VARIATION IN AIRWAY REMODELLING GENES AND THEIR ROLE ON ASTHMA SEVERITY IN CHILDREN AND YOUNG ADULTS

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Abstract

**Background:** Asthma affects approximately 300 million people worldwide\(^1\), 5.2 million of these people live in the UK\(^2\), 1.1 million of these are children\(^2\). Asthma is one of the most common chronic diseases and is the fourth leading cause of morbidity worldwide, and there is no indication of a decline in prevalence\(^3,4\). It is hypothesised that a range of gene-environmental interactions may influence the susceptibility, severity and medication response of asthma in children and young adults\(^5\).

**Methods:** To explore these issues, two studies have been established to create datasets that will describe the phenotypic and genotypic characteristics of children with asthma in the paediatric population across Sussex and Scotland. This thesis is the output of doctoral research using data from these studies (BREATHE and PAGES) that aims to explore the interactions between variants of six genes implicated in airway remodelling and relevant environmental factors and their influence on the severity of asthma in children and young adults.

This thesis is divided by analysis of individual variants. The thesis included one variant of *Chitinase 3-Like-1*, two variants of *Matrix metalloproteinase 9*, two variants of *Matrix metalloproteinase 12*, one variant of *Matrix metalloproteinase 9*, one variant of *Glutathione S-transferase mu-1*, one variant of *Glutathione S-transferase theta-1* and one variant of *Glutathione S-transferase pi-1*. A total of eight variants were investigated. Variants were analysed for effect on multiple proxy measures of asthma severity, including asthma exacerbations, asthma treatment steps, pulmonary function and quality of life. Variants were also analysed for their effect on allergy.

**Results:** The mutant G allele of *CHI3L1* rs4950928 confers protection against asthma-related hospital admissions (OR = 0.72; 95% CI 0.53–0.97; \(P = .035\)), asthma-related exacerbations when exposed to damp (OR = 0.17; 95% CI 0.05–0.56; \(P = .002\)), asthma-related hospital admissions when exposed to damp (OR = 0.12; 95% CI 0.02–1.03; \(P = .025\)), and asthma-related absence when exposed to damp (OR = 0.18; 95% CI 0.05–0.65; \(P = .019\)).

The mutant G allele of *MMP9* rs17576 confers protection against asthma-related exacerbations (OR = 0.68; 95% CI 0.49–0.94; \(P = .018\)), and asthma-related absence (OR = 0.63; 95% CI 0.45–0.88; \(P = .007\)), asthma-related oral steroid intake when exposed to
environmental tobacco smoke (ETS) (OR = 0.44; 95% CI 0.24–0.80; P = .009). In 13-22 year olds the mutant G allele confers protection against asthma-related exacerbations (OR = 0.23; 95% CI 0.10–0.68; P = .006), asthma-related absence (OR = 0.38; 95% CI 0.14–0.99; P = .048), and asthma-related oral steroid intake (OR = 0.28; 95% CI 0.08–0.92; P = .036).

The mutant G allele of MMP9 rs6073983 confers protection against asthma-related exacerbations (OR = 0.53; 95% CI 0.32–0.88; P = .014), asthma-related absence (OR = 0.39; 95% CI 0.23–0.71; P = .002), asthma-related oral steroid intake when exposed to ETS (OR = 0.38; 95% CI 0.15–0.94; P = .032), asthma-related exacerbations when exposed to ETS (OR = 0.28; 95% CI 0.13–0.63; P = .053), addition of preventer medication (OR = 0.48; 95% CI 0.29–0.79; P = .004) and increased asthma severity (OR = 0.49; 95% CI 0.29–0.82; P = .006). In 13-22 year olds the mutant G allele confers greater protection against, addition of preventer medication (OR = 0.36; 95% CI 0.14–0.95; P = .039) and increased asthma severity (OR = 0.36; 95% CI 0.14–0.93; P = .036).

The mutant G allele of MMP12 rs652438 increases the risk of asthma-related exacerbations (OR = 1.51; 95% CI 1.05–2.18; P = .026), asthma-related absence when 13-22 year olds are exposed to ETS (OR = 3.53; 95% CI 1.15–10.80; P = .044), addition of preventer medication (OR = 1.83; 95% CI 1.08–3.10; P = .025) and increased asthma severity (OR = 1.74; 95% CI 1.03–2.96; P = .040).

The merged GSTM1, GSTP1 rs1695 and GSTT1 mutant variant increases the risk of asthma-related hospital admission (OR = 1.51; 95% CI 1.04–2.26; P = .046), asthma-related hospital admissions in 5-12 year olds (OR = 1.88; 95% CI 1.13–3.14; P = .015), addition of preventer medication when 13-22 year olds are exposed to ETS (OR = 2.67; 95% CI 0.91–7.85; P = .029), increased asthma severity when 13-22 year olds are exposed to ETS (OR = 3.19; 95% CI 1.06–9.55; P = .020) and participant reported allergy (OR = 1.47; 95% CI 1.06–2.04; P = .020).

**Conclusion:** This thesis provides clinically relevant findings on the impact of genetic variants in proteins influencing airway remodelling, on asthma severity in children and young adults with asthma. The study increases our understanding of the role of these molecules in causing asthma exacerbations, and the possible interactions with relevant environmental factors. The work could define susceptible population groups among children with asthma and help develop novel, personalised approaches for asthma management.
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Finally, my thanks go to my family and friends for their continuing forbearance, love and encouragement.

Jason Cunningham
Brighton and Sussex Medical School
September 2011
I declare that the research contained in this thesis, unless otherwise formally indicated within the text, is the original work of the author. The thesis has not been previously submitted to these or any other university for a degree, and does not incorporate any material already submitted for a degree.

Signed

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Dated

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### Definitions

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMCase</td>
<td>Acid Mammalian Chitinase</td>
</tr>
<tr>
<td>APC</td>
<td>Antigen Presenting Cell</td>
</tr>
<tr>
<td>ASSM</td>
<td>Airway Smooth Muscle Mass</td>
</tr>
<tr>
<td>BMI</td>
<td>Body Mass Index</td>
</tr>
<tr>
<td>BTS</td>
<td>British Thoracic Society</td>
</tr>
<tr>
<td>CHI</td>
<td>Hospital identification number</td>
</tr>
<tr>
<td>CHI3L1</td>
<td>Chitinase 3-Like-1 also known as cartilage glycoprotein-39</td>
</tr>
<tr>
<td>CHIT1</td>
<td>Chitriosidase 1</td>
</tr>
<tr>
<td>COPD</td>
<td>Chronic Obstructive Pulmonary Disease</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>ECM</td>
<td>Extracellular matrix</td>
</tr>
<tr>
<td>ETS</td>
<td>Environmental tobacco smoke</td>
</tr>
<tr>
<td>FAM</td>
<td>6-carboxy-flourescein</td>
</tr>
<tr>
<td>FEV1</td>
<td>Forced Expiratory Volume in 1 second</td>
</tr>
<tr>
<td>FVC</td>
<td>Forced Vital Capacity</td>
</tr>
<tr>
<td>GSH</td>
<td>Glutathione</td>
</tr>
<tr>
<td>GSTM-1</td>
<td>Glutathione S-Transferase mu-1</td>
</tr>
<tr>
<td>GSTP-1</td>
<td>Glutathione S-Transferase pi-1</td>
</tr>
<tr>
<td>GSTT-1</td>
<td>Glutathione S-Transferase theta-1</td>
</tr>
<tr>
<td>H&amp;E</td>
<td>Hematoxylin and eosin</td>
</tr>
<tr>
<td>HRQOL</td>
<td>Health related quality of life</td>
</tr>
<tr>
<td>IL-8</td>
<td>Inter leukin 8</td>
</tr>
<tr>
<td>IL-13</td>
<td>Inter leukin 13</td>
</tr>
<tr>
<td>MAP kinase</td>
<td>Mitogen active protein kinase</td>
</tr>
<tr>
<td>MGB</td>
<td>Minor groove binder</td>
</tr>
<tr>
<td>MMP</td>
<td>Matrix Metalloproteinase</td>
</tr>
<tr>
<td>MMP1</td>
<td>Matrix Metalloproteinase 1 also known as fibroblast collagenase</td>
</tr>
<tr>
<td>---------------</td>
<td>---------------------------------------------------------------</td>
</tr>
<tr>
<td>MMP3</td>
<td>Matrix Metalloproteinase 3 also known as stromalysin 1</td>
</tr>
<tr>
<td>MMP9</td>
<td>Matrix Metalloproteinase 9 also known as gelatinase B</td>
</tr>
<tr>
<td>MMP12</td>
<td>Matrix Metalloproteinase 12 also known as macrophage elastase</td>
</tr>
<tr>
<td>MMP13</td>
<td>Matrix Metalloproteinase 13 also known as collagenase 3</td>
</tr>
<tr>
<td>NF-κB</td>
<td>Nuclear factor kappa-light-chain-enhancer of activated B cells</td>
</tr>
<tr>
<td>PAGES</td>
<td>Paediatric Asthma Gene Environment Study</td>
</tr>
<tr>
<td>PAQLQ</td>
<td>Paediatric asthma quality of life questionnaire</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase chain reaction</td>
</tr>
<tr>
<td>PEFR</td>
<td>Peak expiratory flow rate</td>
</tr>
<tr>
<td>PI-3K</td>
<td>Phosphoinositide 3-kinase</td>
</tr>
<tr>
<td>pp</td>
<td>% predicted</td>
</tr>
<tr>
<td>ppb</td>
<td>Parts per billion</td>
</tr>
<tr>
<td>QOL</td>
<td>Quality of Life</td>
</tr>
<tr>
<td>RANTES</td>
<td>Regulated upon activation, normal T cell expressed and secreted</td>
</tr>
<tr>
<td>SNP</td>
<td>Single nucleotide polymorphism</td>
</tr>
<tr>
<td>SPT</td>
<td>Skin Prick Test</td>
</tr>
</tbody>
</table>
INTRODUCTION

The introduction to this thesis consists of two chapters, which detail the background and aims of this study. Chapter 1, presents a background to asthma pathogenesis and the impact of asthma on healthcare. Chapter 2 is a background to each gene of interest, relevant research questions, and hypotheses for each variant.
CHAPTER 1

SECTION 1.1

Asthma pathogenesis and management

Asthma is a collection of heterogeneous conditions, characterised by a combination of bronchoconstriction, airway hyper-reactivity, chronic bronchial inflammation, pathological remodelling, with the clinical features of cough, wheeze or breathlessness\(^9\)-\(^{14}\). It affects approximately 300 million people worldwide\(^{15}\), with 5.2 million in the UK alone\(^2\). In the UK 1.1 million children are diagnosed with asthma\(^2\).

To facilitate the understanding of the mechanisms underlying the disease and the separation of asthma into individual disease processes the pathogenesis of asthma needs to be fully explored.

The pathology of asthma consists of chronic airway narrowing and airflow obstruction caused by inflammation and remodelling\(^{16,17}\). The initial pathological trigger of asthma varies depending on the individual, however the universal response is an inflammatory migration to the lung resulting in a hyper-responsive airway\(^{18}\). Airway inflammation in asthma is typically ascribed to a localised increase in eosinophils or neutrophils, an increase in expression of Th2 cytokines, and localisation of mast cells within bundles of airway smooth muscle\(^{19}\). The influx of leukocytes leads to cellular hyperplasia and hypertrophy, eventually resulting in airway remodelling\(^{19}\).

The progressive stages of remodelling in the asthmatic airway epithelium can be categorised into four stages, as described by Davies et al\(^{20}\). The initial stage is insult-induced epithelial damage. This damage results in barrier loss and increased macromolecular permeability. The second stage is the immediate immune response, which is the influx of migrating immune cells and formation of a temporary barrier. At this stage, there is an increase in matrix metalloproteinases (MMP) and collagen, fibroblast proliferation, myofibroblast activation within the epithelium. The third stage is the
interim repair stage. The cell barrier and secretory cell function are restored at this stage. Davies et al.\textsuperscript{20} suggested that primary cell differentiation, matrix remodelling and myofibroblast apoptosis occur at this stage. The final stage of the progress is proliferation and differentiation. Ciliagenesis and secretory function are restored at this stage, along with secondary differentiation and resolution. Repeated damage leads to loss of function through loss of cilia, secretory capacity, hyperplasia, and hypertrophy and thereby progressive airway remodelling.

Characteristic structural changes associated with airway remodelling include increased airway smooth muscle mass (ASMM), increase in number of localised sub-epithelial myofibroblasts and reticula lamina/ basement membrane thickening\textsuperscript{19}. The increase in ASMM is proportional to asthma severity\textsuperscript{21}. The increase in ASMM can be attributed to smooth muscle cell hyperplasia and hypertrophy. Increased ASMM can also be attributed to heightened proliferation, migration and differentiation of peripheral blood fibrocytes in the airway smooth muscle\textsuperscript{16,22}. The number of fibrocytes within the tissue correlates with thickening of the sub-basement membrane and chronic airflow obstruction. As a result of this sub-epithelial progressive fibrosis, chronic asthma sufferers are often unresponsive to steroid based treatments\textsuperscript{22}, making them more prone to exacerbations of asthma.

In adults, remodelling and inflammatory reaction have been linked to clinical severity\textsuperscript{23}. Benayoun et al.\textsuperscript{24} suggested that increased areas of mucus glands, smooth muscle, fibroblast hyperplasia and increased deposits of collagen could differentiate severe from mild asthma\textsuperscript{24}. The accumulation of fibroblasts in the airway is associated with airway remodelling, although the source of the fibroblast hyperplasia has yet to be identified. Airway remodelling has been implicated with persistent airway hyper responsiveness, excessive airway narrowing and fixed airflow obstruction in severe asthma\textsuperscript{25}. However, in children the term remodelling does not necessarily refer to clinical severity, but instead to a series of histopathologic changes\textsuperscript{25}. There is evidence to suggest that eosinophil inflammation, epithelial loss, basement membrane thinking and angiogenesis occur from 3 years of age in asthma, and that inflammation and remodelling occur in parallel, with remodelling the result of exacerbations and not inflammation directly, but as a result of inflammation induced exacerbations\textsuperscript{17,23,25,26}. Interestingly, inflammation and remodelling
are present at 3 years of age but not at 1 year, as such basement membrane thickening is believed to occur in correlation with the development of asthma symptoms and may not be linked to asthma severity\textsuperscript{25-27}. Increased thickness of airway smooth muscle is however, associated with asthma severity\textsuperscript{28}. Children with severe asthma and non-reversible bronchial obstruction experience persistent symptoms alongside persistence of eosinophils and neutrophils in the bronchial intraepithelial area, increased airway smooth muscle and a denser vascular network\textsuperscript{23}. Several methods have been devised to assess remodelling non-invasively. Elevated levels of nitric oxide have been associated with airway hyper responsiveness and remodelling in children and young adults, although further studies are required before nitric oxide can be used as a tool to measure asthma severity. A decline in pulmonary function is suggested to associate with an increase in remodelling, expressed as basement membrane thickening, although evidence is conflicting, and further studies are required\textsuperscript{29}.

Asthma exacerbations represent periods of enhanced airway inflammation and remodelling\textsuperscript{30} and are a marker of pulmonary decline\textsuperscript{17}. Asthma exacerbations are the commonest cause of medical hospital admissions in childhood, these exacerbations have significant effects on quality of life\textsuperscript{31}. Hospital admissions, use of short courses of oral steroids and absence from school, college or work (referred to as ‘absence’ in the rest of the thesis) represent measures of asthma exacerbations\textsuperscript{32-36}. In the BREATHE study these measures have been combined as an overall exacerbation score used to define the frequency of asthma exacerbations and as a measure of asthma severity. Another measure of asthma severity are the BTS (British Thoracic Society) treatment steps (explained below)\textsuperscript{37}. A child moves up to a higher BTS treatment step if asthma control is not adequate on a lower step, and this may be because of uncontrolled symptoms or an excessive need of reliever medication. As such, it may be useful to develop an asthma severity score that incorporates the participant’s drug class adjusted by the extent of regular asthma medication use over the previous six months. The adjusted severity score will provide a cross sectional picture of the participants’ asthma severity.

An important outcome measure of asthma management is the extent of symptoms experienced by the patient as this indicates the level of well-being for the patient\textsuperscript{38-40}. Asthma management comprises of reliever and preventer medications. The first line of
treatment involves the use of reliever inhalers, most commonly a short-acting $\beta_2$ adrenergic receptor agonist such as salbutamol. These serve to reverse the effects of bronchospasm through interaction with the $\beta_2$ adrenergic receptor, immediately reducing bronchospasm in the majority of cases. Preventer medications, for instance glucocorticoids such as beclomethasone dipropionate, are used in conjunction with reliever medications. These have an anti-inflammatory effect and are used to control bronchial inflammation, reducing asthma exacerbations. Oral steroids may be used to manage patients with frequent acute exacerbations of asthma. Patients can also be prescribed long-acting $\beta_2$ adrenergic receptor agonists as a controller medication. Leukotriene-receptor agonists such as montelukast reduce the production of mucus and therefore reduce the frequency and severity of asthma exacerbations. In addition to asthma specific medication anti-histamines and nasal medications are often used to reduce the frequency of exacerbations through the reduction of co-morbidities such as allergic rhinitis.

In the UK paediatric asthma is managed by a stepwise approach as recommended by the British Thoracic Society (BTS) guidelines in the UK\textsuperscript{37}. The steps vary according to three age groups, less than 5 years, 5-12 years and older children (young adults) and adults (Table 1.1.1).
Table 1.1.1 Summary of stepwise approach to asthma management as recommended by the British Thoracic Society (BTS) guidelines

<table>
<thead>
<tr>
<th>Step</th>
<th>In less than 5 year olds:</th>
<th>In 5 -12 year olds:</th>
<th>In young adults and adults:</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Step 1</strong></td>
<td>Prescription of ‘reliever’ medication (short-acting β2-agonists) as required.</td>
<td>Prescription of ‘reliever’ medication (short-acting β2-agonists) as required.</td>
<td>Prescription of ‘reliever’ medication (short-acting β2-agonists) as required.</td>
</tr>
<tr>
<td><strong>Step 2</strong></td>
<td>Addition of regular ‘controller’ therapy, starting with daily use of inhaled steroids (200-400mcg/day) or a leukotriene receptor antagonist.</td>
<td>Addition of regular ‘controller’ therapy, starting with daily use of inhaled steroids (200-400mcg/day).</td>
<td>Addition of regular ‘controller’ therapy, starting with daily use of inhaled steroids. (200-800 mcg/day)</td>
</tr>
<tr>
<td><strong>Step 3</strong></td>
<td>When asthma control when using an inhaled steroid is inadequate, leukotriene receptor antagonists are added e.g. montelukast, or slow-release theophylline. If already using a leukotriene receptor antagonist on step 2 an inhaled steroid is added.</td>
<td>Addition of inhaled long-acting β2 -agonists, e.g. salmeterol. If control is still inadequate, but there are noticeable benefits with inhaled long-acting β2 -agonists, then the inhaled steroid dose in increased to 400 micrograms/day. If no response is discernible with the long-acting β2 –agonist then it is stopped and leukotriene receptor antagonists may be added e.g. montelukast or slow-release theophylline.</td>
<td>Addition of inhaled long-acting β2 -agonists, e.g. salmeterol. If control is still inadequate, but there are noticeable benefits with inhaled long-acting β2 -agonists, then the inhaled steroid dose in increased to 800 micrograms/day. If no response is discernible with the long-acting β2 –agonist then it is stopped and leukotriene receptor antagonists may be added e.g. montelukast. Slow-release theophylline may also be used.</td>
</tr>
<tr>
<td><strong>Step 4</strong></td>
<td>Referral to specialist respiratory paediatrician.</td>
<td>For persistent poor control, the steroid dose is increased to 800 microgrammes/day.</td>
<td>For persistent poor control, the steroid dose can be increased to 2000μg/day or if control is still inadequate leukotriene receptor antagonists can be added.</td>
</tr>
<tr>
<td><strong>Step 5</strong></td>
<td>-</td>
<td>When children are requiring frequent short courses of oral steroids despite the highest recommended dose of inhaled steroids and add-on therapy, regular oral steroids are commenced.</td>
<td>Regular oral steroids are commenced.</td>
</tr>
</tbody>
</table>
For the purpose of the studies in my thesis, the asthma prescribing level was modified to include all age groups under the same steps, as follows: step 0 – no use of inhaled salbutamol on demand within the past month; step 1 - inhaled short-acting β2-agonists e.g. salbutamol on demand; step 2 - regular inhaled steroids plus inhaled salbutamol on demand; step 3 - regular inhaled long-acting β2-agonists e.g. salmeterol plus inhaled steroids with inhaled salbutamol on demand; step 4 - regular inhaled salmeterol plus inhaled steroids plus oral montelukast and/or other add-on medications with inhaled salbutamol on demand.

Asthma control is assessed through the occurrence of day-to-day asthma symptoms, asthma exacerbations, the need for ‘reliever’ treatment with short-acting β2-agonists, and patient quality-of-life.

Quality of Life or Health Related quality of life scores (HRQL) have become a prominent measure in clinical practice and research in asthma\(^{40}\). HRQL measures are representative of the effect of the condition on social, emotional and physical aspects of daily life, as perceived by the patient. In chronic disease, such as asthma an important purpose of treatment is to provide a good quality of life for the patient,\(^{40}\) hence measures of QOL contribute to decisions about management and as a proxy determinant of asthma severity\(^{38}\). We have used the Paediatric Asthma Quality of Life Questionnaire (PAQLQ)\(^ {41}\). Designed to be administered by the parent to the child, PAQLQ produces a Quality of Life (QOL) score reflecting the impact of the child’s asthma on areas of physical and emotional function important to the child.
SECTION 1.2

Impact of Asthma

Paediatric asthma is one of the most common childhood diseases\(^3\). A recent review of asthma prevalence studies by Anandan et al\(^7\) has shown that overall prevalence of paediatric asthma is continuing to increase in the UK\(^7\). Asthma is an increasing burden on our healthcare system and one of the largest burdens on worldwide health care\(^8\). The annual expenditure for asthma in the UK, Germany, and France was approximately €5,500,000,000 in 2006. Almost 48% of this cost is attributed to hospital admission alone\(^9\),\(^10\).

Studies have been performed to identify asthma-susceptibility and asthma-severity genes and also to focus on treatment efficacy in asthma. The BREATHE study, established in Tayside in 2003, is a paediatric pharmacogenetic study created following the completion of the Human Genome Project. The study was created to develop a large population based database with the purpose of identifying specific gene variations which independently, or through environmental interactions, have significant impact on asthma susceptibility, severity or management during childhood. Between, 2006 and 2011 the study has made significant contributions to the field of children’s asthma through the investigation of the role of variations in the genes encoding for the Filaggrin protein (FLG)\(^32,42-44\), the null variation in Glutathione S-transferase M1 & P1\(^45\), the genes encoding for the β2 adrenergic receptor (ADRβ2)\(^33,36,46\), ORMDL3\(^47\), PPAR gamma (PPARG)\(^48\), Matrix metalloproteinase 12 (MMP12)\(^34\), and Chitinase 3-like-1 (CHI3L1 rs4950928)\(^49\) on, asthma phenotype. This line of research has also provided important evidence for significant gene-environment interactions that may contribute to asthma severity in childhood.

The BREATHE study was extended to Sussex in 2008 to explore genetic and clinical variations across the UK. During my period of research I was involved in participant recruitment within Sussex and data analysis of the entire Sussex-Scotland cohort.
A further study, PAGES (the Paediatric Asthma Gene Environment Study)\textsuperscript{50}, was developed across all health care trusts in Scotland and in Brighton, East Sussex. Developed from the BREATHE questionnaire, PAGES focuses on the paediatric environment and its effect on paediatric asthma. The primary outcome measures of PAGES are quantitative measures of asthma severity and QOL. PAGES explores gene-environment interaction to enable better understanding of asthma pathogenesis.

Using data from both studies, the purpose of this thesis is to understand how variations in genes associated with the remodelling process may alter the pathogenesis of asthma to influence the severity of children's asthma. I have studied gene variations which have been associated with asthma and which may interact with each other in a pathway involving the first and second stages of the progressive stages of remodelling as described by Davies \textit{et al.}\textsuperscript{20} (Figure 2.4.1), to influence the severity of asthma. The gene variations which will be discussed are \textit{Chitinase 3-Like-1} (\textit{CHI3L1}) rs4950928\textsuperscript{30,49,51-57} involved in stage 2, \textit{Glutathione S-Transferase μ1} (\textit{GSTM1}) null / ω1 (\textit{GSTP1}) rs1695/ Θ1 (\textit{GSTT1}) null\textsuperscript{58-81}, involved in stage 2, \textit{Matrix metalloproteinase 12} (\textit{MMP12}) rs652438 & rs2276109\textsuperscript{34} involved in stage 2, and \textit{Matrix metalloproteinase 9} (\textit{MMP9}) rs17576 & rs6073983\textsuperscript{82,83} involved in stages 1 and 2.

There are phenotypic differences between asthma in children and young adults\textsuperscript{84,85}. Treatment strategies and the practical issues relating to day-to-day management of asthma also vary between children and young adults\textsuperscript{45}. To factor these phenotypic differences into the analysis, the analysis of asthma severity and lung function was divided by age. Age categorised analysis will also allow us to approximate the effect of the variants on asthma remodelling, based on the assumption that disease onset is within the first five years of life. As the variants tested are all involved in the remodelling process, they were tested for interaction with environmental tobacco smoke and the \textit{Chitinase 3-like-1} variant was tested for interaction with exposure to damp. There is evidence to suggest that a cumulative dose of environmental risk factors, i.e. oxidants from tobacco smoke will lead to comparably increased overall damage over time\textsuperscript{45}. To factor in the effect of time on cumulative dose of environmental exposure the analysis was performed on the sample subdivided by both age and exposure to environmental risk factors.
CHAPTER 2

SECTION 2.1

Chitinase 3-Like-1

2.1.1 Chitin and the Chitinase family

Chitin, $\beta$-(1-4)-poly-N-acetyl-D-glucosamine, is the second most abundant polysaccharide in nature.\textsuperscript{86} The body's immune system is frequently exposed to chitin or chitin oligosaccharides, within the home environment. Chitin can be located on bacteria, viruses, fungi, moulds, parasites, crustaceans and insects. Several groups have attempted to identify common allergens across these organisms\textsuperscript{87-89}. Chitin \textit{per se} is not an allergen, but is a common feature amongst allergenic sources strongly associated with asthma exacerbation, i.e. house dust mites, moulds and cockroaches\textsuperscript{90-93}. Environmental tobacco smoke (ETS) is known to be a trigger of acute asthma exacerbations\textsuperscript{94}. However, tobacco does not contain any form of chitin. In 2008 Seibold \textit{et al}\textsuperscript{95}, reported that tobacco is often contaminated with fungus, resulting in ETS contaminated by chitin polymers. This is potentially another mechanism for ETS induced exacerbations.

It is thought that the chitinase family plays a key role in the identification, destruction and clearance of invading organisms through the identification of chitin and chitin like molecules\textsuperscript{86,96}. Several molecules in the chitinase family have been associated with asthma pathogenesis. Chitinases have been shown to mediate airway inflammation in mouse models of asthma\textsuperscript{95,97}. \textit{Acid mammalian chitinase (AMCase)} and \textit{Macrophage Chitriosidase (CHIT1)} are the only enzymatically active chitinases. \textit{AMCase} is associated with airway hyper-responsiveness, plays a role in Th2 dependant inflammation, and may play a role in monocyte, neutrophil and eosinophil recruitment into the lung\textsuperscript{98}. Tissue from individuals with asthma show increased expression of \textit{AMCase}\textsuperscript{96,99,100}. Increased expression of AMCase has been associated with a decreased responsiveness to
bronchodilators in asthma\textsuperscript{101}. In 2010 Chen Wu \textit{et al}\textsuperscript{89} confirmed that fungal exposure modulates the effect of \textit{CHIT1} SNP’s, increasing the risk of severe asthma exacerbations. \textit{CHIT1} gene expression in BAL fluid is lower in asthmatics, however it is increased up to seven-fold in habitual smokers. It is plausible that this increase in chitinase activity is as a result of chitin from mould spores present within the tobacco\textsuperscript{95}.

\subsection*{2.1.2 Chitinase-3-Like-1}

The gene \textit{CHI3L1} codes for the protein Chitinase 3-Like-1 (CHI3L1), which is expressed in the bronchial sub-mucosa, sub-epithelium, and epithelium and in human smooth muscle cells, alveolar macrophages, neutrophils and other non-respiratory cells\textsuperscript{96,99,100}. \textit{CHI3L1} is 10 exons long and is located on chromosome 1q31-q32. CHI3L1 is a 40-kDa protein.

CHI3L1 has been described as a sentinel chitinase, responsible for picking up and binding to chitin, and triggering an immune mediated response\textsuperscript{101}. CHI3L1 binds with high affinity to chitin and chitin-oligosaccharides. This binding is through a preserved hydrophobic substrate binding cleft\textsuperscript{102}. CHI3L1 is also reported to interact with glycosamines such as heparin and hyaluronan, and to bind to collagen\textsuperscript{96,103}. Chitinase 3-like 1 is only expressed in severe inflammatory conditions and may be correlated to an increase in C-reactive protein\textsuperscript{96}. When expressed it is capable of increasing the rate of mitosis through activation of MAP kinase (mitogen active protein kinase) and PI-3K (phosphoinositide 3-kinase) signalling cascades in fibroblasts, and by working synergistically with insulin like growth factor-1 (Figure 2.1.1). CHI3L1 also plays a role in matrix preservation, through inhibition of cytokine stimulated release of MMP1, MMP3 and MMP13, in CHI3L1 stimulated arterial chondrocytes and skin fibroblasts\textsuperscript{96}. CHI3L1 enhances production of the pro-inflammatory cytokines and chemokines, TNF\textalpha (Tumour necrosis factor alpha), IL-8 (Inter lekin 8), RANTES (regulated upon activation, normal T cell expressed and secreted), eotaxin and increases activity of NF-kB (Nuclear factor kappa-light-chain-enhancer of activated B cells) in the bowel. CHI3L1 also increases bronchial smooth muscle proliferation and migration, an effect that is increased, and positively correlated with CHI3L1 in asthma\textsuperscript{104}. 
Figure 2.1.1 Illustration of molecular interaction initiated by *CHI3L1*
2.1.3 CHI3L1 rs4950928 single nucleotide polymorphism (SNP)

The CHI3L1 rs4950928 SNP is associated with increased serum levels of CHI3L1 and increased affinity for specific binding proteins. Elevation of serum concentrations of CHI3L1 is linked with greater asthma severity, tissue remodelling and pulmonary function. Circulating CHI3L1 concentrations are believed to act as a biomarker for asthma severity, stratified according to treatment stages. The role of CHI3L1 asthma severity in children and young adults, has not been explored.

2.1.4 Chitinase 3-Like-1 hypothesis

**RESEARCH QUESTION:**

Does CHI3L1 (rs4950928) influence the severity of asthma in children and young adults?

**Hypothesis 1-** CHI3L1 (rs4950928) plays a role in the severity of asthma in children and young adults (Section 4.1)

As a sentinel molecule, CHI3L1 binds to its substrate and initiates a severe immune response. Cumulative adhesion of chitin and CHI3L1 substrates in the lung, coupled with inflammation from increased leukocyte infiltration may result in a rapid, severe immune response resulting in asthma exacerbation. It is possible that the CHI3L1 SNP rs4950928, which is expressed in the inflammatory lung, has increased affinity for chitin, thus resulting in higher bronchial epithelial concentrations of chitin. This could increase the exposure of antigen-presenting cells (macrophages and dendritic cells) to chitin, thus mediating a larger TH2 (T-helper 2 cell) and IL-13 (inter leukin 13) mediated immune response associated with asthma severity and airway remodelling.

