	2nd pregnancy	3rd pregnancy	4th pregnancy	5th pregnancy	6th pregnancy	7th pregnancy	8th pregnancy
38.1 What was your age when you became pregnant? *(age in years)* Write number AND shade in ovals	0 1 2 3 4 5 6 7 8 9	0 1 2 3 4 5 6 7 8 9	0 1 2 3 4 5 6 7 8 9	0 1 2 3 4 5 6 7 8 9	0 1 2 3 4 5 6 7 8 9	0 1 2 3 4 5 6 7 8 9	0 1 2 3 4 5 6 7 8 9	0 1 2 3 4 5 6 7 8 9
38.2 Taking 40 **weeks** as the usual length, how long was this pregnancy (counting from your last menstrual period)? Write number AND shade in ovals	0 1 2 3 4 5 6 7 8 9 ○ Don't know	0 1 2 3 4 5 6 7 8 9 ○ Don't know	0 1 2 3 4 5 6 7 8 9 ○ Don't know	0 1 2 3 4 5 6 7 8 9 ○ Don't know	0 1 2 3 4 5 6 7 8 9 ○ Don't know	0 1 2 3 4 5 6 7 8 9 ○ Don't know	0 1 2 3 4 5 6 7 8 9 ○ Don't know	0 1 2 3 4 5 6 7 8 9 ○ Don't know
38.3 Was this a live birth? (i.e not a miscarriage, abortion or stillbirth?)	○ No ○ Yes	○ No ○ Yes	○ No ○ Yes	○ No ○ Yes	○ No ○ Yes	○ No ○ Yes	○ No ○ Yes	○ No ○ Yes
38.4 IF LIVEBIRTH, did you breast-feed the baby or express breast milk for your baby?	○ No ○ Yes	○ No ○ Yes	○ No ○ Yes	○ No ○ Yes	○ No ○ Yes	○ No ○ Yes	○ No ○ Yes	○ No ○ Yes
38.5 IF BREASTFED, for how many **months** did you breastfeed or express breast milk? Write number AND shade in ovals	0 1 2 3 4 5 6 7 8 9	0 1 2 3 4 5 6 7 8 9	0 1 2 3 4 5 6 7 8 9	0 1 2 3 4 5 6 7 8 9	0 1 2 3 4 5 6 7 8 9	0 1 2 3 4 5 6 7 8 9	0 1 2 3 4 5 6 7 8 9	0 1 2 3 4 5 6 7 8 9

Now please make sure that you have signed the Consent Form (page 2) and completed the Family Contact Details Form (pages 11 & 12). Please return this whole booklet in the reply paid envelope provided.
Thank you for your time and contribution to the T.A.S.

The Fourth Follow-up Study of the 1968 Tasmanian Asthma Survey

FAMILY CONTACT DETAILS FORM

We would like to collect the contact details of your family so we can also survey them with a similar questionnaire in the future. Providing their information does not commit them to be involved. You may wish to discuss this with them to make sure they are happy with you passing on their information.

ALL NAMES AND ADDRESSES ARE KEPT STRICTLY CONFIDENTIAL

If you have any questions about this research, contact Cathryn Wharton on 1800 110 711. Please DO NOT separate this page from your booklet. Thank you!

Main study participant details:

Your name: *Family ID:* *ID:*

If this is not correct please provide your current name below:

Current name:

Your address:

If this is not correct please provide your correct address below:

Address:

Street number *Street name* *Suburb* *Postcode*

Home Ph: *Work Ph:* *Mobile Ph:*

Details of your parents:

Name: *ID:*

If this is not correct please provide current name below:

Current name:

Address:

Street number *Street name* *Suburb* *Postcode*

Home Ph: *Work Ph:* *Mobile Ph:*

Name: *ID:*

If this is not correct please provide current name below:

Current name:

Address:

Street number *Street name* *Suburb* *Postcode*

Home Ph: *Work Ph:* *Mobile Ph:*

Details of your brothers and sisters:

Name: *ID:*

If this is not correct please provide current name below:

Current name:

Address:

Street number *Street name* *Suburb* *Postcode*

Home Ph: *Work Ph:* *Mobile Ph:*

Name: *ID:*

If this is not correct please provide current name below:

Current name:

Address:

Street number *Street name* *Suburb* *Postcode*

Home Ph: *Work Ph:* *Mobile Ph:*

Name: *ID:*

If this is not correct please provide current name below:

Current name:

Address:

Street number *Street name* *Suburb* *Postcode*

Home Ph: *Work Ph:* *Mobile Ph:*

Please continue to back page

Name: ID:

If this is not correct please provide current name below:

Current name:

Address:

Street number Street name Suburb Postcode

Home Ph: Work Ph: Mobile Ph:

Name: ID:

If this is not correct please provide current name below:

Current name:

Address:

Street number Street name Suburb Postcode

Home Ph: Work Ph: Mobile Ph:

Name: ID:

If this is not correct please provide current name below:

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Address:

Street number Street name Suburb Postcode

Home Ph: Work Ph: Mobile Ph:

Name: ID:

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Name: ID:

If this is not correct please provide current name below:

Current name:

Address:

Street number Street name Suburb Postcode

Home Ph: Work Ph: Mobile Ph:

Name: ID:

If this is not correct please provide current name below:

Current name:

Address:

Street number Street name Suburb Postcode

Home Ph: Work Ph: Mobile Ph:

Thank you for completing these details.

Do not fold this booklet or remove any of the pages. Please post this completed booklet in the reply paid envelope provided or mail to:

Tasmanian Asthma Study
Department of Public Health
The University of Melbourne
Reply Paid 75370
CARLTON VIC 3053

© NCS Pearson 12650 00 01 02 03 04 05

Participant ID: _____ **Appointment date:** _____ *day month year*

Participant initials: _____ **Centre number:** _____

Participant DOB: _____ *day month year* **Investigator initials:** _____

Sample number: 1

(Centre number: Royal Hobart Hospital= 1, Northern Lung Function Service= 2, Burnie Physiology= 3, Alfred Hospital= 4)

For questions where the subject has to choose one answer, read out the question and all the categories before expecting them to respond. Definitions of ambiguous terms are given in text boxes. To standardise the questionnaire administration across centres, avoid providing any further explanation other than what is given in the questionnaire.

HOME ENVIRONMENT

First I am going to ask few questions about your home and the area where you live.

1. For how many years have you lived in your present home? ☐☐ *(<1 year code as 1)*

2. In which decade was your present home built?

Tick one ☑

- ❒ Before 1940
- ❒ 1941-1960
- ❒ 1961-1970
- ❒ 1971-1980
- ❒ 1981-1990
- ❒ 1990- Present
- ❒ Don't know

3. What is the base structure of your home?

Tick one

- ❒ Concrete slab
- ❒ Stumps and wooden flooring
- ❒ Combination of above
- ❒ Don't know

4. How old is the mattress in your bed?

Tick one

- ❒ Less than 12 months old
- ❒ 1-5 years old
- ❒ More than 5 years old
- ❒ Don't know
- ❒ Not relevant (e.g. waterbed)

5. Is there fitted carpet in the bedroom? ❒ No ❒ Yes

If Yes☞ 5.1 What is the age of the carpet? *Tick one*

- ❒ Less than 12 months old
- ❒ 1 - 5 years old
- ❒ More than 5 years old
- ❒ Don't know

6. On average, how often is your bedroom vacuumed, or if it has a hard floor, how often is it swept or mopped? *Tick one*

- ❒ 5 or more times each week
- ❒ 2 - 4 times each week
- ❒ Once a week
- ❒ Less than once a week but more than once a month
- ❒ Once a month or less

7. On average, how often is your bedroom aired by opening windows for at least for 1-hour? *Tick one*

- ❒ 5 or more times each week
- ❒ 2-4 times each week
- ❒ Once a week
- ❒ Less than once a week but more than once a month
- ❒ Once a month or less

8. Do you keep or own any cats? ❒ No ❒ Yes

If no, go to Q9

If yes ☞ 8.1 How many? Number ☐☐

8.2 Are the cats **allowed** indoors? ❒ No ❒ Yes

If no, go to Q10

If yes ☞ 8.2.1 Are the cats **allowed** in the bedroom? ❒ No ❒ Yes

Now go to Q10

9. Has there been a cat in the house in the last 12 months? ❒ No ❒ Yes ❒ Don't know

AIR POLLUTION

10. During working days (Monday to Friday) is the traffic noise at home so intense that you would have to close the windows so you are not disturbed?

Tick one

- ❒ Constantly
- ❒ Frequently
- ❒ Seldom
- ❒ Never
- ❒ Don't know (never at home during BH)

11. During working days (Monday to Friday), how often do heavy vehicles such as trucks or buses pass your house?

Tick one

- ❒ Constantly
- ❒ Frequently
- ❒ Seldom
- ❒ Never
- ❒ Don't know (never at home during BH)

12. On a scale of 0 to 10, how much is **the area where you live** subjected to woodsmoke in winter? Zero means not at all and 10 means severely affected. *Number* ☐☐

13. On a scale of 0 to 10, how much is **the area where you live** subjected to fog or smog in winter? Zero means not at all and 10 means severely affected. *Number* ☐☐

14. On a scale of 0 to 10, when you are at home, how much are you annoyed by outdoor air pollution from traffic, industry, woodsmoke or other sources if you keep the windows open? Zero means no annoyance and 10 means intolerable annoyance. *Number* ☐☐

Definitions to be used when administering the questions below:

"Attack" or "flare up" of asthma: is a period of time when asthma symptoms are worse or more frequent than usual. One could have asthma symptoms regularly or intermittently without getting attacks or flare-ups. The attacks tend to occur after a cold/sore throat or during an 'allergen season'.

Chronic Bronchitis/COPD/COAD/emphysema: cough and sputum for at least 3 months for 2 successive years.

ASTHMA

Now I am going to ask few questions about few respiratory conditions that you may or may not have.

15. Have you ever had asthma? ❒ No ❒ Yes

If no, go to Q16 page 7

If yes continue

15.1 Was this confirmed by a doctor? ❒ No ❒ Yes

If yes 15.2 How old were you when this was confirmed? ☐☐ *years old*

15.3 How old were you when you had your most recent symptoms of asthma? ☐☐ *years old*

Symptom Severity

15.4. Have you been woken from your sleep by your asthma?

last 12 months		*last 1 month*	
❒ No	❒ Yes	❒ No	❒ Yes

If Yes to last month:

15.4.1 How many nights were you woken from sleep by your asthma in the last week?

☐☐ *Number of nights*

15.5. Have you had asthma symptoms when you wake in the morning?

last 12 months		*last 1 month*	
❒ No	❒ Yes	❒ No	❒ Yes

If Yes to last month:

15.5.1 How many mornings in the last week? ☐☐ *Number of mornings*

15.6 Have you been limited in any of the following activities because of asthma?

Tick the appropriate boxes in each category:	*last 12 months*		*last 1 month*	
15.6.1 All activities	❒ No	❒ Yes	❒ No	❒ Yes
15.6.2 When dressing	❒ No	❒ Yes	❒ No	❒ Yes
15.6.3 Walking on level ground	❒ No	❒ Yes	❒ No	❒ Yes

15.6.4	Hurrying on level ground	❒ No	❒ Yes	❒ No	❒ Yes
15.6.5	Walking up stairs or up hills	❒ No	❒ Yes	❒ No	❒ Yes
15.6.6	Active sports	❒ No	❒ Yes	❒ No	❒ Yes
15.6.7	Other (Specify) _____	❒ No	❒ Yes	❒ No	❒ Yes

☞ *If Yes to any of 15.6.1 to 15.6.7, go to 15.7. If all were No, do 15.6.8 first.*

15.6.8 Would you agree or disagree with the following statement:

	last 12 months		*last month*	
"My asthma has not limited any of my activities."	❒ agree	❒ disagree	❒ agree	❒ disagree

15.7. How frequent have your asthma symptoms (of any severity) been?

Tick one

- ❒ No asthma in the past 12 months
- ❒ Asthma symptoms in the past 12 months but not in the last month
- ❒ Asthma symptoms in the last month, but not frequent (less than once per week)
- ❒ Frequent (once per week or more but not daily) in the last 1 month
- ❒ Persistent (daily)

☞ *If 'No asthma in the past 12 months', go to Q15.14. If any asthma in last 12 months continue*

15.8. How frequent have your asthma attacks/flare ups been over the past 12 months?

An "attack" or "flare up" of asthma is a period of time when asthma symptoms are worse or more frequent than usual. One could have asthma symptoms regularly or intermittently without getting attacks or flare-ups.