CHI3L1 is a susceptibility gene for asthma. Increased quantities of CHI3L1 in the serum and the lungs have been associated with asthma severity in an adult sample and can be used as a biomarker for asthma and decline in lung function. Increased quantities of serum CHI3L1 has been observed in Chinese patients with asthma, and the level has been correlated with asthma exacerbations. CHI3L1 SNP’s were investigated through a
genome wide association study of serum CHI3L1 levels, known to be elevated in patients with severe asthma\textsuperscript{107}.

Using the asthma severity score and exacerbation score described in Section 3.7 the BREATHE cohort was investigated to determine if the CHI3L1 rs4950928 variant plays a role in asthma severity. The sample was subsequently categorised by age, and the analysis repeated to account for and identify if the rs4950928 variant was affected by variation in asthma phenotype between children and young adults.

**Hypothesis 2- CHI3L1 (rs4950928) effects the severity of asthma in children and young adults through influencing airway remodelling and leukocyte infiltration into the lung (Section 4.2)**

Through NF-\(\kappa\)B pathway up-regulation, CHI3L1 increases leukocyte migration (Figure 2.1.1)\textsuperscript{51}. The rs4950928 polymorphism may up-regulate the efficiency of this pathway, which would result in greater quantities of leukocyte migration. As a by-product of this effect TNF-\(\alpha\) secretion would be increased. This could possibly lead to significant levels of airway smooth muscle hypertrophy\textsuperscript{108}. An increase in CHI3L1 levels has been associated with a decline in lung function in adults\textsuperscript{30}.

There is previous evidence of a decline in lung function in adults with increased CHI3L1, to determine if this effect was present in children and young adults from the merged BREATHE and PAGES cohorts, the rs4950928 variant was tested for an association with PEFR, FEV1, FVC, FEV1/FVC and bronchodilator reversibility of FEV1.

To investigate the influence of the rs4950928 variant on remodelling, the rs4950928 variant was tested for association with PEFR, FEV1, FVC, FEV1/FVC as proxy markers of obstruction, bronchodilator reversibility of FEV1 as a surrogate for smooth muscle hyperplasia and exhaled nitric oxide as a surrogate for eosinophil infiltration.

In order to investigate the influence of the rs4950928 variant on phenotypic difference between asthma in children and young adults, and on remodelling over time, and based on the assumption that asthma symptoms began in the first five years of life, the overall structural change, categorised by age (described in 3.7) was measured by proxy. PEFR, FEV1, FVC, FEV1/FVC were used as proxy markers of obstruction, bronchodilator
reversibility of FEV1 as a surrogate for smooth muscle hyperplasia and exhaled nitric oxide as a surrogate for eosinophil infiltration.

**Hypothesis 3- CHI3L1 (rs4950928) interacts with environmental tobacco smoke (ETS) exposure and exposure to damp to influence the severity of asthma (Section 4.3).**

Environmental tobacco smoke and damp are risk factors for asthma severity\textsuperscript{60,90,94,109-114}. Mould from household damp and fungi growing on tobacco plants contain chitin\textsuperscript{95} and the exposure to these environmental risk factors may increase airway sensitivity to chitin. This could lead to an increased risk of exacerbations of asthma in individuals with the rs4950928 variant. Exhaled nitric oxide is increased in the airways of adult smokers with asthma, and is a marker for atopic asthma.\textsuperscript{111} Environmental tobacco smoke increases eotaxin production, changing the inflammatory phenotype of the asthmatic lung and further increasing eosinophil and neutrophil infiltration\textsuperscript{115}. Young adults who smoke or are exposed to smoke have a higher risk of current asthma\textsuperscript{116,117}

This is the first study to investigate the impact of interactions between the rs4950928 variant, environmental tobacco smoke exposure and damp exposure on asthma severity. Environmental tobacco smoke increases CHIT1 activity and expression and is a risk factor for asthma exacerbations, asthma severity and pulmonary function. There is some evidence to suggest the presence of fungi in environmental tobacco smoke, this may interact with CHI3L1 to influence asthma severity. Damp is also a risk factor for asthma severity. CHI3L1 may be crucial to the relationship between asthma severity and damp exposure. Using the asthma severity score and exacerbation score described in Section 3.7 the BREATHE cohort was investigated to determine if the rs4950928 variant interacts with environmental tobacco smoke to influence asthma severity. The PAGES cohort was investigated to determine if the rs4950928 variant interacts with damp exposure to influence asthma severity. There is evidence of variation in asthma phenotypes between children and young adults. There is also evidence that the risk of asthma is elevated in young adults exposed to tobacco smoke. This could be due to a cumulative accumulation of environmental risk factors over time. The sample was subsequently categorised by age, and the analysis repeated.
There is evidence to suggest that a cumulative dose of environmental risk factors, i.e. oxidants from tobacco smoke will lead to comparably increased overall damage over time\textsuperscript{45}. To determine the effect of environmental risk factors on remodelling the rs4950928 variant was tested for an association with PEFR, FEV1, FVC, FEV1/FVC and bronchodilator reversibility of FEV1. The rs4950928 variant may increase inflammation in the lung through signalling mechanisms. Exposure to environmental risk factors may increase activity of CHI3L1 and increase leukocyte infiltration. Environmental tobacco smoke increases eotaxin production. CHI3L1 rs4950928 may also increase eotaxin production (Figure 2.1.1). It is plausible to suggest that the rs4950928 variant may interact with exposure to environmental tobacco smoke to increase leukocyte infiltration into the lung. To test the plausibility of this hypothesis the rs4950928 variant was tested for an interaction with environmental tobacco smoke and with damp. To factor in phenotypic variation and cumulative accumulation of risk factors the sample was subsequently categorised by age.

**Hypothesis 4- The genetic variant rs4950928 is associated with sensitivity and/or allergy to chitin containing allergens (Section 4.3)**

It is possible that the \textit{CHI3L1} SNP rs4950928, which is expressed in the inflammatory lung, increases the affinity of CHI3L1 for chitin, thus resulting in higher bronchial epithelial concentrations of chitin. This could increase the exposure of antigen-presenting cells (APCs) (macrophages and dendritic cells) to chitin, thus mediating a larger TH2 (T-helper 2 cell) and IL-13 (Inter leukin 13) mediated immune response associated with asthma severity and airway remodelling.

Rathcke \textit{et al.} \textsuperscript{23} investigated the \textit{CHI3L1} polymorphisms in a sample of 6514 Danish adults. Of this sample 540 had asthma, 300 of whom were defined as atopic asthma. In contrast to other studies, these researchers observed an association between the minor G allele, atopic asthma and self-reported physician diagnosed asthma.

To determine if \textit{CHI3L1} rs4950928 increases antigen exposure to APCs, therefore leading to increased allergen sensitisation, the frequency of participant reported house dust mite allergy and skin prick test ascertained sensitivity to \textit{Aspergillus fumigatus} and \textit{Alternaria alternans} was compared with the rs4950928 variant.
Hypothesis 5- *CHI3L1* (rs4950928) influences the quality of life of children and young adults with asthma (Section 4.3)

Asthma exacerbations, increased asthma severity and reduced lung function have been shown to have a detrimental impact on quality of life\(^{38,118}\).

If the rs4950928 variant plays a role on asthma severity, it is plausible that there will be an impact on quality of life. To explore the validity of this risk factor the presence of rs4950928 variant was compared with mean quality of life scores derived from the Paediatric Asthma Quality of Life Questionnaire.
Figure 2.1.2 Illustration of chromosomal location of rs4950928 modified from genecards.org\textsuperscript{119} and NCBI dbSNP\textsuperscript{120}
SECTION 2.2.

Matrix Metalloproteinases

2.2.1 The Matrix metalloproteinase family

The matrix metalloproteinase (MMP) family is composed of 25 zinc containing enzymes with distinct structures, but related function\(^{22}\). The MMP genes have been mapped to chromosomes 10, 11, 14, 16, and 22\(^{121}\). Members of the MMP family cause the degradation of the extra cellular matrix (ECM). Each MMP is principally responsible for degradation of particular ECM components\(^{22}\). The MMP family is also responsible for the migration of leukocytes into and out of the lung. In the absence of the MMP family, lethal asphyxiation would occur due to an inability to clear allergic inflammatory cells\(^{122}\).
Figure 2.2.1. Illustration of MMP12 and MMP9 normal immediate response pathway
2.2.2 Matrix Metalloproteinase 9 (MMP9)

*MMP9* (gelatinase B) encodes for MMP9, a 92KDa protease. The primary substrate of MMP9 is denatured collagen (gelatin)\(^1\)\(^2\). MMP9 is synthesised under inflammatory conditions by neutrophils, eosinophils, alveolar macrophages and epithelial cells. MMP9 is the dominant MMP in the lungs and its expression correlates with eosinophil count in the bronchial mucosa\(^1\)\(^2\). The primary role of MMP9 is to control the trafficking of inflammatory cells\(^1\)\(^5\). MMP9 acts downstream of Th2 cytokine signalling and can control T cell response to allergens.

MMP9 targets tight junctions increasing transepithelial conductance and leading to anoikis\(^5\)\(^1\). Anoikis is apoptosis induced through cell detachment. Following detachment, the surrounding epithelial cells have been observed to stretch and flatten to cover the area vacated by the detached cells\(^1\)\(^2\). It is plausible, based on the hypothesis of Davies et al\(^2\)\(^0\), that MMP9 plays a role in sensitisation, through barrier loss and in remodelling, through the formation of a temporary epithelial barrier\(^2\)\(^0\). Sputum concentrations of MMP9 directly relate to airway inflammation and are simultaneously inversely associated with airway thickening\(^1\)\(^2\)\(^5\).

MMP9 is increased in the airways of asthmatic patients\(^1\)\(^2\)\(^6\) and is increased during asthma exacerbation\(^1\)\(^2\)\(^7\). Two *MMP9* variants (G2127T and G5546A) were associated with a risk of developing atopic asthma in a cohort of Japanese children and young adults with atopic asthma\(^1\)\(^2\)\(^8\). One *MMP9* variant, the C1562T SNP has been investigated and was found to be in complete linkage disequilibrium with G2127T. This functional promoter polymorphism decreases protein-DNA interaction leading to increased protease activity. The T allele results in 3.5 times greater MMP9 activity as reported by luciferase reporter assay\(^1\)\(^5\), which is likely to result in increased remodelling.
2.2.3 MMP9 rs17576 and rs6073983 single nucleotide polymorphisms (SNP) (Figure 2.2.2)

In a sample of German children recruited to the ISAAC study the rs2664538 (recoded to rs17576) variant has been associated with increased risk of non-atopic wheeze and non-atopic asthma\(^8\). The rs3918241 (in linkage disequilibrium with rs6073983) variant was associated with decreased expiratory flow in non-atopic children with asthma\(^8\). Both the rs17576 and rs3918241 variants are associated with decreased MMP9 expression and activity.

The roles of the rs17576 and rs6073983 MMP9 SNPs on childhood asthma severity and its interactions with environmental triggers, has not been explored.

2.2.4 Matrix Metalloproteinase 9 hypothesis

MMP9 plays a role in anoikosis and macrophage differentiation. Through the loss of the epithelial barrier it is plausible there is increased allergen exposure to antigen-presenting cells (APCs) (macrophages and dendritic cells) to allergens, thus mediating a larger TH2 (T-helper 2 cell) and IL-13 (Inter leukin 13) mediated immune response leading to greater asthma severity and increased airway remodelling.

**RESEARCH QUESTION:**

Is there an association between the presence of MMP9 gene variants, rs17576 and rs6073983, and severity of asthma in children and young adults?

**Hypothesis 1- Genetic variants of MMP9 influence the severity of asthma in children and young adults (Section 5.1)**

An increased expression of MMP9 has been detected in the lungs of individuals with asthma during periods of exacerbation. Both variants may decrease MMP9 expression and activity. Through the targeting of tight junctions, MMP9 leads to anoikis. It is plausible that variations in MMP9 play a role in allergen sensitisation through barrier loss, leading to increased leukocyte activity. Further through aiding leukocyte migration, variation in MMP9 could play a role in the development of the hyper-responsive lung leading to increased cell damage and cell death. Remodelling may occur through repeated formation of a temporary epithelial barrier\(^4\), ultimately increasing the severity
of asthma in children and young adults. The rs17576 variant has been associated with increased risk of non-atopic wheeze and non-atopic asthma.\textsuperscript{82}

Both variants will be investigated in the BREATHE cohort to determine if either variant plays a role in asthma severity as measured by the asthma severity score and exacerbation score described in Section 3.7. To determine the effect of age, both variants were subsequently subcategorised and the analysis repeated.

**Hypothesis 2- Genetic variants of MMP9 influence pathological remodelling via structural changes in the lung (Section 5.2)**

The primary role of MMP9 is to control the trafficking of inflammatory cells\textsuperscript{15}. MMP9 acts downstream of Th2 cytokine signalling and can control T cell response to allergens. Through the targeting of tight junctions, MMP9 leads to anoikis. It is plausible that variations in \textit{MMP9} also play a role in allergen sensitisation through barrier loss and alteration of T cell signalling, which could lead to increased leukocyte activity. Further, through aiding leukocyte migration, variation in \textit{MMP9} could play a role in the development of the hyper-responsive lung leading to increased cell damage and cell death. Thus, leading to remodelling through repeated formation of a temporary epithelial barrier\textsuperscript{4}, increasing the severity of asthma in children and young adults.

There is previous evidence of a decline in lung function in the form of decreased expiratory flow with the rs3918241 (in linkage disequilibrium with the rs6073983 variant)\textsuperscript{82}. To determine if this effect was present with either variant in children and young adults from the merged BREATHE and PAGES cohorts, both variants were tested for an association with PEFR, FEV1, FVC, FEV1/FVC and bronchodilator reversibility of FEV1. To investigate the influence of either variant on remodelling, both variants were tested for association with PEFR, FEV1, FVC, FEV1/FVC as proxy markers of obstruction, bronchodilator reversibility of FEV1 as a surrogate for smooth muscle hyperplasia and exhaled nitric oxide as a surrogate for eosinophil infiltration.

The sample was subsequently categorised by age(Section 3.7) in order to investigate the influence of either variant on phenotypic difference between asthma in children and young adults and on airway remodelling over time. The analysis was based on the
assumption that asthma symptoms began in the first five years of life, and was measured by proxy through the measurement of PEFR, FEV1, FVC, FEV1/FVC, bronchodilator reversibility of FEV1 and exhaled nitric oxide.

**Hypothesis 3- Environmental tobacco smoke (ETS) interacts with genetic variants of MMP9 to influence the severity of asthma (Section 5.3).**

Environmental tobacco smoke is a risk factor for asthma severity.\(^{60,90,94,109-114}\) Exhaled nitric oxide is increased in the airways of adult smokers with asthma, and is a marker for atopic asthma.\(^{111}\) Environmental tobacco smoke increases eotaxin production, changing the inflammatory phenotype of the asthmatic lung and further increasing eosinophil and neutrophil infiltration.\(^{115}\) Environmental oxidants cause cellular damage. MMP9 facilitates leukocyte infiltration and is related to remodelling in response to pulmonary damage. MMP9 activity is increased by tobacco smoke.\(^{129}\) Increased activity of MMP9 may have an anti-fibrotic effect increasing the permeability of the ECM.\(^{130}\) Young adults who smoke or are exposed to smoke have a higher risk of current asthma.\(^{116,117}\)

This is the first study to investigate the impact of interactions between the MMP9 variants and environmental tobacco smoke exposure on asthma severity. Using the asthma severity score and exacerbation score described in Section 3.7 the BREATHE cohort was investigated to determine if either variant interacts with environmental tobacco smoke to influence asthma severity. There is evidence of variation in asthma phenotypes between children and young adults. There is also evidence that the risk of asthma is elevated in young adults exposed to tobacco smoke. This could be due to a cumulative accumulation of environmental risk factors over time. The sample was subsequently categorised by age, and the analysis repeated.

A cumulative dose of environmental risk factors, i.e. oxidants from tobacco smoke may lead to comparably increased overall damage over time.\(^{45}\) To determine the effect of environmental risk factors on asthma severity, as measured through airway remodelling in the form of basement membrane thickening and smooth muscle hyperplasia, both variants were tested for an association with PEFR, FEV1, FVC, FEV1/FVC and bronchodilator reversibility of FEV1.
MMP9 variants may influence inflammation in the lung and exposure to environmental tobacco smoke may interact with either variant to influence leukocyte infiltration. To test the plausibility of this hypothesis both variants were tested for an interaction with environmental tobacco smoke. To factor in phenotypic variation and cumulative accumulation of risk factors the sample was subsequently categorised by age.

Hypothesis 4 - Genetic variations of MMP9 are associated with allergy and atopic sensitivity in children and young adults with asthma (Section 5.3)

MMP9 controls the trafficking of inflammatory cells and is synthesised by neutrophils, eosinophils, alveolar macrophages and epithelial cells. MMP9 controls T cell response to allergens and its levels are directly correlated with eosinophil concentrations. MMP9 plays a role in anoikosis and macrophage differentiation. It is plausible that variations in MMP9 also play a role in allergen sensitisation through barrier loss and alteration of T cell signalling, which could lead to increased leukocyte activity. Through the loss of the epithelial barrier it is plausible there is increased allergen exposure to antigen-presenting cells (APCs) (macrophages and dendritic cells) to allergens, thus mediating a larger TH2 (T-helper 2 cell) and IL-13 (inter leukin 13) mediated immune response leading to greater asthma severity and increased airway remodelling.

Two MMP9 variants (G2127T and G5546A) were associated with a risk of developing atopic asthma in a cohort of Japanese children and young adults with atopic asthma. The rs17576 variant has been associated with increased risk of non-atopic wheeze and non-atopic asthma.

To determine if genetic variation in MMP9 increases exposure to APCs, therefore leading to increased antigen exposure, the frequency of participant reported allergy and the frequency of positive skin prick test sensitivity was compared with both variants (Section 3.7).
Hypothesis 5- Genetic variation of \textit{MMP9} influence the quality of life of children and young adults with asthma (Section 5.3)

If either variant influences asthma severity, then there may be an impact on quality of life. As such both variants were investigated to determine if there influence quality of life as derived from the Paediatric Asthma Quality of Life Questionnaire.
Figure 2.2.2 Illustration of chromosomal location of rs17576 and rs6073983 modified from genecards.org\textsuperscript{131} and NCBI dbSNP\textsuperscript{132,133}.
2.2.5 Matrix Metalloproteinase 12 (MMP12)

*MMP12* (macrophage elastase) is translated as a 1.8Kb transcript which encodes for a 470 amino acid pro-enzyme. The pro-enzyme is comprised of a 9KDa amino terminal propeptide domain, a 12KDa catalytic domain, bearing the zinc based binding site and a 23KDa Haemopoexin-like carboxy terminal. The active MMP12 enzyme consists of interconnected domains, these are a protease domain and an ancillary domain, connected by a flexible proline rich hinge peptide.

The primary substrate of MMP12 is elastin. However, it also degrades, although to a lesser extent; fibronectin, fibrilin-1, laminin, entactin, type IV collagen fragments, chondrin sulphate, heparin sulphate, proteoglycans, vibronectin, myelin basic protein, α1-antitrypsin, tissue factor pathway inhibitor, plasminogen and N-cadherin. These are constituents and inter cellular binding molecules within the ECM. MMP12 can activate MMP2 and MMP3, which in-turn activate MMP 1 and MMP9, leading to the degradation of other ECM constituents (Collagen I, Collagen III, Collagen IV, Collagen V and gelatin).

MMP12 is associated with macrophage infiltration in inflammatory diseases. MMP12 is synthesised under inflammatory conditions, through macrophage activation, but also from epithelial cells, smooth muscle cells and endothelial cells in the alveolar walls. Upon activation MMP12, stored in cytoplasmic vesicles, is secreted and has a continuing effect for up to 24 hours following insult (Figure 2.2.1). Human macrophage MMP12 levels can be increased by IL-1β (Inter leukin-1β) and TNF-α. Following allergen exposure MMP12 is implemental in IL-13 induced allergic inflammation, controlling infiltration of eosinophils and macrophages to the airways.

MMP12 has been associated with macrophage dependant inflammatory diseases, including asthma and reduced lung function as a result of smoking related lung injury.
2.2.6 MMP12 rs652438 and rs2276109 single nucleotide polymorphisms (SNP) (Figure 2.2.3)

Members of a collaborative group have recently associated the rs652438 variation with exacerbation risk in a cohort of Scottish children\textsuperscript{34}. However, the recruitment of the extended BREATHE cohort together with PAGES offers a unique opportunity to confirm the earlier findings in a larger cohort. It also offers the opportunity to explore other related gene-clinical associations. The rs2276109 variant is a transition substitution SNP which occurs in the coding region. The G allele has been positively associated with FEV1 in children with asthma\textsuperscript{142}. This finding has not been validated in any other study\textsuperscript{34}.

2.2.7 Matrix Metalloproteinase 12 hypothesis

MMP12 is synthesised under inflammatory conditions, through macrophage activation, but also from epithelial cells, smooth muscle cells and endothelial cells in the alveolar walls. MMP12 plays a role in matrix turnover through the inhibition of elastin breakdown.

The rs652438 variant is a missense transition substitution SNP which occurs in the promoter region, affecting the haemopexin domain of \textit{MMP12}. The SNP alters expression and substrate binding through alteration of the flexibility of the MMP12 binding site. The A allele is approximately 3 times as active as the G allele. As such, the A allele increases the rate and quantity of macrophage migration into the lung in comparison to the G allele\textsuperscript{143}. Reduced activity of MMP12 will reduce MMP12 mediated expression of MMP1, MMP2 and as such MMP1 and MMP9 (Figure 2.2.1)\textsuperscript{137,138}. A reduction in MMP concentrations within the lung may reduce the breakdown and turnover of the ECM, leading to gradual airway thickening and remodelling, increasing the severity of asthma in children and young adults. The A allele of the rs2276109 variant is associated with greater binding activity for activator protein 1, resulting in higher MMP12 promoter activity. The increase in activity may increase matrix turnover to compensate for increased airway thickening and remodelling in the asthmatic airway.
**RESEARCH QUESTION:**

Is there an association between *MMP12* gene variants rs2276109 and rs652438 and severity of asthma in children and young adults?

**Hypothesis 1 - Genetic variants of *MMP12* influence the severity of asthma in children and young adults (Section 6.1)**

*MMP12* is synthesised under inflammatory conditions, through macrophage activation, but also from epithelial cells, smooth muscle cells and endothelial cells in the alveolar walls. *MMP12* plays a role in matrix turnover through the inhibition of elastin breakdown. Which subsequently results in remodelling, through repeated thickening of the epithelial barrier, ultimately increasing the severity of asthma in children and young adults. Variations in *MMP12* have been associated with asthma exacerbations in Scottish children.

Using the asthma severity score and exacerbation score described in Section 3.7 the BREATHE cohort was investigated to determine if *MMP12* is a risk factor in asthma severity.

**Hypothesis 2 - Genetic variants of *MMP12* effect the severity of asthma in children and young adults through influencing airway remodelling and leukocyte infiltration into the lung (Section 6.2)**

*MMP12* may promote fibrosis through inhibition of downstream MMP’s (Figure 2.4.1). The rs652438 variant reduces MMP12 activity. Reduced activity of MMP12 will reduce MMP12 mediated expression of MMP1, MMP2 and as such MMP1 and MMP9. A reduction in MMP concentrations within the lung may reduce the breakdown and turnover of the ECM, leading to gradual airway thickening and remodelling. The effect of the rs2276109 variant on MMP12 expression has not been explored. The G allele has been positively associated with FEV1 in children with asthma. It is plausible to suggest that the rs2276109 variant alters the expression of MMP12, perhaps increasing expression and therefore heightening matrix turnover to compensate for increased airway thickening and remodelling in the asthmatic airway.
Mukhopadhyay et al\textsuperscript{34} associated the G allele and reduced FEV1 in COPD, however did not investigate the effect of either variant on measures of lung function in asthma due to the normality of lung function measures in the paediatric UK population as measured from the BREATHE cohort. We have now increased the population size of the lung function cohort and may be able to discern differences between lung function samples. We have also investigated the effect of bronchodilator reversibility of FEV1 and exhaled nitric oxide. To determine if this effect was present with either variant in children and young adults from the merged BREATHE and PAGES cohorts, both variants were tested for an association with PEFR, FEV1, FVC, FEV1/FVC and bronchodilator reversibility of FEV1. To determine the influence of either variant on remodelling, both variants were tested for association with PEFR, FEV1, FVC, FEV1/FVC as proxy markers of obstruction, bronchodilator reversibility of FEV1 as a surrogate for smooth muscle hyperplasia and exhaled nitric oxide as a surrogate for eosinophil infiltration.

The rate of remodelling cannot be directly assessed in this sample. To investigate the influence of either variant on phenotypic difference between asthma in children and young adults and on remodelling over time, based on the assumption that asthma symptoms began in the first five years of life, the overall structural change, categorised by age (described in 3.7), was measured by proxy through the measurement of PEFR, FEV1, FVC, FEV1/FVC, bronchodilator reversibility of FEV1 and exhaled nitric oxide.

**Hypothesis 3- Environmental tobacco smoke (ETS) interacts with genetic variants of MMP12 to influence the severity of asthma (Section 6.3)**

MMP12 has been implicated in tobacco smoke induced inflammation in COPD\textsuperscript{145}. MMP12 has been associated with macrophage dependant inflammatory diseases, including asthma\textsuperscript{22} and reduced lung function as a result of smoking related lung injury\textsuperscript{121}. Environmental tobacco smoke is a risk factor for asthma severity\textsuperscript{60,90,94,109-114} and causes damage to the lung through exposure to free radical oxidants, leading to inflammation and remodelling in the lung. MMP12 is synthesised under inflammatory conditions, through macrophage activation. Macrophage activity and expression is increased by environmental tobacco smoke\textsuperscript{146}. MMP12 plays a role in matrix turnover through the inhibition of elastin break down. Interaction with environmental tobacco smoke may
result in increased severity through IL-13 mediated airway inflammation and remodelling. Young adults who smoke or are exposed to smoke have a higher risk of current asthma\textsuperscript{116,117}. Both variants are known to have an effect on lung function.

The BREATHE cohort was investigated to determine if either variant interacts with environmental tobacco smoke to influence asthma severity as measured by the asthma severity score and exacerbations core (Section 3.7). To investigate the hypothesis of accumulation of environmental risk factors over time, the sample was subsequently categorised by age, and the analysis repeated.

The variants were also investigated for an association with PEFR, FEV1, FVC, FEV1/FVC and bronchodilator reversibility of FEV1To determine the effect of environmental risk factors on remodelling . MMP9 variants may influence inflammation in the lung and exposure to environmental tobacco smoke may interact with either variant to influence leukocyte infiltration. To test the plausibility of this hypothesis both variants were tested for an interaction with environmental tobacco smoke. To factor in phenotypic variation and cumulative accumulation of risk factors the sample was subsequently categorised by age.

**Hypothesis 4- Genomic variants of MMP12 play a role in the development of allergy and atopic sensitivity in children and young adults with asthma (Section 6.3)**

Following allergen exposure, MMP12 is implemental in IL-13 induced allergic inflammation, controlling infiltration of eosinophils and macrophages to the airways\textsuperscript{34}. MMP12 is released from macrophages following insult and increases MMP9 concentrations. MMP9 controls T cell response to allergens and its levels are directly correlated with eosinophil concentrations. MMP9 plays a role in anoikosis and macrophage differentiation. The increase in active macrophages may also lead to increased macrophage exposure to allergens, thus mediating a larger TH2 and IL-13 mediated immune response. To determine if genetic variants of MMP9 influence allergic sensitisation, the frequency of participant reported allergy and the frequency of positive skin prick test sensitivity was compared with both variants (Section 3.7).
Hypothesis 5- Genetic variants of \textit{MMP12} influence the quality of life of children and young adults with asthma (Section 6.3)

To explore the role either variant may have on quality of life both variants were compared with mean quality of life scores derived from the Paediatric Asthma Quality of Life Questionnaire.
Figure 2.2.3 Illustration of chromosomal location of rs2276109 and rs652438 modified from Genecards.org\textsuperscript{147} and NCBI dbSNP\textsuperscript{148,149}.
SECTION 2.3

Glutathione-S-Transferases

2.3.1 The GST family

The *Glutathione S-transferase (GST)* gene family codes for the GST enzymes, a group of phase II detoxification enzymes. The GST enzymes are a fundamental component of the cell damage defence system\(^6^0\). GSTs are expressed in the bronchial wall\(^6^0\). GSTs function as an antioxidant defence through reactive oxygen species metabolism. They repair damaged reactive oxygen species and play a role in detoxification of xenobiotics, for instance tobacco smoke carcinogens\(^6^7\).

Detoxification occurs through the secretion of Glutathione (GSH). GSH is a tripeptide thiol that is induced by GSTs. GSH functions to reduce the organic peroxides, protecting the airway from lipid peroxidation. GSH homeostasis is altered in children with severe asthma\(^6^2\).

GSTs induce GSH to rapidly eliminate reactive oxidation species, therefore protecting from tissue damage\(^7^6\). The loss of homeostatic balance between oxidative forces and antioxidant defence is believed to be the cause of forced oxidative injury, contributing to the severity of asthma. The increase in reactive oxidative species in asthma is reflected in the increase in activity of eosinophils and macrophages in peripheral blood\(^1^5^0\).

2.3.2 Glutathione-S-Transferase μ1 (GSTM-1), ω1 (GSTP-1 rs1695), Θ1 (GSTT-1) and Asthma

*GST-mu 1* spans a 6 Kb region, localised at Ch1p13\(^6^0\). *GSTM-1* is expressed in the lungs and has been implicated in the pathogenesis of asthma\(^6^8\) (Figure 2.3.1). The *GSTM-1* homozygous deletion or null variant is associated with a reduction in enzymatic activity, increased susceptibility to respiratory disease and poor lung function in children\(^6^0\). Children who possess the *GSTM-1* null variant and have been exposed to ETS *in utero* have an increased risk of early onset asthma, asthma with current symptoms, persistent
asthma, lifetime wheezing, wheezing with exercise, wheezing requiring medication and hospital admissions\cite{45,68,76,150}.

GST-pi 1 spans a 3 Kb region, localised at Ch11q13. GSTP1 is expressed in the lungs and has been implicated in the pathogenesis of asthma\cite{68} (Figure 2.5.1). The GSTP1 rs1695 variant alters electrophilic binding specificity, reducing the efficiency of enzyme activity\cite{151,152}. GSTP1 represents 90% of GST activity in the airway\cite{153}. The rs1695 variant is a missense transition substitution. The rs1695 variant is associated with asthma susceptibility and increased asthma severity in 13-21 year olds exposed to environmental tobacco smoke. The rs1695 variant is also associated with FVC and FEV1 deficits in children with asthma\cite{64}.

GST-theta 1 spans a 44 Kb region, localised at Ch1p13. GSTT-1 is expressed in the lungs and has been implicated in the pathogenesis of asthma\cite{58} (Figure 2.3.1). The GSTT-1 homozygous deletion or null variant is associated with a reduction in enzymatic activity, increased susceptibility to respiratory disease and poor lung function in children. Children who possess the GSTT-1 null variant and have been exposed to ETS \textit{in utero} have an increased risk of significant decrements in lung function\cite{154}.

Preliminary studies by our group were undertaken on the first 504 recruits from a Scottish cohort\cite{45}. My analysis for this thesis will aim to confirm the pilot data in the extended dataset of 867 BREATHE recruits and the extended lung function data set of 690 BREATHE and PAGES recruits. The signal from our preliminary analysis underlines the need for this definitive analysis to confirm or refute the extent of the influence of the GSTM-1, GSTP-1 AND GSTT-1 polymorphisms on clinical phenotype in children with asthma.

2.3.3 GST mutant variant hypothesis

For the purpose of this investigation the three mutant variants of GSTM-1 null, GSTP-1 rs1695 and GSTT-1 null were combined. The mutant variant consists of one or more mutant variant of GSTM-1 null, GSTP-1 rs1695 and GSTT-1 null, representing a reduction in enzymatic activity. GSH homeostasis is altered in children with severe asthma\cite{55}. The loss of homeostatic balance between oxidative forces and antioxidant defence is believed to be the cause of forced oxidative injury, attributing to the severity of asthma through
remodelling and inflammation as reflected by the increase in activity of eosinophils and macrophages in peripheral blood\textsuperscript{57}.

**RESEARCH QUESTION:**

Do GST mutant variants influence the severity of asthma in children and young adults?

**Hypothesis 1** - GST mutant variants increase the severity of asthma in children and young adults (Section 7.1)

GSTM1, GSTP1 and GSTT1 are fundamental components of the cell damage defence system. GSTs induce GSH to rapidly eliminate reactive oxidation species, therefore protecting from tissue damage. The loss of homeostatic balance between oxidative forces and antioxidant defence is believed to be the cause of forced oxidative injury, attributing to the severity of asthma.

GSTM\textsuperscript{-1} and \textit{GSTT1} null are associated with increased susceptibility to respiratory disease\textsuperscript{60} and in children exposed to ETS with asthma severity and reduced lung function\textsuperscript{45,68,76,150,154}. The rs1695 variant is associated with asthma severity in 13-21 year olds exposed to environmental tobacco smoke. Using the asthma severity score and exacerbation score described in Section 3.7 the BREATHE cohort was investigated to determine if the combined GST mutant variants play a role in asthma severity. There is previous evidence of phenotypic variance between age groups with the GST gene family. The sample was subsequently categorised by age, and the analysis repeated to account for and identify if the combined mutant variants effect on asthma severity was affected by variation in asthma phenotype between children and young adults.

**Hypothesis 2** GST mutant variants increase the severity of asthma in children and young adults through airway remodelling and increased leukocyte infiltration into the lung (Section 7.2)

The GST mutant variants are associated with a reduction in enzymatic activity, increased susceptibility to respiratory disease and poor lung function in children. The loss of homeostatic balance between oxidative forces and antioxidant defence is believed to be the cause of forced oxidative injury, attributing to the severity of asthma. The increase in
reactive oxidative species in asthma is reflected in the increase in activity of eosinophils and macrophages in peripheral blood.

**GSTM-1 null** is associated with poor lung function in children\(^6^0\). The rs1695 variant is associated with FVC and FEV1 deficits in children with asthma\(^6^4\). Children who possess the **GSTT-1 null** variant and have been exposed to ETS *in utero* have an increased risk of significant decrements in lung function\(^1^5^4\). To the best of this author’s knowledge none of the GST variants have been investigated for an effect on bronchodilator reversibility of FEV1 or exhaled nitric oxide in a sample of children and young adults with asthma. To determine if this effect was present with the combined mutant variant in children and young adults from the larger merged sample from the BREATHE and PAGES cohorts, the mutant variants was tested for an association with PEFR, FEV1, FVC, FEV1/FVC, bronchodilator reversibility of FEV1 and mean exhaled nitric oxide.

To investigate the influence of the combined mutant variant on phenotypic difference between asthma in children and young adults, based on the assumption that asthma symptoms began in the first five years of life, the variants were investigated with associations with PEFR, FEV1, FVC, FEV1/FVC, bronchodilator reversibility of FEV1 and exhaled nitric oxide categorised by age.

**Hypothesis 3- GST mutant variants interact with environmental tobacco smoke (ETS) exposure to increase the severity of asthma (Section 7.3)**

Environmental tobacco smoke is a risk factor for asthma severity\(^6^0,9^0,9^4,1^0^9-1^1^4\). The GST enzymes are a fundamental component of the cell damage defence system\(^6^0\). GSTs function as an antioxidant defence through reactive oxygen species metabolism. They repair damaged reactive oxygen species and play a role in detoxification of xenobiotics, for instance tobacco smoke carcinogens\(^6^7\). The absence of a functioning cell damage defence system with the GST mutant variant may lead increased cell damage, and as such inflammation and remodelling. Exhaled nitric oxide is increased in the airways of adult smokers with asthma, and is a marker for atopic asthma.\(^1^1^1\) Children who possess the GST mutant variants and have been exposed to ETS *in utero* have an increased risk of early onset asthma, asthma with current symptoms, persistent asthma, life time wheezing, wheezing with exercise, wheezing requiring medication and hospital admissions. Using
the asthma severity score and exacerbation score described in Section 3.7 the BREATHE cohort was investigated to determine if the mutant variant interacts with environmental tobacco smoke to influence asthma severity. There is variation in GST asthma phenotypes between children and young adults. There is also evidence that the risk of asthma is elevated in young adults exposed to tobacco smoke. This could be due to a cumulative accumulation of environmental risk factors over time. To investigate this hypothesis, the sample was subsequently categorised by age, and the analysis repeated.

A cumulative dose of environmental risk factors, i.e. oxidants from tobacco smoke may lead to comparably increased overall damage over time. To determine the effect of environmental tobacco smoke on pulmonary function as a proxy of remodelling the mutant variant was tested for an association with PEFR, FEV1, FVC, FEV1/FVC and bronchodilator reversibility of FEV1. To factor in phenotypic variation and cumulative accumulation of risk factors the sample was subsequently categorised by age.

**Hypothesis 4-** GST mutant variants infer allergy and atopic sensitivity in children and young adults with asthma (Section 6.3)

GSTM1 null, GSTP1 rs1695 and GSTT1 null have been associated with risk of atopic asthma. GSTT1 null has been significantly associated with atopy. The loss of homeostatic balance between oxidative forces and antioxidant defence is believed to be the cause of forced oxidative injury, leading to increased circulating eosinophil and macrophage concentrations. This could plausibly lead to increased antigen sensitisation, resulting in the development of atopy.

To determine if genetic GST mutant variants increase exposure to APCs, therefore leading to increased antigen exposure, the frequency of participant reported allergy and the frequency of positive skin prick test sensitivity was compared with both variants (Section 7.3).
Hypothesis 5- *GST* mutant variants impair the quality of life of children and young adults with asthma (Section 7.3)

If the mutant variant influences asthma severity, it is plausible that there will be an impact on quality of life. To explore this the mutant variant was compared with quality of life scores derived from the Paediatric Asthma Quality of Life Questionnaire.
Figure 2.3.1 Illustration of chromosomal location of GSTM1, GSTT1 and GSTP1 rs1695 modified from Genecards.org\textsuperscript{6-8} and NCBI dbSNP\textsuperscript{155}
SECTION 2.4
Pathogenesis of target genes

Figure 2.4.1 Illustration of hypothesised pathogenesis of candidate genes
<table>
<thead>
<tr>
<th>Variant</th>
<th>Mutation type</th>
<th>Mutation location</th>
<th>Protein change with mutant allele</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHI3L1</td>
<td>C/G transversion substitution</td>
<td>Promoter region</td>
<td>Increased protein levels and increased protein affinity</td>
<td>56</td>
</tr>
<tr>
<td>MMP9</td>
<td>Missense A/G transition substitution</td>
<td>Coding region</td>
<td>Believed to be partial loss of function within proteinase therefore decrease in levels/ function</td>
<td>156</td>
</tr>
<tr>
<td>MMP9</td>
<td>A/T transversion substitution</td>
<td>Coding region</td>
<td>Believed to be decrease protein levels/ function</td>
<td>None</td>
</tr>
<tr>
<td>MMP12</td>
<td>A/G transition substitution</td>
<td>Coding region</td>
<td>Binding activity for activator protein 1, therefore reduced MMP12 activity</td>
<td>157</td>
</tr>
<tr>
<td>MMP12</td>
<td>Missense A/G transition substitution</td>
<td>Promoter region</td>
<td>Alters expression and binding of the protein through alteration of the binding site. Therefore reducing activity.</td>
<td>141</td>
</tr>
<tr>
<td>GSTM1 null</td>
<td>null</td>
<td>Homozygous deletion</td>
<td>No Protein</td>
<td>60</td>
</tr>
<tr>
<td>GSTP1 null</td>
<td>Missense A/G transition substitution</td>
<td>Promoter region</td>
<td>Alters electrophilic binding specificity reducing efficiency of enzyme activity</td>
<td>151,152</td>
</tr>
<tr>
<td>GSTT1 null</td>
<td>null</td>
<td>Homozygous deletion</td>
<td>No Protein</td>
<td>154</td>
</tr>
</tbody>
</table>

Table 2.4.1 Summary of gene mutations and associated protein changes

The six target genes, *Chitinase 3-like-1* (CHI3L1), *Matrix metalloproteinase 12* (MMP12), *Matrix metalloproteinase 9* (MMP9) and *Glutathione-S-Transferase mu-1* (GSTM1), *Glutathione-S-Transferase pi-1* (GSTP1), *Glutathione-S-Transferase theta-1* (GSTT1) and their corresponding gene variations influence the second stage of the remodelling pathway (Figure 2.1.1, 2.2.1, 2.4.1). Anoikis induced by MMP9126 may also play a role in stage one (initial sensitisation). To explore the roles of these target genes in asthma pathogenesis they will be investigated in a structured, hypothesis based manner. Each variant will be investigated for each hypothesis pertaining to an aspect of asthma severity pathogenesis and the analysis will be presented in sections 1 to 3. As a whole, the hypotheses will determine the extent of each variant’s influence on asthma severity.
Materials and Methods

Two studies will be presented in this thesis. The BREATHE study has successfully genotyped 1539 participants, 364 from Sussex and 1175 from Scotland. PAGES (Paediatric Asthma Gene Environment Study) has successfully genotyped 552 participants, 15 from Sussex and 364 participants from sites across Scotland. Procedures in both studies were standardized across all sites. If more than four genotype assays failed to amplify the participants sample was excluded from the study.

‘BREATHE,’ received approval by the Tayside Committee on Medical Research and Ethics. PAGES, received approval by the Cornwall and Plymouth Committee of Medical Research Ethics. Written informed consent was obtained from the patient and/or parent/guardian in both studies as relevant in compliance with the Helsinki accord.158-160

The BREATHE study will be described in detail in Section 3.1 and PAGES in Section 3.2. Additional methods including pulmonary function, saliva collection, genotyping and data analysis will be discussed under separate sub headings in chapter 3.
CHAPTER 3

SECTION 3.1

BREATHE

A cross sectional study of the role of genetic variation in children’s asthma

Clinical records were examined for children and young adults between the ages of 3-22, with physician diagnosis of asthma invited to participate in this study. Those with any other severe congenital abnormality or systemic disease e.g. cystic fibrosis were not invited to participate.

Participants were recruited in Sussex, England from October 2008 and Tayside and Dumfries, Scotland from June 2003.

The participant dataset includes demographic, anthropometric and clinical details from a total of 1539 individuals attending 5 secondary care, paediatric asthma clinics in Sussex, 2 secondary care paediatric asthma clinics in Tayside, 29 primary care clinics in Tayside and 1 secondary care clinic in Dumfries. 364 individuals were recruited from secondary care clinics in the Royal Alexandra Children’s Hospital, Princess Royal Hospital, Eastbourne District General Hospital, Hastings Conquest Hospital and Worthing and Southlands Hospital, Sussex. 1175 individuals were recruited from secondary care clinics in Ninewells Hospital and Medical School, and Perth Royal Infirmary, Scotland.

Unique identifying demographic data was collected from participants in the form of the participant’s date of birth, address and hospital identification number (CHI). Informed consent was provided by a parent and where appropriate the participant. For children over the age of 16 parental consent was not required, but participant consent was only accepted providing the researcher was under the full belief that the participant fully understood the study and its implications. Following informed consent, a detailed history was taken from the participant with the assistance of their parent or guardian. Anthropometric data in the form of height (cm), weight (kg) and calculated BMI was recorded. Participants were asked to remove shoes and heavy outer clothing prior to
height and weight measurements. Clinical data in the form of personal history of asthma, allergies, eczema, rhinitis, exposure to smoke and animals; and family history of atopic disease was recorded. Reported reactions to allergies included urticaria and wheeze was recorded, as was the participants report of diagnostically undertaken skin prick testing and/or specific IgE (commonly referred to as RAST) assay and the corresponding results.

Exposure to the environment and asthma inducing triggers were deduced from participant report. The participant’s medication was recorded along with an assessment of the participant’s inhaler technique. The frequency of bronchodilator use to relieve symptoms, number of episodes of asthma-related hospital admissions, number of days of absence (from school, college or work) due to worsening of asthma and short courses of oral steroids required for asthma attacks over the previous six months was also recorded.

(BREATHE Questionnaire Appendix 1)

Pulmonary function was performed on children over the age of 5 who were not currently experiencing an acute exacerbation of asthma. A saliva sample for genotyping was collected from all participants.

All samples and records were anonymised and identified with a unique research number. Patient consent and research data were stored in two separate password secured databases. Patient consent databases were stored on respective NHS Trust databases. Anonymised questionnaire data was entered into matching databases in Scotland and Sussex. Scottish data was entered by Ms. Inez Murrie, Dr Tahania Ismail and Dr Kaninika Basu. All data from questionnaires in Sussex was entered into the Sussex BREATHE questionnaire database by myself. Hard copies of consent forms and questionnaires were stored separately in locked filing cabinets in secure locations on NHS property. In Sussex original copies of questionnaires and consent were stored in the Royal Alexandra Children’s Hospital. Respective trusts maintain a record of consent for their participants. Data entry was monitored by Dr Shrabani Chakraborty for quality control through random selection and crosschecking of questionnaire entry.
SECTION 3.2

PAGES (Paediatric Asthma Gene Environment Study)

*A cross sectional study creating a database to investigate gene-environment interactions in children with asthma* (Appendix 4)

Clinical records were examined for children between the ages of two and sixteen, under the care of a respiratory paediatrician. Children with any other severe congenital abnormality or systemic disease e.g. cystic fibrosis, broncho-pulmonary dysplasia, cerebral palsy were not invited to participate.

Participants were recruited in England from Brighton and in Scotland from Inverness, Elgin, Aberdeen, Dundee, Perth, Stirling, Kirkcaldy, Paisley, Glasgow, Wishaw, Edinburgh, Kilmarnock, Melrose and Dumfries from March 2008.

The data set includes demographic, anthropometric and clinical details from 552 children attending 1 secondary asthma clinic in Sussex and 15 secondary care clinics across Scotland. 15 participants were recruited from the secondary care clinics in the Royal Alexandra Children’s Hospital, Sussex. 364 participants were from secondary care asthma clinics in Scotland.

In Sussex, participants and their parents were approached for consent in the secondary care asthma clinic. Eligible participants received the parent and child information leaflet, letter of invitation, consent form and asthma questionnaire. Following receipt of informed consent an appropriate time was arranged for an assessment.

The assessment (Figure 3.2.1) included completion of age specific dietary questionnaire and quality of life questionnaire, skin prick allergy testing measurements of height, weight, and lung function, including bronchodilator induced reversibility, and the collection of saliva samples for salivary cotinine analysis and genotyping.

Participants and their parents were asked to ensure that if feasible participants would avoid using their short-acting beta agonists for 6 hours prior to the assessment and long acting beta agonists for 12 hours prior to the assessment.
The PAGES asthma questionnaire has been developed using questions from the BREATHE study\textsuperscript{33,161} with additional questions relating to asthma control (the Child Asthma Control Test\textsuperscript{®}, used with permission) and environmental exposures (from Biobank) (Appendix 2). Additional variables included eczema medication and self reported food allergies. Questions were asked on the participant’s home environment, including the length of time in current accommodation, the type of accommodation, the appliance types in the home and the presence of damp in the household. Questions were also asked on the impact of the parent’s place of work and exercise on the participant’s asthma symptoms. A four week and six month history of parental opinion on control, and number of exacerbations measured by number of courses of oral steroids, hospital admission and school absences were also recorded in the PAGES asthma questionnaire.

PAGES used The Paediatric Asthma Quality of Life Questionnaire (PAQLQ)\textsuperscript{41} (Appendix 3). Designed to be administered by the parent to the child, PAQLQ produces a Quality of Life (QOL) score reflecting the impact of the child’s asthma on areas of physical and emotional function important to the child. Three topic areas are addressed within the 23 questions included in the questionnaire; activity limitation, symptoms and emotional function. Each question has a score ranging from 1 to 7 and QOL score is derived from the mean score for each topic area, in addition to the total mean score.

The dietary questionnaire used is the Scottish Collaborative Group semi-quantitative food frequency questionnaire version C1 http://www.foodfrequency.org, specifically designed for use in children. There are two versions, one for young adults (> 11 years of age) and the other for children. The aim of the dietary questionnaire was to estimate the daily intake of a range of nutrients in children. The questionnaire includes 166 commonly ingested foods or drinks. The questionnaire is designed to estimate the proportion and frequency of dietary intake determined by personal estimation based on the guidelines at the front of the questionnaire.

For the purpose of this study, PAGES will be used to supplement the analysis undertaken on the BREATHE study. Asthma severity and exacerbation data has not been merged with data collected for the BREATHE cohort because of the variation in collection techniques, in that questionnaires from PAGES are completed by participants and their parent/guardian rather than by a researcher.
Pulmonary function data will be merged between the two studies, creating a pulmonary function cohort of 1187 cases. Bronchodilator reversibility, Nitric Oxide, parentally reported allergy, parentally reported exposure to damp in conjunction with parentally reported exacerbations, skin prick sensitivities and quality of life data will be used in this study.
Figure 3.2.1: Illustration of PAGES participant interview process including the questions asked prior to skin prick testing. The interview is held in a clinical area and the skin prick test carried out with the assistance of clinical staff. The diagram is used as a prompt during the interview.
SECTION 3.3

Assessment of pulmonary function

Spirometry was undertaken using the Microlab Mark 3 spirometer (MicroMedical, Cardinal Healthcare, 7000 Cardinal Place, Dublin). The spirometer was calibrated before each clinic using a one litre syringe. A calibration error of ± 3% was considered acceptable. The spirometer was used to measure peak expiratory flow rate (PEFR), forced expiratory volume in 1 second (FEV₁), forced vital capacity (FVC) and FEV₁/FVC ratio. All percentage predicted values were calculated using participant age, sex and height. A disposable mouthpiece was used with each participant.\(^{162,163}\)

Participants were instructed to take a deep breath and forcibly exhale into the mouth piece, continuing exhalation to achieve maximum exhalation. A graphic incentive from the Microlab Mark 3 Spirometer was used to encourage maximum exhalation. At least three successive attempts were performed. If the participant was experiencing difficulty in achieving a satisfactory technique the participant was re-trained and had a further three attempts, therefore a maximum of six attempts were undertaken.

Pulmonary function data was collected from a single consult. The best result with correct technique was selected\(^{164,165}\). Assessment was not performed on participants suffering from exacerbations or other acute illness. The lung function test results were expressed as a percentage of that predicted using the data of Rosenthal \textit{et al}.\(^{166}\)

Bronchodilator reversibility- \textit{PAGES} study

Following satisfactory spirometry assessment, the patient was administered 200µg of salbutamol from a pressurised metered dose inhaler in conjunction with a large volume spacer device (Volumatic®, GlaxoSmithKline, UK). After fifteen minutes another spirometry, as above was performed to ascertain the bronchodilator induced reversibility, defined as change in FEV1 by 15%.
SECTION 3.4

Skin prick technique and reagents

Skin prick testing to determine sensitivity to allergens was performed on the children who participated in PAGES. Eight allergens were tested as part of the PAGES protocol. A positive and negative control were used to compare the allergic reactions. Allergens were manufactured by Alk Abello Ltd, (Alk Abelló Ltd, c/o UDG, Amber Park, Berristow Lane, South Normanton, Derbyshire).

- Positive control- histamine 10mg/ml (ALK001)
- Negative control- 0.9% saline(ALK002)
- *Aspergillus fumigatus* (ALK405)
- *Alternaria alternans* (ALK402)
- Cat hair and dander (ALK555)
- Dog hair and dander (ALK553)
- Grass Mix (208,214,218,223,225,228) (ALK299)
- House Dust Mite *D. pteronyssinus* (ALK503)
- Hen’s Egg (ALK701)
- Peanut (ALK762)

Testing was performed under clinical supervision, in a paediatric clinical setting, with a readily available crash trolley. Testing was withheld in participants who had taken antihistamines within 72 hours and who had previous history of anaphylaxis to allergens.

Parents and participants were asked to refrain from using antihistamines for 72 hours, and oil based skin creams for 24 hours prior to the assessment. The area chosen for the test was free of eczema or any other skin condition which could have affected the test results. The chosen area was cleaned and the allergens were applied using Alk Abello lancets (001 606) and the reactions were measured after 15 minutes.

The longest diameter of the weal and the longest diameter of the erythema were measured in millimetres. A positive test was defined as a weal ≥ 3mm in longest diameter. In cases of dermatographism, positivity is defined as greater than the negative control. If the case of an adverse reaction such as anaphylaxis a member of the clinical
staff was present with ready access to clinical support, a crash trolley and epinephrine. If any results were positive, it was the responsibility of the member of clinical staff to decide if the participant needs referral to the specialist allergy clinic.

Figure 3.4.1: Illustration of skin prick testing technique
SECTION 3.5

Saliva collection for DNA

Saliva was collected from all participants. Prior to the Oragene method used throughout all sites, a mouthwash was also used for collection of BREATHE samples in Scotland. Mouthwash was used until July, 2008 on 1075 participants.

Figure 3.5.1: H&E stained saliva slides, illustrating the source of DNA within saliva.
Oragene® DNA is a non-invasive DNA collection kit (http://www.dnagenotek.com). The median DNA yield from 2ml Oragene® saliva samples is about 110 microgram. The DNA from saliva is stable in this kit for up to 5 years in room temperature. The stability is achieved with proprietary reagents that inactivate bacteria and nucleases in saliva and minimize chemical hydrolysis of DNA.

Saliva was collected in one of two ways, depending on the age, parental and child choice and assessment of ability to produce and cooperation by the researcher. Children either donated saliva directly into the Oragene-DNA collection pot (Ref- OG-250, DNA Genotek Inc, Ottawa, Canada), or saliva was collected through the Genotek saliva collection for young children kit (Ref- CS-1, DNA Genotek Inc, Ottawa, Canada).

If participants were donating directly into the collection pot, they were instructed to spit directly into the container, to the specified mark. Participants were encouraged to have a drink of water to aid in saliva production, prior to specimen collection.
When using the saliva kit for young children a minimum of five swabs were collected. The swabs were rotated in the space between gum and cheek. Each swab was left in the mouth for approximately 15 seconds. During this period the participant was asked to keep his/her mouth closed, and ‘suck their cheeks’ to encourage increased salivation. The swabs were then cut by the researcher using the scissors provided by the young person collection kit, ensuring the bud dropped directly into the Genotek saliva collection pot.

Once saliva was collected, the preservative containing lid was screwed on to break the reagent seal, and the pot rotated five times. Each pot was labelled with the patient’s initials, the study number and date of sample on the sample lid, the container was labelled research number specific bar-coded sticker.

Mouthwash method (Scotland)

After ensuring the participant had not eaten or cleaned their teeth immediately before providing the sample, the participant was encouraged to swirl 4-5mls of clean tap water around their mouth for 20-30 seconds. The participant was instructed to spit the sample
into a universal container. The container was sealed and labelled as described previously. If the participant had eaten the test was not performed for a further 20 minutes.
SECTION 3.6

Genotyping

The following procedures were performed in the Biomedical Research Institute, University of Dundee, under the supervision of Professor Colin Palmer and Dr Roger Tavendale. I purified and quantified 150 samples collected from the participants from Sussex. I amplified, ran and interpreted 1152 samples for 8 SNP assays.

Purification

Extraction was performed using the Oragene DNA purification kit. The following method was used:

1. Samples incubated for 2 hours in 50°C air incubator to ensure adequate DNA release and permanent nuclease inactivation
2. 500µl of sample transferred to a labelled 1.5ml micro centrifuge tube using a Gilson pipette with autoclaved 1ml tips
3. 20µl of Oragene DNA purifier(OG-L2P) added to the sample
4. Sample vortexed for several seconds until the sample was visibly turbid
5. Samples incubated on ice for ten minutes.
6. Samples centrifuged for 5 minutes at 13,000 rpm
7. Resultant supernatant transferred into fresh micro centrifuge tube using fresh pipette tips
8. Pellet discarded
9. A proportional volume of 95% ethanol was added to the supernatant
10. Sample inverted gently 10 times until DNA visible as a small clot
11. The samples were allowed to stand for 10 minutes to enable complete DNA precipitation
12. Samples placed in micro centrifuge, hinge on the outer edge of the centrifuge rim and centrifuged for 2 minutes at 13,000 rpm
13. Supernatant removed using a fine point Pasteur pipette, care taken not to dislodge the pellet by running the pipette along the opposite slope to the hinge
14. 250µl of 70% ethanol added to the pellet and left for 1 minute
15. Ethanol removed completely using a Pasteur pipette
16. 100µl of DNA buffer added to the pellet and vortexed until pellet dissolved
17. Samples stored at 4°C

**DNA quantification**

DNA was quantified using the Hoechst DNA staining methodology.

**Amplification**

Amplification was performed in 10µl wells. Each well contained allele specific primers and AmpliTaq Gold® PCR Master Mix (Applied Biosystems). Each well contains 2.25µl of DNase free water diluted sample at a 1 to 20ng dilution, 0.25µl of primer mix, 2.5µl of AmpliTaq Gold® PCR Master Mix, comprising 1.5mM MgCl2, 10nmol of each dNTP and 1 unit AmpliTaq Gold DNA polymerase. Amplification was performed using thermal cyclers. Cycles consisted of three water baths, two of 95°C and one at 65°C. Allelic discrimination assays required 60 cycles to amplify, with the exception of the GSTP1 assay, which required 35 cycles. Real time PCR assays required 40 cycles to amplify.

**SNP assays**

**CHI3L1 rs4950928**

Genotyping for the functional promoter C/G transversion substitution rs4950928 was performed using a Taqman SNP genotyping assay c_27832042_10 (Applied Biosystems Europe, Warrington, UK). The context sequence used was

5’-ATATACCTGTCCACTCCACTCCCC[C/G]ACGCGGCAAACCAGCCCTTTTATGG-3’.

The C allele was tagged with VIC and the G allele was tagged with FAM

**MMP9 rs17576**

Genotyping for missense A/G transition substitution rs17576 was performed using a Taqman SNP genotyping assay c_11655953_10 (Applied Biosystems Europe, Warrington, UK). The primer sequence used was
5’-CTCCTCGCCCGACTCTACACC[A/G]GGACGGCAATGCTGATGGGAAACCC-3’.

The A allele was tagged with VIC and the G allele was tagged with FAM

**MMP9 rs6073983**

Genotyping for the promoter A/T transversion substitution rs6073983 was performed using a Taqman SNP genotyping assay c_30627092_10 (Applied Biosystems Europe, Warrington, UK). The primer sequence used was

5’-AGGTGAAAGTCAGGCATCAG[T/A]GAGAAAATCTCTGAGTATTCCAAT-3’.

The A allele was tagged with VIC and the T allele was tagged with FAM

**MMP12 rs2276109**

Genotyping for the coding C/T transition substitution rs2276109 was performed using a Taqman SNP genotyping assay c_15880589_10 (Applied Biosystems Europe, Warrington, UK). The primer sequence used was

5’-AGATGATAAGGGGATGATATCACT[A/G]TGAGTCACTCATAGGATTCATATTC-3’.

The C allele was tagged with FAM and the T allele was tagged with FAM

**MMP12 rs652438**

Genotyping for the coding C/T transition substitution rs652438 was performed using a Taqman SNP genotyping assay c_785907_10 (Applied Biosystems Europe, Warrington, UK). The context sequence used was

5’-AGATGACAAAATACTGGTTAATTAGCA[A/G]TTTAAGACCAGGCAAATTATCCC-3’.

The C allele was tagged with VIC and the T allele was tagged with FAM

**GSTM-1 null**

Genotyping for GSTM1 null was performed using a real time PCR. The in-house designed primers were manufactured by Qiagen and the probes were manufactured by Eurogentec.

The forward primer used was 5’-CTTGGAGGAACCTCCCTGAAAAG-3’.
The reverse primer used was 5’-TGGAACTCCATAACACGTGA-3’.

The FAM probe was 5’-AAGCGGCATGGTTTGCAGG-3’.

The absence of amplified GSTM1 product was indicative of GSTM1 null genotype.

**GSTP-1 rs1695**

Genotyping for missense A/G transition substitution rs1695 was performed using a Taqman SNP genotyping assay c_3237198_20 (Applied Biosystems Europe, Warrington, UK). The context sequence used was,

5’-CGTGGAGGACCTCGCTGCAAATAC[A/G]TCTCCCTCATCTACAACCAACTATGT-3’.

The A allele was tagged with VIC and the G allele was tagged with FAM

**GSTT-1 null**

Genotyping for GSTT1 null was performed using real time PCR.

The forward primer used was 5’-GTGCAAACACCTCCTGGAGAT-3’.

The reverse primer used was 5’-AGTCTTGCCCTTCAGAATGA-3’.

The FAM probe was 5’-ATGCTGCCCATCCCTGCC-3’.

The absence of amplified GSTT1 product was indicative of GSTT1 null genotype.

**Interpretation**

Allelic discrimination assays were performed post PCR on an Applied Biosystems 7700 sequence detection system (Figure 3.6.1). Real time PCR assays were performed on either an Applied Biosystems 7500 or 7900 Real time PCR system (Figure 3.6.2).

TaqMan SNP genotyping assays consist of two context sequence amplifying primers and two allele specific TaqMan Minor Groove Binder (MGB) probes. Each allele specific probe contains a reporter dye at the 5’ end. The reporter dye for allele 1 is VIC ®. The reporter dye for allele 2 is FAM (6-carboxy-fluorescein). At the 3’ end of each allele specific probe there is a non-fluorescent quencher. The quencher serves to increase the accuracy of
allelic discrimination by suppressing fluorescence from the reporter dye, whilst the probe is intact.

During the assay each probe anneals to a specific complimentary sequence between the forward and reverse primer sites. Hybridized probes are cleaved by AmpliTaq Gold® polymerase, separating the reporter dye from the quencher dye. Incomplete hybridization, or mismatches between the probe and the target are dislodged by AmpliTaq Gold® polymerase without cleaving the probe. Fluorescence was recorded by the Applied Biosystems 7700 sequence detection system. For each sample, VIC® fluorescence only indicates homozygosity of allele 1, FAM fluorescence only indicates homozygosity of allele 2 and fluorescence from both VIC® and FAM indicates heterozygosity. Each plate contained negative controls in the form of ‘NTC’ wells, absent of any DNA.

Real time PCR assays were undertaken on null variants. The presence of absence of variant was determined by the degree of FAM florescence from the sample, using Tamra (bound to the 3’ end of the probe) as a reference. Fluorescence was recorded by either the Applied Biosystems 7500 or 7900 real time PCR system.
Figure 3.6.1 Illustration of interpreted 96 well plate allelic discrimination assay, performed at Biomedical Research Institute, Dundee

![Alloic Discrimination](image1)

Figure 3.6.2 Illustration of complete automated 384 well plate real time PCR assay, performed at Biomedical Research Institute, Dundee

![Delta Rn vs Cycle](image2)
Statistics

The statistical analysis undertaken in this thesis was driven by the research questions and individual hypotheses described in Chapter 2 and in each discussion chapter. A p value <0.05 was defined as indicating statistical significance throughout. Adjustments for multiple testing have not been made because of the complications of multiple outcome measures. There is much controversy in the scientific community as to the pro’s and con’s of adjusting for multiple testing. The argument can be classified into two groups. The classical view maintains the priority of minimising the risk of type I error through multiple testing adjustments. In contrast the rational analysis view argue that by reducing the chance of making a type I error, the chance of a type II error is increased. Feise\textsuperscript{167} advocates a reorder of interpretation priorities. In saying a reduction in the importance of a p value in favour of the effect size, validity and quality of findings. In saying if the data is not clinically worthwhile statistical significance is moot. This may mean by not correcting for multiple testing there is an increased risk of incorrectly identifying an association where in fact there is none\textsuperscript{167}, however by regarding all findings as tentative until they are corroborated, evaluating effect size, clinical significance in terms of minimum clinically important differences, biological plausibility and by assessing the quality of the study the risk of Type I error will be minimised. It is also important to externally validate findings. In saying findings should be biologically plausible and generalisable to the population, as such the inclusion criteria of a study and the quality of the study is vital to produce valid, representative findings. When interpreting results it is therefore important to relate results to the applicable population, bearing in-mind the representativeness of the analysed sample, minimising the risk of type I error.
All statistical analysis was performed using SPSS for Windows, version 17 (SPSS Inc, Chicago, Ill.).