Tick one

- ❒ None in the past 12 months
- ❒ 3 or less in the past 12 months
- ❒ 4 or more but less than monthly
- ❒ More than monthly in the last 12 months
- ❒ More than weekly or persistent
- ❒ Not sure

15.9. Have you had an episode of asthma which has made you unable to speak or severe enough to limit your speech to only 1 or 2 words between breaths?

last 12 months		*last 1 month*	
❒ No	❒ Yes	❒ No	❒ Yes

15.10. On average, how would **you** rate the severity of your asthma?

	last 12 months tick one	*last 1 month tick one*
Not severe at all	❒	❒
Mild	❒	❒
Moderate	❒	❒
Severe	❒	❒
Not sure	❒	❒

Events

15.11. Have you lost any days from work, school or usual activities because of your asthma?

	last 12 months		*last 1 month*	
	❒ No	❒ Yes	❒ No	❒ Yes
If yes ☞: 15.11.1. How many?				
	last 12 months		*last 1 month*	

15.12. Have you had an attack or symptoms of asthma that was so bad, you needed to call your general practitioner, ambulance, emergency locum or 24 hour clinic?

	last 12 months		*last 1 month*	
	❒ No	❒ Yes	❒ No	❒ Yes
If yes ☞ 15.12.1. How many?				
	last 12 months		*last 1 month*	

15.13. Have you had an attack or symptoms of asthma that was so bad you had to go to a hospital emergency or casualty department?

	last 12 months		*last 1 month*	
	❒ No	❒ Yes	❒ No	❒ Yes
If yes ☞ 15.13.1. How many?				
	last 12 months		*last 1 month*	

15.14. Have you ever been admitted to a hospital because of your asthma? ❒ No ❒ Yes

✋ *If no, go to Q16*

If yes ☞ 15.14.1 In the past 12 months? ❒ No ❒ Yes

If yes ☞ 15.14.1.1 How many times in the last 12 months? ☐☐

Number

15.14.1.2 How many times in the last 1 month? ☐☐

Number

15.15. Have you ever had an attack or symptoms of asthma which resulted in an admission to a hospital intensive care unit? ❒ No ❒ Yes

COPD/COAD/Chronic Bronchitis/EMPHYSEMA

16. Have you ever had Chronic Obstructive Pulmonary Disease/Chronic Obstructive Airways Disease/Chronic Bronchitis/Emphysema?
Signs of these illnesses are cough and sputum for at least 3 months for 2 years in a row.

❒ No ❒ Yes

If no, go to Q17

If yes continue

16.1 Was this confirmed by a doctor? ❒ No ❒ Yes

If yes ☞ 16.2 How old were you when this was confirmed? ☐☐ *years old*

FAMILY PREDISPOSITION

Interviewers note: Encourage respondents to give answers to the best of their knowledge. Don't read out the "Don't Know' option, and avoid using it whenever possible. If they don't know the health status of all of their siblings (or children), record their answers for the ones they do know about. If they don't know about any of their siblings (or children), then use 'Don't Know.'

I am now going to ask about the respiratory conditions of your family members. It is possible that you are not aware of these details but respond according to what you know about your family.

17. Has your biological mother ever had self reported or doctor diagnosed:

17.1 Asthma? ❒ No ❒ Yes ❒ Don't know

17.2 COPD, COAD, chronic bronchitis or emphysema? ❒ No ❒ Yes ❒ Don't know

18. Has your biological father ever had self reported or doctor diagnosed:

18.1 Asthma? ❒ No ❒ Yes ❒ Don't know

18.2 COPD, COAD, chronic bronchitis or emphysema? ❒ No ❒ Yes ❒ Don't know

19. Do you, or did you, have any biological brothers or sisters? ❒ No ❒ Yes ❒ Don't know
This includes half-brothers and half-sisters, but not step-brothers or step-sisters.

✋ *If No, go to Q20 If Yes continue*

19.1 How many? ☐☐ Number ❒ Don't know

☞ How many of your biological brothers or sisters have ever had self reported or doctor diagnosed:

19.2 Asthma? ☐☐ Number ❒ Don't know

19.3 COPD, COAD, chronic bronchitis, emphysema? ☐☐ Number ❒ Don't know

20. Do you, or did you, have any biological children? ❒ No ❒ Yes

✋ *If No, go to Q21 If Yes continue*

20.1 How many? ☐☐ Number

20.2. How many of them have ever had self reported or doctor diagnosed asthma?

☐☐ Number ❒ Don't know

21. Has any member of your family or close relatives died from asthma? By family and close relatives I mean children, parents, siblings, nephews, nieces, grand parents, first cousins, uncles and aunts ❒ No ❒ Yes ❒ Don't know

If Yes: 21.1 How many? ☐☐ Number

22. Has any member of your family or close relatives died from COPD/COAD/Chronic Bronchitis/Emphysema? ❒ No ❒ Yes ❒ Don't know

If Yes: 22.1 How many? ☐☐ Number

When administering Q23 and Q25:

First ask the main question as given below i.e. "Have you used any inhaled medicines to help your breathing in the last 12 months?"

If the response is yes, get the participant to tell what medication/s he/she has used and find what group of drugs the participant's drug belongs to from the medication list provided with this questionnaire.

Then administer the sub questions under each medication (i.e. if a ***Short Acting beta-2-agonist inhaler is used complete 23.1 to 23.1.2.3)***

MEDICINES AND INHALERS

23. Have you used any inhaled medicines to help your breathing in the last 12 months? ❒ No ❒ Yes

If No, go to Q24 page 18. If Yes, continue

Which of the following have been used in the last 12 months?

23.1 Short Acting beta-2-agonist inhalers? ❒ No ❒ Yes

If no go to Q 23.2. If Yes continue

Which one? _____

Which type of inhaler device do you use? _____

Strength/dose per puff (mcg)? _____

23.1.1 In the last 12 months, have you used them for any of the following circumstances?

23.1.1.1 For relief of symptoms and/or as needed to prevent predictable symptoms such as exercise induced asthma ❒ No ❒ Yes

If Yes: Average number of puffs per month ☐☐

23.1.1.2 For flare-ups or attacks ❒ No ❒ Yes

If Yes: Number of flare ups for which this medication was used in last 12 months ☐☐

Average number of days for which this medication was used at each flareup ☐☐

Average number of puffs per day during flare ups ☐☐

23.1.1.3 Regularly i.e daily (whether symptoms present or not) ❒ No ❒ Yes

If Yes: Average number of puffs per day ☐☐

23.1.2 Specifically, in the last 1 month, have you used them for any of the following circumstances?

23.1.2.1 For relief of symptoms and/or as needed to prevent predictable symptoms such as exercise induced asthma ❒ No ❒ Yes

If Yes: Average number of puffs per month ☐☐

23.1.2.2 For flare-ups or attacks ❒ No ❒ Yes

If Yes: Number of flare ups for which this medication was used in last 12 months ☐☐

Average number of days for which this medication was used at each flareup ☐☐

Average number of puffs per day during flare ups ☐☐

23.1.2.3 Regularly i.e daily (whether symptoms present or not) ❒ No ❒ Yes

If Yes: Average number of puffs per day ☐☐

23.2 Long Acting beta-2-agonists? ❒ No ❒ Yes

If no go to Q 23.3. If Yes continue

Which one? _____

Which type of inhaler device do you use? _____

Strength/dose per puff (mcg)? _____

23.2.1 In the last 12 months, have you used them for any of the following circumstances?

23.2.1.1 For relief of symptoms and/or as needed to prevent predictable symptoms such as exercise induced asthma ❒ No ❒ Yes

If Yes: Average number of puffs per month ☐☐

23.2.1.2 For flare-ups or attacks ❒ No ❒ Yes

If Yes: Number of flare ups for which this medication was used in last 12 months ☐☐

Average number of days for which this medication was used at each flareup ☐☐

Average number of puffs per day during flare ups ☐☐

23.2.1.3 Regularly i.e daily (whether symptoms present or not) ❒ No ❒ Yes

If Yes: Average number of puffs per day ☐☐

23.2.2 Specifically, in the last 1 month, have you used them for any of the following circumstances?

23.2.2.1 For relief of symptoms and/or as needed to prevent predictable symptoms such as exercise induced asthma ❒ No ❒ Yes

If Yes: Average number of puffs per month ☐☐

23.2.2.2 For flare-ups or attacks ❒ No ❒ Yes

If Yes: Number of flare ups for which this medication was used in last 12 months ☐☐

Average number of days for which this medication was used at each flareup ☐☐

Average number of puffs per day during flare ups ☐☐

23.2.2.3 Regularly i.e daily (whether symptoms present or not) ❒ No ❒ Yes

If Yes: Average number of puffs per day ☐☐

23.3 Anticholinergic Inhalers? ❒ No ❒ Yes

If no go to Q 23.4 If Yes continue

Which one? _____

Which type of inhaler device do you use? _____

Strength/dose per puff (mcg)? _____

23.3.1 In the last 12 months, have you used them for any of the following circumstances?

23.3.1.1 For relief of symptoms and/or as needed to prevent predictable symptoms such as exercise induced asthma ❒ No ❒ Yes

If Yes: Average number of puffs per month ☐☐

23.3.1.2 For flare-ups or attacks ❒ No ❒ Yes

If Yes: Number of flare ups for which this medication was used in last 12 months ☐☐

Average number of days for which this medication was used at each flareup ☐☐

Average number of puffs per day during flare ups ☐☐

23.3.1.3 Regularly i.e daily (whether symptoms present or not) ❒ No ❒ Yes

If Yes: Average number of puffs per day ☐☐

23.3.2 Specifically, in the last 1 month, have you used them for any of the following circumstances?

23.3.2.1 For relief of symptoms and/or as needed to prevent predictable symptoms such as exercise induced asthma ❒ No ❒ Yes

If Yes: Average number of puffs per month ☐☐

23.3.2.2 For flare-ups or attacks ❒ No ❒ Yes

If Yes: Number of flare ups for which this medication was used in last 12 months ☐☐