All power calculations were performed using PS Power and Sample Size Calculations, Version 3.0 (Department of Biostatistics, Vanderbilt University).

**Characteristics and principal outcomes**

**Age**

To investigate the effect of time as a proximal measure of exposure to environmental factors and as an approximate of time with asthma symptoms, age was categorised into three groups using BTS guidelines for patient management (less than 5 years; 5-12 years; 13-22 years). For the purpose of investigating pulmonary function the less than 5 years age group was excluded due to the unreliability of spirometry in these children.

**Modified BTS asthma treatment steps**

The asthma prescribing status was determined in accordance with the British Thoracic Society (BTS) guidelines 2002\(^{37}\) for physician-led management of asthma, as follows: step 0- no use of inhaled salbutamol on demand within the past month; step 1: inhaled salbutamol on demand; step 2: regular inhaled steroids plus inhaled salbutamol on demand; step 3: regular inhaled salmeterol plus inhaled steroids with salbutamol on demand; step 4: regular inhaled salmeterol plus inhaled steroids plus oral montelukast with inhaled salbutamol on demand.

For the purpose of analysis the modified BTS asthma treatment steps have been converted into a binary score, salbutamol only (steps 0 & 1) and the addition of preventer medication (steps 2-4).

The use of inhaled short-acting β-agonists (bronchodilators) was categorised as follows: 0, rarely or never required; 1, required few times a week but less than once daily; 2, required daily; 3, excessive use over a 24-hour period on a regular basis. Each category is based on a standard, 200µg dose of salbutamol inhaler or equivalent dose of an alternative bronchodilator.
Asthma severity score

Bronchodilator use adjusted modified BTS asthma treatment steps

An asthma severity score was derived from bronchodilator corrected drug class. The asthma severity score ranges from 0-5, where 0 is equivalent to mild asthma and 5 corresponds to severe asthma. The score is derived from a composite calculation, adjusting drug class by the frequency of bronchodilator use. For the purpose of developing this score, bronchodilator use was converted into rare-occasional use (0&1) and daily use (2&3). The use of bronchodilator on a daily basis infers an increase of participant drug class + 1.

For analysis the Asthma severity score has been converted into a binary score, mild asthma (0 & 1) and moderate to severe asthma (steps 2-5). Throughout the text the outcome measure of the asthma severity score is referred to as drug associated asthma severity. The outcome measure of the binary modified treatment steps will be referred to as use of preventer medication.

Exacerbation score

An exacerbation score was derived from combined yes/no responses for any of the three measures of exacerbations over a 6 month period of reporting. The measures of asthma exacerbation are defined as,

- Hospital admission due to asthma exacerbation.
- Nursery, school, college or work absence due to asthma exacerbations. Throughout this document this will be referred to as ‘absence’.
- Intake of oral steroids due to asthma exacerbation.
The total asthma exacerbation response was calculated as any of these measures during the same period of time and grouped as present or absent, this measure has been validated through publication as a measure of asthma severity\textsuperscript{35,161}.

**Reported allergy/ sensitivity score**

The number of allergies for each participant was identified through participant report of allergies. Sensitivity was ascertained in some cases using skin prick testing for eight different allergens. The total number of reported allergies was derived for each participant, this is represented as a ‘total numerical allergy score.’ This score was then converted to a binary (Yes 1 or more allergies/No 0 allergies) ‘allergy score’ used in analysis. The total number of sensitivities was derived for each participant, this is represented as a ‘total numerical sensitivity score.’ This score was then converted to a binary (Yes 1 or more sensitivities/No 0 sensitivities) ‘sensitivity score’ used in analysis.

**Exhaled nitric oxide categorised by clinical relevance**

Exhaled nitric oxide was tested as a continuous variable using univariate analysis and as a categorised variable to determine the clinical relevance of any findings in accordance with the British Thoracic Society (BTS) guidelines 2002\textsuperscript{37} for physician-led management of asthma, as follows: less than 25ppb: normal exhaled nitric oxide; 25-49 ppb: elevated exhaled nitric oxide; greater than 50ppb: clinically elevated exhaled nitric oxide.

**Techniques**

1. **Asthma severity**

For the purpose of analysis asthma severity was derived from proxy measures, the asthma exacerbation score and asthma severity score. Analysis was undertaken on the BREATHE study only. Only successfully genotyped cases were used. Binary logistic regression was used to calculate odds ratios and P values, corrected for age and sex.

For age categorised analysis, cases were categorised by age and analysed through a split file to obtain individual odds ratios and P values, corrected for sex. Cases were also tested
for interactions between exposure to smoke or damp and genetic variation. Odds ratios for interactions were obtained through reversal of direction of analyses.

2. **Pulmonary function**

PEFR, FEV1, FVC, FEV1/FVC and bronchodilator reversibility of FEV1 will be used as proxy measures of airway remodelling. PEFR, FEV1, FVC and FEV1/FVC will be used to approximate obstruction or basement membrane thickening and bronchodilator reversibility will be used to approximate airway smooth muscle hyperplasia. Analysis was undertaken on merged pulmonary function data from the BREATHE study and PAGES. Only successfully genotyped cases were used. Univariate analysis of variance was used to calculate P values, corrected for age and sex.

For age categorised analysis, cases were categorised by age and analysed through a split file to obtain individual odds ratios and P values, corrected for sex. Cases were also tested for interactions between exposure to smoke or damp and genetic variation.

3. **Exhaled nitric oxide categorised by clinical relevance**

Exhaled nitric oxide will be used as a proxy measure of pulmonary eosinophil infiltration. Only successfully genotyped cases were used. The effect of nitric oxide as a continuous variable was tested using univariate analysis of variance was used to calculate P values, corrected for age and sex. For categorised analysis of clinical relevance, multinomial logistic regression was used to calculate odds ratios and P values, corrected for age and sex. The reference category was ‘normal exhaled nitric oxide.’ Odds ratios and p values are representative of;

- Normal exhaled nitric oxide v elevated exhaled nitric oxide
- Normal exhaled nitric oxide v clinically elevated exhaled nitric oxide

4. **Reported allergy/ sensitivity score**

Analysis was undertaken on the BREATHE study and PAGES. Only successfully genotyped cases were used. Binary logistic regression was used to calculate odds ratios and P values, corrected for age and sex.

5. **Quality of life**
Analysis was undertaken on PAGES only. Only successfully genotyped cases were used. Univariate analysis of variance was used to calculate P values, corrected for age and sex.

*If the frequency of genotypes from categorised cases became to diffuse, the analysis was excluded due to insufficient cases.*

**Power calculations**

Calculation were undertaken on the most broad variables for each analysis; Overall exacerbations, FEV1, Bronchodilator reversibility, Nitric Oxide, Skin prick test, Overall quality of life. Exposure to environmental tobacco smoke or damp was not adjusted by genotype, but instead by exposure within the whole population.

1. **Dichotomous Data**

Sample size was calculated assuming an uncorrected chi-square approach at 90% power and 95% confidence. The calculation was adjusted by genotype distribution and reported effects (exposure) for each population.

2. **Comparison of Means**

Sample size was calculated assuming T-test approach, at 90% power and 95% confidence. The calculation was adjusted by genotype distribution and standard deviation.

3. **Sub-Categorisation**

Data was sub categorised by age, environmental tobacco smoke exposure and exposure to damp. For sub categorisation by age, the sample size was not adjusted. Where the data was investigated for an interaction, as in for environmental tobacco smoke exposure and exposure to damp, the calculated sample size was multiplied by four.

4. **Minimum clinically important difference**

An estimate of the minimum clinically important difference was required for Overall exacerbations, FEV1, Bronchodilator reversibility, Nitric Oxide, Skin prick test, quality of life.
The minimum clinically important difference for asthma exacerbations was 10%. The measure of overall asthma exacerbations was used in favour of any of the other measures of asthma exacerbations or asthma severity due to the breadth of the measure. As such small differences may be deemed clinically important in other measures, for instance in hospital admissions due to asthma exacerbations a small change difference between populations could have a large impact on the cost of healthcare.

The minimum clinically important difference for FEV1 was 12% in accordance with the BTS guidelines for bronchodilator reversibility37.

The minimum clinically important difference for bronchodilator reversibility was 12% in accordance with BTS guidelines37.

The minimum clinically important difference for Nitric oxide was 25ppb. This coincides with the difference between clinically elevated and non-elevated Nitric oxide as described by the BTS guidelines37.

The minimum clinically important difference for skin prick test was 10%.

The minimum clinically important difference for quality of life was 0.5 in accordance with the Juniper guidelines41.
RESULTS

The next four chapters in this thesis present the results for each of the gene variants analysed, grouped by gene of interest. Each results chapter presented in this thesis is in a comparable format, based on the hypotheses described in Chapter 2. The aim of the hypothesis in this thesis is to answer the relevant research question for each gene variant. The hypotheses individually assess the effect of gene variants on aspects of the pathogenesis of asthma severity. Hypothesis 1 deduces if the variant is directly associated with asthma exacerbations or asthma medication use as proxy markers of asthma severity. Hypothesis 2 deduces if the variant is directly associated with a change in pulmonary function as proxy markers of obstruction, eosinophil infiltration and smooth muscle hyperplasia. Hypothesis 3 looks at the influence of a common environmental toxin, in the form of environmental tobacco smoke and interaction with the variant to discern an association with both asthma severity in the form of asthma exacerbations or medication use, and remodelling measured by pulmonary function measures. Hypothesis 4 investigated the effect of the variant on reported allergy and allergen sensitivity measured through skin prick testing. Hypothesis 5 explores the influence the variant may have on QOL.

We know that asthma phenotype differs with age, as such in an attempt at identifying age specific effects a sub group analysis was performed on Hypothesis 1, 2 and 3. The first purpose of dividing by age was to discern an association with the variants and the known change in asthma from childhood to young adult. The second purpose was to attempt to approximate the extent of remodelling over the course of asthma progression, based on the assumption that asthma onset was in the first 5 years of life. For the purpose of clarity and to answer each hypothesis clearly, the results chapters presented in this thesis have been presented as a hypothesis staged analysis.

Each results chapter has been divided into three sections, addressing the individual hypothesis through separate analyses. Hypothesis 1 will be addressed in section 1. Hypothesis 2 will be addressed in section 2. Hypothesis 3, 4 and 5 will be addressed sequentially in section 3 of each results chapter. Variants were grouped by a standard dominance model. To remain consistent all analysis was undertaken to compare the
wildtype variant of each gene to the mutant variant. This may mean that in the presence of an environmental risk factor, the protective effect of a variable will increase, due to comparative increase in risk, with the risk variant, also exposed to the environmental risk factor.
CHAPTER 4

SECTION 4.1

Relationship between CHI3L1 (rs4950928) and asthma severity in a sample of UK children and young adults recruited to the BREATHE study

Sample demographics

1385 young individuals with physician diagnosed asthma derived from both primary and secondary care were successfully genotyped for CHI3L1 (rs4950928). Individuals range from 3-22 years of age (mean 9.7 years, SD4.1). 58.8% (n=815) are male. 34.4% (n=477) of participants were exposed to tobacco smoke in their home environment over the previous six months (Table 4.1.1).

Over the previous six months 44.5% (n=768) of the 1385 participants had at least one exacerbation, either requiring a hospital admission, a short course of oral steroids, an absence or a combination of these. 17.4% of participants had at least one hospital admission in the previous 6 months. 28.8% had been prescribed a short course of oral steroids. 36.9% reported absence due to an exacerbation of asthma.
Table 4.1.1: Characteristics of study participants with asthma and the rs4950928 SNP in the BREATHE study (n=1385)

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (years)</strong></td>
<td>Range, 3-22 (mean, 9.7; SD, 4.1)</td>
</tr>
<tr>
<td><strong>Sex (males: females)</strong></td>
<td>815 (58.8%): 570 (41.2%)</td>
</tr>
<tr>
<td><strong>CHI3L1 functional promoter SNP rs4950928 (%)</strong> (CC: CG:GG)</td>
<td>858 (62.0%): 463 (33.4%): 64 (4.6%)</td>
</tr>
<tr>
<td><strong>Exposure to tobacco smoke (yes/no)</strong></td>
<td>477/908 (34.4%)</td>
</tr>
<tr>
<td><strong>Reported allergy to HDM (yes/no)</strong></td>
<td>133/1252 (9.6%)</td>
</tr>
<tr>
<td><strong>Mean % predicted FEV1 (SD) (n=932)</strong></td>
<td>95.9 (14.8)</td>
</tr>
<tr>
<td><strong>Mean % predicted FVC (SD) (n=931)</strong></td>
<td>92.9 (13.9)</td>
</tr>
<tr>
<td><strong>Mean FEV1/ FVC (SD) (n=931)</strong></td>
<td>87.2 (8.5)</td>
</tr>
<tr>
<td><strong>Modified BTS asthma treatment steps * (n=1385)</strong></td>
<td>0=34; 1=2330; 2=743; 3=196; 4=179</td>
</tr>
<tr>
<td><strong>Inhaled bronchodilator use † (n=1385)</strong></td>
<td>0=159; 1=928; 2=212; 3=86</td>
</tr>
<tr>
<td><strong>Asthma severity score ‡ (n=1385)</strong></td>
<td>0=32; 1=226; 2=605; 3=279; 4=167; 5=76</td>
</tr>
<tr>
<td><strong>Hospital admissions (yes/ no) over previous 6 months due to exacerbations (n=1385)</strong></td>
<td>241/ 1144 (17.4%)</td>
</tr>
<tr>
<td><strong>Oral steroid intake (yes/ no) over previous 6 months due to exacerbations (n=1385)</strong></td>
<td>399/986 (28.8%)</td>
</tr>
<tr>
<td><strong>Absence (yes/ no) over previous 6 months due to exacerbations (n=1385)</strong></td>
<td>511/874 (36.9%)</td>
</tr>
<tr>
<td><strong>Overall exacerbations (yes/ no) over previous 6 months ‡ (n=1385)</strong></td>
<td>617/768 (44.5%)</td>
</tr>
</tbody>
</table>

**Keys:**
- *Step 1= no/occasional use of inhaled salbutamol; Step2= step1+ inhaled steroids; step 3= step2+ inhaled long acting β2 without montelukast; step 4= step 3 + montelukast
- † Inhaled bronchodilator use: 0= none, 1= occasional, 2= daily, 3= excessive use
- ‡ Asthma severity score (bronchodilator use adjusted modified treatment steps): A scale where 0=mild and 5=severe
- ◊ Defined as any one of the following in previous 6 months: absence, courses of oral steroids, or hospital admissions
The 1385 recruited participants were genotyped for the functional promoter SNP rs4950928. The CC genotype was present in 62% (n=858) of participants. The -131G allele (CG or GG) was present in 38% (n=527) of participants.

Among the participants with the CC genotype, 46.4% (n=398) reported at least one marker of asthma exacerbations in the previous six months. 19.4% (n=166) reported hospital admission, 37.9% (n=325) absence and 29.9% (n=257) use of oral steroids (Table 4.1.2; Figure 4.1.1). 17.8% (n=153) suffer from severe asthma (severity score steps 4&5) (Table 4.1.3). 64.3% (n=552) suffer from moderate asthma (severity score steps 2&3). Among the participants with the -131G allele, 41.6% (n=219) reported an exacerbation in the previous six months. 14.2% (n=75) reported hospital admission, 35.3% (n=186) reported absence and 26.9% (n=142) reported use of oral steroids for asthma exacerbation (Table 4.1.2; Figure 4.1.1). 17.1% (n=90) suffer from severe asthma (severity score steps 4&5). 62.9% (n=332) suffer from moderate asthma (severity score steps 2&3) (Table 4.1.3).
**CHI3L1 (rs4950928) and asthma severity**

<table>
<thead>
<tr>
<th></th>
<th>CHI3L1 functional promoter SNP rs4950928</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CG/GG (n=527)</td>
</tr>
<tr>
<td></td>
<td>CC (n=858)</td>
</tr>
<tr>
<td></td>
<td>OR (95% CI)</td>
</tr>
<tr>
<td></td>
<td>P value</td>
</tr>
<tr>
<td>Hospital admission due to asthma exacerbations (Y)</td>
<td>75 (14.2%)</td>
</tr>
<tr>
<td>Absence due to asthma exacerbations (Y)</td>
<td>186 (35.3%)</td>
</tr>
<tr>
<td>Oral steroid intake due to asthma exacerbations (Y)</td>
<td>142 (26.9%)</td>
</tr>
<tr>
<td>Overall asthma exacerbations (Y)</td>
<td>219 (41.6%)</td>
</tr>
<tr>
<td>Modified treatment steps</td>
<td>Addition of preventer medication (Y)</td>
</tr>
<tr>
<td>Asthma severity score</td>
<td>Moderate to severe (Y)</td>
</tr>
</tbody>
</table>

**Table 4.1.2:** Overall effect of CHI3L1 functional promoter SNP rs4950928 genotype (dominant and mutant varieties) on hospital admissions, oral steroid intake and absences due to asthma exacerbations, overall asthma exacerbations, modified treatment steps and asthma severity in children and young adults recruited to the BREATHE study

Key: *: p<0.05; OR: Odds ratio; CI: Confidence interval; Y: Yes; Absence: Absence in form of school absence, college absence and work absence; Modified treatment steps: Salbutamol only-Step 1; Addition of preventer medication- Step2-4
Asthma severity score: (bronchodilator use adjusted Modified treatment steps): 0&1=mild, 2-5=moderate to severe
P values were calculated by binary logistic regression corrected for age and sex.
The homozygous C allele (CC) was associated with hospital admissions due to exacerbations \((p=0.035)\) when compared to the dominant G allele (CG or GG). The odds ratio for CG/GG when compared to CC was 0.72, indicating that the minor allele may have a protective role which reduces the need for asthma-related hospital admissions.

We did not observe any significant association between \(CHI3L1\) rs4950928 and overall asthma exacerbations, or asthma-related absences, or oral steroid intake due to asthma exacerbations over the previous six months (Table 4.1.2; Figure 4.1.1). We subsequently categorised by age and repeated the analysis, however no association was identified, this data is not included.

Through proxy measures, we tested for gene-dosage effect using the modified BTS treatment steps, categorised into salbutamol and addition of preventer medication. We also tested for an association with risk of severity using the asthma severity score, categorised into mild and moderate to severe (Table 4.1.2). However, no significant effect was observed. We subsequently categorised by age and repeated the analysis, however we found no association, this data is not included.
Figure 4.1.1 Illustration of Overall effect of CHI3L1 functional promoter SNP rs4950928 genotype (dominant and mutant varieties) on hospital admissions, oral steroid intake and absences due to asthma exacerbations, and overall asthma exacerbations, in children and young adults recruited to the BREATHE study.
SECTION 4.2

Relationship between CHI3L1 (rs4950928) and measures of pulmonary function in a sample of UK children and young adults recruited to the BREATHE study and PAGES

CHI3L1 (rs4950928) and pulmonary function

<table>
<thead>
<tr>
<th>CHI3L1 functional promoter SNP rs4950928</th>
<th>CG/GG</th>
<th>CC</th>
<th>n</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ppPEFR [n] (sd)</td>
<td>[396]</td>
<td>[657]</td>
<td>1053</td>
<td>0.743</td>
</tr>
<tr>
<td></td>
<td>87.7 (17.2)</td>
<td>87.5 (17.1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ppFEV1[n] (sd)</td>
<td>[406]</td>
<td>[668]</td>
<td>1074</td>
<td>0.098</td>
</tr>
<tr>
<td></td>
<td>95.2 (14.4)</td>
<td>96.8 (16.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ppFVC [n] (sd)</td>
<td>[406]</td>
<td>[667]</td>
<td>1073</td>
<td>0.040*</td>
</tr>
<tr>
<td></td>
<td>93.7 (14.2)</td>
<td>95.4 (15.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FEV1/FVC [n] (sd)</td>
<td>[406]</td>
<td>[667]</td>
<td>1073</td>
<td>0.600</td>
</tr>
<tr>
<td></td>
<td>86.3 (9.0)</td>
<td>86.2 (8.6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean change in ppFEV1 post bronchodilator [n] (sd)</td>
<td>[47]</td>
<td>[78]</td>
<td>125</td>
<td>0.124</td>
</tr>
<tr>
<td></td>
<td>6.2 (7.0)</td>
<td>4.5 (5.8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean Nitric oxide (ppb) [n] (sd)</td>
<td>[57]</td>
<td>[100]</td>
<td>157</td>
<td>0.656</td>
</tr>
<tr>
<td></td>
<td>34.7 (27.6)</td>
<td>35.7 (35.7)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Nitric oxide categorised by clinical relevance

| Clinical elevated exhaled nitric oxide (>50 ppb) (Y) | 22/71 (30.1%) | 32/139 (23.0%) | 55 | 0.301 |
| Elevated exhaled nitric oxide (25-40 ppb) (Y)       | 9/71 (12.7%)  | 28/139 (20.1%) | 37 | 0.549 |

Table 4.2.1: Overall effect of CHI3L1 functional promoter SNP rs4950928 genotype (dominant and mutant varieties) on mean % predicted lung function in participants recruited to the BREATHE study and PAGES

Key: *: p<0.05; pp: % predicted; ppb: parts per billion; FEV1: Forced expiratory volume in 1 second; FVC: Forced vital capacity
P-values were calculated by univariate analysis of variance corrected for age and sex
P-values for Nitric oxide categorised by clinical relevance were calculated by multinomial logistic regression corrected for age and sex

We tested for an effect of CHI3L1 rs4950928 on measures of pulmonary function (Table 4.2.1; Figure 4.2.1). The C allele (CC) was associated with increased mean % predicted FVC (p=0.040) when compared to the minor G allele (CG or GG). No significant effect was observed with any other investigated measures of pulmonary function.
Figure 4.2.1 Illustration of Overall effect of CHI3L1 functional promoter SNP rs4950928 genotype (dominant and mutant varieties) on % predicted PEFR, FEV1, FVC and FEV1/FVC, in children and young adults recruited to the BREATHE study and PAGES
### Table 4.2.2: Overall effect of CHI3L1 functional promoter SNP rs4950928 genotype (dominant and mutant varieties) on mean % predicted lung function categorised by age in participants recruited to the BREATHE study and PAGES

<table>
<thead>
<tr>
<th>CHI3L1 functional promoter SNP rs4950928</th>
<th>5-12 years</th>
<th></th>
<th>13-22 years</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CG/GG</td>
<td>CC</td>
<td>CG/GG</td>
<td>CC</td>
</tr>
<tr>
<td>Mean ppPEFR (sd)</td>
<td>85.5 (17.3)</td>
<td>84.8 (16.1)</td>
<td>0.543</td>
<td>91.5 (16.5)</td>
</tr>
<tr>
<td></td>
<td>253</td>
<td>471</td>
<td>-</td>
<td>143</td>
</tr>
<tr>
<td>Mean ppFEV1 (sd)</td>
<td>95.6 (14.3)</td>
<td>95.7 (14.9)</td>
<td>0.884</td>
<td>94.5 (14.5)</td>
</tr>
<tr>
<td></td>
<td>260</td>
<td>479</td>
<td>-</td>
<td>146</td>
</tr>
<tr>
<td>Mean ppFVC (sd)</td>
<td>93.1 (13.9)</td>
<td>93.1 (14.2)</td>
<td>0.618</td>
<td>94.7 (14.8)</td>
</tr>
<tr>
<td></td>
<td>260</td>
<td>479</td>
<td>-</td>
<td>146</td>
</tr>
<tr>
<td>FEV1/FVC (sd)</td>
<td>86.4 (8.4)</td>
<td>86.3 (8.5)</td>
<td>0.795</td>
<td>86.1 (10.1)</td>
</tr>
<tr>
<td></td>
<td>260</td>
<td>479</td>
<td>-</td>
<td>146</td>
</tr>
<tr>
<td>Mean change in ppFEV1 post bronchodilator (sd)</td>
<td>6.5 (6.2)</td>
<td>4.4 (5.7)</td>
<td>0.108</td>
<td>5.3 (9.6)</td>
</tr>
<tr>
<td></td>
<td>36</td>
<td>59</td>
<td>-</td>
<td>11</td>
</tr>
<tr>
<td>Nitric oxide (ppb) (sd)</td>
<td>29.2 (22.8)</td>
<td>34.8 (34.7)</td>
<td>0.378</td>
<td>49.9 (34.4)</td>
</tr>
<tr>
<td></td>
<td>42</td>
<td>76</td>
<td>-</td>
<td>15</td>
</tr>
</tbody>
</table>

Key: *: p<0.05; pp: % predicted; ppb: parts per billion; FEV1: Forced expiratory volume in 1 second; FVC: Forced vital capacity
P-values were calculated by univariate analysis of variance corrected for sex
We tested for an association between \textit{CHI3L1} rs4950928 and measures of pulmonary function, categorised by age. The -131C allele (CC) was associated with increased mean \% predicted FEV1 ($p=0.08$) and FVC ($p=0.04$) in the 13-22 years category, when compared to the minor -131G allele (CG or GG). No significant effect was observed with any other investigated measures of pulmonary function.
Figure 4.2.2 Illustration of Overall effect of \textit{CHI3L1} functional promoter SNP rs4950928 genotype (dominant and mutant varieties) on % predicted PEFR, FEV1, FVC and FEV1/FVC, categorised by age (5-12 years and 13-22 years), in children and young adults recruited to the BREATHE study and PAGES.
SECTION 4.3

Relationship between CHI3L1 (rs4950928) and environmental factors in a sample of UK children and young adults

CHI3L1 (rs4950928) and exposure to environmental tobacco smoke

1385 individuals recruited to the BREATHE study were tested for an interaction between the rs4950928 genotype and environmental tobacco smoke on exacerbations. The CC genotype was present in 63.1% (n=1123) of participants. 32.5% (n=365) were exposed to environment tobacco smoke. The -131G allele (CG or GG) was present in 36.9% (n=661) of participants. 33.6% (n=222) were exposed to environmental tobacco smoke.

We did not observe any significant association between CHI3L1 rs4950928 and overall asthma exacerbations, or asthma-related hospital admissions, or asthma-related absences, or oral steroid intake due to asthma exacerbations over the previous six months (Table 4.3.1). We tested the gene-dosage effect for the risk allele for each severity score step for long-term control of asthma (Table 4.3.1). No significant effect was observed when the risk allele was tested for each severity step. We subsequently categorised by age and repeated the analysis, however no association was identified, this data is not included.
<table>
<thead>
<tr>
<th></th>
<th>CHI3L1 functional promoter SNP rs4950928</th>
<th></th>
<th></th>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Exposed to tobacco smoke</td>
<td>Not exposed to tobacco smoke</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>CG/ GG (n=184)</td>
<td>CC (n=293)</td>
<td>OR (95%CI)</td>
<td>CG/ GG (n=343)</td>
<td>CC (n=565)</td>
<td>OR (95%CI)</td>
<td>P Value for interaction</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hospital admission due to asthma exacerbations (Y)</td>
<td>31 (16.9%)</td>
<td>73 (24.9%)</td>
<td>0.62 (0.38-1.01)</td>
<td>93 (16.5%)</td>
<td>44 (12.8%)</td>
<td>0.79 (0.53-1.17)</td>
<td>0.456</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Absence due to asthma exacerbations (Y)</td>
<td>74 (40.2%)</td>
<td>129 (44.0%)</td>
<td>0.88 (0.60-1.29)</td>
<td>196 (34.7%)</td>
<td>112 (32.7%)</td>
<td>0.96 (0.71-1.28)</td>
<td>0.727</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oral steroid intake due to asthma exacerbations (Y)</td>
<td>59 (32.1%)</td>
<td>99 (33.8%)</td>
<td>0.95 (0.64-1.42)</td>
<td>158 (28.0%)</td>
<td>83 (24.2%)</td>
<td>0.86 (0.63-1.92)</td>
<td>0.687</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Overall asthma exacerbations (Y)</td>
<td>90 (48.9%)</td>
<td>150 (51.2%)</td>
<td>0.94 (0.64-1.38)</td>
<td>248 (43.9%)</td>
<td>129 (37.6%)</td>
<td>0.80 (0.61-1.07)</td>
<td>0.518</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asthma severity score</td>
<td>Moderate to severe</td>
<td>146 (79.3%)</td>
<td>244 (83.3%)</td>
<td>461 (81.6%)</td>
<td>276 (80.5%)</td>
<td>461 (81.6%)</td>
<td>0.96 (0.68-1.35)</td>
<td>0.493</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 4.3.1: Overall effect of CHI3L1 functional promoter SNP rs4950928 genotype (dominant and mutant varieties) on hospital admissions, oral steroid intake and absences due to asthma exacerbations, overall asthma exacerbations and asthma severity, categorised by exposure to tobacco smoke in children and young adults recruited to the BREATHE study

Key: OR: Odds ratio; CI: Confidence interval
Asthma severity score: (bronchodilator use adjusted modified treatment steps): 0&1=mild, 2-5=moderate to severe
P value for interaction and odds ratios were calculated by binary logistic regression corrected for age, and sex
Table 4.3.2: Overall effect of CHI3L1 functional promoter SNP rs4950928 genotype (dominant and mutant varieties) on mean % predicted lung function categorised by age and exposure to environmental tobacco smoke

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Exposed to environmental tobacco smoke</th>
<th>Not exposed to environmental tobacco smoke</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5-12 P Value</td>
<td>13-22 P Value</td>
</tr>
<tr>
<td>CHI3L1</td>
<td>CG/GG</td>
<td>CC</td>
</tr>
<tr>
<td>Mean ppPEFR</td>
<td>84.9 (17.5)</td>
<td>84.8 (17.0)</td>
</tr>
<tr>
<td>n</td>
<td>80</td>
<td>136</td>
</tr>
<tr>
<td>Mean ppFEV1</td>
<td>95.9 (15.8)</td>
<td>94.8 (15.7)</td>
</tr>
<tr>
<td>(sd)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ppFVC</td>
<td>93.5 (15.2)</td>
<td>93.6 (14.9)</td>
</tr>
<tr>
<td>(sd)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FEV1/FVC</td>
<td>86.3 (8.6)</td>
<td>85.9 (9.0)</td>
</tr>
<tr>
<td>(sd)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>80</td>
<td>137</td>
</tr>
</tbody>
</table>

Table 4.3.2: Overall effect of CHI3L1 functional promoter SNP rs4950928 genotype (dominant and mutant varieties) on mean % predicted lung function categorised by age and exposure to environmental tobacco smoke

Key: *: P<0.05; pp: % predicted; ppb: parts per billion; FEV1: Forced expiratory volume in 1 second; FVC: Forced vital capacity

P-values for interaction were calculated by univariate analysis of variance corrected for sex
We tested for an interaction between CHI3L1 rs4950928 and environmental tobacco exposure on measures of pulmonary function, categorised by age. (Table 4.3.2; Figure 4.3.1). No overall significant interaction was observed with any measures of pulmonary function, however, both FEV1 not exposed to ETS (p=0.023) and FVC not exposed to ETS (p=0.004) in the 13-22 years were significantly associated with comparably increased lung function in homozygous CC sample when compared to the CG/GG sample.
Figure 4.3.1 Illustration of Overall effect of CHI3L1 functional promoter SNP rs4950928 genotype (dominant and mutant varieties) on % predicted PEFR, FEV1, and FVC, categorised by age (5-12 years and 13-22 years) and exposure to environmental tobacco smoke (ETS- = no exposure; ETS+= exposed) in children and young adults recruited to the BREATHE study and PAGES
**CHI3L1 (rs4950928) and exposure to damp**

405 individuals recruited PAGES were tested for an interaction between the rs4950928 genotype and exposure to damp on exacerbations in individuals with asthma (Table 4.3.3; Figure 4.3.2). The CC genotype was present in 66.9% (n=271) of participants. 16.2% (n=44) were exposed to damp. The -131G allele (CG or GG) was present in 33.1% (n=134) of participants. 13.4% (n=18) were exposed damp.