Average number of days for which this medication was used at each flareup ☐☐

Average number of puffs per day during flare ups ☐☐

23.3.2.3 Regularly i.e daily (whether symptoms present or not) ❒ No ❒ Yes

If Yes: Average number of puffs per day ☐☐

23.4 Inhaled Steroids? ❒ No ❒ Yes

If no go to Q 23.5 If Yes continue

Which one? _____

Which type of inhaler device do you use? _____

Strength/dose per puff (mcg)? _____

23.4.1 In the last 12 months, have you used them for any of the following circumstances?

23.4.1.1 For relief of symptoms and/or as needed to prevent predictable symptoms such as exercise induced asthma ❒ No ❒ Yes

If Yes: Average number of puffs per month ☐☐

23.4.1.2 For flare-ups or attacks ❒ No ❒ Yes

If Yes: Number of flare ups for which this medication was used in last 12 months ☐☐

Average number of days for which this medication was used at each flareup ☐☐

Average number of puffs per day during flare ups ☐☐

23.4.1.3 Regularly i.e daily (whether symptoms present or not) ❒ No ❒ Yes

If Yes: Average number of puffs per day ☐☐

23.4.2 Specifically, in the last 1 month, have you used them for any of the following circumstances?

23.4.2.1 For relief of symptoms and/or as needed to prevent predictable symptoms such as exercise induced asthma ❒ No ❒ Yes

If Yes: Average number of puffs per month ☐☐

23.4.2.2 For flare-ups or attacks ❒ No ❒ Yes

If Yes: Number of flare ups for which this medication was used in last 12 months ☐☐

Average number of days for which this medication was used at each flareup ☐☐

Average number of puffs per day during flare ups ☐☐

23.4.2.3 Regularly i.e daily (whether symptoms present or not) ❒ No ❒ Yes

If Yes: Average number of puffs per day ☐☐

23.5 Inhaled cromoglycate/nedocromil? ❒ No ❒ Yes

If no go to Q 23.6 If Yes continue

Which one? _____

Which type of inhaler device do you use? _____

Strength/dose per puff (mcg)? _____

23.5.1 In the last 12 months, have you used them for any of the following circumstances?

23.5.1.1 For relief of symptoms and/or as needed to prevent predictable symptoms such as exercise induced asthma ❒ No ❒ Yes

If Yes: Average number of puffs per month ☐☐

23.5.1.2 For flare-ups or attacks ❒ No ❒ Yes

If Yes: Number of flare ups for which this medication was used in last 12 months ☐☐

Average number of days for which this medication was used at each flareup ☐☐

Average number of puffs per day during flare ups ☐☐

23.5.1.3 Regularly i.e daily (whether symptoms present or not) ❒ No ❒ Yes

If Yes: Average number of puffs per day ☐☐

23.5.2 Specifically, in the last 1 month, have you used them for any of the following circumstances?

23.5.2.1 For relief of symptoms and/or as needed to prevent predictable symptoms such as exercise induced asthma ❒ No ❒ Yes

If Yes: Average number of puffs per month ☐☐

23.5.2.2 For flare-ups or attacks ❒ No ❒ Yes

If Yes: Number of flare ups for which this medication was used in last 12 months ☐☐

Average number of days for which this medication was used at each flareup ☐☐

Average number of puffs per day during flare ups ☐☐

23.5.2.3 Regularly i.e daily (whether symptoms present or not) ❒ No ❒ Yes

If Yes: Average number of puffs per day ☐☐

23.6 Inhaled Compound Bronchodilators? ❒ No ❒ Yes

If no go to Q 23.7 If Yes continue

Which one? _____

Which type of inhaler device do you use? _____

Strength/dose per puff (mcg)? _____

23.6.1 In the last 12 months, have you used them for any of the following circumstances?

23.6.1.1 For relief of symptoms and/or as needed to prevent predictable symptoms such as exercise induced asthma ❒ No ❒ Yes

If Yes: Average number of puffs per month ☐☐

23.6.1.2 For flare-ups or attacks ❒ No ❒ Yes

If Yes: Number of flare ups for which this medication was used in last 12 months ☐☐

Average number of days for which this medication was used at each flareup ☐☐

Average number of puffs per day during flare ups ☐☐

23.6.1.3 Regularly i.e daily (whether symptoms present or not) ❒ No ❒ Yes

If Yes: Average number of puffs per day ☐☐

23.6.2 Specifically, in the last 1 month, have you used them for any of the following circumstances?

23.6.2.1 For relief of symptoms and/or as needed to prevent predictable symptoms such as exercise induced asthma ❒ No ❒ Yes

If Yes: Average number of puffs per month ☐☐

23.6.2.2 For flare-ups or attacks ❒ No ❒ Yes

If Yes: Number of flare ups for which this medication was used in last 12 months ☐☐

Average number of days for which this medication was used at each flareup ☐☐

Average number of puffs per day during flare ups ☐☐

23.6.2.3 Regularly i.e daily (whether symptoms present or not) ❒ No ❒ Yes

If Yes: Average number of puffs per day ☐☐

23.7 Inhaled combination of steroids and long acting beta 2 agonist? ❒ No ❒ Yes

If no go to Q 23.8 If Yes continue

Which one? _____

Which type of inhaler device do you use? _____

Strength/dose per puff (mcg)? _____

23.7.1 In the last 12 months, have you used them for any of the following circumstances?

23.7.1.1 For relief of symptoms and/or as needed to prevent predictable symptoms such as exercise induced asthma ❒ No ❒ Yes

If Yes: Average number of puffs per month ☐☐

23.7.1.2 For flare-ups or attacks ❒ No ❒ Yes

If Yes: Number of flare ups for which this medication was used in last 12 months ☐☐

Average number of days for which this medication was used at each flareup ☐☐

Average number of puffs per day during flare ups ☐☐

23.7.1.3 Regularly i.e daily (whether symptoms present or not) ❒ No ❒ Yes

If Yes: Average number of puffs per day ☐☐

23.7.2 Specifically, in the last 1 month, have you used them for any of the following circumstances?

23.7.2.1 For relief of symptoms and/or as needed to prevent predictable symptoms such as exercise induced asthma ❒ No ❒ Yes

If Yes: Average number of puffs per month ☐☐

23.7.2.2 For flare-ups or attacks ❒ No ❒ Yes

If Yes: Number of flare ups for which this medication was used in last 12 months ☐☐

Average number of days for which this medication was used at each flareup ☐☐

Average number of puffs per day during flare ups ☐☐

23.7.2.3 Regularly i.e daily (whether symptoms present or not) ❒ No ❒ Yes

If Yes: Average number of puffs per day ☐☐

23.8 Other inhaled medication? ❒ No ❒ Yes

If no go to Q 24 page 18. If Yes continue

Which one? _____

Which type of inhaler device do you use? _____

Strength/dose per puff (mcg)? _____

23.8.1 In the last 12 months, have you used them for any of the following circumstances?

23.8.1.1 For relief of symptoms and/or as needed to prevent predictable symptoms such as exercise induced asthma ❒ No ❒ Yes

If Yes: Average number of puffs per month ☐☐

23.8.1.2 For flare-ups or attacks ❒ No ❒ Yes

If Yes: Number of flare ups for which this medication was used in last 12 months ☐☐

Average number of days for which this medication was used at each flareup ☐☐

Average number of puffs per day during flare ups ☐☐

23.8.1.3 Regularly i.e daily (whether symptoms present or not) ❒ No ❒ Yes

If Yes: Average number of puffs per day ☐☐

23.8.2 Specifically, in the last 1 month, have you used them for any of the following circumstances?

23.8.2.1 For relief of symptoms and/or as needed to prevent predictable symptoms such as exercise induced asthma ❒ No ❒ Yes

If Yes: Average number of puffs per month ☐☐

23.8.2.2 For flare-ups or attacks ❒ No ❒ Yes

If Yes: Number of flare ups for which this medication was used in last 12 months ☐☐

Average number of days for which this medication was used at each flareup ☐☐

Average number of puffs per day during flare ups ☐☐

23.8.2.3 Regularly i.e daily (whether symptoms present or not) ❒ No ❒ Yes

If Yes: Average number of puffs per day ☐☐

24. Have you ever used inhaled steroids to help your breathing? ❒ No ❒ Yes

If No go to Q 25, if Yes continue

24.1 At what age did you start using inhaled steroids? ☐☐ years

24.2 In the past 5 years, how many months would you have used inhaled steroids on most days? ☐☐ months

25. Have you used any pills, capsules, tablets or medicines, other than inhaled medicines to help your breathing at any time in the last 12 months? ❒ No ❒ Yes

If No go to Q 26 page 22, if Yes continue

Which of the following have been used in the last 12 months?

25.1 Oral theophyllines ❒ No ❒ Yes

If no go to Q25.2 If Yes continue

Which one? _____

Strength/dose per tablet (mg)? _____

25.1.1 In the last 12 months, have you used them for any of the following circumstances?

25.1.1.1 For relief of symptoms and/or as needed to prevent predictable symptoms such as exercise induced asthma ❒ No ❒ Yes

If Yes: Average number of tablets per month ☐☐

25.1.1.2 For flare-ups or attacks ❒ No ❒ Yes

If Yes: Number of flare ups for which this medication was used in last 12 months ☐☐

Average number of days for which this medication was used at each flareup ☐☐

Average number of tablets per day during flare ups ☐☐

25.1.1.3 Regularly i.e daily (whether symptoms present or not) ❒ No ❒ Yes

If Yes: Average number of tablets per day ☐☐

25.1.2 Specifically, in the last 1 month, have you used them for any of the following circumstances?

25.1.2.1 For relief of symptoms and/or as needed to prevent predictable symptoms such as exercise induced asthma ❒ No ❒ Yes

If Yes: Average number of tablets per month ☐☐

25.1.2.2 For flare-ups or attacks ❒ No ❒ Yes

If Yes: Number of flare ups for which this medication was used in last 12 months ☐☐

Average number of days for which this medication was used at each flareup ☐☐

Average number of tablets per day during flare ups ☐☐

25.1.2.3 Regularly i.e daily (whether symptoms present or not) ❒ No ❒ Yes

If Yes: Average number of tablets per day ☐☐

25.2 Oral Steroids? ❒ No ❒ Yes

If no go to Q25.3 If Yes continue

Which one? _____

Strength/dose per tablet (mg)? _____

25.2.1 In the last 12 months, have you used them for any of the following circumstances?

25.2.1.1 For relief of symptoms and/or as needed to prevent predictable symptoms such as exercise induced asthma ❒ No ❒ Yes

If Yes: Average number of tablets per month ☐☐

25.2.1.2 For flare-ups or attacks ❒ No ❒ Yes

If Yes: Number of flare ups for which this medication was used in last 12 months ☐☐

Average number of days for which this medication was used at each flareup ☐☐

Average number of tablets per day during flare ups ☐☐

25.2.1.3 Regularly i.e daily (whether symptoms present or not) ❒ No ❒ Yes

If Yes: Average number of tablets per day ☐☐

25.2.2 Specifically, in the last 1 month, have you used them for any of the following circumstances?

25.2.2.1 For relief of symptoms and/or as needed to prevent predictable symptoms such as exercise induced asthma ❒ No ❒ Yes

If Yes: Average number of tablets per month ☐☐

25.2.2.2 For flare-ups or attacks ❒ No ❒ Yes

If Yes: Number of flare ups for which this medication was used in last 12 months ☐☐

Average number of days for which this medication was used at each flareup ☐☐

Average number of tablets per day during flare ups ☐☐

25.2.2.3 Regularly i.e daily (whether symptoms present or not) ❒ No ❒ Yes

If Yes: Average number of tablets per day ☐☐

25.3 Oral Anti-leukotrienes? ❒ No ❒ Yes

If no go to Q25.4 If Yes continue

Which one? _____

Strength/dose per tablet (mg)? _____

25.3.1 In the last 12 months, have you used them for any of the following circumstances?

25.3.1.1 For relief of symptoms and/or as needed to prevent predictable symptoms such as exercise induced asthma ❒ No ❒ Yes

If Yes: Average number of tablets per month ☐☐

25.3.1.2 For flare-ups or attacks ❒ No ❒ Yes

If Yes: Number of flare ups for which this medication was used in last 12 months ☐☐

Average number of days for which this medication was used at each flareup ☐☐

Average number of tablets per day during flare ups ☐☐

25.3.1.3 Regularly i.e daily (whether symptoms present or not) ❒ No ❒ Yes

If Yes: Average number of tablets per day ☐☐

25.3.2 Specifically, in the last 1 month, have you used them for any of the following circumstances?

25.3.2.1 For relief of symptoms and/or as needed to prevent predictable symptoms such as exercise induced asthma ❒ No ❒ Yes

If Yes: Average number of tablets per month ☐☐

25.3.2.2 For flare-ups or attacks ❒ No ❒ Yes

If Yes: Number of flare ups for which this medication was used in last 12 months ☐☐

Average number of days for which this medication was used at each flareup ☐☐

Average number of tablets per day during flare ups ☐☐

25.3.2.3 Regularly i.e daily (whether symptoms present or not) ❒ No ❒ Yes

If Yes: Average number of tablets per day ☐☐

25.4 Other Oral Medications? ❒ No ❒ Yes

25.4.1 List _____

_____

_____

26. Have you ever been prescribed home oxygen therapy? ❒ No ❒ Yes

If no go to Q27 If Yes continue

26.1 Are you currently using oxygen therapy at home? ❒ No ❒ Yes

26.2 For how many years have you been using oxygen therapy at home? ☐☐ years

26.3 How have you used oxygen therapy during the last month?

26.3.1 For relief of symptoms or when needed ❒ No ❒ Yes

26.3.2 For flare-ups or attacks ❒ No ❒ Yes

26.3.3 Regularly, on a daily basis ❒ No ❒ Yes

27. Have you ever had an influenza vaccination? ❒ No ❒ Yes

If yes 27.1 Have you been vaccinated for influenza in the last 12months? ❒ No ❒ Yes

28. Have you ever had a pneumonia vaccination? ❒ No ❒ Yes

If yes: 28.1 Have you been vaccinated for pneumonia in the last 5 years? ❒ No ❒ Yes

29. Have you ever been vaccinated or desensitised for allergy? ❒ No ❒ Yes

If yes: 29.1 Have you been vaccinated for allergy in the last 12 months? ❒ No ❒ Yes

30. Have you had any other injections to help your breathing at any time in the last 12 months?

❒ No ❒ Yes

If yes: 30.1 What injections? _____

_____

Thank you for completing this questionnaire as part of the Tasmanian Asthma Study 36 year follow-up study.

37680

TAHS
Tasmanian Longitudinal Health Study

Follow up of the Tasmanian Longitudinal Health Study from first to sixth decade

STUDY BOOKLET

Participant ID:	
Family ID:	
Appointment Date:	Day / Month / Year
Testing Scientist Number:	
Testing Centre ID:	
Barometric pressure (mmHg):	○ mmHg ○ hPa
Room temperature (Celsius):	.

QUESTIONNAIRE COMPLETION INSTRUCTIONS

Please answer each question the best you can. Please use a BLACK or DARK BLUE pen.

Please shade the circles or squares completely (do not tick or cross)

● ■

Write clearly within the boxes

A	B	C		1	2	3

Write clearly within each space

PLEASE WRITE IN CAPITAL LETTERS

- circles are provided where only one choice is permitted.
- squares indicate that multiple responses are permitted.

If you make a mistake, or want to change any of your shaded responses, please place a cross through the incorrect response ✖ and then shade the correct response ●

For written responses, please cross out your incorrect response and write your new response just above or below the one you have crossed out.

I	N	C	O	R	R	E	C	T

CORRECT

PART 1

SECTION A: BACKGROUND INFORMATION

A1. In which country:

	Australia	Other	Please specify	Don't know	Office Use only
Were you born	○	○		○	
Was your mother born	○	○		○	
Was your father born	○	○		○	
Was your mother's mother born	○	○		○	
Was your mother's father born	○	○		○	
Was your father's mother born	○	○		○	
Was your father's father born	○	○		○	

A2. What is the highest educational or vocational qualification that you have completed?

- ○ Grade 1 to 6
- ○ Grade 7 to 9
- ○ Grade 10 or 11
- ○ Grade 12 or equivalent (e.g. Higher School Certificate)
- ○ Trade/Apprenticeship (e.g. hairdresser, electrician, plumber)
- ○ Certificate or Diploma (e.g. child care, technician etc)
- ○ University degree (e.g. Bachelor)
- ○ Higher University degree (e.g. Graduate Diploma, Masters, PhD)

A3. Are you currently employed (including self-employed)?

- ○ Yes → ○ Full time ○ Part time ○ Casual
- ○ No
- ○ No, studying
- ○ No, retired

A4. What is/was your main occupation?

- ○ Manager or administrator (e.g. magistrate, general manager, school principal, director of nursing)
- ○ Professional (e.g. scientist, nurse, allied health professional, teacher, artist)
- ○ Associate professional (e.g. technician, manager, police officer, small business owner)
- ○ Tradesperson or related worker (e.g. hairdresser, gardener, florist)
- ○ Advanced clerical or service worker (e.g. secretary, flight attendant, law clerk, personal assistant)
- ○ Intermediate clerical, sales, service worker (e.g. administration worker, child care worker, nursing assistant, hospitality worker)
- ○ Intermediate production or transport worker (e.g. machine operator, bus driver, sewing machinist)
- ○ Elementary clerical, sales or service worker (e.g. filing/mail clerk, parking inspector, sales assistant, housekeeper)
- ○ Labourer or related worker (e.g. cleaner, factory worker, farm hand, kitchen hand)
- ○ House person

A5. What is your usual work pattern?

- ○ Daytime - no shifts
- ○ Rotating shifts with nights
- ○ Rotating shifts without nights
- ○ Permanent nights
- ○ Other (please specify)

A6. What is your current marital status?
○ De facto ○ Divorced ○ Married ○ Never married ○ Separated but not divorced ○ Widowed

A7. What is your current postcode? | | | | |

SECTION B: HOME ENVIRONMENT AND PETS

B1. Which types of heating do you use at home? (Please choose **ALL** that apply)
☐ Gas ducted central heating
☐ Coal or wood fire
☐ Gas room heater
☐ Electric heater (e.g. radiator, fan or dimplex-type)
☐ Other central heating (e.g. electric, hydronic, slab floor)
☐ Reverse cycle air-conditioning
☐ Oil Heater
☐ Other (please specify)
☐ No heating

B2. What kind of stove do you mostly use for cooking?
○ Gas ○ Electric ○ Coal, coke or wood ○ Other (please specify)

B2a. Do you have an exhaust fan over the stove?
○ No → **Please go to question B3**
○ Yes

B2b. When cooking how often do you use the fan?
○ All of the time ○ Some of the time ○ None of the time

B2c. Does the fan take the fumes outside the house?
○ No ○ Yes

B3. Has there ever been mould or mildew on any surface, other than food, in your home?
○ No → **Please go to question B4**
○ Yes

B3a. Which rooms have been affected? (Please choose **ALL** that apply)
☐ Bathrooms ☐ Living rooms ☐ Your bedroom ☐ Kitchen ☐ Other bedrooms ☐ Any other area/s

B3b. Has there ever been mould or mildew on any surface, other than food, in your home in the last 12 months?
○ No ○ Yes

B4. How old is the mattress on your bed?
○ Less than 12 months old ○ 1 to 5 years old ○ More than 5 years old ○ Don't know ○ Not relevant (e.g. waterbed)

B5. Is there fitted carpet in the bedroom?
○ No → **Please go to question B6**
○ Yes

B5a. What is the age of the carpet?
○ Less than 12 months old ○ 1 to 5 years old ○ More than 5 years old ○ Don't know

B6. Do you keep or own any cats?
○ No → **Please go to question B7**
○ Yes → **B6a. How many cats?** | |

B6b. Are the cats allowed indoors?
○ No → **Please go to question B7**
○ Yes

B6c. Are the cats allowed in the bedroom?
○ No ○ Yes

B7. Has there been a cat in the house <u>in the last 12 months</u>?
○ No ○ Yes ○ Don't know

B8. Do you keep or own any dogs?
○ No → **Please go to question B9**
○ Yes → **B8a. How many dogs?** ☐☐

B8b. Are the dogs <u>allowed</u> indoors?
○ No → **Please go to question B9**
○ Yes

B8c. Are the dogs <u>allowed</u> in the bedroom?
○ No ○ Yes

B9. Has there been a dog in the house <u>in the last 12 months</u>?
○ No ○ Yes ○ Don't know

SECTION C: CHILDHOOD ENVIRONMENT

C1. What term best describes the place you lived most of the time when you were under the age of five years?
○ Farm ○ Country town ○ Suburb of a city ○ Inner city ○ Don't know

C2. How many of your brothers, sisters or other children regularly slept in your bedroom before you were five years old, not including yourself?
☐☐

C3. Did you have a serious respiratory infection before the age of five years?
○ No ○ Yes ○ Don't know

C4. Did you go to school, pre-school, kindergarten, or a day care centre before the age of five years?
○ No ○ Yes

C5. At what age did you first attend a school, pre-school, kindergarten, or day care?
☐☐ age in years

	During the first year of your life?			When you were aged 1 to 4 years?			When you were aged 5 to 15 years?		
	No	**Yes**	**Don't know**	**No**	**Yes**	**Don't know**	**No**	**Yes**	**Don't know**
C6. Did your father smoke	○	○	○	○	○	○	○	○	○
C7. Did your mother smoke	○	○	○	○	○	○	○	○	○
C8. Was there a cat in your home	○	○	○	○	○	○	○	○	○
C9. Was there a dog in your home	○	○	○	○	○	○	○	○	○
C10. Did you have carpet (or a rug) covering the floor in your bedroom	○	○	○	○	○	○	○	○	○
C11. As a child did you live on a farm that was run by your family	○	○	○	○	○	○	○	○	○
C12. As a child did you have regular contact with farm animals e.g. horses, cows, pigs, sheep and/or poultry *(by regular contact we mean at least once a week playing, riding or cleaning the animals or the area that they are housed)*	○	○	○	○	○	○	○	○	○
C13. As a child did you consume any farm milk	○	○	○	○	○	○	○	○	○

C14 What was the main type of heating your home had when you were under the age of five years?
(Please choose **ALL** that apply)

- ☐ Gas ducted central heating
- ☐ Coal or wood fire
- ☐ Gas room heater
- ☐ Electric heater (e.g. radiator, fan or dimplex-type)
- ☐ Other central heating (e.g. electric, hydronic, slab floor)
- ☐ Reverse cycle air-conditioning
- ☐ Oil Heater
- ☐ Other (please specify)
- ☐ No heating

C15. When you were a child, how often did you eat vegetables that your family (or friends) had grown?