The -131G allele (CG/GG) interacted with damp exposure to exert a protective effect on overall exacerbations (p=0.002; OR 0.17), hospital admissions (p=0.025; OR 0.12) and absence (p=0.019; OR 0.18) due to exacerbations when compared to the C allele (CC). These results indicate that the minor allele may interact with exposure to damp to have a protective role, reducing asthma-related exacerbations, particularly in the form of absence and oral steroid intake due to exacerbations when compared to the homozygous (CC) risk allele.

We did not observe any significant interaction between CHI3L1 rs4950928, damp and asthma-related use of oral steroids (p=0.094; OR 0.28) over the previous six months (Table 4.3.3; Figure 4.3.2).

We could not test for gene-dosage effect for the risk allele for each treatment step (Modified treatment steps) or severity score step for long-term control of asthma due to insufficient cases.
### Table 4.3.3: Overall effect of CHI3L1 functional promoter SNP rs4950928 genotype (dominant and mutant varieties) on hospital admissions, oral steroid intake and absences due to asthma exacerbations, overall asthma exacerbations and asthma severity, categorised by exposure to damp in children and young adults recruited to PAGES

**Key**: *: p<0.05; OR: Odds ratio; CI: Confidence interval

*P* values for interaction and odds ratios for exacerbations were calculated by binary logistic regression corrected for age, and sex

<table>
<thead>
<tr>
<th></th>
<th>Exposed to damp</th>
<th>Not exposed to damp</th>
<th>OR (95%CI)</th>
<th>OR (95%CI)</th>
<th>P Value for interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hospital admission due to asthma exacerbations (Y)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CG/GG (n=20)</td>
<td>1 (5.0%)</td>
<td>13 (28.3%)</td>
<td>0.12 (0.02-1.03)</td>
<td>24 (20.3%)</td>
<td>35 (14.8%)</td>
</tr>
<tr>
<td>CC (n=46)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Absence due to asthma exacerbations (Y)</strong></td>
<td>4 (20.0%)</td>
<td>27 (58.7%)</td>
<td>0.18 (0.05-0.65)</td>
<td>53 (44.9%)</td>
<td>112 (47.5%)</td>
</tr>
<tr>
<td><strong>Oral steroid intake due to asthma exacerbations (Y)</strong></td>
<td>3 (15.0%)</td>
<td>18 (39.1%)</td>
<td>0.28 (0.07-1.12)</td>
<td>49 (41.5%)</td>
<td>103 (43.6%)</td>
</tr>
<tr>
<td><strong>Overall asthma exacerbations (Y)</strong></td>
<td>5 (25.0%)</td>
<td>31 (67.4%)</td>
<td>0.17 (0.05-0.56)</td>
<td>76 (64.4%)</td>
<td>141 (59.7%)</td>
</tr>
</tbody>
</table>
Figure 4.3.2 Illustration of Overall effect of CHI3L1 functional promoter SNP rs4950928 genotype (dominant and mutant varieties) on hospital admissions, oral steroid intake and absences due to asthma exacerbations, and overall asthma exacerbations, categorised by exposure to damp (damp- = no exposure; damp + = exposed) in children and young adults recruited to PAGES.
**CHI3L1 (rs4950928) and atopy**

1790 individuals recruited to the BREATHE study and PAGES were tested for an association between the rs4950928 SNP and reported allergy to house dust mite (Table 4.3.4). 95 individuals recruited to PAGES were tested for an association between the rs4950928 SNP and sensitivity to *Aspergillus fumigatus* and *Alternaria alternans*.

We did not observe any significant association between *CHI3L1* rs4950928 and reported allergy to house dust mite (OR 0.81 (95%CI 0.58-1.12); p=0.197), sensitivity to *Dermatophagoides pteronyssinus* (OR 2.09 (95%CI 0.80-5.48); p=0.131), sensitivity to *Aspergillus fumigatus* (OR 1.59 (95%CI 0.53-4.72); p=0.406) or sensitivity to *Alternaria alternans* (OR 2.19 (95%CI 0.63-7.67); p=0.217) (Table 4.3.4).
Table 4.3.4: Overall effect of CHI3L1 functional promoter SNP rs4950928 genotype (dominant and mutant varieties) on participant reported allergy to house dust mite and skin prick test identified sensitivity to Dermatophagoides pteronyssinus, Aspergillus fumigatus and Alternaria alternans

<table>
<thead>
<tr>
<th></th>
<th>CG/GG</th>
<th>CC</th>
<th>OR (95% CI)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reported house dust mite allergy (Y)</td>
<td>59/661 (8.9%)</td>
<td>120/1129 (10.6%)</td>
<td>0.81 (0.58-1.12)</td>
<td>0.197</td>
</tr>
<tr>
<td>Sensitivity to <em>Dermatophagoides pteronyssinus</em> (Y)</td>
<td>23/31 (74.2%)</td>
<td>37/64 (57.8%)</td>
<td>2.09 (0.80-5.48)</td>
<td>0.131</td>
</tr>
<tr>
<td>Sensitivity to <em>Aspergillus fumigatus</em> (Y)</td>
<td>7/30 (23.3%)</td>
<td>10/63 (15.9%)</td>
<td>1.59 (0.53-4.72)</td>
<td>0.406</td>
</tr>
<tr>
<td>Sensitivity to <em>Alternaria alternans</em> (Y)</td>
<td>6/31 (19.4%)</td>
<td>6/63 (9.5%)</td>
<td>2.19 (0.63-7.67)</td>
<td>0.217</td>
</tr>
</tbody>
</table>

Key: OR: Odds ratio; CI: Confidence interval

P-values and odds ratios were calculated by binary logistic regression corrected for age and sex.
**CHI3L1** (rs4950928) and quality of life

208 individuals recruited to PAGES were tested for an association between the rs4950928 SNP and mean quality of life scores derived from the Paediatric Asthma Quality of Life Questionnaire.

We did not observe any significant association between **CHI3L1** rs4950928 and mean activity score, mean symptoms score, mean emotions score, or mean overall score) (Table 4.3.5).

<table>
<thead>
<tr>
<th></th>
<th>CHI3L1 functional promoter SNP rs4950928</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>CG/GG (n=68)</td>
<td>CC (n=143)</td>
<td>P value</td>
</tr>
<tr>
<td>Mean activity score (sd)</td>
<td>4.7 (1.7)</td>
<td>4.7 (1.7)</td>
<td>0.726</td>
<td></td>
</tr>
<tr>
<td>Mean symptoms score (sd)</td>
<td>5.0 (1.6)</td>
<td>5.2 (1.6)</td>
<td>0.460</td>
<td></td>
</tr>
<tr>
<td>Mean emotion score (sd)</td>
<td>5.4 (1.7)</td>
<td>5.4 (1.6)</td>
<td>0.596</td>
<td></td>
</tr>
<tr>
<td>Mean overall score (sd)</td>
<td>5.1 (1.6)</td>
<td>5.2 (1.5)</td>
<td>0.521</td>
<td></td>
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</tbody>
</table>

Table 4.3.5: Overall effect of **CHI3L1** functional promoter SNP rs4950928 genotype (dominant and mutant varieties) on mean quality of life scores derived from the Paediatric Asthma Quality of Life Questionnaire.

P-values were calculated by univariate analysis of variance corrected for age and sex.
CHAPTER 5

Results of **MMP9** (rs17576 & rs6073983)

SECTION 5.1

Relationship between **MMP9** SNP rs17576 and rs6073983 and asthma severity in a sample of children and young adults recruited to the BREATHE study

Sample Demographics rs17576

1452 young individuals with physician diagnosed asthma derived from both primary and secondary care were successfully genotyped for **MMP9** (rs17576) (Table 5.1.1). Individuals range from 3-22 years of age (mean 9.9 years, SD 4.1). 59.0% (n=856) are male. 34.6% (n=503) of participants were exposed to tobacco smoke in their environment over the previous six months.

Over the previous six months 44.6% (n=648) of the 1452 participants had at least one exacerbation, either requiring a hospital admission, a short course of oral steroids, an absence or a combination of these. In some participants, the asthma exacerbation(s) resulted in more than one of these events. 17.6% of participants had at least one hospital admission in the previous 6 months. 28.9% had been prescribed a short course of oral steroids. 37.0% reported absence due to asthma exacerbation.
Table 5.1.1: Characteristics of study participants with asthma and the rs17576 SNP in the BREATHE study (n=1452)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Description</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>Range, 3-22 (mean,9.9; SD, 4.1)</td>
<td></td>
</tr>
<tr>
<td>Sex (males: females)</td>
<td>856 (59.0%):596 (41.0%)</td>
<td></td>
</tr>
<tr>
<td>MMP9 rs17576(%) (AA: AG:GG)</td>
<td>582 (40.1%): 677 (46.6%): 193 (13.3%)</td>
<td></td>
</tr>
<tr>
<td>Exposure to tobacco smoke (yes/no)</td>
<td>503/949 (34.6%)</td>
<td></td>
</tr>
<tr>
<td>Reported allergy (yes/no)</td>
<td>266/1186 (18.3%)</td>
<td></td>
</tr>
<tr>
<td>Mean % predicted FEV1 (SD) (n=973)</td>
<td>95.9 (14.9)</td>
<td></td>
</tr>
<tr>
<td>Mean % predicted FVC (SD) (n=972)</td>
<td>92.9 (13.9)</td>
<td></td>
</tr>
<tr>
<td>Mean FEV1/ FVC (SD) (n=972)</td>
<td>0.87 (8.5)</td>
<td></td>
</tr>
<tr>
<td>Modified BTS asthma treatment steps * (n=1452)</td>
<td>1=276; 2=787; 3=209; 4=180</td>
<td></td>
</tr>
<tr>
<td>Inhaled bronchodilator use † (n=1452)</td>
<td>0=168; 1=974; 2=224; 3=86</td>
<td></td>
</tr>
<tr>
<td>Asthma severity score ‡ (n=1452)</td>
<td>0=34; 1=233; 2=640; 3=298; 4=171; 5=76</td>
<td></td>
</tr>
<tr>
<td>Hospital admissions (yes/ no) over previous 6 months due to exacerbations (n=1452)</td>
<td>256/1196 (17.6%)</td>
<td></td>
</tr>
<tr>
<td>Oral steroid intake (yes/ no) over previous 6 months due to exacerbations (n=1452)</td>
<td>420/1032 (28.9%)</td>
<td></td>
</tr>
<tr>
<td>Absence (yes/ no) over previous 6 months due to exacerbations (n=1452)</td>
<td>537/915 (37.0%)</td>
<td></td>
</tr>
<tr>
<td>Overall exacerbations (yes/ no) over previous 6 months ‡ (n=1452)</td>
<td>648/804 (44.6%)</td>
<td></td>
</tr>
</tbody>
</table>

Keys: *Step 1= no/occasional use of inhaled salbutamol; Step2= step1+ inhaled steroids; step 3= step2+ inhaled long acting β2 without montelukast; step 4= step 3 + montelukast
†Inhaled bronchodilator use: 0= none, 1= occasional, 2= daily, 3= excessive use
‡Asthma severity score: (bronchodilator use adjusted modified treatment steps): A scale where 0=mild and 5=severe
◊ Defined as any one of the following in previous 6 months: absence, courses of oral steroids, or hospital admissions
The 1452 recruited participants were genotyped for the missense Transition Substitution SNP rs17576. The AA genotype was present in 40.1% (n=582) of participants. 46.6% (n=677) are heterozygous for the AG genotype. The -836G allele was homozygous in 13.3% (n=193) of participants.

Among the participants with the AA/AG genotype, 45.7% (n=575) reported at least one measure for asthma exacerbations in the previous six months. 17.6% (n=221) reported hospital admission, 38.2% (n=481) absence and 29.3% (n=369) use of oral steroids (Table 5.1.3; Figure 5.1.1). 81.9% (n=1031) suffer from moderate to severe asthma (severity score steps 2-5) (Table 5.1.5). 18.1% (n=228) suffer from mild asthma (severity score steps 0&1). Among the participants with the -836G allele, 37.8% (n=73) reported an exacerbation in the previous six months. 18.1% (n=35) reported hospital admission, 29.0% (n=56) reported absence and 26.4% (n=51) reported use of oral steroids due to exacerbation of asthma. 79.8% (n=154) suffer from moderate to difficult to manage asthma (severity score steps 2-5). 20.2% (n=39) suffer from mild asthma (severity score steps 0&1).

**Sample Demographics rs6073983**

1439 young individuals with physician diagnosed asthma derived from both primary and secondary care were successfully genotyped for MMP9 (rs6073983) (Table 5.1.2). Individuals range from 3-22 years of age (mean 9.9 years, SD4.1). 59.2% (n=852) are male. 34.7% (n=499) of participants were exposed to tobacco smoke in their environment over the previous six months.

Over the previous six months 44.6% (n=642) of the 1439 participants had at least one exacerbation, either requiring a hospital admission, a short course of oral steroids, an absence or a combination of two or more of these. 17.4% of participants had at least one hospital admission in the previous 6 months. 28.9% had been prescribed a short course of oral steroids. 37.0% reported absence due to asthma exacerbation.
### Table 5.1.2: Characteristics of study participants with asthma and the rs6073983 SNP in the BREATHE study (n=1439)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>n 1439</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>Range, 3-22 (mean, 9.9; SD, 4.1)</td>
</tr>
<tr>
<td>Sex (males: females)</td>
<td>852 (59.2%):587 (40.8%)</td>
</tr>
<tr>
<td>MMP9 rs6073983(%) (AA: AT:TT)</td>
<td>863 (60.0%):499 (34.7%): 77 (5.4%)</td>
</tr>
<tr>
<td>Exposure to smoke (yes/no) (1439)</td>
<td>499/940 (34.7%)</td>
</tr>
<tr>
<td>Reported allergy (yes/no) (1439)</td>
<td>266/1173 (18.5%)</td>
</tr>
<tr>
<td>Mean % predicted FEV1 (SD) (n=966)</td>
<td>95.8 (14.8)</td>
</tr>
<tr>
<td>Mean % predicted FVC (SD) (n-965)</td>
<td>92.9 (13.9)</td>
</tr>
<tr>
<td>Mean FEV1/ FVC (SD) (n-965)</td>
<td>0.87 (8.5)</td>
</tr>
<tr>
<td>Modified BTS asthma treatment steps * (n=1439)</td>
<td>1=276; 2=778; 3=207; 4=178</td>
</tr>
<tr>
<td>Inhaled bronchodilator use † (n=1439)</td>
<td>0=167; 1=964; 2=222; 3=86</td>
</tr>
<tr>
<td>Asthma severity score ‡ (n=1398)</td>
<td>0=35; 1=232; 2=632; 3=295; 4=170; 5=75</td>
</tr>
<tr>
<td>Hospital admissions (yes/ no) over previous 6 months due to exacerbations (n=1439)</td>
<td>251/1188 (17.4%)</td>
</tr>
<tr>
<td>Oral steroid intake (yes/ no) over previous 6 months due to exacerbations (n=1439)</td>
<td>416/1023 (28.9%)</td>
</tr>
<tr>
<td>Absence (yes/ no) over previous 6 months due to exacerbations (n=1439)</td>
<td>532/907 (37.0%)</td>
</tr>
<tr>
<td>Overall exacerbations (yes/ no) over previous 6 months ◊ (n=1439)</td>
<td>642/797 (44.6%)</td>
</tr>
</tbody>
</table>

**Keys:**
*Step 1= no/occasional use of inhaled salbutamol; Step2= step1+ inhaled steroids; step 3= step2+ inhaled long acting β2 without montelukast; step 4= step 3 + montelukast

†Inhaled bronchodilator use: 0= none, 1= occasional, 2= daily, 3= excessive use

‡Asthma severity score: (bronchodilator use adjusted modified treatment steps): A scale where 0=mild and 5=severe

◊ Defined as any one of the following in previous 6 months: absence, courses of oral steroids, or hospital admissions
The 1439 recruited participants were genotyped for the transversion substitution SNP rs6073983. The AA genotype was present in 60.0% (n=863) of participants. 34.7% (n=499) are heterozygous for the AT genotype. The T allele was homozygous in 5.4% (n=77) of participants.

Among the participants with the AA/AT genotype, 45.3% (n=617) reported at least one measure for asthma exacerbations in the previous six months. 17.6% (n=239) reported hospital admission, 37.9% (n=516) absence and 29.1% (n=396) use of oral steroids (Table 5.1.4; Figure 5.1.2). 82.1% (n=1118) suffer from moderate to severe asthma (severity score steps 2-5 (Table 5.1.6). 17.9% (n=244) suffer from mild asthma (severity score steps 0&1). Among the participants homozygous for the T allele, 32.5% (n=25) reported an exacerbation in the previous six months. 15.6% (n=12) reported hospital admission, 20.8% (n=16) reported a absence and 25.9% (n=20) reported use of oral steroids due to exacerbation of asthma. 70.1% (n=54) suffer from moderate to difficult to manage asthma (severity score steps 2-5). 29.9% (n=23) suffer from mild asthma (severity score steps 0&1).
- **MMP9**(rs17576) and asthma exacerbations

<table>
<thead>
<tr>
<th></th>
<th>MMP9 SNP rs17576</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GG (n=193)</td>
</tr>
<tr>
<td>Hospital admission due to asthma exacerbations (Y)</td>
<td>35 (18.1%)</td>
</tr>
<tr>
<td>Absence due to asthma exacerbations (Y)</td>
<td>56 (29.0%)</td>
</tr>
<tr>
<td>Oral steroid intake due to asthma exacerbations (Y)</td>
<td>51 (26.4%)</td>
</tr>
<tr>
<td>Overall asthma exacerbations (Y)</td>
<td>73 (37.8%)</td>
</tr>
<tr>
<td>Modified treatment steps</td>
<td>Addition of preventer medication (Y)</td>
</tr>
<tr>
<td>Asthma severity score</td>
<td>Moderate to severe (Y)</td>
</tr>
</tbody>
</table>

Table 5.1.3: Overall effect of **MMP9** SNP rs17576 genotype (dominant and mutant varieties) on hospital admissions, oral steroid intake, absences due to asthma exacerbations, overall asthma exacerbations, modified treatment steps and asthma severity in children and young adults recruited to the BREATHE study

Key: *: p<0.05; OR: Odds ratio; CI: Confidence interval

P values were calculated by binary logistic regression corrected for age and sex.
The homozygous G allele was associated with absence due to exacerbations ($p=0.007$) and overall exacerbations ($p=0.018$). When compared to the dominant A allele (AA/AG) the odds ratio for GG in absence was 0.63, and in overall exacerbations was 0.68 indicating that the minor allele (GG) may have a protective role reducing the number of asthma exacerbations, particularly those requiring absence (Table 5.1.3/ Figure 5.1.1).

We did not observe any association between the risk allele and hospital admissions due to exacerbations or oral steroid intake due to asthma exacerbations over the previous six months.

Through proxy measures, we tested for gene-dosage effect using the modified BTS treatment steps, categorised into salbutamol and addition of preventer medication. We also tested for an association with risk of severity using the asthma severity score, categorised into mild and moderate to severe (Table 5.1.3). However, no significant effect was observed. We subsequently categorised by age and repeated the analysis, however we found no association, this data is not included.
Figure 5.1.1 Illustration of Overall effect of *MMP9* rs17576 genotype (dominant and mutant varieties) on hospital admissions, oral steroid intake and absences due to asthma exacerbations, and overall asthma exacerbations, in children and young adults recruited to the BREATHE study.
Table 5.1.4: Overall effect of MMP9 SNP rs17576 genotype (dominant and mutant varieties) on hospital admissions, oral steroid intake, absences due to asthma exacerbations, and overall asthma exacerbations in age categorised children and young adults recruited to the BREATHE study

<table>
<thead>
<tr>
<th>Age</th>
<th>Hospital admission</th>
<th>Absence</th>
<th>Oral steroid intake</th>
<th>Overall asthma exacerbations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GG</td>
<td>AA/AG</td>
<td>GG</td>
<td>AA/AG</td>
</tr>
<tr>
<td>3-4 years</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Yes (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>12 (37.5)</td>
<td>54 (31.2)</td>
<td>15 (46.9)</td>
<td>90 (52.0)</td>
</tr>
<tr>
<td></td>
<td>n</td>
<td>32</td>
<td>173</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>OR (95%CI)</td>
<td>1.34 (0.61-2.94)</td>
<td>0.81 (0.38-1.73)</td>
<td>1.35 (0.64-2.88)</td>
</tr>
<tr>
<td></td>
<td>P Value</td>
<td>0.470</td>
<td>0.588</td>
<td>0.433</td>
</tr>
<tr>
<td>5-12 years</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Yes (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>22 (19.1)</td>
<td>144 (18.2)</td>
<td>36 (31.3)</td>
<td>320 (40.5)</td>
</tr>
<tr>
<td></td>
<td>n</td>
<td>115</td>
<td>790</td>
<td>115</td>
</tr>
<tr>
<td></td>
<td>OR (95%CI)</td>
<td>1.06 (0.64-1.75)</td>
<td>0.67 (0.44-1.02)</td>
<td>0.90 (0.58-1.39)</td>
</tr>
<tr>
<td></td>
<td>P Value</td>
<td>0.817</td>
<td>0.062</td>
<td>0.622</td>
</tr>
<tr>
<td>13-22 years</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Yes (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 (2.2)</td>
<td>23 (7.8)</td>
<td>5 (10.9)</td>
<td>71 (23.9)</td>
</tr>
<tr>
<td></td>
<td>n</td>
<td>46</td>
<td>296</td>
<td>46</td>
</tr>
<tr>
<td></td>
<td>OR (95%CI)</td>
<td>0.25 (0.03-1.93)</td>
<td>0.38 (0.14-0.99)</td>
<td>0.28 (0.08-0.92)</td>
</tr>
<tr>
<td></td>
<td>P Value</td>
<td>0.186</td>
<td>0.048*</td>
<td>0.036*</td>
</tr>
</tbody>
</table>

Key: *: p<0.05; OR: Odds ratio; CI: Confidence interval

P values were calculated by binary logistic regression corrected for age and sex.
In the 13-22 years age group the homozygous G allele was associated with absence due to asthma exacerbations ($p=0.048$), oral steroid intake due to asthma exacerbations ($p=0.036$) and overall exacerbations ($p=0.006$). When compared to the dominant A allele (AA/AG) the odds ratio for GG in absence was 0.38, in oral steroid intake was 0.28, and in overall exacerbations was 0.23 indicating that the minor allele (GG) may have a protective role reducing the number of asthma exacerbations (Table 5.1/ Figure 5.1.2).

We did not observe any association between the risk allele and hospital admissions due to exacerbations or oral steroid intake due to asthma exacerbations over the previous six months.
Figure 5.1.2 Illustration of Overall effect of \textit{MMP9} rs17576 genotype (dominant and mutant varieties) on hospital admissions, oral steroid intake and absences due to asthma exacerbations, and overall asthma exacerbations, in 13-22 year old participants recruited to the BREATHE study
**MMP9(rs607393) and asthma exacerbations**

<table>
<thead>
<tr>
<th></th>
<th>MMP9 SNP rs6073983</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TT (n=77)</td>
</tr>
<tr>
<td>Hospital admission due to asthma exacerbations (Y)</td>
<td>12 (15.6%)</td>
</tr>
<tr>
<td>Absence due to asthma exacerbations (Y)</td>
<td>16 (20.8%)</td>
</tr>
<tr>
<td>Oral steroid intake due to asthma exacerbations (Y)</td>
<td>20 (25.9%)</td>
</tr>
<tr>
<td>Overall asthma exacerbations (Y)</td>
<td>25 (32.5%)</td>
</tr>
<tr>
<td>Modified treatment steps</td>
<td>Addition of preventer medication (Y)</td>
</tr>
<tr>
<td>Asthma severity score</td>
<td>Moderate to severe (Y)</td>
</tr>
</tbody>
</table>

**Table 5.1.5:** Overall effect of MMP9 SNP rs6073983 genotype (dominant and mutant varieties) on hospital admissions, oral steroid intake, absences due to asthma exacerbations, overall asthma exacerbations, modified treatment steps and asthma severity in children and young adults

Key: *: p<0.05; OR: Odds ratio; CI: Confidence interval
P values were calculated by binary logistic regression corrected for age and sex
The homozygous T allele was associated with absence due to exacerbations (p=0.002) and overall exacerbations (p=0.014). When compared to the dominant A allele (AA/AT) the odds ratio for TT in absence was 0.39, and in overall exacerbations was 0.53 indicating that the minor allele (TT) may have a protective role reducing the number of asthma exacerbations, particularly those requiring absence (Table 5.1.5/ Figure 5.1.3).

We did not observe any association between the risk allele and hospital admissions due to exacerbations or oral steroid intake due to asthma exacerbations over the previous six months. We subsequently categorised by age and repeated the analysis, however no association was identified, this data is not included.

Through proxy measures, we tested for gene-dosage effect using the modified BTS treatment steps, categorised into salbutamol and addition of preventer medication. We also tested for an association with risk of severity using the asthma severity score, categorised into mild and moderate to severe for rs6073983 (Table 5.1.5; Figure 5.1.4). The homozygous T allele was associated with reduction of preventer medications in modified treatment steps (p=0.004) and moderate to severe asthma as classified by the asthma severity score (p=0.006). When compared to the dominant A allele (AA/AT) the odds ratio for TT in modified treatment steps was 0.48, and in severity was 0.49 indicating that the minor allele (TT) may have a protective role in a gene-dose relationship and with drug associated severity with this SNP.
Figure 5.1.3 Illustration of Overall effect of *MMP9* rs6073983 genotype on hospital admissions, oral steroid intake and absences due to asthma exacerbations, and overall asthma exacerbations, in children and young adults recruited to the BREATHE study.
Figure 5.1.4 Illustration of Overall effect of MMP9 SNP rs6073983 genotype (dominant and mutant varieties) on modified treatment steps and asthma severity in children and young adults recruited to the BREATHE study.
<table>
<thead>
<tr>
<th></th>
<th>MMP9 SNP rs6073983</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TT</td>
<td>AA/AT</td>
<td>OR (95%CI)</td>
<td>P value</td>
<td></td>
</tr>
<tr>
<td>3-4 years</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Modified treatment steps</td>
<td>Addition of preventer medication (Y)</td>
<td>10/12 (83.3%)</td>
<td>165/188 (87.8%)</td>
<td>0.68 (0.14-3.31)</td>
<td>0.631</td>
</tr>
<tr>
<td>Asthma severity score</td>
<td>Moderate to severe (Y)</td>
<td>10/12 (83.3%)</td>
<td>166/188 (88.3%)</td>
<td>0.65 (0.13-3.18)</td>
<td>0.597</td>
</tr>
<tr>
<td>5-12 years</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Modified treatment steps</td>
<td>Addition of preventer medication (Y)</td>
<td>34/47 (72.3%)</td>
<td>709/851 (83.3%)</td>
<td>0.53 (0.27-1.02)</td>
<td>0.059</td>
</tr>
<tr>
<td>Asthma severity score</td>
<td>Moderate to severe (Y)</td>
<td>35/47 (74.5%)</td>
<td>715/851 (84.0%)</td>
<td>0.56 (0.28-1.10)</td>
<td>0.093</td>
</tr>
<tr>
<td>13-22 years</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Modified treatment steps</td>
<td>Addition of preventer medication (Y)</td>
<td>9/18 (50.0%)</td>
<td>236/323 (73.1%)</td>
<td>0.36 (0.14-0.95)</td>
<td>0.039*</td>
</tr>
<tr>
<td>Asthma severity score</td>
<td>Moderate to severe (Y)</td>
<td>9/18 (50.0%)</td>
<td>237/323 (73.4%)</td>
<td>0.36 (0.14-0.93)</td>
<td>0.036*</td>
</tr>
</tbody>
</table>

Table 5.1.6 Overall effect of MMP9 SNP rs6073983 genotype (dominant and mutant varieties) on modified treatment steps and asthma severity on age categorised participants recruited to the BREATHE study

Key: *: p<0.05; OR: Odds ratio; CI: Confidence interval

Modified treatment steps: Salbutamol only-Step 1; Addition of preventer medication- Step2-4
Asthma severity score: (bronchodilator use adjusted Modified treatment steps): 0&1-mild, 2=moderate to severe
P-values and odds ratios were calculated by binary logistic regression corrected for age and sex
Through proxy measures, we tested for gene-dosage effect using the modified BTS treatment steps, categorised into salbutamol and addition of preventer medication. We also tested for an association with risk of severity using the asthma severity score, categorised into mild and moderate to severe for rs6073983, categorised by age (Table 5.1.6; Figure 5.1.5). In 13-22 year olds the homozygous T allele was associated with reduction of preventer medications in modified treatment steps \((p=0.039)\) and moderate to severe asthma as classified by the asthma severity score \((p=0.036)\). When compared to the dominant A allele (AA/AT) the odds ratio for TT in modified treatment steps was 0.36, and in severity was 0.36 indicating that the minor allele (TT) may have a protective role in a gene-dose relationship and with drug associated severity with this SNP.
Figure 5.1.5 Illustration of Overall effect of MMP9 SNP rs6073983 genotype (dominant and mutant varieties) on modified treatment steps and asthma severity in 13-22 year old participants recruited to the BREATHE study.
SECTION 5.2

Relationship between *MMP9* SNP rs17576 and rs6073983 and measures of pulmonary function in a sample of UK children and young adults recruited to the BREATHE study and PAGES

*MMP9* (rs17576) and pulmonary function

<table>
<thead>
<tr>
<th></th>
<th><em>MMP9</em> SNP rs17576</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>GG</td>
<td>AA/AG</td>
<td>n</td>
<td>P value</td>
</tr>
<tr>
<td>Mean ppPEFR [n] (sd)</td>
<td>[139]</td>
<td>87.9 (16.1)</td>
<td>[958]</td>
<td>87.4 (17.2)</td>
<td>1101</td>
</tr>
<tr>
<td>Mean ppFEV1 [n] (sd)</td>
<td>[139]</td>
<td>96.4 (14.8)</td>
<td>[980]</td>
<td>96.1 (15.5)</td>
<td>1123</td>
</tr>
<tr>
<td>Mean ppFVC [n] (sd)</td>
<td>[139]</td>
<td>93.8 (15.5)</td>
<td>[979]</td>
<td>94.8 (14.8)</td>
<td>1122</td>
</tr>
<tr>
<td>FEV1/FVC [n] (sd)</td>
<td>[139]</td>
<td>86.8 (8.7)</td>
<td>[979]</td>
<td>86.2 (8.8)</td>
<td>1122</td>
</tr>
<tr>
<td>Mean change in ppFEV1 post</td>
<td>[13]</td>
<td>5.7 (5.2)</td>
<td>[116]</td>
<td>5.0 (6.5)</td>
<td>129</td>
</tr>
<tr>
<td>bronchodilator [n] (sd)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean Nitric oxide [n] (ppb)</td>
<td>[17]</td>
<td>28.1 (21.4)</td>
<td>[144]</td>
<td>35.5 (33.7)</td>
<td>161</td>
</tr>
<tr>
<td>(sd)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Nitric oxide categorised by clinical relevance

|                              |          |          |          |          |          |
| Clinically elevated exhaled nitric oxide (>50ppb) (Y) | 4/22   | (22.7%) | 50/195  | (25.6%) | 54   | 0.791    |
| Elevated exhaled nitric oxide (25-49ppb) (Y)         | 5/22  | (18.2%) | 35/195  | (17.9%) | 40   | 0.581    |

Table 5.2.1: Overall effect of *MMP9* SNP rs17576 genotype (dominant and mutant varieties) on mean % predicted lung function in participants recruited to the BREATHE study and PAGES

Key: pp: % predicted; ppb: parts per billion; FEV1: Forced expiratory volume in 1 second; FVC: Forced vital capacity P-values were calculated by univariate analysis of variance corrected for sex