- ○ Never/rarely
- ○ Every week
- ○ Several times a week
- ○ Daily
- ○ Several times a day

C16. Currently, how often do you eat potatoes or vegetables that you (or your family or friends) have grown yourself?

- ○ Never
- ○ Rarely
- ○ Almost every week in summer/autumn season
- ○ Almost daily
- ○ Almost daily in summer/autumn season

SECTION D: SMOKING AND ALCOHOL USE

D1. In your lifetime, have you smoked at least 100 cigarettes or equal amounts of cigars, pipes or any tobacco product?

- ○ No → **Please go to question D2**
- ○ Yes

D1a. How old were you when you started smoking? ☐☐ age in years

D1b. Do you currently smoke (within the last 4 weeks)?

- ○ Not at all → **Please go to question D1d**
- ○ Yes, daily → On average how much (per day) do you currently smoke? ☐☐ no. of cigarettes
- ○ Yes, at least weekly → On average how much (per week) do you currently smoke? ☐☐ no. of cigarettes
- ○ Yes, less than weekly → On average how much (per month) do you currently smoke? ☐☐ no. of cigarettes

D1c.

D1d. How old were you when you stopped smoking?

☐☐ age in years

D1e. On average, during periods when you smoked, how much did you smoke (total number of cigarettes or equivalent product)? *Provide the average number per day or per week or per month.*

☐☐ ○ Per day ○ Per week ○ Per month

D2. Not counting yourself, how many people in your household currently smoke regularly (most days of the week) inside the house?

☐☐ number of people

D3. On average, how many hours per day are you exposed to other people's tobacco smoke (home as well as outside home)?

☐☐ hours per day

D4. Over the last 12 months, how often did you drink beer, wine and/or spirits?

	Never	Less than once a month	1 to 3 times per month	1 day per week	2 days per week	3 days per week	4 days per week	5 days per week	6 days per week	Every day
Beer (low alcohol)	○	○	○	○	○	○	○	○	○	○
Beer (full strength)	○	○	○	○	○	○	○	○	○	○
Red wine	○	○	○	○	○	○	○	○	○	○
White wine (including sparkling wines)	○	○	○	○	○	○	○	○	○	○
Fortified wines, port, sherry etc	○	○	○	○	○	○	○	○	○	○
Spirits, liqueurs, etc	○	○	○	○	○	○	○	○	○	○

D5. Over the last 12 months, on days when you were drinking, how many glasses of beer, wine and/or spirits did you usually drink?

	1	2	3	4	5	6	7	8	9	10 or more
Beer (low alcohol)	○	○	○	○	○	○	○	○	○	○
Beer (full strength)	○	○	○	○	○	○	○	○	○	○
Red wine	○	○	○	○	○	○	○	○	○	○
White wine (including sparkling wines)	○	○	○	○	○	○	○	○	○	○
Fortified wines, port, sherry etc	○	○	○	○	○	○	○	○	○	○
Spirits, liqueurs, etc	○	○	○	○	○	○	○	○	○	○
Total number of glasses	○	○	○	○	○	○	○	○	○	○

D6. Over the last 12 months, what was the maximum number of glasses of beer, wine and/or spirits that you drank in 24 hours?

	1	2	3	4	5	6	7	8	9	10 or more
Total number of glasses	○	○	○	○	○	○	○	○	○	○

SECTION E: SUN EXPOSURE AND AIR POLLUTION

E1. If you went out in the sun without any protection in summer for 30 minutes during the middle of the day would you:
○ Just burn and not tan afterwards ○ Burn first and then tan afterwards ○ Not burn at all, just tan

E2. How many hours <u>a day</u> do you spend outdoors on a usual work and non-work day?
Include, for example, the time you spent in the garden, using public transport, walking outdoors, or any other work and non-work related outdoor activities.

If you are currently not working put '0' for a usual work day and fill in your time spent outdoors for a usual non-work day.

	Usual work day		Usual non-work day	
	Hours	Minutes	Hours	Minutes
Time outdoors in summer	☐☐	☐☐	☐☐	☐☐
Time outdoors in winter	☐☐	☐☐	☐☐	☐☐

	Constantly	Frequently	Seldom	Never	Don't know (never at home during working hours)
E3. During working days (Monday to Friday) is the traffic noise at home so intense that you would have to close the windows so you are not disturbed	○	○	○	○	○
E4. During working days (Monday to Friday), how often do heavy vehicles such as trucks or buses pass your house?	○	○	○	○	○

SECTION F: SLEEPINESS

F1. How likely are you to doze off or fall asleep in the following situations, in contrast to feeling just tired?

	Would never doze	Slight chance of dozing	Moderate chance of dozing	High chance of dozing
Sitting and reading	○	○	○	○
Watching TV	○	○	○	○
Sitting, inactive in a public place (e.g. a theatre or a meeting)	○	○	○	○
As a passenger in a car for an hour without a break	○	○	○	○
Lying down to rest in the afternoon when circumstances permit	○	○	○	○
Sitting and talking to someone	○	○	○	○
Sitting quietly after lunch without alcohol	○	○	○	○
In a car, while stopped for a few minutes in the traffic	○	○	○	○

F2. Do you snore?

○ No → **Please go to question F5**

○ Yes

F2a. Your snoring is:

○ Slightly louder than breathing ○ Louder than talking

○ As loud as talking ○ Very loud - can be heard in adjacent rooms

F3. How often do you snore?

○ Nearly every day ○ 3 to 4 times a week ○ 1 to 2 times a week ○ 1 to 2 times a month ○ Never or nearly never

F4. Has your snoring ever bothered other people?

○ No ○ Yes

F5. Has anyone noticed that you stop breathing during your sleep?

○ Nearly every day ○ 3 to 4 times a week ○ 1 to 2 times a week ○ 1 to 2 times a month ○ Never or nearly never

F6. During your waking time, do you feel tired, fatigued, or not up to par?

○ Nearly every day ○ 3 to 4 times a week ○ 1 to 2 times a week ○ 1 to 2 times a month ○ Never or nearly never

F7. How often do you feel tired or fatigued after your sleep?

○ Nearly every day ○ 3 to 4 times a week ○ 1 to 2 times a week ○ 1 to 2 times a month ○ Never or nearly never

F8. Have you ever nodded off or fallen asleep while driving a vehicle?

○ No → **Please go to question F9**

○ Yes

F8a. How often does this occur?

○ Nearly every day ○ 3 to 4 times a week ○ 1 to 2 times a week ○ 1 to 2 times a month ○ Never or nearly never

F9. Do you have high blood pressure?

○ No ○ Yes ○ Don't know

SECTION G: PHYSICAL ACTIVITY AND DIET

Think about all the vigorous activities that you did in the last 7 days. Vigorous physical activities refer to activities that take hard physical effort and make you breathe much harder than normal. Think only about those physical activities that you did for at least 10 minutes at a time.

G1. During the last 7 days, on how many days did you do vigorous physical activities like heavy lifting, digging, aerobics, or fast bicycling?

☐ days per week ○ No vigorous physical activities → **Please go to question G3**

G2. How much time did you usually spend doing vigorous physical activities on one of those days?

Hours ☐☐ Minutes ☐☐ ○ Don't know/Not sure

Think about all the moderate activities that you did in the last 7 days. Moderate activities refer to activities that take moderate physical effort and make you breathe somewhat harder than normal. Think only about those physical activities that you did for at least 10 minutes at a time.

G3. During the last 7 days, on how many days did you do moderate physical activities like carrying light loads, bicycling at a regular pace, or doubles tennis? Do not include walking.

☐ days per week ○ No moderate physical activities → **Please go to question G5**

G4. How much time did you usually spend doing moderate physical activities on one of those days?

Hours ☐☐ Minutes ☐☐ ○ Don't know/Not sure

Think about the time you spent walking in the last 7 days. This includes at work and at home, walking to travel from place to place, and any other walking that you have done solely for recreation, sport, exercise, or leisure.

G5. During the last 7 days, on how many days did you walk for at least 10 minutes at a time?

☐ days per week ○ No walking → **Please go to question G7**

G6. How much time did you usually spend walking on one of those days?

Hours ☐☐ Minutes ☐☐ ○ Don't know/Not sure

This question is about the time you spent sitting on weekdays during the last 7 days. Include time spent at work, at home, while doing course work and during leisure time. This may include time spent sitting at a desk, visiting friends, reading, or sitting or lying down to watch television.

G7. During the last 7 days, how much time did you spend sitting on a week day?

Hours ☐☐ Minutes ☐☐ ○ Don't know/Not sure

G8. During a normal week how many hours a day (24 hours) do you watch television?

○ Don't watch TV
○ Less than 1 hour
○ More than 1 hour but less than 3 hours
○ More than 3 hours but less than 5 hours
○ 5 hours or more

G9. How often do you:

	6+ times per week	3 to 5 times per week	1 to 2 times per week	Less than once a week	Never
Eat fried food with batter or breadcrumb coating	○	○	○	○	○
Eat gravy, cream sauces or cheese sauces	○	○	○	○	○
Add butter, margarine, oil or sour cream to vegetables, rice, spaghetti	○	○	○	○	○
Eat vegetables that are fried or roasted with fat or oil	○	○	○	○	○
Eat meat pies, sausages, salami, burgers or bacon	○	○	○	○	○
Eat pastries, cakes, sweet biscuits or croissants	○	○	○	○	○
Eat hot chips	○	○	○	○	○

G10. How is your meat usually cooked?

○ Fried ○ Grilled/roasted with added fat/oil ○ Eat meat occasionally or never
○ Stewed ○ Grilled/roasted without fat/oil

G11. How do you spread butter/margarine on bread?

○ Thickly ○ Medium ○ Thinly ○ Never use butter/margarine

G12. How often do you eat:

	6+ times per week	3 to 5 times per week	1 to 2 times per week	Less than once a week	Never
Chocolate or sweet snack bars	○	○	○	○	○
Crisps, corn chips or nuts	○	○	○	○	○
Cream	○	○	○	○	○
More than a small serve of ice cream	○	○	○	○	○
More than a small piece of cheese (exclude low fat cheese)	○	○	○	○	○

G13. What type of milk do you use on breakfast cereal or in cooking?

○ Cow ○ Goat ○ Soy

G13a. What form of milk in G13 do you consume?

○ Condensed or evaporated ○ Full cream ○ Full cream and reduced fat ○ Reduced fat ○ Skim

G14. How much skin on chicken do you eat?

○ Most or all of the skin ○ Some of the skin ○ None of the skin /I am vegetarian

G15. How much of the fat on meat do you eat?

○ Most or all of the fat ○ Some of the fat ○ None of the fat/I am vegetarian

G16. How many serves* do you usually eat each day of: *(*serve is what fits into the palm of your hand)*

	I don't eat fruit/ vegetables	Less than 1 serving per day	1 serving per day	2 servings per day	3 servings per day	4+ servings per day
Fruit (fresh, canned, frozen)	○	○	○	○	○	○
Vegetables (fresh, canned, frozen)	○	○	○	○	○	○

SECTION H: GENERAL HEALTH

H1. Which of the following dental hygiene tools do you use, and how often?