P-values for Nitric oxide categorised by clinical relevance were calculated by multinomial logistic regression corrected for age and sex

We tested for an effect between *MMP9* rs17576 and measures of pulmonary function (Table 5.2.1). No significant effect was observed with any investigated measure of pulmonary function.
**MMP9 (rs6073983) and pulmonary function**

<table>
<thead>
<tr>
<th>MMP9 SNP rs6073983</th>
<th>TT</th>
<th>AA/AT</th>
<th>n</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ppPEFR [n] (sd)</td>
<td>[56] 84.9 (17.4)</td>
<td>[1030] 87.5 (17.1)</td>
<td>1086</td>
<td>0.314</td>
</tr>
<tr>
<td>Mean ppFEV1 [n] (sd)</td>
<td>[56] 95.0 (15.1)</td>
<td>[1051] 96.1 (15.4)</td>
<td>1111</td>
<td>0.664</td>
</tr>
<tr>
<td>Mean ppFVC [n] (sd)</td>
<td>[56] 92.7 (14.8)</td>
<td>[1050] 94.7 (14.9)</td>
<td>1110</td>
<td>0.385</td>
</tr>
<tr>
<td>FEV1/FVC [n] (sd)</td>
<td>[56] 87.2 (8.5)</td>
<td>[1050] 86.2 (8.8)</td>
<td>1110</td>
<td>0.490</td>
</tr>
<tr>
<td>Mean change in ppFEV1 post bronchodilator [n] (sd)</td>
<td>[4] 7.00 (5.2)</td>
<td>[120] 5.0 (6.5)</td>
<td>124</td>
<td>0.235</td>
</tr>
<tr>
<td>Mean Nitric oxide [n] (ppb) (sd)</td>
<td>[7] 25.8 (19.0)</td>
<td>[147] 34.9 (33.4)</td>
<td>154</td>
<td>0.502</td>
</tr>
</tbody>
</table>

Nitric oxide categorised by clinical relevance

| Clinically elevated exhaled nitric oxide (>50ppb) (Y) | 1/7 (14.3%) | 50/147 (32.0%) | 51 | 0.260   |
| Elevated exhaled nitric oxide (25-49ppb) (Y) | 3/7 (42.9%) | 36/147 (24.4%) | 39 | 0.680   |

**Table 5.2.2: Overall effect of MMP9 SNP rs6073983 genotype (dominant and mutant varieties) on mean % predicted lung function in participants recruited to the BREATHE study and PAGES**

Key: pp: % predicted; ppb: parts per billion; FEV1: Forced expiratory volume in 1 second; FVC: Forced vital capacity
P-values were calculated by univariate analysis of variance corrected for sex
P-values for Nitric oxide categorised by clinical relevance were calculated by multinomial logistic regression corrected for age and sex

We tested for an effect between MMP9 rs6073983 and measures of pulmonary function (Table 5.2.2). No significant effect was observed with any investigated measure of pulmonary function.
**MMP9 (rs17576) and pulmonary function categorised by age**

<table>
<thead>
<tr>
<th><strong>MMP9 SNP rs17576</strong></th>
<th>5-12 years</th>
<th>P value</th>
<th>13-22 years</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GG (sd)</td>
<td>AA/AG (sd)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ppPEFR</td>
<td>85.0 (15.4)</td>
<td>84.9 (16.5)</td>
<td>0.656</td>
<td>94.2 (16.0)</td>
</tr>
<tr>
<td>n</td>
<td>94</td>
<td>664</td>
<td>-</td>
<td>45</td>
</tr>
<tr>
<td>Mean ppFEV1</td>
<td>95.5 (15.1)</td>
<td>95.7 (14.6)</td>
<td>0.731</td>
<td>98.3 (14.3)</td>
</tr>
<tr>
<td>Mean ppFVC</td>
<td>92.5 (15.0)</td>
<td>93.6 (13.8)</td>
<td>0.636</td>
<td>96.7 (16.2)</td>
</tr>
<tr>
<td>FEV1/FVC (sd)</td>
<td>86.3 (8.8)</td>
<td>86.4 (8.5)</td>
<td>0.993</td>
<td>88.0 (8.3)</td>
</tr>
<tr>
<td>n</td>
<td>94</td>
<td>680</td>
<td>-</td>
<td>45</td>
</tr>
<tr>
<td>Mean change in ppFEV1</td>
<td>6.3 (5.6)</td>
<td>5.0 (6.1)</td>
<td>0.462</td>
<td>3.7 (3.4)</td>
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<td>post bronchodilator</td>
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<td>(sd)</td>
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<tr>
<td>n</td>
<td>10</td>
<td>88</td>
<td>-</td>
<td>3</td>
</tr>
<tr>
<td>Nitric oxide (ppb)</td>
<td>28.5 (22.8)</td>
<td>32.9 (31.8)</td>
<td>0.713</td>
<td>26.3 (17.3)</td>
</tr>
<tr>
<td>(sd)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>14</td>
<td>106</td>
<td>-</td>
<td>3</td>
</tr>
</tbody>
</table>

**Table 5.2.3: Overall effect of MMP9 SNP rs17576 genotype (dominant and mutant varieties) on mean % predicted lung function categorised by age in participants recruited to the BREATHE study and PAGES**

Key: pp: % predicted; ppb: parts per billion; FEV1: Forced expiratory volume in 1 second; FVC: Forced vital capacity

P-values were calculated by univariate analysis of variance corrected for sex

We tested for an effect between MMP9 rs17576 and measures of pulmonary function, categorised by age (Table 5.2.3). No significant effect was observed with any investigated measures of pulmonary function.
MMP9 (rs6073983) and pulmonary function categorised by age

Table 5.2.4: Overall effect of MMP9 SNP rs6073983 genotype (dominant and mutant varieties) on mean % predicted lung function categorised by age in participants recruited to the BREATHE study and PAGES

<table>
<thead>
<tr>
<th>MMP9 SNP rs6073983</th>
<th>5-12 years</th>
<th>P value</th>
<th>13-22 years</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TT</td>
<td>AA/AT</td>
<td>TT</td>
<td>AA/AT</td>
</tr>
<tr>
<td>Mean ppPEFR (sd)</td>
<td>79.9 (14.9)</td>
<td>85.1 (16.4)</td>
<td>0.085</td>
<td>93.9 (18.1)</td>
</tr>
<tr>
<td>n</td>
<td>36</td>
<td>713</td>
<td>-</td>
<td>56</td>
</tr>
<tr>
<td>Mean ppFEV1 (sd)</td>
<td>94.0 (15.1)</td>
<td>95.7 (14.6)</td>
<td>0.485</td>
<td>96.8 (15.5)</td>
</tr>
<tr>
<td>Mean ppFVC (sd)</td>
<td>91.8 (12.9)</td>
<td>93.5 (14.1)</td>
<td>0.460</td>
<td>94.3 (17.9)</td>
</tr>
<tr>
<td>FEV1/FVC (sd)</td>
<td>86.4 (8.9)</td>
<td>86.4 (8.5)</td>
<td>0.890</td>
<td>88.6 (7.6)</td>
</tr>
<tr>
<td>n</td>
<td>36</td>
<td>729</td>
<td>-</td>
<td>20</td>
</tr>
<tr>
<td>Mean change in ppFEV1 post bronchodilator (sd)</td>
<td>8.5 (8.3)</td>
<td>5.0 (0.4)</td>
<td>0.433</td>
<td>5.5 (1.7)</td>
</tr>
<tr>
<td>n</td>
<td>2</td>
<td>93</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>Nitric oxide (ppb) (sd)</td>
<td>22.6 (18.7)</td>
<td>32.4 (31.5)</td>
<td>0.395</td>
<td>45.0 (-)</td>
</tr>
<tr>
<td>n</td>
<td>6</td>
<td>110</td>
<td>-</td>
<td>1</td>
</tr>
</tbody>
</table>

Key: pp: % predicted; ppb: parts per billion; FEV1: Forced expiratory volume in 1 second; FVC: Forced vital capacity
P-values were calculated by univariate analysis of variance corrected for sex

We tested for an effect between MMP9 rs6073983 and measures of pulmonary function, categorised by age (Table 5.2.4). No significant effect was observed with any investigated measures of pulmonary function.
SECTION 5.3

Relationship between *MMP9* (rs17576 & rs6073983) and environmental factors in a sample of UK children and young adults

*MMP9* (rs17576) and exposure to environmental tobacco smoke

1452 individuals recruited to the BREATHE study were tested for an interaction between the rs17576 genotype and environmental tobacco smoke on exacerbations in individuals with asthma. The dominant A allele (AA/AG) was present in 86.7% (n=1259) of participants. 33.96% (n=428) were exposed to environment tobacco smoke. The -836G allele (GG) was homozygous in 13.3% (n=193) of participants. 38.9% (n=75) were exposed to environmental tobacco smoke.

When compared to the dominant A allele (AA/AG) the homozygous G allele was associated with oral steroids intake for asthma exacerbations when exposed to environmental tobacco smoke (p=0.009; OR 0.44) (Table 5.3.1; Figure 5.3.1). We did not observe any association between the SNP and overall exacerbations, hospital admissions due to exacerbations or absence due to exacerbations when exposed to environmental tobacco smoke over the previous six months. We tested the gene-dosage effect for the risk allele for each modified treatment step and severity score step for long-term control of asthma (Table 5.3.1). However, no significant effect was observed. No significant effect was observed when the risk allele was tested for each severity step. We subsequently categorised by age and repeated the analysis, however no association was identified, this data is not included.
### Table 5.3.1: Overall effect of MMP9 SNP rs17576 genotype (dominant and mutant varieties) on hospital admissions, oral steroid intake and absences due to asthma exacerbations, overall asthma exacerbations and asthma severity, categorised by exposure to tobacco smoke in children and young adults recruited to the BREATHE study

<table>
<thead>
<tr>
<th></th>
<th>Exposed to tobacco smoke</th>
<th>Not exposed to tobacco smoke</th>
<th>P Value for interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GG (n=75)</td>
<td>AA/AG (n=428)</td>
<td>OR (95%CI)</td>
</tr>
<tr>
<td>Hospital admission due to asthma exacerbations (Y)</td>
<td>13 (17.3%)</td>
<td>95 (22.2%)</td>
<td>0.61 (0.32-1.18)</td>
</tr>
<tr>
<td>Absence due to asthma exacerbations (Y)</td>
<td>24 (32.0%)</td>
<td>189 (44.2%)</td>
<td>0.51 (0.30-0.87)</td>
</tr>
<tr>
<td>Oral steroid intake due to asthma exacerbations (Y)</td>
<td>16 (21.3%)</td>
<td>148 (34.6%)</td>
<td>0.44 (0.24-0.80)</td>
</tr>
<tr>
<td>Overall asthma exacerbations (Y)</td>
<td>28 (37.3%)</td>
<td>205 (47.9%)</td>
<td>0.45 (0.27-0.76)</td>
</tr>
<tr>
<td>Asthma severity score</td>
<td>Moderate to severe (Y)</td>
<td>62 (82.7%)</td>
<td>345 (80.6%)</td>
</tr>
</tbody>
</table>

Key: *: p<0.05; OR: Odds ratio; CI: Confidence interval
Asthma severity score: (bronchodilator use adjusted modified treatment steps): 0&1=mild, 2-5=moderate to severe
P values and odds ratios for exacerbations were calculated by binary logistic regression corrected for age, and sex
Figure 5.3.1 Illustration of Overall effect of MMP9 SNP rs17576 genotype (dominant and mutant varieties) on hospital admissions, oral steroid intake and absences due to asthma exacerbations, and overall asthma exacerbations, categorised by exposure to environmental tobacco smoke (ETS- = not exposed; ETS+ = exposed) in children and young adults recruited to the BREATHE study.
Table 5.3.2: Overall effect of MMP9 SNP rs17576 genotype (dominant and mutant varieties) on mean % predicted lung function categorised by age and exposure to environmental tobacco smoke in children and young adults recruited to the BREATHE study and PAGES

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Exposed to environmental tobacco smoke</th>
<th>Not exposed to environmental tobacco smoke</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5-12</td>
<td>P Value</td>
</tr>
<tr>
<td>MMP9 rs17576</td>
<td>GG</td>
<td>AA/AG</td>
</tr>
<tr>
<td>Mean ppPEFR (sd)</td>
<td>83.4 (16.2)</td>
<td>84.8 (16.9)</td>
</tr>
<tr>
<td>Mean ppFEV1 (sd)</td>
<td>94.7 (15.9)</td>
<td>95.6 (15.7)</td>
</tr>
<tr>
<td>Mean ppFVC (sd)</td>
<td>93.1 (14.3)</td>
<td>93.8 (14.8)</td>
</tr>
<tr>
<td>FEV1/FVC (sd)</td>
<td>86.2 (10.2)</td>
<td>86.1 (8.7)</td>
</tr>
<tr>
<td>N</td>
<td>32</td>
<td>195</td>
</tr>
</tbody>
</table>

Table 5.3.2: Overall effect of MMP9 SNP rs17576 genotype (dominant and mutant varieties) on mean % predicted lung function categorised by age and exposure to environmental tobacco smoke in children and young adults recruited to the BREATHE study and PAGES

Key: pp: % predicted; ppb: parts per billion; FEV1: Forced expiratory volume in 1 second; FVC: Forced vital capacity

P-values were calculated by univariate analysis of variance corrected for sex

We tested for an interaction between MMP9 rs17576 and measures of pulmonary function, categorised by age and exposure to environmental tobacco smoke. Bronchodilator reversibility and exhaled nitric oxide were excluded due to insufficient cases (Table 5.3.2). No significant effect was observed with any investigated measures of pulmonary function.
**MMP9 (rs6073983) and exposure to environmental tobacco smoke**

1439 individuals recruited to the BREATHE study were tested for an interaction between the rs6073983 genotype and environmental tobacco smoke on exacerbations in individuals with asthma. The dominant A allele (AA/AT) was present in 94.6% (n=1362) of participants. 34.2% (n=466) were exposed to environmental tobacco smoke. The T allele (TT) was homozygous in 5.4% (n=77) of participants. 42.9% (n=33) were exposed to environmental tobacco smoke.

When compared to the dominant A allele (AA/AT) the homozygous T allele (TT) was associated with intake of oral steroids due to asthma exacerbations when exposed to environmental tobacco smoke over the previous six months (p=0.032; OR 0.38) (Table 5.3.3; Figure 5.3.2). We did not observe any association between the SNP and overall exacerbations, hospital admissions due to exacerbations or absence due to exacerbations when exposed to environmental tobacco smoke over the previous six. We tested the gene-dosage effect for the risk allele for each modified treatment step and severity score step for long-term control of asthma (Table 5.3.3). However, no significant effect was observed. No significant effect was observed when the risk allele was tested for each severity step. We subsequently categorised by age and repeated the analysis, however no association was identified, this data is not included.
<table>
<thead>
<tr>
<th>MMP9 SNP rs6073983</th>
<th>Exposed to tobacco smoke</th>
<th>Not exposed to tobacco smoke</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TT (n=33)</td>
<td>AA/AT (n=466)</td>
</tr>
<tr>
<td>Hospital admission due to asthma exacerbations (Y)</td>
<td>4 (12.1%)</td>
<td>103 (22.1%)</td>
</tr>
<tr>
<td>Absence due to asthma exacerbations (Y)</td>
<td>7 (21.2%)</td>
<td>205 (43.9%)</td>
</tr>
<tr>
<td>Oral steroid intake due to asthma exacerbations (Y)</td>
<td>6 (18.2%)</td>
<td>157 (33.7%)</td>
</tr>
<tr>
<td>Overall asthma exacerbations (Y)</td>
<td>9 (27.3%)</td>
<td>241 (51.7%)</td>
</tr>
<tr>
<td>Asthma severity score</td>
<td>Moderate to severe (Y)</td>
<td>26 (78.8%)</td>
</tr>
</tbody>
</table>

Table 5.3.3: Overall effect of MMP9 SNP rs6073983 genotype (dominant and mutant varieties) on hospital admissions, oral steroid intake and absences due to asthma exacerbations, overall asthma exacerbations and asthma severity, categorised by exposure to tobacco smoke in children and young adults recruited to the BREATHE study.

Key: *: p<0.05; OR: Odds ratio; CI: Confidence interval

Asthma severity score: (bronchodilator use adjusted modified treatment steps): 0&1-mild, 2-5-moderate to severe

P values and odds ratios for exacerbations were calculated by binary logistic regression corrected for age, and sex.
Figure 5.3.2 Illustration of Overall effect of MMP9 SNP rs6073983 genotype (dominant and mutant varieties) on hospital admissions, oral steroid intake and absences due to asthma exacerbations, and overall asthma exacerbations, categorised by exposure to environmental tobacco smoke (ETS- = not exposed; ETS+ = exposed) in children and young adults recruited to the BREATHE study and PAGES.
Table 5.3.4: Overall effect of MMP9 SNP rs6073983 genotype (dominant and mutant varieties) on mean % predicted lung function categorised by age and exposure to environmental tobacco smoke recruited to the BREATHE study and PAGES

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Exposed to environmental tobacco smoke</th>
<th></th>
<th>Not exposed to environmental tobacco smoke</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5-12</td>
<td>13-22</td>
<td>P Value</td>
<td>5-12</td>
</tr>
<tr>
<td>MMP9 SNP rs6073983</td>
<td>TT</td>
<td>AA/AT</td>
<td>0.062</td>
<td>TT</td>
</tr>
<tr>
<td>Mean ppPEFR</td>
<td>77.3 (13.1)</td>
<td>85.1 (16.9)</td>
<td>89.3 (20.3)</td>
<td>89.1 (19.5)</td>
</tr>
<tr>
<td>N</td>
<td>16</td>
<td>209</td>
<td>8</td>
<td>125</td>
</tr>
<tr>
<td>Mean ppFEV1</td>
<td>94.0 (14.5)</td>
<td>95.5 (15.8)</td>
<td>98.2 (24.0)</td>
<td>95.3 (20.8)</td>
</tr>
<tr>
<td>N</td>
<td>16</td>
<td>210</td>
<td>8</td>
<td>127</td>
</tr>
<tr>
<td>Mean ppFVC</td>
<td>92.2 (10.8)</td>
<td>93.8 (15.0)</td>
<td>100.9 (24.3)</td>
<td>98.4 (19.4)</td>
</tr>
<tr>
<td>N</td>
<td>16</td>
<td>210</td>
<td>8</td>
<td>127</td>
</tr>
<tr>
<td>FEV1/FVC</td>
<td>86.3 (8.8)</td>
<td>86.0 (8.9)</td>
<td>84.7 (6.4)</td>
<td>83.3 (10.4)</td>
</tr>
</tbody>
</table>

Table 5.3.4: Overall effect of MMP9 SNP rs6073983 genotype (dominant and mutant varieties) on mean % predicted lung function categorised by age and exposure to environmental tobacco smoke recruited to the BREATHE study and PAGES

Key: pp: % predicted; FEV1: Forced expiratory volume in 1 second; FVC: Forced vital capacity

P-values were calculated by univariate analysis of variance corrected for age and sex

We tested for an interaction between MMP9 rs6073983 and measures of pulmonary function, categorised by age and exposure to environmental tobacco smoke. Bronchodilator reversibility and exhaled nitric oxide were excluded due to insufficient cases (Table 5.3.4). No significant effect was observed with any investigated measures of pulmonary function.
**MMP9 (rs17576) and atopy**

1576 individuals recruited to the BREATHE study and PAGES were tested for an association between the rs17576 SNP and any reported allergies (Table 5.3.5). 329 individuals recruited to PAGES were tested for an association between the rs17576 SNP and any confirmed sensitivity to a selection of tested allergens.

We did not observe any significant association between rs17576 and reported allergy or confirmed sensitivity.

<table>
<thead>
<tr>
<th><strong>MMP9 SNP rs175763</strong></th>
<th>GG</th>
<th>AA/AG</th>
<th>OR (95% CI)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reported allergy (Y)</td>
<td>28/194 (14.4%)</td>
<td>262/1382 (18.9%)</td>
<td>0.72 (0.47-1.10)</td>
<td>0.133</td>
</tr>
<tr>
<td>Positive SPT result to any allergen (Y)</td>
<td>8/33 (24.2%)</td>
<td>75/296 (25.3%)</td>
<td>0.93 (0.39-2.19)</td>
<td>0.871</td>
</tr>
</tbody>
</table>

**Table 5.3.5: Overall effect of MMP9 SNP rs17576 genotype (dominant and mutant varieties) on participant reported allergy and skin prick test identified sensitivity**

Key: OR: Odds ratio; CI: Confidence interval
P-values and odds ratios were calculated by binary logistic regression corrected for age and sex
MMP9 (rs6073983) and atopy

1853 individuals recruited to the BREATHE study and PAGES were tested for an association between the rs6073983 SNP and any reported (Table 5.3.6). 414 individuals recruited to PAGES were tested for an association between the rs6073983 SNP and any confirmed sensitivity to a selection of tested allergens.

We did not observe any significant association between rs6073983 and reported allergy or confirmed sensitivity.

<table>
<thead>
<tr>
<th>MMP9 SNP rs6073983</th>
<th>TT</th>
<th>AA/AT</th>
<th>OR (95% CI)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reported allergy (Y)</td>
<td>16/93 (17.2%)</td>
<td>318/1760 (18.1%)</td>
<td>0.95 (0.55-1.65)</td>
<td>0.856</td>
</tr>
<tr>
<td>Positive SPT result to any allergen (Y)</td>
<td>2/16 (12.5%)</td>
<td>80/398 (20.1%)</td>
<td>0.56 (0.12-2.77)</td>
<td>0.500</td>
</tr>
</tbody>
</table>

Table 5.3.6: Overall effect of MMP9 SNP rs6073983 genotype (dominant and mutant varieties) on participant reported allergy and skin prick test identified sensitivity

Key: OR: Odds ratio; CI: Confidence interval; SPT: Skin prick test
P-values and odds ratios were calculated by binary logistic regression corrected for age and sex.
**MMP9 (rs17576) and quality of life**

214 individuals recruited to PAGES were tested for an association between the rs17576 SNP and mean quality of life scores derived from the Paediatric Asthma Quality of Life Questionnaire.

We did not observe any significant association between rs17576 and mean activity score, mean symptoms score, mean emotions score or mean overall score (Table 5.3.7).

<table>
<thead>
<tr>
<th></th>
<th>MMP9 SNP rs17576</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GG (n=26)</td>
<td>AA/AG (n=191)</td>
<td>P value</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean activity score (sd)</td>
<td>4.9 (1.7)</td>
<td>4.7 (1.7)</td>
<td>0.489</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean symptoms score (sd)</td>
<td>5.5 (1.4)</td>
<td>5.1 (1.6)</td>
<td>0.500</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean emotion score (sd)</td>
<td>5.4 (1.7)</td>
<td>5.4 (1.6)</td>
<td>0.132</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean overall score (sd)</td>
<td>5.3 (1.5)</td>
<td>5.1 (1.6)</td>
<td>0.281</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 5.3.7: Overall effect of **MMP9 SNP rs17576** genotype (dominant and mutant varieties) on mean quality of life scores derived from the Paediatric Asthma Quality of Life Questionnaire.

P-values were calculated by univariate analysis of variance corrected for age and sex
**MMP9 (rs6073983) and quality of life**

205 individuals recruited to PAGES were tested for an association between the rs6073983 SNP and mean quality of life scores derived from the Paediatric Asthma Quality of Life Questionnaire.

We did not observe any significant association between rs6073983 and mean activity score, mean symptoms score, mean emotions score or mean overall score (Table 5.3.8).

<table>
<thead>
<tr>
<th>MMP9 SNP rs6073983</th>
<th>TT (n=9)</th>
<th>AA/AT (n=199)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean activity score (sd)</td>
<td>5.0 (1.7)</td>
<td>4.7 (1.7)</td>
<td>0.759</td>
</tr>
<tr>
<td>Mean symptoms score (sd)</td>
<td>5.7 (0.9)</td>
<td>5.1 (1.6)</td>
<td>0.313</td>
</tr>
<tr>
<td>Mean emotion score (sd)</td>
<td>5.7 (1.5)</td>
<td>5.4 (1.6)</td>
<td>0.820</td>
</tr>
<tr>
<td>Mean overall score (sd)</td>
<td>5.5 (1.2)</td>
<td>5.1 (1.6)</td>
<td>0.622</td>
</tr>
</tbody>
</table>

Table 5.3.8: Overall effect of MMP9 SNP rs6073983 genotype (dominant and mutant varieties) on mean quality of life scores derived from the Paediatric Asthma Quality of Life Questionnaire.

P-values were calculated by univariate analysis of variance corrected for age and sex.
CHAPTER 6

Results of MMP12 (rs2276109 & rs652438)

SECTION 6.1

Relationship between MMP12 SNP rs2276109 and rs652438 and asthma severity in a sample of children and young adults recruited to the BREATHE study

Sample Demographics rs2276109

1440 young individuals with physician diagnosed asthma derived from both primary and secondary care were successfully genotyped for MMP12 (rs2276109). Individuals range from 3-22 years of age (mean 9.9 years, SD4.1). 58.9% (n=848) are male. 34.9% (n=502) of participants were exposed to tobacco smoke in their environment over the previous six months (Table 6.1.1).

Over the previous six months 44.7% (n=643) of the 1440 participants had at least one exacerbation, either requiring a hospital admission, a short course of oral steroids, an absence or a combination of two or more of these. 17.7% of participants had at least one hospital admission in the previous 6 months. 28.9% had been prescribed a short course of oral steroids. 36.9% reported absence due to asthma exacerbation.
Table 6.1.1: Characteristics of study participants with asthma and the rs2276109 SNP in the BREATHE study (n=1440)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>Range, 3-22 (mean, 9.9; SD, 4.1)</td>
</tr>
<tr>
<td>Sex (males: females)</td>
<td>848 (58.9%):592 (41.1%)</td>
</tr>
<tr>
<td>MMP12 rs652438(%) (AA: AG:GG)</td>
<td>1121 (77.8%):304 (21.1%):15 (1.0%)</td>
</tr>
<tr>
<td>Exposure to smoke (yes/no) (1440)</td>
<td>502/938 (34.9%)</td>
</tr>
<tr>
<td>Reported allergy (yes/no) (1440)</td>
<td>266/1174 (18.5%)</td>
</tr>
<tr>
<td>Mean % predicted FEV1 (SD) (n=969)</td>
<td>95.7 (15.1)</td>
</tr>
<tr>
<td>Mean % predicted FVC (SD) (n=968)</td>
<td>92.9 (14.2)</td>
</tr>
<tr>
<td>Mean FEV1/ FVC (SD) (n=968)</td>
<td>0.87 (8.6)</td>
</tr>
<tr>
<td>Modified BTS asthma treatment steps * (n=1440)</td>
<td>1=277; 2=780; 3=206; 4=177</td>
</tr>
<tr>
<td>Inhaled bronchodilator use † (n=1440)</td>
<td>0=166; 1=963; 2=223; 3=88</td>
</tr>
<tr>
<td>Asthma severity score ‡ (n=1440)</td>
<td>0=34; 1=233; 2=633; 3=296; 4=169; 5=75</td>
</tr>
<tr>
<td>Hospital admissions (yes/ no) over previous 6 months due to exacerbations (n=1440)</td>
<td>255/1185 (17.7%)</td>
</tr>
<tr>
<td>Oral steroid intake (yes/ no) over previous 6 months due to exacerbations (n=1440)</td>
<td>416/1024 (28.9%)</td>
</tr>
<tr>
<td>Absence (yes/ no) over previous 6 months due to exacerbations (n=1440)</td>
<td>531/909 (36.9%)</td>
</tr>
<tr>
<td>Overall exacerbations (yes/ no) over previous 6 months (n=1440)</td>
<td>643/797 (44.7%)</td>
</tr>
</tbody>
</table>

Keys: *Step 1= no/occasional use of inhaled salbutamol; Step2= step1+ inhaled steroids; step 3= step2+ inhaled long acting β2 without montelukast; step 4= step 3 + montelukast
†Inhaled bronchodilator use: 0= none, 1= occasional, 2= daily, 3= excessive use
‡Asthma severity score: (bronchodilator use adjusted modified treatment steps): A scale where 0=mild and 5=severe
◊ Defined as any one of the following in previous 6 months: absence, courses of oral steroids, or hospital admissions
The 1440 recruited participants were genotyped for the transition substitution SNP rs2276109. The AA genotype was present in 77.8% (n=1121) of participants. 21.1% (n=304) are heterozygous for the AG genotype. The G allele was homozygous in 1.0% (n=15) of participants.

Among the participants with the AA genotype, 46.8% (n=502) reported at least one measure for asthma exacerbations in the previous six months. 17.9% (n=201) reported hospital admission, 36.6% (n=410) absence and 28.9% (n=324) use of oral steroids (Table 6.1.3). 81.9% (n=918) suffer from moderate to difficult to manage asthma (severity score steps 2-5 (Table 6.1.5). 18.1% (n=203) suffer from mild asthma (severity score steps 0&1).

Among the participants with the G allele, 44.2% (n=141) reported an exacerbation in the previous six months. 16.9% (n=54) reported hospital admission, 37.9% (n=121) reported an absence and 28.8% (n=92) reported use of oral steroids due to exacerbation of asthma. 79.9% (n=255) suffer from moderate to difficult to manage asthma (severity score steps 2-5). 20.1% (n=64) suffer from mild asthma (severity score steps 0&1).

**Sample Demographics rs652438**

1422 young individuals with physician diagnosed asthma derived from both primary and secondary care were successfully genotyped for **MMP12** (rs652438) (Table 6.1.2). Individuals range from 3-22 years of age (mean 10.0 years, SD4.1). 58.7% (n=835) are male. 34.7% (n=493) of participants were exposed to tobacco smoke in their environment over the previous six months.

Over the previous six months 44.4% (n=631) of the 1422 participants had at least one exacerbation, either requiring a hospital admission, a short course of oral steroids, an absence or a combination two or more of these. 17.6% of participants had at least one hospital admission in the previous 6 months. 28.9% had been prescribed a short course of oral steroids. 36.6% reported absence due to asthma exacerbation.
<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong></td>
<td>Range, 3-22 (mean,10.0; SD, 4.1)</td>
</tr>
<tr>
<td><strong>Sex (males: females)</strong></td>
<td>835 (58.7%):587 (41.3%)</td>
</tr>
<tr>
<td><strong>MMP12 rs652438(%) (AA: AG:GG)</strong></td>
<td>1285(90.4%):132 (9.3%):5 (0.4%)</td>
</tr>
<tr>
<td><strong>Exposure to smoke (yes/no)</strong> (1422)</td>
<td>493/929 (34.7%)</td>
</tr>
<tr>
<td><strong>Reported allergy (yes/no)</strong> (1422)</td>
<td>264/1158 (18.6%)</td>
</tr>
<tr>
<td><strong>Mean % predicted FEV1 (SD) (n=961)</strong></td>
<td>95.7 (15.1)</td>
</tr>
<tr>
<td><strong>Mean % predicted FVC (SD) (n-960)</strong></td>
<td>92.8 (14.3)</td>
</tr>
<tr>
<td><strong>Mean FEV1/ FVC (SD) (n-960)</strong></td>
<td>0.87 (8.6)</td>
</tr>
<tr>
<td><strong>Modified BTS asthma treatment steps * (n=1422)</strong></td>
<td>1=277; 2=767; 3=202; 4=176</td>
</tr>
<tr>
<td><strong>Inhaled bronchodilator use † (n=1422)</strong></td>
<td>0=163; 1=952; 2=220; 3=87</td>
</tr>
<tr>
<td><strong>Asthma severity score ‡ (n=1422)</strong></td>
<td>0=33; 1=234; 2=623; 3=290; 4=167; 5=75</td>
</tr>
<tr>
<td><strong>Hospital admissions (yes/ no) over previous 6 months due to exacerbations (n=1422)</strong></td>
<td>250/1172 (17.6%)</td>
</tr>
<tr>
<td><strong>Oral steroid intake (yes/ no) over previous 6 months due to exacerbations (n=1422)</strong></td>
<td>411/1011 (28.9%)</td>
</tr>
<tr>
<td><strong>Absence (yes/ no) over previous 6 months due to exacerbations (n=1422)</strong></td>
<td>520/902 (36.6%)</td>
</tr>
<tr>
<td><strong>Overall exacerbations (yes/ no) over previous 6 months ‡ (n=1422)</strong></td>
<td>631/791 (44.4%)</td>
</tr>
</tbody>
</table>

**Table 6.1.2: Characteristics of study participants with asthma and the rs652438 SNP in the BREATHE study (n=1422)**

**Keys:**
*Step 1= no/occasional use of inhaled salbutamol; Step2= step1+ inhaled steroids; step 3= step2+ inhaled long acting β2 without montelukast; step 4= step 3 + montelukast
†Inhaled bronchodilator use: 0= none, 1= occasional, 2= daily, 3= excessive use
‡Asthma severity score: (bronchodilator use adjusted modified treatment steps): A scale where 0=mild and 5=severe
◊ Defined as any one of the following in previous 6 months: absence, courses of oral steroids, or hospital admissions
The 1422 recruited participants were genotyped for the transition substitution SNP rs652438. The AA genotype was present in 90.4% (n=1285) of participants. 9.3% (n=132) are heterozygous for the AG genotype. The G allele was homozygous in 0.4% (n=5) of participants.

Among the participants with the AA genotype, 43.5% (n=559) reported at least one measure for asthma exacerbations in the previous six months. 17.7% (n=227) reported hospital admission, 36.2% (n=465) absence and 28.2% (n=363) use of oral steroids (Table 6.1.4; Figure 6.1.1). 80.5% (n=1035) suffer from moderate to difficult to manage asthma (severity score steps 2-5 (Table 6.1.6). 19.5% (n=250) suffer from mild asthma (severity score steps 0&1). Among the participants with the G allele, 52.5% (n=72) reported an exacerbation in the previous six months. 16.8% (n=23) reported hospital admission, 40.1% (n=55) reported an absence and 13.1% (n=48) reported use of oral steroids due to exacerbation of asthma. 87.6% (n=120) suffer from moderate to difficult to manage asthma (severity score steps 2-5). 12.4% (n=17) suffer from mild asthma (severity score steps 0&1).
**MMP12 (rs2276109) and asthma exacerbations**

<table>
<thead>
<tr>
<th></th>
<th>MMP12 SNP rs2276109</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AG/GG (n=319)</td>
</tr>
<tr>
<td>Hospital admission due to asthma exacerbations (Y)</td>
<td>54 (16.9%)</td>
</tr>
<tr>
<td>Absence due to asthma exacerbations (Y)</td>
<td>121 (37.9%)</td>
</tr>
<tr>
<td>Oral steroid intake due to asthma exacerbations (Y)</td>
<td>92 (28.8%)</td>
</tr>
<tr>
<td>Overall asthma exacerbations (Y)</td>
<td>141 (44.2%)</td>
</tr>
<tr>
<td>Modified treatment steps</td>
<td>Addition of preventer medication (Y)</td>
</tr>
<tr>
<td>Asthma severity score</td>
<td>Moderate to severe (Y)</td>
</tr>
</tbody>
</table>

Table 6.1.3: Overall effect of MMP12 SNP rs2276109 genotype (dominant and recessive varieties) on hospital admissions, oral steroid intake, absences due to asthma exacerbations, and overall asthma exacerbations, modified treatment steps and asthma severity in children and young adults recruited to the BREATHE study

Key: OR: Odds ratio; CI: Confidence interval

P values were calculated by binary logistic regression corrected for age and sex.
We did not observe any association between the risk allele and overall exacerbations, hospital admissions due to exacerbations, absence due to exacerbations or oral steroid intake for asthma exacerbations over the previous six months (Table 6.1.3). We subsequently categorised by age and repeated the analysis, however no association was identified, this data is not included.

Through proxy measures, we tested for gene-dosage effect using the modified BTS treatment steps, categorised into salbutamol and addition of preventer medication. We also tested for an association with risk of severity (Table 6.1.3). However, no significant effect was observed indicating that there is not a gene-dose relationship with this SNP. No significant effect was observed when the risk allele was tested for each severity step, indicating there is no relationship with drug-associated severity with this SNP. We subsequently categorised by age and repeated the analysis, however no association was identified, this data is not included.
### MMP12 (rs652438) and asthma exacerbations

<table>
<thead>
<tr>
<th></th>
<th>MMP12 SNP rs652438</th>
<th></th>
<th>OR (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AG/GG (n=137)</td>
<td>AA (n=1285)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hospital admission due to asthma exacerbations (Y)</td>
<td>23 (16.8%)</td>
<td>227 (17.7%)</td>
<td>0.96 (0.59-1.55)</td>
<td>0.858</td>
</tr>
<tr>
<td>Absence due to asthma exacerbations (Y)</td>
<td>55 (40.1%)</td>
<td>465 (36.2%)</td>
<td>1.21 (0.84-1.76)</td>
<td>0.302</td>
</tr>
<tr>
<td>Oral steroid intake due to asthma exacerbations (Y)</td>
<td>48 (30.0%)</td>
<td>363 (28.2%)</td>
<td>1.42 (0.97-2.07)</td>
<td>0.072</td>
</tr>
<tr>
<td>Overall asthma exacerbations (Y)</td>
<td>72 (52.6%)</td>
<td>559 (43.5%)</td>
<td>1.51 (1.05-2.18)</td>
<td>0.026*</td>
</tr>
<tr>
<td>Modified treatment steps</td>
<td>Addition of preventer medication (Y)</td>
<td>120 (87.6%)</td>
<td>1025 (79.8%)</td>
<td>1.83 (1.08-3.10)</td>
</tr>
<tr>
<td>Asthma severity score</td>
<td>Moderate to severe (Y)</td>
<td>120 (87.6%)</td>
<td>1035 (80.5%)</td>
<td>1.74 (1.03-2.96)</td>
</tr>
</tbody>
</table>

**Table 6.1.4: Overall effect of MMP12 SNP rs652438 genotype (dominant and recessive varieties) on hospital admissions, oral steroid intake, absences due to asthma exacerbations, overall asthma exacerbations, modified treatment steps and asthma severity in children and young adults**

Key: *: p<0.05; OR: Odds ratio; CI: Confidence interval

P values were calculated by binary logistic regression corrected for age and sex.
The homozygous A allele was associated with overall exacerbations (p=0.026). When compared to the homozygous A allele (AA) the odds ratio for AG/GG in overall exacerbations was 1.51 indicating that the minor allele (AG/GG) may play a role in increasing the risk of asthma exacerbations (Table 6.1.4/ Figure 6.1.1).

We did not observe any association between the risk allele and hospital admissions due to exacerbations or absence due to exacerbations or oral steroid intake for asthma exacerbations over the previous six months. We subsequently categorised by age and repeated the analysis, however no association was identified, this data is not included.

Through proxy measures, we tested for gene-dosage effect using the modified BTS treatment steps, categorised into salbutamol and addition of preventer medication. We also tested for an association with risk of severity. The homozygous A allele was associated with addition of preventer medications in modified treatment steps (p=0.025) and moderate to severe asthma as classified by the asthma severity score (p=0.040) when compared to the dominant G allele. The odds ratio for AG/GG in modified treatment steps was 1.83, and in severity was 1.74 indicating that the minor allele (AG/GG) may have a role in a gene-dose relationship and with drug associated severity with this SNP (Table 6.1.4; Figure 6.1.2). We subsequently categorised by age and repeated the analysis, however no association was identified, this data is not included.
Figure 6.1.1 Illustration of Overall effect of *MMP12* rs652438 genotype on hospital admissions, oral steroid intake and absences due to asthma exacerbations, and overall asthma exacerbations, in children and young adults recruited to the BREATHE study.
Figure 6.1.2 Illustration of Overall effect of MMP12 SNP rs652438 genotype (dominant and mutant varieties) on modified treatment steps and asthma severity in children and young adults recruited to the BREATHE study.

Asthma severity score
- Mild: AG/GG 10%, AA 10%
- Moderate to severe: AG/GG 30%, AA 30%
- p-value: 0.040

Modified treatment steps
- Salbutamol only: AG/GG 5%, AA 5%
- Addition of preventer medication: AG/GG 25%, AA 25%
- Modified treatment steps: AG/GG 40%, AA 40%
- p-value: 0.025
SECTION 6.2

Relationship between *MMP12* SNP rs2276109 and rs652438 and measures of pulmonary function in a sample of UK children and young adults recruited to the BREATHE study and PAGES

*MMP12* (rs2276109) and pulmonary function

<table>
<thead>
<tr>
<th></th>
<th>MMP12 SNP rs2276109</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AG/GG</td>
<td>AA</td>
<td>n</td>
<td>P value</td>
</tr>
<tr>
<td>Mean ppPEFR [n] (sd)</td>
<td>[248]</td>
<td>[843]</td>
<td>1091</td>
<td>0.741</td>
</tr>
<tr>
<td></td>
<td>87.2(16.6)</td>
<td>87.3(17.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ppFEV1 [n] (sd)</td>
<td>[254]</td>
<td>[859]</td>
<td>1113</td>
<td>0.784</td>
</tr>
<tr>
<td></td>
<td>95.8(14.9)</td>
<td>96.0(15.8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ppFVC [n]sd)</td>
<td>[254]</td>
<td>[859]</td>
<td>1112</td>
<td>0.991</td>
</tr>
<tr>
<td></td>
<td>94.8(14.6)</td>
<td>94.6(15.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FEV1/FVC [n] (sd)</td>
<td>[254]</td>
<td>[859]</td>
<td>1112</td>
<td>0.993</td>
</tr>
<tr>
<td></td>
<td>86.2(8.8)</td>
<td>86.8(8.8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean change in ppFEV1 post bronchodilator [n] (sd)</td>
<td>[28]</td>
<td>[99]</td>
<td>127</td>
<td>0.834</td>
</tr>
<tr>
<td></td>
<td>5.0(4.9)</td>
<td>5.1(6.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean Nitric oxide [n] (ppb) (sd)</td>
<td>[35]</td>
<td>[124]</td>
<td>159</td>
<td>0.345</td>
</tr>
<tr>
<td></td>
<td>42.3(38.2)</td>
<td>33.0(30.9)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Nitric oxide categorised by clinical relevance

<table>
<thead>
<tr>
<th></th>
<th>Clinically elevated exhaled nitric oxide (&gt;50ppb) (Y)</th>
<th>Elevated exhaled nitric oxide (25-49ppb) (Y)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>15/35 (42.9%)</td>
<td>8/35 (22.9%)</td>
</tr>
<tr>
<td></td>
<td>39/124 (31.5%)</td>
<td>32/124 (25.8%)</td>
</tr>
<tr>
<td></td>
<td>54</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>0.142</td>
<td>0.746</td>
</tr>
</tbody>
</table>

Table 6.2.1: Overall effect of *MMP12* SNP rs2276109 genotype (dominant and mutant varieties) on mean % predicted lung function

Key: pp: % predicted; ppb: parts per billion; FEV1: Forced expiratory volume in 1 second; FVC: Forced vital capacity

P-values were calculated by univariate analysis of variance corrected for sex

P-values for Nitric oxide categorised by clinical relevance were calculated by multinomial logistic regression corrected for age and sex

We tested for an effect between *MMP12* rs2276109 and measures of pulmonary function (Table 6.2.1). No significant effect was observed with any investigated measure of pulmonary function.
### MMP12 (rs652438) and pulmonary function

We tested for an effect between *MMP12* rs652438 and measures of pulmonary function. The homozygous A (AA) allele was associated with reduced mean % predicted PEFR (p=0.013), FEV1 (P=0.037), FVC (p=0.020) when compared to the dominant G allele (AG or GG) (Table 6.2.2; Figure 6.2.1). No significant effect was observed with FEV1/FVC or with Mean change in FEV1 post bronchodilator or with mean Nitric oxide (ppb).

#### Table 6.2.2: Overall effect of *MMP12* SNP rs652438 genotype (dominant and mutant varieties) on mean % predicted lung function

<table>
<thead>
<tr>
<th></th>
<th>AG/GG</th>
<th>AA</th>
<th>n</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ppPEFR [n] (sd)</td>
<td>110 [91.3(20.0)]</td>
<td>975 [86.9(16.7)]</td>
<td>1085</td>
<td>0.013*</td>
</tr>
<tr>
<td>Mean ppFEV1 [n] (sd)</td>
<td>114 [98.6(19.2)]</td>
<td>993 [95.6(15.1)]</td>
<td>1107</td>
<td>0.037*</td>
</tr>
<tr>
<td>Mean ppFVC [n] (sd)</td>
<td>114 [97.3(19.1)]</td>
<td>992 [94.3(14.6)]</td>
<td>1106</td>
<td>0.020*</td>
</tr>
<tr>
<td>FEV1/FVC [n] (sd)</td>
<td>114 [86.4(19.3)]</td>
<td>992 [86.2(8.8)]</td>
<td>1106</td>
<td>0.919</td>
</tr>
<tr>
<td>Mean change in ppFEV1 post bronchodilator [n] (sd)</td>
<td>12 [5.8(5.4)]</td>
<td>117 [5.0(6.4)]</td>
<td>129</td>
<td>0.674</td>
</tr>
<tr>
<td>Mean Nitric oxide [n] (ppb)(sd)</td>
<td>18 [30.4(26.0)]</td>
<td>144 [35.5(33.4)]</td>
<td>162</td>
<td>0.514</td>
</tr>
</tbody>
</table>

**Nitric oxide categorised by clinical relevance**

| Clinically elevated exhaled nitric oxide (>50ppb) (Y) | 6/18 (33.3%) | 49/144 (34.0%) | 55 | 0.937   |
| Elevated exhaled nitric oxide (25-49ppb) (Y)        | 4/18 (22.2%) | 36/144 (25.0%) | 40 | 0.682   |

**Key:** *: p<0.05; pp: % predicted; ppb: parts per billion; FEV1: Forced expiratory volume in 1 second; FVC: Forced vital capacity

P-values were calculated by univariate analysis of variance corrected for sex

P-values for Nitric oxide categorised by clinical relevance were calculated by multinomial logistic regression corrected for age and sex.
Figure 6.2.1 Illustration of Overall effect of MMP12 SNP rs652438 genotype (dominant and mutant varieties) on % predicted PEFR, FEV1, FVC and FEV1/FVC, in children and young adults recruited to the BREATHE study and PAGES.
**MMP12 (rs2276109) and pulmonary function categorised by age**

<table>
<thead>
<tr>
<th>MMP12 SNP rs2276109</th>
<th>5-12 years</th>
<th>P Value</th>
<th>13-22 years</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AG/GG</td>
<td>AA</td>
<td>AG/GG</td>
<td>AA</td>
</tr>
<tr>
<td>Mean ppPEFR (sd)</td>
<td>84.8 (16.3)</td>
<td>84.7 (16.7)</td>
<td>0.983</td>
<td>92.2 (16.3)</td>
</tr>
<tr>
<td>N</td>
<td>169</td>
<td>583</td>
<td>-</td>
<td>79</td>
</tr>
<tr>
<td>Mean ppFEV1 (sd)</td>
<td>95.4 (15.2)</td>
<td>95.5 (14.9)</td>
<td>0.712</td>
<td>96.8 (14.4)</td>
</tr>
<tr>
<td>Mean ppFVC (sd)</td>
<td>93.3 (14.5)</td>
<td>93.3 (14.3)</td>
<td>0.994</td>
<td>98.0 (14.3)</td>
</tr>
<tr>
<td>FEV1/FVC (sd)</td>
<td>86.4 (8.2)</td>
<td>86.4 (8.6)</td>
<td>0.795</td>
<td>85.8 (10.0)</td>
</tr>
<tr>
<td>N</td>
<td>173</td>
<td>596</td>
<td>-</td>
<td>81</td>
</tr>
<tr>
<td>Mean change in ppFEV1 post bronchodilator (sd)</td>
<td>6.4 (5.5)</td>
<td>4.9 (6.2)</td>
<td>0.467</td>
<td>2.7 (2.8)</td>
</tr>
<tr>
<td>N</td>
<td>18</td>
<td>78</td>
<td>-</td>
<td>10</td>
</tr>
<tr>
<td>Nitric oxide (ppb) (sd)</td>
<td>44.9 (40.5)</td>
<td>29.9 (27.8)</td>
<td>0.031*</td>
<td>37.4 (34.4)</td>
</tr>
<tr>
<td>N</td>
<td>23</td>
<td>95</td>
<td>-</td>
<td>12</td>
</tr>
</tbody>
</table>

**Table 6.2.3: Overall effect of MMP12 SNP rs2276109 genotype (dominant and mutant varieties) on mean % predicted lung function categorised by age in participants recruited to the BREATHE study and PAGES**

Key: pp: % predicted; ppb: parts per billion; FEV1: Forced expiratory volume in 1 second; FVC: Forced vital capacity  
P-values were calculated by univariate analysis of variance corrected for sex

We tested for an effect between MMP12 rs2276109 and measures of pulmonary function, categorised by age (Table 6.2.3). In the 5-12 year old category the homozygous A (AA) allele was associated with reduced nitric oxide (p=0.031) when compared to the dominant G allele (AG or GG). No significant effect was observed with any other investigated measures of pulmonary function.
**MMP12 (rs652438) and pulmonary function categorised by age**

<table>
<thead>
<tr>
<th>MMP12 SNP rs652438</th>
<th>5-12 years</th>
<th>P Value</th>
<th>13-22 years</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>AG/GG</td>
<td>AA</td>
<td></td>
<td>AG/GG</td>
<td>AA</td>
</tr>
<tr>
<td>Mean ppPEFR (sd)</td>
<td>87.5 (16.6)</td>
<td>0.191</td>
<td>98.3 (23.9)</td>
<td>0.043*</td>
</tr>
<tr>
<td>N</td>
<td>72</td>
<td>673</td>
<td>-</td>
<td>38</td>
</tr>
<tr>
<td>Mean ppFEV1 (sd)</td>
<td>96.6 (14.5)</td>
<td>0.404</td>
<td>102.5 (26.1)</td>
<td>0.028*</td>
</tr>
<tr>
<td>Mean ppFVC (sd)</td>
<td>94.3 (13.5)</td>
<td>0.501</td>
<td>103.7 (25.9)</td>
<td>0.009*</td>
</tr>
<tr>
<td>FEV1/FVC (sd)</td>
<td>86.9 (8.7 )</td>
<td>0.591</td>
<td>85.3 (10.5)</td>
<td>0.691</td>
</tr>
<tr>
<td>N</td>
<td>76</td>
<td>685</td>
<td>-</td>
<td>38</td>
</tr>
<tr>
<td>Mean change in ppFEV1 post bronchodilator (sd)</td>
<td>5.2 (3.4)</td>
<td>0.953</td>
<td>6.9 (8.8)</td>
<td>4.6 (7.2)</td>
</tr>
<tr>
<td>N</td>
<td>8</td>
<td>90</td>
<td>-</td>
<td>4</td>
</tr>
<tr>
<td>Nitric oxide (ppb) (sd)</td>
<td>26.8 (23.3)</td>
<td>0.502</td>
<td>37.7 (31.8)</td>
<td>42.1 (38.3)</td>
</tr>
<tr>
<td>N</td>
<td>12</td>
<td>109</td>
<td>-</td>
<td>6</td>
</tr>
</tbody>
</table>

Table 6.2.4: Overall effect of MMP12 SNP rs652438 genotype (dominant and mutant varieties) on mean % predicted lung function categorised by age in participants recruited to the BREATHE study and PAGES

Key:*: p<0.05; pp: % predicted; ppb: parts per billion; FEV1: Forced expiratory volume in 1 second; FVC: Forced vital capacity

P-values were calculated by univariate analysis of variance corrected for sex

We tested for an effect between MMP12 rs652438 and measures of pulmonary function, categorised by age (Table 6.2.4; Figure 6.2.2). In the 13-22 years age category, the homozygous A (AA) allele was associated with reduced mean % predicted PEFR (p=0.043), FEV1 (P=0.028), FVC (p=0.009) when compared to the dominant G allele (AG or GG) (Table 6.2.4; Figure 6.2.2). No significant effect was observed with FEV1/FVC.
Figure 6.2.2 Illustration of Overall effect of MMP12 SNP rs652438 genotype (dominant and mutant varieties) on % predicted PEFR, FEV1, FVC and FEV1/FVC, categorised by age (5-12 years and 13-22 years), in children and young adults recruited to the BREATHE study and PAGES.
SECTION 6.3

Relationship between MMP12 (rs2276109 & rs652438) and environmental factors in a sample of UK children and young adults

MMP12 (rs2276109) and exposure to environmental tobacco smoke

1440 individuals recruited to the BREATHE study were tested for an interaction between the rs2276109 genotype and environmental tobacco smoke on exacerbations in individuals with asthma. The dominant A allele (AA) was present in 77.9% (n=1121) of participants. 34.8% (n=390) were exposed to environment tobacco smoke. The G allele (AG/GG) was homozygous in 22.1% (n=319) of participants. 35.1% (n=112) were exposed to environmental tobacco smoke (Table 6.3.1).

We did not observe any association between the SNP and overall exacerbations, hospital admissions due to exacerbations, absence due to exacerbations or oral steroid intake due to asthma exacerbations over the previous six months (Table 6.6.1; Figure 6.6.1).

Through proxy measures, we tested for gene-dosage effect using the modified BTS treatment steps, categorised into salbutamol and addition of preventer medication. We also tested for an association with risk of severity (Table 6.3.1). No significant effect was observed when the risk allele was tested for each severity step, indicating there is no relationship with drug-associated severity with this SNP. We subsequently categorised by age and repeated the analysis, however no association was identified, this data is not included.
**Table 6.3.1: Overall effect of MMP12 SNP rs2276109 genotype (dominant and mutant varieties) on hospital admissions, oral steroid intake and absences due to asthma exacerbations, overall asthma exacerbations and asthma severity, categorised by exposure to tobacco smoke in children and young adults recruited to the BREATHE study**

<table>
<thead>
<tr>
<th></th>
<th>Exposed to tobacco smoke</th>
<th>Not exposed to tobacco smoke</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AG/GG (n=112)</td>
<td>AA (n=390)</td>
</tr>
<tr>
<td>Hospital admission due to asthma exacerbations (Y)</td>
<td>24 (21.4%)</td>
<td>84 (21.5%)</td>
</tr>
<tr>
<td></td>
<td>OR (95%CI)</td>
<td>1.14 (0.67-1.94)</td>
</tr>
<tr>
<td></td>
<td>AG/GG (n=207)</td>
<td>AA (n=731)</td>
</tr>
<tr>
<td>Absence due to asthma exacerbations (Y)</td>
<td>30 (14.5%)</td>
<td>117 (16.0%)</td>
</tr>
<tr>
<td></td>
<td>OR (95%CI)</td>
<td>0.88 (0.57-1.38)</td>
</tr>
<tr>
<td>Oral steroid intake due to asthma exacerbations (Y)</td>
<td>76 (36.7%)</td>
<td>244 (33.4%)</td>
</tr>
<tr>
<td></td>
<td>OR (95%CI)</td>
<td>1.17 (0.84-1.62)</td>
</tr>
<tr>
<td>Overall asthma exacerbations (Y)</td>
<td>90 (43.5%)</td>
<td>305 (41.7%)</td>
</tr>
<tr>
<td></td>
<td>OR (95%CI)</td>
<td>1.09 (0.79-1.49)</td>
</tr>
<tr>
<td>Asthma severity score</td>
<td>Moderate to severe (Y)</td>
<td>166 (80.2%)</td>
</tr>
<tr>
<td></td>
<td>OR (95%CI)</td>
<td>0.89 (0.61-1.33)</td>
</tr>
</tbody>
</table>

*P* values and odds ratios for exacerbations were calculated by binary logistic regression corrected for age, and sex.

Key: OR: Odds ratio; CI: Confidence interval

Asthma severity score: (bronchodilator use adjusted modified treatment steps):0 & 1 = mild, 2 - 5 = moderate to severe
Table 6.3.2: Overall effect of MMP12 SNP rs2276109 genotype (dominant and mutant varieties) on mean % predicted lung function categorised by age and exposure to environmental tobacco smoke in children and young adults recruited to the BREATHE study and PAGES

Key: pp: % predicted; ppb: parts per billion; FEV1: Forced expiratory volume in 1 second; FVC: Forced vital capacity
P-values were calculated by univariate analysis of variance corrected for sex
We tested for an interaction between measures of pulmonary function exposure to environmental tobacco smoke and MMP12 rs2276109, categorised by age. Bronchodilator reversibility and exhaled nitric oxide were excluded due to insufficient cases (Table 6.3.2). No significant effect was observed with any investigated measures of pulmonary function.
**MMP12 (rs652438) and exposure to environmental tobacco smoke**

1422 individuals recruited to the BREATHE study were tested for an interaction between the rs652438 genotype and environmental tobacco smoke on exacerbations in individuals with asthma. The dominant A allele (AA) was present in 90.4% (n=1285) of participants. 34.7% (n=446) were exposed to environment tobacco smoke. The G allele (AG/GG) was homozygous in 9.6% (n=137) of participants. 34.3% (n=47) were exposed to environmental tobacco smoke (Table 6.3.3).

We did not observe any association between the SNP and overall exacerbations, hospital admissions due to exacerbations, absence due to exacerbations or oral steroid intake due to asthma exacerbations over the previous six months (Table 6.3.3; Figure 6.6.4).

Through proxy measures, we tested for gene-dosage effect using the modified BTS treatment steps, categorised into salbutamol and addition of preventer medication. We also tested for an association with risk of severity. (Table 6.3.3). No significant effect was observed when the risk allele was tested for each severity step, indicating there is no relationship with drug-associated severity with this SNP.
### Table 6.3.3: Overall effect of MMP12 SNP rs652438 genotype (dominant and mutant varieties) on hospital admissions, oral steroid intake and absences due to asthma exacerbations, overall asthma exacerbations and asthma severity, categorised by exposure to tobacco smoke in children and young adults recruited to the BREATHE study

<table>
<thead>
<tr>
<th></th>
<th>Exposed to tobacco smoke</th>
<th>Not exposed to tobacco smoke</th>
<th>OR (95%CI)</th>
<th>OR (95%CI)</th>
<th>P Value for interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AG/GG (n=47)</td>
<td>AA (n=446)</td>
<td>AG/GG (n=90)</td>
<td>AA (n=839)</td>
<td></td>
</tr>
<tr>
<td>Hospital admission</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>due to asthma</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>exacerbations (Y)</td>
<td>7 (14.9%)</td>
<td>99 (22.2%)</td>
<td>0.60 (0.25-1.42)</td>
<td>128 (15.3%)</td>
<td>1.24 (0.69-2.24)</td>
</tr>
<tr>
<td>Absence due to</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>asthma exacerbations</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Y)</td>
<td>22 (46.8%)</td>
<td>185 (41.5%)</td>
<td>1.29 (0.69-2.41)</td>
<td>280 (33.4%)</td>
<td>1.18 (0.75-1.88)</td>
</tr>
<tr>
<td>Oral steroid intake</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>due to asthma</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>exacerbations (Y)</td>
<td>17 (36.2%)</td>
<td>144 (32.3%)</td>
<td>1.23 (0.64-2.34)</td>
<td>219 (26.1%)</td>
<td>1.54 (0.96-2.47)</td>
</tr>
<tr>
<td>Overall asthma</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>exacerbations (Y)</td>
<td>27 (57.4%)</td>
<td>217 (48.7%)</td>
<td>1.53 (0.81-2.89)</td>
<td>342 (40.8%)</td>
<td>1.53 (0.97-2.39)</td>
</tr>
<tr>
<td>Asthma severity score</td>
<td>Moderate to severe (Y)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>44 (93.6%)</td>
<td>356 (79.8%)</td>
<td>3.84 (1.16-12.72)</td>
<td>679 (80.9%)</td>
<td>1.29 (0.71-2.36)</td>
</tr>
</tbody>
</table>

Key: OR: Odds ratio; CI: Confidence interval
Asthma severity score: (bronchodilator use adjusted modified treatment steps): 0&1=mild, 2-5=moderate to severe
P values and odds ratios for exacerbations were calculated by binary logistic regression corrected for age, and sex
<table>
<thead>
<tr>
<th>MMP12 rs652438</th>
<th>3-4 years</th>
<th>5-12 years</th>
<th>13-22 years</th>
</tr>
</thead>
<tbody>
<tr>
<td>ETS+ AG/GG</td>
<td>3 (33.3%)</td>
<td>21 (41.8%)</td>
<td>15 (37.5%)</td>
</tr>
<tr>
<td>ETS+ AA</td>
<td>23 (39.7%)</td>
<td>64 (24.2%)</td>
<td>12 (9.7%)</td>
</tr>
<tr>
<td>ETS- AG/GG</td>
<td>4 (5.7%)</td>
<td>10 (16.1%)</td>
<td>2 (9.5%)</td>
</tr>
<tr>
<td>ETS- AA</td>
<td>33 (28.5%)</td>
<td>87 (16.1%)</td>
<td>8 (4.4%)</td>
</tr>
<tr>
<td>Hospital admission</td>
<td>ETS+ AG/GG</td>
<td>4 (44.4%)</td>
<td>33 (56.9%)</td>
</tr>
<tr>
<td>ETS+ AA</td>
<td>5 (71.4%)</td>
<td>55 (47.4%)</td>
<td></td>
</tr>
<tr>
<td>ETS- AG/GG</td>
<td>4 (44.4%)</td>
<td>30 (51.7%)</td>
<td></td>
</tr>
<tr>
<td>ETS- AA</td>
<td>6 (85.7%)</td>
<td>49 (42.2%)</td>
<td></td>
</tr>
<tr>
<td>Absence</td>
<td>ETS+ AG/GG</td>
<td>7 (116) 9</td>
<td></td>
</tr>
<tr>
<td>ETS+ AA</td>
<td>11 (116) 9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ETS- AG/GG</td>
<td>5 (58)   7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ETS- AA</td>
<td>11 (116) 7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oral steroid intake</td>
<td>ETS+ AG/GG</td>
<td>7 (116) 9</td>
<td></td>
</tr>
<tr>
<td>ETS+ AA</td>
<td>11 (116) 9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ETS- AG/GG</td>
<td>5 (58)   7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ETS- AA</td>
<td>11 (116) 7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall asthma exacerbations</td>
<td>ETS+ AG/GG</td>
<td>9 (116) 7</td>
<td></td>
</tr>
<tr>
<td>ETS+ AA</td>
<td>11 (116) 7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ETS- AG/GG</td>
<td>5 (58)   7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ETS- AA</td>
<td>11 (116) 7</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 6.3.4 Overall effect of MMP12 SNP rs652438 genotype (dominant and mutant varieties) on hospital admissions, oral steroid intake and absences due to asthma exacerbations, overall asthma exacerbations and asthma severity, categorised by age and exposure to tobacco smoke in children and young adults recruited to the BREATHE study and PAGES

Key: *: p<0.05; OR: Odds ratio; CI: Confidence interval ETS+: exposed to environmental tobacco smoke; ETS- Not exposed to environmental tobacco smoke. P values and odds ratios were calculated by binary logistic regression corrected for age, and sex.
The dominant G allele (AG/GG) was associated with absence due to asthma exacerbations (p=0.044) in 13-22 year olds, exposed to environmental tobacco smoke. When compared to the homozygous A allele (AA) the odds ratio for AG/GG in absence was 3.53 indicating that the G may be a risk factor for asthma exacerbations, particularly those requiring absence (Table 6.3.4/ Figure 6.3.1).