	Never/rarely	Once a week	Once a day	Twice a day	More than twice a day
Tooth brush	○	○	○	○	○
Fluoride dental paste	○	○	○	○	○
Dental floss	○	○	○	○	○
Tooth picks	○	○	○	○	○
Mouth wash	○	○	○	○	○

H2. Over the last two weeks how often have you been bothered by the following problems?

	Not at all	Several days	More than half the days	Nearly every day
Feeling nervous, anxious or on edge	○	○	○	○
Not being able to stop or control worrying	○	○	○	○
Worrying too much about different things	○	○	○	○
Trouble relaxing	○	○	○	○
Being so restless that it is hard to sit still	○	○	○	○
Becoming easily annoyed or irritable	○	○	○	○
Feeling afraid as if something awful might happen	○	○	○	○

H3. Over the last two weeks how often have you been bothered by the following problems?

	Not at all	Several days	More than half the days	Nearly every day
Little interest or pleasure in doing things	○	○	○	○
Feeling down, depressed or hopeless	○	○	○	○
Trouble falling or staying asleep or sleeping too much	○	○	○	○
Feeling tired or having little energy	○	○	○	○
Poor appetite or overeating	○	○	○	○
Feeling bad about yourself - or that you are a failure or have let yourself or your family down	○	○	○	○
Trouble concentrating on things, such as reading the newspaper or watching television	○	○	○	○
Moving or speaking so slowly that other people could have noticed? Or the opposite - being so fidgety or restless that you have been moving around a lot more than usual	○	○	○	○
Thoughts that you would be better off dead or of hurting yourself in some way	○	○	○	○

H4. Has a doctor ever told you that you have/had any of the following conditions? (Please choose **ALL** that apply)

- ☐ Angina, heart attack or myocardial infarction
- ☐ Anxiety
- ☐ Cancer
- ☐ Celiac disease
- ☐ Crohn's disease/ulcerative colitis
- ☐ Depression
- ☐ Diabetes or high sugar levels in the blood or urine
- ☐ Gastro-oesophageal Reflux Disease (GORD)
- ☐ High blood pressure or Hypertension
- ☐ High levels of cholesterol/triglycerides
- ☐ Lupus/Systemic Lupus Erythematosus
- ☐ Multiple Sclerosis
- ☐ Other Psychiatric/mental health problems
- ☐ Rheumatoid arthritis
- ☐ Thyroid problems
- ☐ Transient ischemic attack (TIA) or a stroke
- ☐ Type 1 Diabetes Mellitus
- ☐ None of the above

Thank you for your help with Part 1 of the questionnaire

PART 2

SECTION J: ECZEMA

J1. Have you ever had eczema or any kind of skin allergy?
- ○ No → **Please go to question J2**
- ○ Yes

J1a. Was this eczema or skin allergy confirmed by a doctor?
○ No ○ Yes

J2. Have you ever had an itchy rash that was coming and going for at least 6 months?
- ○ No → **Please go to question J3**
- ○ Yes

J2a. How old were you when you first had this itchy rash? ☐☐ age in years

J2b. Have you had this itchy rash in the last 12 months?
○ No ○ Yes

J2c. Has this rash at any time affected any of the following places: (Please choose **ALL** that apply)
☐ Folds of the elbows ☐ Behind the knees ☐ In front of the ankles
☐ Under the buttocks ☐ Around the neck, ears or eyes ☐ None of the above

J3. Have you used any medicines including creams and ointments for rash or eczema during the last 12 months?
○ No ○ Yes

SECTION K: HAY FEVER AND RHINOSINUSITIS

K1. Have you ever had hay fever or nasal allergies (that is sneezing, running or blocked nose when you do not have a cold or the flu)?
- ○ No → **Please go to question K2**
- ○ Yes

K1a. Have you had this problem in the last 12 months?
○ No ○ Yes

K1b. Was this problem accompanied by itchy or watery eyes?
○ No ○ Yes

K1c. How old were you when you first had hay fever or nasal allergies? ☐☐ age in years

K2. Has your nose been blocked for more than 12 weeks during the last 12 months?
○ No ○ Yes

K3. Have you had pain or pressure around the forehead, nose or eyes for more than 12 weeks during the last 12 months?
○ No ○ Yes

K4. Have you had discoloured nasal discharge or discoloured mucus in the throat for more than 12 weeks during the last 12 months?
○ No ○ Yes

K5. Has your sense of smell been reduced or absent for more than 12 weeks during the last 12 months?
○ No ○ Yes

K6. Has a doctor ever told you that you have chronic sinusitis?
○ No ○ Yes

SECTION L: SYMPTOMS OF ALLERGY

L1. When you are near animals, such as cats, dogs, or horses; near feathers, including pillows, quilts or doonas; or in a dusty part of the house, do you ever: (Please choose **ALL** that apply)
☐ Start to cough ☐ Start to wheeze ☐ Get a feeling of tightness in the chest ☐ Start to feel short of breath
☐ Get a runny or stuffy nose or start to sneeze ☐ Get itchy or watery eyes ☐ None of the above

L2. When you are near trees, grass or flowers, or when there is a lot of pollen about, do you ever:
(Please choose **ALL** that apply)
☐ Start to cough ☐ Start to wheeze ☐ Get a feeling of tightness in the chest ☐ Start to feel short of breath
☐ Get a runny or stuffy nose or start to sneeze ☐ Get itchy or watery eyes ☐ None of the above

L2a. ***If yes to any of the above*** **- At which time of the year does this happen?** (Please choose **ALL** that apply)
☐ Winter ☐ Spring ☐ Summer ☐ Autumn

SECTION M: FOOD ALLERGY

M1. Have you ever had any food allergies?

○ No ⟶ **Please go to Section N**

○ Yes ⟶ **Please complete the Food Allergy table below**

FOOD ALLERGY TABLE

What foods are you allergic to? *(Please choose **ALL** that apply)*	☐ Peanut	☐ Tree nut (specify)	☐ Shellfish	☐ Fish	☐ Cow's milk	☐ Egg	☐ Wheat	☐ Sesame	☐ Other (specify)
Was the allergy confirmed by a doctor?	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
At what age did you develop the allergy?	☐☐ years	☐☐ years	☐☐ years	☐☐ years	☐☐ years	☐☐ years	☐☐ years	☐☐ years	☐☐ years
What was the reaction? *(Please choose **ALL** that apply)*	☐ A rash or itchy skin ☐ Diarrhoea or vomiting ☐ Runny or stuffy nose ☐ Severe headaches ☐ Breathless ☐ Other reaction (specify)	☐ A rash or itchy skin ☐ Diarrhoea or vomiting ☐ Runny or stuffy nose ☐ Severe headaches ☐ Breathless ☐ Other reaction (specify)	☐ A rash or itchy skin ☐ Diarrhoea or vomiting ☐ Runny or stuffy nose ☐ Severe headaches ☐ Breathless ☐ Other reaction (specify)	☐ A rash or itchy skin ☐ Diarrhoea or vomiting ☐ Runny or stuffy nose ☐ Severe headaches ☐ Breathless ☐ Other reaction (specify)	☐ A rash or itchy skin ☐ Diarrhoea or vomiting ☐ Runny or stuffy nose ☐ Severe headaches ☐ Breathless ☐ Other reaction (specify)	☐ A rash or itchy skin ☐ Diarrhoea or vomiting ☐ Runny or stuffy nose ☐ Severe headaches ☐ Breathless ☐ Other reaction (specify)	☐ A rash or itchy skin ☐ Diarrhoea or vomiting ☐ Runny or stuffy nose ☐ Severe headaches ☐ Breathless ☐ Other reaction (specify)	☐ A rash or itchy skin ☐ Diarrhoea or vomiting ☐ Runny or stuffy nose ☐ Severe headaches ☐ Breathless ☐ Other reaction (specify)	☐ A rash or itchy skin ☐ Diarrhoea or vomiting ☐ Runny or stuffy nose ☐ Severe headaches ☐ Breathless ☐ Other reaction (specify)
Are you still allergic to it?	○ Yes ○ No ○ Don't know	○ Yes ○ No ○ Don't know	○ Yes ○ No ○ Don't know	○ Yes ○ No ○ Don't know	○ Yes ○ No ○ Don't know	○ Yes ○ No ○ Don't know	○ Yes ○ No ○ Don't know	○ Yes ○ No ○ Don't know	○ Yes ○ No ○ Don't know
Have you been prescribed an Epipen/Anapen?	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No

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SECTION N: RESPIRATORY SYMPTOMS

N1. Have you, at anytime in your life, suffered from attacks of asthma of wheezy breathing?
(Regard asthma and wheezy breathing as being much the same thing for this question.)

○ No → **Please go to question N2**
○ Yes

N1a. How old were you when you had your first attack of asthma or wheezy breathing?

☐☐ age in years

N1b. How old were you when you had your most recent attack of asthma or wheezy breathing?

☐☐ age in years

N1c. Have you had an attack of asthma or wheezy breathing in the last 12 months?
○ No ○ Yes

N1d. Have you taken any medicines including inhalers or tablets for asthma or wheezy breathing in the last 12 months?
○ No ○ Yes

N2. Have you had wheezing or whistling in your chest in the last 12 months?
(Wheezing means a whistling sound, however high or low pitched and however faint.)

○ No → **Please go to question N3**
○ Yes

N2a. Have you been at all breathless when the wheezing noise was present?
○ No ○ Yes

N2b. Have you had this wheezing or whistling when you did not have a cold?
○ No ○ Yes

N3. Have you, at any time in the last 12 months?

	No	Yes
Had an attack of shortness of breath at rest	○	○
Had an attack of shortness of breath after exercise	○	○
Woken due to a feeling of tightness in your chest	○	○
Been woken at night by an attack of shortness of breath	○	○

SECTION O: SNORING AND SLEEPINESS

O1. During the last month, do you or have you been told you snore loudly in sleep?

○ No → **Please go to question O2**
○ Don't know
○ Yes

O1a. On average, how often?
○ Rarely, less than once a week ○ 1 to 2 times per week ○ 3 to 4 times per week
○ 5 to 7 times per week ○ Don't know

O2. During the last month, do you or have you been told you snort or gasp in sleep?

○ No → **Please go to question O3**
○ Don't know
○ Yes

O2a. On average, how often?
○ Rarely, less than once a week ○ 1 to 2 times per week ○ 3 to 4 times per week
○ 5 to 7 times per week ○ Don't know

O3. During the last month, do you or have you been told you choke or stop breathing in sleep?
- ○ No → **Please go to question O4**
- ○ Don't know
- ○ Yes

O3a. On average, how often?
- ○ Rarely, less than once a week
- ○ 1 to 2 times per week
- ○ 3 to 4 times per week
- ○ 5 to 7 times per week
- ○ Don't know

O4. During the last month, have you had excessive sleepiness during the day?
○ No ○ Yes

O5. Has your doctor ever told you that you have or had obstructive sleep apnoea?
- ○ No → **Please go to question O6**
- ○ Yes

O5a. How old were you when you were told you had obstructive sleep apnoea? ☐☐ age in years

O5b. How old were you when you had your first overnight sleep study? ☐☐ age in years

O5c. Are you currently being treated for obstructive sleep apnoea with any of the following?
(Please choose **ALL** that apply)
☐ CPAP ☐ Surgery ☐ Weight loss ☐ Oral splint ☐ Positional device ☐ Other

O6. How many hours in 24 do you sleep? ☐☐ hours

SECTION P: COUGH AND PHLEGM

P1. Have you at any time in your life suffered from attacks of bronchitis or attacks of cough with sputum (phlegm) in the chest ("loose" or "rattly" cough)?
- ○ No → **Please go to question P2**
- ○ Yes

P1a. How long is it since the last attack?
- ○ Within the last 6 months
- ○ Within the last year but longer than the last 6 months
- ○ Less or equal to 2 years but more than 1 year
- ○ More than 2 years

P1b. At what age did these attacks begin? ☐☐ age in years

P2. Do you usually cough when you do not have a cold?
- ○ No → **Please go to question P3**
- ○ Yes

P2a. Are there months in which you cough on most days?
- ○ No → **Please go to question P3**
- ○ Yes

P2b. Do you cough on most days for at least three months of each year?
○ No ○ Yes

P2c. For how many years have you had this cough?
○ Less than 2 years ○ 2 to 5 years ○ More than 5 years

P3. Do you usually have phlegm in your chest when you do not have a cold?
- ○ No → **Please go to question P4**
- ○ Yes

P3a. Are there months in which you have phlegm in your chest on most days?
- ○ No → **Please go to question P4**
- ○ Yes

P3b. Do you bring up this phlegm on most days for at least three months of each year?
○ No ○ Yes

P3c. For how many years have you had this phlegm?
○ Less than 2 years ○ 2 to 5 years ○ More than 5 years

P4. Have you, at anytime in your life, suffered from cough with phlegm in the chest (with or without a cold)?

○ No ⟶ **Please go to question Q1**

○ Yes

P4a. Have you had this cough with phlegm on most days for at least three months and for two years in a row?

○ No

○ Yes

SECTION Q: CHEST COLDS AND CHEST ILLNESSES

Q1. If you get a cold, does it usually go to your chest? *(Usually means more than half of the time)*

○ No

○ Yes

○ Do not get colds

Q2. During the past three years, have you had any chest illnesses that have kept you off work, indoors at home, or in bed?

○ No ⟶ **Please go to question Q3**

○ Yes

Q2a. Did you produce phlegm with any of the chest illnesses?

○ No

○ Yes

Q2b. In the last three years, how many such illnesses, with increased phlegm, did you have which lasted a week or more? *(if none enter zero)*

☐☐ number of illnesses

Q3. Have you ever had Pneumonia?

○ No ⟶ **Please go to question Q4**

○ Yes

Q3a. Was this confirmed by a doctor?

○ No

○ Yes

Q3b. At what age did you first have it?

☐☐ age in years

Q3c. How many times have you had it?

☐☐ number of times

Q3d. Have you ever been hospitalised for Pneumonia?

○ No

○ Yes

Q4. Have you ever been hospitalised for any other chest illness? ○ No ○ Yes

If Yes, please specify diagnosis (see Code Sheet)	Office Use only	Age at first occurrence	Number of occurrences
1.	☐☐	☐☐ years	☐☐ number
2.	☐☐	☐☐ years	☐☐ number
3.	☐☐	☐☐ years	☐☐ number

SECTION R: ASTHMA

R1. Have you ever had asthma?
- ○ No → **Please go to Section S**
- ○ Yes

R2. How old were you when you had your first symptoms of asthma? ☐☐ age in years

R3. Was this confirmed by a doctor?
- ○ No → **Please go to question R4**
- ○ Yes

R3a. How old were you when this was confirmed? ☐☐ age in years

R4. How old were you when you had your most recent symptoms of asthma? ☐☐ age in years

R5. Have you been woken from your sleep by your asthma?

Last 12 months: ○ No ○ Yes

Last 1 month: ○ No ○ Yes → **R5a. How many nights were you woken from sleep by your asthma in the last week?** ☐ number of nights

R6. Have you had asthma symptoms when you wake in the morning?

Last 12 months: ○ No ○ Yes

Last 1 month: ○ No ○ Yes → **R6a. How many mornings in the last week?** ☐ number of mornings

R7. Have you been limited in any of the following activities because of asthma?

	Last 12 months		Last 1 month	
	No	**Yes**	**No**	**Yes**
All activities	○	○	○	○
When dressing	○	○	○	○
Walking on level ground	○	○	○	○
Hurrying on level ground	○	○	○	○
Walking up stairs or up hills	○	○	○	○
Active sports	○	○	○	○
Other (please specify) ☐	○	○	○	○

If you answered Yes to <u>ANY</u> statements in question R7 → Please go to question R9
If you answered No to <u>ALL</u> statements in question R7 → Please answer question R8 before continuing

R8. Would you agree or disagree with the following statement:

My asthma has not limited any of my activities:

Last 12 months: ○ Agree ○ Disagree

Last 1 month: ○ Agree ○ Disagree

R9. How frequent have your asthma symptoms (of any severity) been?
- ○ No asthma in the past 12 months
- ○ Asthma symptoms in the past 12 months but not in the last month
- ○ Asthma symptoms in the last month, but not frequent (less than once per week)
- ○ Frequent (once per week or more but not daily) in the last 1 month
- ○ Persistent (daily)

R10. How frequent have your asthma attacks/flare ups been over the past 12 months?

An "attack" or "flare up" of asthma is a period of time when asthma symptoms are worse or more frequent than usual. One could have asthma symptoms regularly or intermittently without getting attacks or flare-ups.

○ None in the past 12 months ○ 3 or less in the past 12 months ○ 4 or more but less than monthly
○ More than monthly in the last 12 months ○ More than weekly or persistent ○ Not sure

R11. Have you had an episode of asthma which has made you unable to speak or severe enough to limit your speech to only 1 or 2 words between breaths?

Last 12 months: ○ No ○ Yes
Last 1 month: ○ No ○ Yes

R12. On average, how would <u>you</u> rate the severity of your asthma?

	Not severe at all	Mild	Moderate	Severe	Not sure
Last 12 months	○	○	○	○	○
Last 1 month	○	○	○	○	○

R13. Have you lost any days from work, school or usual activities because of your asthma?

Last 12 months: ○ No ○ Yes → **R13a. How many?** ☐☐☐ days

Last 1 month: ○ No ○ Yes → **R13b. How many?** ☐☐ days

R14. Have you had an attack or symptoms of asthma that was so bad, you needed to call your general practitioner, ambulance, emergency locum or 24 hour clinic?

Last 12 months: ○ No ○ Yes → **R14a. How many?** ☐☐☐ number

Last 1 month: ○ No ○ Yes → **R14b. How many?** ☐☐ number

R15. Have you had an attack or symptoms of asthma that was so bad you had to go to a hospital emergency or casualty department?

Last 12 months: ○ No ○ Yes → **R15a. How many?** ☐☐☐ number

Last 1 month: ○ No ○ Yes → **R15b. How many?** ☐☐ number

R16. Have you ever been admitted to a hospital because of your asthma?

○ No → **Please go to question R17**

○ Yes

R16a. In the past 12 months?

○ No → **Please go to question R17**

○ Yes

R16b. How many times in the last 12 months? ☐☐ number

R16c. How many times in the last 1 month? ☐☐ number

R17. Have you ever had an attack or symptoms of asthma that resulted in an admission to a hospital intensive care unit?

○ No ○ Yes

R18. How frequently have you seen the following health professionals for your asthma in the last 12 months?
(enter zero if not at all)

1) **General Practitioner** *(number of times in the last 12 months)* ☐☐ number
2) **Respiratory specialist** *(number of times in the last 12 months)* ☐☐ number
3) **Nurse/Asthma educator** *(number of times in the last 12 months)* ☐☐ number
4) **Pharmacist** *(number of times in the last 12 months)* ☐☐ number

R19. Have you ever been given a demonstration on the correct use of your metered dose inhaler?

○ No → **Please go to question R20**

○ Yes

R19a. In the last 12 months?

○ No

○ Yes

R20. Has your doctor ever checked your inhaler technique?

○ No → **Please go to question R21**

○ Yes

R20a. In the last 12 months?

○ No

○ Yes

R21. Do you have written instructions from your doctor on how to manage your asthma if it gets worse or if you have an attack?

○ No → **Please go to question R22**

○ Yes

R21a. In the last 12 months?

○ No

○ Yes

R22. Has your doctor given you a verbal plan telling you how to manage your asthma if it gets worse or if you have an attack?

○ No → **Please go to question R23**

○ Yes

R22a. In the last 12 months?

○ No

○ Yes

R23. Do you have a peak flow meter of your own?

○ No → **Please go to question R24**

○ Yes

R23a. How often have you used it in the last 3 months?

○ Never

○ Some days

○ Most days

R24. Has your doctor ever measured your breathing in his/her surgery (including peak flows/spirometry/bronchodilator response)?

○ No → **Please go to Section S**

○ Yes

R24a. In the last 12 months?

○ No

○ Yes

SECTION S: MEDICINES AND INHALERS (see Medication Code List)

S1. Have you used any inhaled medicines to help your breathing in the last 12 months?
○ No → **Please go to question S2** ○ Yes → **Please continue with the questions below**

Which have been used in the last 12 months?	Medication 1	Medication 2	Medication 3	Medication 4
a) Which one?				
b) Which type of inhaler device?				
c) Strength/dose per puff (mcg)?				
d) Are you currently using this medication?	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
e) How long have you been using this medication?	○ Days ○ Months ○ Years	○ Days ○ Months ○ Years	○ Days ○ Months ○ Years	○ Days ○ Months ○ Years
f) In the last 12 months, how have you used them:				
When needed - *Average number of puffs per month*	(puff/mth)	(puff/mth)	(puff/mth)	(puff/mth)
In short courses - *Number of courses in last 12 months*	(number)	(number)	(number)	(number)
- *Average number of puffs per day during flare-up*	(puff/day)	(puff/day)	(puff/day)	(puff/day)
- *Average number of days of flare-up*	(days)	(days)	(days)	(days)
Continuously - *Average number of puffs per day*	(puff/day)	(puff/day)	(puff/day)	(puff/day)
Not at all	○ Not at all	○ Not at all	○ Not at all	○ Not at all
g) In the last month, how have you used them:				
When needed - *Average number of puffs per month*	(puff/mth)	(puff/mth)	(puff/mth)	(puff/mth)
In short courses - *Number of courses in last 12 months*	(number)	(number)	(number)	(number)
- *Average number of puffs per day during flare-up*	(puff/day)	(puff/day)	(puff/day)	(puff/day)
- *Average number of days of flare-up*	(days)	(days)	(days)	(days)
Continuously - *Average number of puffs per day*	(puff/day)	(puff/day)	(puff/day)	(puff/day)
Not at all	○ Not at all	○ Not at all	○ Not at all	○ Not at all
h) Is this medication the same type as prescribed by your doctor?	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
i) Are you taking this medication at the same dose as prescribed by your doctor?	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
j) If No to h) or i) why not the same: *(Please choose **ALL** that apply)*	☐ Cost ☐ Side effects ☐ Symptoms resolved ☐ Forget to take ☐ Other (specify)	☐ Cost ☐ Side effects ☐ Symptoms resolved ☐ Forget to take ☐ Other (specify)	☐ Cost ☐ Side effects ☐ Symptoms resolved ☐ Forget to take ☐ Other (specify)	☐ Cost ☐ Side effects ☐ Symptoms resolved ☐ Forget to take ☐ Other (specify)

S2. Have you used any pills, capsules, tablets or medicines, other than inhaled medicines to help your breathing at any time in the last 12 months?

○ No → **Please go to question S3** ○ Yes → **Please continue with the questions below**

Which have been used in the last 12 months?	Medication 1	Medication 2	Medication 3	Medication 4
a) Which one?				
b) Strength/dose per tablet (mg)?				
c) Are you currently using this medication?	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
d) How long have you been using this medication?	○ Days ○ Months ○ Years	○ Days ○ Months ○ Years	○ Days ○ Months ○ Years	○ Days ○ Months ○ Years
e) In the last 12 months, how have you used them:				
When needed - *Average number of tablets per month*	(tab/mth)	(tab/mth)	(tab/mth)	(tab/mth)
In short courses - *Number of courses in last 12 months*	(number)	(number)	(number)	(number)
- *Average number of tablets per day during flare-up*	(tab/day)	(tab/day)	(tab/day)	(tab/day)
- *Average number of days of flare-up*	(days)	(days)	(days)	(days)
Continuously - *Average number of tablets per day*	(tab/day)	(tab/day)	(tab/day)	(tab/day)
Not at all	○ Not at all	○ Not at all	○ Not at all	○ Not at all
f) In the last month, how have you used them:				
When needed - *Average number of tablets per month*	(tab/mth)	(tab/mth)	(tab/mth)	(tab/mth)
In short courses - *Number of courses in last 12 months*	(number)	(number)	(number)	(number)
- *Average number of tablets per day during flare-up*	(tab/day)	(tab/day)	(tab/day)	(tab/day)
- *Average number of days of flare-up*	(days)	(days)	(days)	(days)
Continuously - *Average number of tablets per day*	(tab/day)	(tab/day)	(tab/day)	(tab/day)
Not at all	○ Not at all	○ Not at all	○ Not at all	○ Not at all
g) Is this medication the same type as prescribed by your doctor?	○ Yes ○ No ○ Don't know	○ Yes ○ No ○ Don't know	○ Yes ○ No ○ Don't know	○ Yes ○ No ○ Don't know
h) Are you taking this medication at the same dose as prescribed by your doctor?	○ Yes ○ No ○ Don't know	○ Yes ○ No ○ Don't know	○ Yes ○ No ○ Don't know	○ Yes ○ No ○ Don't know
i) If No to g) or h) why not the same: *(Please choose **ALL** that apply)*	☐ Cost ☐ Side effects ☐ Symptoms resolved ☐ Forget to take ☐ Other (specify)	☐ Cost ☐ Side effects ☐ Symptoms resolved ☐ Forget to take ☐ Other (specify)	☐ Cost ☐ Side effects ☐ Symptoms resolved ☐ Forget to take ☐ Other (specify)	☐ Cost ☐ Side effects ☐ Symptoms resolved ☐ Forget to take ☐ Other (specify)

S3. Have you ever used inhaled steroids to help your breathing?

○ No → **Please go to question S4**

○ Yes

S3a. At what age did you start using inhaled steroids? ☐☐ years

S3b. In the past 5 years, how many months would you have used inhaled steroids on most days? ☐☐ months

S4. Have you ever been prescribed home oxygen therapy?

○ No → **Please go to question S5**

○ Yes

S4a. Are you currently using oxygen therapy at home?

○ No ○ Yes

S4b. For how many years have you been using oxygen therapy at home? ☐☐ years

S4c. How have you used oxygen therapy during the last month?

1) **For relief of symptoms or when needed** ○ No ○ Yes
2) **For flare-ups or attacks** ○ No ○ Yes
3) **Regularly, on a daily basis** ○ No ○ Yes

S5. Have you ever had an influenza vaccination?

○ No → **Please go to question S6**

○ Yes

S5a. Have you been vaccinated for influenza in the last 12 months?

○ No ○ Yes

S6. Have you ever had a pneumonia vaccination?

○ No → **Please go to question S7**

○ Yes

S6a. Have you been vaccinated for pneumonia in the last 5 years?

○ No ○ Yes

S7. Have you ever been vaccinated or desensitised for allergy?

○ No → **Please go to question S8**

○ Yes

S7a. Have you been vaccinated for allergy in the last 12 months?

○ No ○ Yes

S8. Have you had any other injections to help your breathing at any time in the last 12 months?

○ No ○ Yes (please specify) ☐

SECTION T: SCREENING QUESTIONNAIRE FOR LAB TESTING

T1. Have you used a puffer or inhaler in the last 24 hours? ○ No ○ Yes

If Yes, what inhaler(s) did you use and how many hours ago was the last dose taken?	Office Use only	Number of hours
Inhaler 1.	☐☐	☐☐☐ hours
Inhaler 2.	☐☐	☐☐☐ hours
Inhaler 3.	☐☐	☐☐☐ hours

T2. Have you taken any medication for breathing (other than inhalers) in the last 24 hours? ○ No ○ Yes

If Yes, which medication(s) did you take and how many hours ago was the last dose taken?	Office Use only	Number of hours
Medication 1.		hours
Medication 2.		hours
Medication 3.		hours

T3. Have you had a cigarette (or any other tobacco product) in the last 24 hours?
○ No → **Please go to question T4**
○ Yes
T3a. How many hours ago was your last smoke? hours

T4. Have you taken an antihistamine (a medication for allergy including hay fever) or cough medicine in the last 72 hours?
○ No ○ Yes

If Yes, which medication(s) did you take and how many hours ago was the last dose taken?	Office Use only	Number of hours
Medication 1.		hours
Medication 2.		hours
Medication 3.		hours

T5. Have you taken any medication for high blood pressure or a heart condition, or used eye drops to treat glaucoma in the last 72 hours? ○ No ○ Yes

If Yes, which medication(s) did you take and how many hours ago was the last dose taken?	Office Use only	Number of hours
Medication 1.		hours
Medication 2.		hours
Medication 3.		hours

T6. Have you had a respiratory infection in the last 3 weeks?
○ No → **Please go to question T7**
○ Yes
T6a. How many days ago did it end? days

T7. Have you ever had an anaphylactic reaction or been treated with an Epipen?
○ No → **Please go to Section U**
○ Yes
T7a. Were you admitted to hospital for this anaphylaxis?
○ No ○ Yes
T7b. Did you react to any of the following: (Please choose **ALL** that apply)

☐ Alternaria	☐ Cow's milk	☐ House dust mite	☐ Penicillium
☐ Aspergillus	☐ Egg	☐ Mixed grasses	☐ Rye grass
☐ Cat	☐ Hormodendrum	☐ Peanut	☐ Shellfish

T7c At what age did you have this reaction?
○ Under 18 ○ Over 18

SECTION U: LABORATORY DATA SHEET

U1. SKIN PRICK TEST DATA

	1st diam	2nd diam		1st diam	2nd diam		1st diam	2nd diam
Negative control			Penicillium			Peanut		
House dust mite			Aspergillus			Shellfish		
Cat			Rye grass			Cow's milk		
Hormodendrum			Mixed grasses			Positive control		
Alternaria			Egg white					

ANTHROPOMETRIC DATA

U2.	**Height (cm):** Range 00-300 cm	.	cm
U3.	**Weight (kg):** Range 00-200.0 kg	.	kg
U4.	**Neck measurements (cm):** Range 00-70.0 cm	1. . cm	2. . cm
U5.	**Waist measurements (cm):** Range 00-200.0 cm	1. . cm	2. . cm
U6.	**Hip measurements (cm):** Range 00-200.0 cm	1. . cm	2. . cm

SPIROMETRY
Pre-Bronchodilator

		Blow 1	Blow 2	Blow 3
U7.	**FVC (litres)** Range 00-10.00 L	.	.	.
U8.	**FEV_1 (litres)** Range 00-10.00 L	.	.	.
U9.	**FEV_1 /FVC (%)** Range 00-100%	.	.	.

SPIROMETRY
Post-Bronchodilator

		Blow 1	Blow 2	Blow 3
U10.	**FVC (litres)** Range 00-10.00 L	.	.	.
U11.	**FEV_1 (litres)** Range 00-10.00 L	.	.	.
U12.	**FEV_1 /FVC (%)** Range 00-100%	.	.	.

SPIROMETRY
BEST Values Pre and Post BD

		Pre-BD	Post-BD
U13.	**FVC (litres)** Range 00-10.00 L	.	.
U14.	**FEV_1 (litres)** Range 00-10.00 L	.	.
U15.	**FEV_1 /FVC (%)** Range 00-100%	.	.
U16.	**PEF (litres/sec)** Range 00-20.00 L/sec	.	.
U17.	**$FEF_{25-75\%}$ (litres/sec)** Range 00-10.00 L/sec	.	.
U18.	**$FEF_{50\%}$ (litres/sec)** Range 00-10.00 L/sec	.	.
U19.	**$FIF_{50\%}$ (litres/sec)** Range 00-10.00 L/sec	.	.

HB AND COHB

U20. Hb Concentration g/dl ___.___

U21. COHb % ___.___

SINGLE BREATH DLCO (post-BD)

		Measurement 1	Measurement 2
U22.	**DLCO (uncorrected)** (ml/min/mmHg)	___.___	___.___
U23.	**DLCO (corrected)** (ml/min/mmHg)	___.___	___.___
U24.	**VA (litres)**	___.___	___.___
U25.	**DLCO/VA (uncorrected)** (ml/min/mmHg/L)	___.___	___.___
U26.	**DLCO/VA (corrected)** (ml/min/mmHg/L)	___.___	___.___
U27.	**Inspiratory VC (L)**	___.___	___.___

APNEALINK STUDY

Instructions: Complete 1 form for each Home ApneaLink test attempted.

U28. Date of test ___ / ___ / ____ (Day / Month / Year)

U29. Serial Number of ApneaLink _____ _____ _____ _____

U30. Serial Number of Pulse Oximetry _____ _____ _____

U31. Recording Duration: ___ Hours ___ Minutes

U32. Evaluation Period Duration: ___ Hours ___ Minutes

If evaluation period is less than 4 hours the study must be repeated

U33. Please indicate why evaluation period is less than 4 hours.

- ○ UNK, slept 4 or more
- ○ Unable to sleep due to AL - complete question U33
- ○ Subject unsure if device was working or set-up correctly - complete question U33
- ○ Normally sleeps less than 4 hours
- ○ Unable to sleep unrelated to AL
- ○ Other (please specify) - consider completing question U33

U34. ApneaLink Test Results:

○ No Data ⟶ **Please go to question U33**

		Result			Result	
a)	**AHI**	___	e/hr	**f) Average saturation**	___	%
b)	**RI** (Risk Indicator)	___		**g) Lowest saturation**	___	%
c)	**Apnea Index**	___	e/hr	**h) Baseline saturation**	___	%
d)	**Hypopnea Index**	___	e/hr	**i) Cumulative time saturation ≤ 90%**	___	mins
e)	**ODI** (Oxygen desaturation index)	___	e/hr	**j) CSR probable**	○ Yes ○ No	

U35. Were there any hardware, software, technical or user interface problems related to the ApneaLink?

○ No ○ Yes ⟶ **Please complete a Technical Observation CRF**