We did not observe any association between the risk allele and any other form of exacerbation when separated by age categories and exposure to environmental tobacco smoke over the previous six months.
Figure 6.3.1 Illustration of Overall effect of *MMP12* rs652438 genotype on hospital admissions, oral steroid intake and absences due to asthma exacerbations, and overall asthma exacerbations, in 13-22 year olds with asthma, exposed to environmental tobacco smoke (ETS- = no exposure; ETS+= exposed) and recruited to the BREATHE study.
Table 6.3.5 Overall effect of MMP12 SNP rs652438 genotype (dominant and mutant varieties) on mean % predicted lung function categorised by age and exposure to environmental tobacco smoke in children and young adults recruited to the BREATHE study and PAGES

Key: *: p<0.05; pp: % predicted; ppb: parts per billion; FEV1: Forced expiratory volume in 1 second; FVC: Forced vital capacity

P-values were calculated by univariate analysis of variance corrected for sex

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Exposed to environmental tobacco smoke</th>
<th>Not exposed to environmental tobacco smoke</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5-12</td>
<td>P Value</td>
</tr>
<tr>
<td>MMP9 rs652438</td>
<td>AG/GG</td>
<td>AA</td>
</tr>
<tr>
<td>Mean ppPEFR</td>
<td>88.8 (13.6)</td>
<td>84.2 (17.5)</td>
</tr>
<tr>
<td>n</td>
<td>18</td>
<td>206</td>
</tr>
<tr>
<td>Mean ppFEV1</td>
<td>96.7 (17.0)</td>
<td>95.1 (15.7)</td>
</tr>
<tr>
<td>(sd)</td>
<td>94.6 (15.6)</td>
<td>93.4 (15.1)</td>
</tr>
<tr>
<td>Mean ppFVC</td>
<td>85.4 (10.5)</td>
<td>86.1 (8.7)</td>
</tr>
<tr>
<td>(sd)</td>
<td>155</td>
<td>206</td>
</tr>
</tbody>
</table>
We tested for an association between *MMP12* rs652438 and measures of pulmonary function, categorised by age. Bronchodilator reversibility and exhaled nitric oxide were excluded due to insufficient cases (Table 6.3.5). In the 13-22 years age category, exposed to environmental tobacco smoke, the homozygous A (AA) allele was associated with reduced mean % predicted PEFR (*p*=0.015), FEV1 (*p*=0.030), FVC (*p*=0.004) when compared to the minor G allele (AG or GG) (Table 6.3.5 Figure 6.3.2. No significant effect was observed between the SNP, any other investigated measure of pulmonary function in any of the other categories.
Figure 6.3.2 Illustration of Overall effect of MMP12 SNP rs652438 genotype (dominant and mutant varieties) on % predicted PEFR, FEV1, and FVC categorised by age (5-12 years and 13-22 years) and exposure to environmental tobacco smoke (ETS- = no exposure; ETS+ = exposed) in children and young adults recruited to the BREATHE study and PAGES
**MMP12 (rs2276109) and atopy**

1576 individuals recruited to the BREATHE study and PAGES were tested for an association between the rs2276109 SNP and any reported (Table 6.3.6). 424 individuals recruited to PAGES were tested for an association between the rs2276109 SNP and any confirmed sensitivity to a selection of tested allergens.

We did not observe any significant association between rs2276109 and reported allergy or confirmed sensitivity.

<table>
<thead>
<tr>
<th></th>
<th>MMP12 SNP rs2276109</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AG/GG</td>
</tr>
<tr>
<td>Reported allergy (Y)</td>
<td>67/420</td>
</tr>
<tr>
<td></td>
<td>(15.9%)</td>
</tr>
<tr>
<td>Positive SPT result to any allergen (Y)</td>
<td>18/101</td>
</tr>
<tr>
<td></td>
<td>(17.8%)</td>
</tr>
</tbody>
</table>

Table 6.3.6 Overall effect of MMP12 SNP rs2276109 genotype (dominant and mutant varieties) on participant reported allergy and skin prick test identified sensitivity

Key: OR: Odds ratio; CI: Confidence interval
P-values and odds ratios were calculated by binary logistic regression corrected for age and sex
**MMP12 (rs652438) and atopy**

1853 individuals recruited to the BREATHE study and PAGES were tested for an association between the rs652438 SNP and any reported (Table 6.3.7). 414 individuals recruited to PAGES were tested for an association between the rs652438 SNP and any confirmed sensitivity to a selection of tested allergens.

We did not observe any significant association between rs652438 and reported allergy or confirmed sensitivity.

<table>
<thead>
<tr>
<th></th>
<th>MMP12 SNP rs652438</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AG/GG</td>
</tr>
<tr>
<td>Reported allergy (Y)</td>
<td></td>
</tr>
<tr>
<td>42/183 (22.9%)</td>
<td>293/1665 (17.6%)</td>
</tr>
<tr>
<td>Positive SPT result to any allergen (Y)</td>
<td>9/46 (19.6%)</td>
</tr>
</tbody>
</table>

**Table 6.3.7: Overall effect of MMP12 SNP rs652438 genotype (dominant and mutant varieties) on participant reported allergy and skin prick test identified sensitivity**

Key: OR: Odds ratio; CI: Confidence interval; SPT: Skin prick test

P-values and odds ratios were calculated by binary logistic regression corrected for age and sex
**MMP12 (rs2276109) and quality of life**

214 individuals recruited to PAGES were tested for an association between the rs2276109 SNP and mean quality of life scores derived from the Paediatric Asthma Quality of Life Questionnaire.

We did not observe any significant association between rs2276109 and mean activity score, mean symptoms score, mean emotions score or mean overall score (Table 6.3.8).

<table>
<thead>
<tr>
<th></th>
<th>MMP12 SNP rs2276109</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AG/GG (n=53)</td>
</tr>
<tr>
<td>Mean activity score (sd)</td>
<td>5.2 (1.8)</td>
</tr>
<tr>
<td>Mean symptoms score (sd)</td>
<td>5.3 (1.6)</td>
</tr>
<tr>
<td>Mean emotion score (sd)</td>
<td>5.5 (1.6)</td>
</tr>
<tr>
<td>Mean overall score (sd)</td>
<td>6.3 (1.6)</td>
</tr>
</tbody>
</table>

**Table 6.3.8: Overall effect of MMP12 SNP rs2276109 genotype (dominant and mutant varieties) on mean quality of life scores derived from the Paediatric Asthma Quality of Life Questionnaire.**

P-values were calculated by univariate analysis of variance corrected for age and sex.
**MMP12 (rs652438) and quality of life**

205 individuals recruited to PAGES were tested for an association between the rs652438 SNP and mean quality of life scores derived from the Paediatric Asthma Quality of Life Questionnaire.

We did not observe any significant association between rs652438 and mean activity score, mean symptoms score, mean emotions score or mean overall score (Table 6.3.9).

<table>
<thead>
<tr>
<th></th>
<th>MMP12 SNP rs652438</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AG/GG (n=25)</td>
</tr>
<tr>
<td>Mean activity score (sd)</td>
<td>4.7 (1.7)</td>
</tr>
<tr>
<td>Mean symptoms score (sd)</td>
<td>5.0 (1.6)</td>
</tr>
<tr>
<td>Mean emotion score (sd)</td>
<td>5.4 (1.8)</td>
</tr>
<tr>
<td>Mean overall score (sd)</td>
<td>5.0 (1.6)</td>
</tr>
</tbody>
</table>

**Table 6.3.9:** Overall effect of MMP12 SNP rs652438 genotype (dominant and mutant varieties) on mean quality of life scores derived from the Paediatric Asthma Quality of Life Questionnaire.

P-values were calculated by univariate analysis of variance corrected for age and sex.
CHAPTER 7

Results of GST mutant variants

SECTION 7.1

Relationship between merged GSTM1 null/ GSTP1 rs1695/ GSTT1 null and asthma severity in a sample of UK children and young adults recruited to the BREATHE study

Sample demographics

867 young individuals with physician-diagnosed asthma derived from both primary and secondary care were successfully genotyped for GSTM1 null, GSTP1 rs1695 and GSTT1 null (Table 7.1.1). Individuals range from 3-22 years of age (mean 9.8 years, SD4.0). 60.3% (n=523) are male. 35.8% (n=310) of participants were exposed to tobacco smoke in their home environment over the previous six months.

Over the previous six months 47.2% (n=409) of the 867 participants had at least one exacerbation, either requiring a hospital admission, a short course of oral steroids, an absence or a combination of these. 18.5% of participants had at least one hospital admission in the previous 6 months. 29.6% had been prescribed a short course of oral steroids. 40.0% reported absence due to an exacerbation of asthma.
Table 7.1.1: Characteristics of study participants with GST mutant variant and asthma in the BREATHE study (n=867)

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>Range, 3-22 (mean, 9.6; SD, 4.0)</td>
</tr>
<tr>
<td>Sex (males: females)</td>
<td>523 (60.3%): 344 (39.7%)</td>
</tr>
<tr>
<td>GSTM1: GSTM1 null (%)</td>
<td>404 (46.8%): 460 (53.2%)</td>
</tr>
<tr>
<td>GSTP1 rs1695 A/A:A/G:G/G (%)</td>
<td>355(40.9%): 406(46.8%): 106(12.2%)</td>
</tr>
<tr>
<td>GSTT1: GSTT1 null (%)</td>
<td>629 (82.8%): 131 (17.2%)</td>
</tr>
<tr>
<td>Merged GSTM1 null:GSTP G/G:GSTT null (W/T: Mutant)(%)</td>
<td>265 (30.6%): 602 (69.4%)</td>
</tr>
<tr>
<td>Exposure to tobacco smoke (yes/no)</td>
<td>310/557 (35.8%)</td>
</tr>
<tr>
<td>Reported allergy (yes/no)</td>
<td>182/685 (21.0%)</td>
</tr>
<tr>
<td>Mean % predicted FEV1 (SD) (n=553)</td>
<td>96.9 (15.1)</td>
</tr>
<tr>
<td>Mean % predicted FVC (SD) (n=552)</td>
<td>94.6 (13.8)</td>
</tr>
<tr>
<td>Mean FEV1/ FVC (SD) (n=552)</td>
<td>86.3 (9.0)</td>
</tr>
<tr>
<td>Modified BTS asthma treatment steps * (n=867)</td>
<td>0=33; 1=121; 2=441; 3=140; 4=132</td>
</tr>
<tr>
<td>Inhaled bronchodilator use † (n=867)</td>
<td>0=113; 1=544; 2=150; 3=60</td>
</tr>
<tr>
<td>Asthma severity score ‡ (n=867)</td>
<td>0=31; 1=117; 2=350; 3=190; 4=121; 5=58</td>
</tr>
<tr>
<td>Hospital admissions (yes/ no) over previous 6 months due to exacerbations (n=867)</td>
<td>160/ 707 (18.5%)</td>
</tr>
<tr>
<td>Oral steroid intake (yes/ no) over previous 6 months due to exacerbations (n=867)</td>
<td>257/610 (29.6%)</td>
</tr>
<tr>
<td>Absence (yes/ no) over previous 6 months due to exacerbations (n=867)</td>
<td>347/520 (40.0%)</td>
</tr>
<tr>
<td>Overall exacerbations (yes/ no) over previous 6 months (n=867)</td>
<td>409/458 (47.2%)</td>
</tr>
</tbody>
</table>

Keys: *Step 1= no/occasional use of inhaled salbutamol; Step2= step1+ inhaled steroids; step 3= step2+ inhaled long acting β2 without montelukast; step 4= step 3 + montelukast †Inhaled bronchodilator use: 0= none, 1= occasional, 2= daily, 3= excessive use 1Asthma severity score: (bronchodilator use adjusted modified treatment steps): A scale where 0=mild and 5=severe
The 867 recruited participants were genotyped for \textit{GSTM1} null, \textit{GSTP1} and \textit{GSTT1} null. The mutant variants of each genotype were merged to produce a combined mutant genotype. The genotypes were split into wildtype and mutant variants. The wildtype variants were defined as one or more wildtype (\textit{GSTM1}+, \textit{GSTP1} A allele (A/A or A/G) and \textit{GSTT1}+) variant. The mutant variant was defined as one or more mutant (\textit{GSTM1} null, \textit{GSTP1} G/G and \textit{GSTT1} null) variants. The wildtype variant was present in 30.6\% (n=265) of participants. The mutant variant was present in 69.4\% (n=602) of participants.

Among the participants with the wildtype variant, 43.0\% (n=114) reported at least one marker of asthma exacerbations in the previous six months: 14.0\% (n=37) reported hospital admission, 37.7\% (n=100) absence and 26.0\% (n=69) use of oral steroids (Table 7.1.2). 20.0\% (n=53) suffer from severe asthma (severity score steps 4&5) (Table 7.1.4). 61.1\% (n=162) suffer from moderate asthma (severity score steps 2&3). Among the participants with mutant variant, 49.0\% (n=295) reported an exacerbation in the previous six months. 20.4\% (n=123) reported hospital admission, 41.0\% (n=247) reported absence and 31.2\% (n=188) reported use of oral steroids for asthma exacerbation (Table 7.1.2). 20.9\% (n=126) suffer from severe asthma (severity score steps 4&5). 62.8\% (n=378) suffer from moderate asthma (severity score steps 2&3) (Table 7.1.4).
**GST mutant variants and asthma severity**

<table>
<thead>
<tr>
<th>Mutant (n=607)</th>
<th>WT (n=265)</th>
<th>OR (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hospital admission due to asthma exacerbations (Y)</td>
<td>123 (20.3%)</td>
<td>37 (13.9%)</td>
<td>1.51 (1.01-2.26)</td>
</tr>
<tr>
<td>Absence due to asthma exacerbations (Y)</td>
<td>247 (40.7%)</td>
<td>100 (37.7%)</td>
<td>1.11 (0.82-1.50)</td>
</tr>
<tr>
<td>Oral steroid intake due to asthma exacerbations (Y)</td>
<td>188 (30.9%)</td>
<td>69 (26.0%)</td>
<td>1.24 (0.89-1.72)</td>
</tr>
<tr>
<td>Overall asthma exacerbations (Y)</td>
<td>295 (48.6%)</td>
<td>114 (43.0%)</td>
<td>1.22 (0.91-1.65)</td>
</tr>
<tr>
<td>Modified treatment steps</td>
<td>Addition of preventer medication (Y)</td>
<td>500 (83.1%)</td>
<td>213 (80.4%)</td>
</tr>
<tr>
<td>Asthma severity score</td>
<td>Moderate to severe (Y)</td>
<td>504 (83.7%)</td>
<td>215 (81.1%)</td>
</tr>
</tbody>
</table>

Table 7.1.2: Overall effect of *Merged GSTM1/GSTP1/GSTT1* genotype (wildtype (W/T) and mutant varieties) on hospital admissions, oral steroid intake and absences due to asthma exacerbations, and overall asthma exacerbations, in children and young adults recruited to the BREATHE study

Key: *: p<0.05; OR: Odds ratio; CI: Confidence interval W/T: Wild type variants of GSTM1, GSTT1 and GSTP1 rs1695
Mutant: at least one mutant variant in the form of GSTM1 or GSTT1 null or GSTP1 rs1695 variant

P values were calculated by binary logistic regression corrected for age and sex

The mutant variant is associated with hospital admissions (p=0.046) when compared to the wildtype variant. The odds ratio for mutant variant when compared to the wildtype variant was 1.51, indicating that the mutant variant may play a role in increasing the mutant of asthma exacerbations (Table 7.1.2/ Figure 7.1.1). We did not observe any significant association between mutant variant and overall asthma exacerbations (OR 1.22 (95%CI 0.89-1.72); p=0.184), or asthma-related absences (OR 1.11 (95%CI 0.82-1.50); p=0.506), or oral steroid intake due to asthma exacerbations (OR 1.24 (95%CI 0.64-1.09); p=0.196) over the previous six months (Table 7.1.2).

Through proxy measures, we tested for gene-dosage effect using the modified BTS treatment steps, categorised into salbutamol and addition of preventer medication. We also tested for an association with risk of severity (Table 7.1.2). However, no significant
effect was observed, indicating the absence of a gene-treatment step relationship with this variant. No significant effect was observed when the null allele was tested for each severity step, indicating there is no relationship with drug-associated severity with this variant.
Figure 7.1.1 Illustration of Overall effect of GST mutant variant on hospital admissions, oral steroid intake and absences due to asthma exacerbations, and overall asthma exacerbations, in children and young adults recruited to the BREATHE study.
Table 7.1.3: Overall effect of *Merged GSTM1/GSTP1/GSTT1* genotype (wildtype (W/T) and mutant varieties) on hospital admissions, oral steroid intake and absences due to asthma exacerbations, and overall asthma exacerbations, in age categorised children and young adults recruited to the BREATHE study

<table>
<thead>
<tr>
<th></th>
<th>Hospital admission</th>
<th>Absence</th>
<th>Oral steroid intake</th>
<th>Overall asthma exacerbations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mutant</td>
<td>W/T</td>
<td>Mutant</td>
<td>W/T</td>
</tr>
<tr>
<td>3-4 years</td>
<td>Yes</td>
<td>31 (30.7%)</td>
<td>9 (40.9%)</td>
<td>49 (48.5%)</td>
</tr>
<tr>
<td></td>
<td>n</td>
<td>101</td>
<td>22</td>
<td>101</td>
</tr>
<tr>
<td></td>
<td>OR (95%CI)</td>
<td>0.66 (0.25-1.70)</td>
<td>0.66 (0.26-1.68)</td>
<td>1.21 (0.47-3.08)</td>
</tr>
<tr>
<td></td>
<td>P Value</td>
<td>0.384</td>
<td>0.378</td>
<td>0.697</td>
</tr>
<tr>
<td>5-12 years</td>
<td>Yes</td>
<td>77 (20.4%)</td>
<td>22 (12.0%)</td>
<td>212 (56.2%)</td>
</tr>
<tr>
<td></td>
<td>n</td>
<td>377</td>
<td>183</td>
<td>377</td>
</tr>
<tr>
<td></td>
<td>OR (95%CI)</td>
<td>1.88 (1.13-3.14)</td>
<td>1.15 (0.80-1.64)</td>
<td>1.12 (0.76-1.67)</td>
</tr>
<tr>
<td></td>
<td>P Value</td>
<td>0.015*</td>
<td>0.455</td>
<td>0.559</td>
</tr>
<tr>
<td>13-22 years</td>
<td>Yes</td>
<td>15 (12.1%)</td>
<td>6 (10.0%)</td>
<td>91 (73.4%)</td>
</tr>
<tr>
<td></td>
<td>n</td>
<td>124</td>
<td>60</td>
<td>124</td>
</tr>
<tr>
<td></td>
<td>OR (95%CI)</td>
<td>1.18 (0.43-3.25)</td>
<td>1.28 (0.61-2.67)</td>
<td>1.54 (0.69-3.44)</td>
</tr>
<tr>
<td></td>
<td>P Value</td>
<td>0.746</td>
<td>0.510</td>
<td>0.290</td>
</tr>
</tbody>
</table>

Table 7.1.3: Overall effect of *Merged GSTM1/GSTP1/GSTT1* genotype (wildtype (W/T) and mutant varieties) on hospital admissions, oral steroid intake and absences due to asthma exacerbations, and overall asthma exacerbations, in age categorised children and young adults recruited to the BREATHE study.

Key: *: p<0.05; OR: Odds ratio; CI: Confidence interval W/T: Wild type variants of GSTM1, GSTT1 and GSTP1 rs1695
Mutant: at least one mutant variant in the form of GSTM1 or GSTT1 null or GSTP1 rs1695 variant
P values were calculated by binary logistic regression corrected for age and sex.

In 5-12 year olds with asthma the mutant variant is associated with hospital admissions due to asthma exacerbations (p=0.015) when compared to the wildtype variant. The odds ratio for mutant variant when compared to the wildtype variant was 1.88, indicating that the mutant variant may play a role in increasing the mutant of asthma exacerbations (Table 7.1.3/ Figure 7.1.2). We did not observe any significant association between mutant variant and any other measure of exacerbation in any other age category.
Figure 7.1.2 Illustration of Overall effect of GST mutant variant on hospital admissions, oral steroid intake and absences due to asthma exacerbations, and overall asthma exacerbations, in 5-12 year olds recruited to the BREATHE study.
SECTION 7.2

Relationship between GST mutant variant and measures of pulmonary function in a sample of UK children and young adults recruited to the BREATHE study and PAGES

GST mutant variant and pulmonary function

<table>
<thead>
<tr>
<th>Merged GSTM1/GSTP1/GSTT1</th>
<th>Mutant</th>
<th>W/T</th>
<th>n</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ppPEFR [n] (sd)</td>
<td>[466]</td>
<td>88.5 (17.3)</td>
<td>[211]</td>
<td>89.2 (16.3)</td>
</tr>
<tr>
<td>Mean ppFEV1 [n] (sd)</td>
<td>[473]</td>
<td>96.3 (14.8)</td>
<td>[217]</td>
<td>98.1 (15.2)</td>
</tr>
<tr>
<td>Mean ppFVC [n] (sd)</td>
<td>[472]</td>
<td>96.7 (14.1)</td>
<td>[217]</td>
<td>97.0 (13.9)</td>
</tr>
<tr>
<td>FEV1/FVC [n] (sd)</td>
<td>[472]</td>
<td>84.6 (9.2)</td>
<td>[217]</td>
<td>85.9 (9.0)</td>
</tr>
<tr>
<td>Mean change in ppFEV1 post bronchodilator [n] (sd)</td>
<td>[89]</td>
<td>5.2 (6.5)</td>
<td>[30]</td>
<td>4.8 (4.9)</td>
</tr>
<tr>
<td>Mean Nitric oxide [n] (ppb) (sd)</td>
<td>[105]</td>
<td>33.9 (32.6)</td>
<td>[40]</td>
<td>33.1 (31.5)</td>
</tr>
</tbody>
</table>

Nitric oxide categorised by clinical relevance

- Clinically elevated exhaled nitric oxide (>50ppb) (Y): 35/105 (33.3%) vs 11/40 (27.5%); 46; 0.401
- Elevated exhaled nitric oxide (25–49ppb) (Y): 20/105 (19.1%) vs 13/40 (32.5%); 33; 0.316

Table 7.2.1: Overall effect of Merged GSTM1/GSTP1/GSTT1 genotype (wildtype (W/T) and mutant varieties) on mean % predicted lung function

Key: pp: % predicted; ppb: parts per billion; FEV1: Forced expiratory volume in 1 second; FVC: Forced vital capacity; W/T: Wild type variants of GSTM1, GSTT1 and GSTP1 rs169; Mutant: at least one mutant variant in the form of GSTM1 or GSTT1 null or GSTP1 rs1695 variant; P-values were calculated by univariate analysis of variance corrected for sex

P-values for Nitric oxide categorised by clinical relevance were calculated by multinomial logistic regression corrected for age and sex

We tested for an effect between GST mutant variant and measures of pulmonary function (Table 7.2.1). No significant effect was observed with any investigated measures of pulmonary function.
**GST mutant variant and pulmonary function categorised by age**

<table>
<thead>
<tr>
<th>Merged GSTM1/ GSTP1/ GSTT1</th>
<th>5-12 years</th>
<th>P Value</th>
<th>13-22 years</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mutant</td>
<td>W/T</td>
<td>Mutant</td>
<td>W/T</td>
</tr>
<tr>
<td>Mean ppPEFR (sd)</td>
<td>86.3 (17.0)</td>
<td>87.2 (15.8)</td>
<td>0.539</td>
<td>92.7 (16.9)</td>
</tr>
<tr>
<td>N</td>
<td>339</td>
<td>151</td>
<td>-</td>
<td>69</td>
</tr>
<tr>
<td>Mean ppFEV1 (sd)</td>
<td>96.0 (14.5)</td>
<td>98.0 (15.1)</td>
<td>0.180</td>
<td>97.3 (15.8)</td>
</tr>
<tr>
<td>Mean ppFVC (sd)</td>
<td>95.4 (14.2)</td>
<td>95.6 (13.5)</td>
<td>0.832</td>
<td>100.4 (13.3)</td>
</tr>
<tr>
<td>FEV1/FVC (sd)</td>
<td>84.9 (8.8)</td>
<td>86.4 (8.8)</td>
<td>0.119</td>
<td>83.8 (10.2)</td>
</tr>
<tr>
<td>N</td>
<td>343</td>
<td>155</td>
<td>-</td>
<td>129</td>
</tr>
<tr>
<td>Mean change in ppFEV1 post bronchodilator (sd)</td>
<td>5.3 (6.3)</td>
<td>5.2 (4.4)</td>
<td>0.835</td>
<td>4.9 (7.5)</td>
</tr>
<tr>
<td>N</td>
<td>69</td>
<td>21</td>
<td>-</td>
<td>20</td>
</tr>
<tr>
<td>Nitric oxide (ppb) (sd)</td>
<td>32.0 (31.9)</td>
<td>31.9 (27.9)</td>
<td>0.895</td>
<td>39.1 (34.9)</td>
</tr>
<tr>
<td>N</td>
<td>84</td>
<td>26</td>
<td>-</td>
<td>21</td>
</tr>
</tbody>
</table>

**Table 7.2.2: Overall effect of Merged GSTM1/GSTP1/GSTT1 genotype (wildtype (W/T) and mutant varieties) on mean % predicted lung function categorised by age in participants recruited to the BREATHE study and PAGES**

Key: pp: % predicted; ppb: parts per billion; FEV1: Forced expiratory volume in 1 second; FVC: Forced vital capacity W/T: Wild type variants of GSTM1, GSTT1 and GSTP1 rs1695; Mutant: at least one mutant variant in the form of GSTM1 or GSTT1 null or GSTP1 rs1695 variant

P-values were calculated by univariate analysis of variance corrected for sex

We tested for an association between GST mutant variant and measures of pulmonary function, categorised by age (Table 7.2.2). No significant effect was observed with any investigated measures of pulmonary function.
SECTION 7.3

Relationship between GST mutant variant and environmental factors in a sample of UK children and young adults

GST mutant variant and exposure to environmental tobacco smoke

867 individuals recruited to the BREATHE study were tested for an interaction between the GST mutant variant and environmental tobacco smoke on exacerbations (Table 7.3.1). The GST wildtype variant was present in 30.3% (n=265) of participants. 35.1% (n=93) were exposed to environment tobacco smoke. The GST mutant variant was present in 69.4% (n=602) of participants. 36.1% (n=217) were exposed to environmental tobacco smoke.

We did not observe any significant association between the GST mutant variant when compared to the wildtype variant and overall asthma exacerbations, or asthma-related hospital admissions, or asthma-related absences, or oral steroid intake due to asthma exacerbations over the previous six months (Table 7.3.1). Through proxy measures, we tested for an association with risk of severity (Table 7.3.1). However, no significant effect was observed indicating that there is not a gene-dose relationship with this variant. No significant effect was observed when the null allele was tested for each severity step, indicating there is no relationship with drug-associated severity with this variant, in this sample.
<table>
<thead>
<tr>
<th></th>
<th>Merged GSTM1/GSTP1/GSTT1</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th>P Value for interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Exposed to tobacco smoke</td>
<td>Not exposed to tobacco smoke</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mutant (n=217)</td>
<td>W/T (n=93)</td>
<td>OR (95%CI)</td>
<td>Mutant (n=385)</td>
<td>W/T (n=172)</td>
<td>OR (95%CI)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hospital admission due to asthma exacerbations (Y)</td>
<td>53 (24.4%)</td>
<td>13 (13.9%)</td>
<td>1.92 (0.98-3.77)</td>
<td>70 (18.2%)</td>
<td>24 (13.9%)</td>
<td>1.29 (0.78-2.15)</td>
<td>0.356</td>
<td></td>
</tr>
<tr>
<td>Absence due to asthma exacerbations (Y)</td>
<td>105 (48.4%)</td>
<td>38 (40.9%)</td>
<td>1.31 (0.79-2.15)</td>
<td>142 (36.9%)</td>
<td>62 (36.1%)</td>
<td>0.99 (0.68-1.46)</td>
<td>0.397</td>
<td></td>
</tr>
<tr>
<td>Oral steroid intake due to asthma exacerbations (Y)</td>
<td>73 (33.6%)</td>
<td>24 (25.8%)</td>
<td>1.41 (0.81-2.44)</td>
<td>115 (29.9%)</td>
<td>45 (26.2%)</td>
<td>1.51 (0.77-1.73)</td>
<td>0.567</td>
<td></td>
</tr>
<tr>
<td>Overall asthma exacerbations (Y)</td>
<td>122 (56.2%)</td>
<td>41 (44.1%)</td>
<td>1.57 (0.95-2.59)</td>
<td>173 (44.9%)</td>
<td>73 (42.4%)</td>
<td>1.06 (0.73-1.53)</td>
<td>0.212</td>
<td></td>
</tr>
<tr>
<td>Asthma severity score</td>
<td>Moderate to severe (Y)</td>
<td>180 (82.9%)</td>
<td>75 (80.6%)</td>
<td>1.15 (0.61-2.15)</td>
<td>324 (84.2%)</td>
<td>140 (81.4%)</td>
<td>1.21 (0.76-1.95)</td>
<td>0.892</td>
</tr>
</tbody>
</table>

Table 7.3.1: Overall effect of Merged GSTM1/GSTP1/GSTT1 genotype (wildtype (W/T) and mutant varieties) on hospital admissions, oral steroid intake and absences due to asthma exacerbations, overall asthma exacerbations and asthma severity, categorised by exposure to tobacco smoke in children and young adults recruited to the BREATHE study.

Key: OR: Odds ratio; CI: Confidence interval W/T: Wild type variants of GSTM1, GSTT1 and GSTP1 rs1695; Mutant: at least one mutant variant in the form of GSTM1 or GSTT1 null or GSTP1 rs1695 variant

Asthma severity score: (bronchodilator use adjusted modified treatment steps): 0=1-mild, 2=moderate to severe

P values and odds ratios were calculated by binary logistic regression corrected for age, and sex.
We did not observe any significant association between mutant variant and any other measure of exacerbation in any age category (Table 7.3.2).
Table 7.3.3: Overall effect of *Merged GSTM1/GSTP1/GSTT1* genotype (wildtype (W/T) and mutant varieties) on modified treatment steps and asthma severity, categorised by age and exposure to tobacco smoke in children and young adults recruited to the BREATHE study

<table>
<thead>
<tr>
<th></th>
<th>5-12 years</th>
<th>13-22 years</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Modified treatment steps</td>
<td>Addition of preventer medication</td>
</tr>
<tr>
<td><strong>Merged GSTM1/ GSTP1/GSTT1</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-12 years</td>
<td>Modified treatment steps</td>
<td>Addition of preventer medication</td>
</tr>
<tr>
<td>Asthma severity score</td>
<td>Moderate to severe</td>
<td>113 (82.5%)</td>
</tr>
<tr>
<td>13-22 years</td>
<td>Modified treatment steps</td>
<td>Addition of preventer medication</td>
</tr>
<tr>
<td>Asthma severity score</td>
<td>Moderate to severe</td>
<td>40 (81.6%)</td>
</tr>
</tbody>
</table>

Key: *: p<0.05; OR: Odds ratio; CI: Confidence interval; W/T: Wild type variants of GSTM1, GSTT1 and GSTP1 rs1695; Mutant: at least one mutant variant in the form of GSTM1 or GSTT1 null or GSTP1 rs1695 variant; ETS+: exposed to environmental tobacco smoke; ETS-: Not exposed to environmental tobacco smoke; Asthma severity score: (bronchodilator use adjusted modified treatment steps): 0=1-mild, 2=moderate to severe. P values and odds ratios were calculated by binary logistic regression corrected for age, and sex.
Through proxy measures, we tested for gene-dosage effect using the modified BTS treatment steps, categorised into salbutamol and addition of preventer medication. We also tested for an association with risk of severity. In 13-22 year olds exposed to environmental tobacco smoke with asthma the mutant variants are associated with addition of preventer medications in modified treatment steps (p=0.029) and moderate to severe asthma as classified by the asthma severity score (p=0.020). When compared to the wildtype variant the odds ratio for mutant variants in modified treatment steps was 2.67, and in severity was 3.19 indicating that mutant variants may have a role in a gene-dose relationship and with drug associated severity (Table 7.3.3; Figure 7.3.1). We did not observe any significant association between mutant variants and any other measure of exacerbation in any other age category, indicating a lack of association between the mutant variants and these variables.
Figure 7.3.1 Illustration of Overall effect of GST mutant variants on modified treatment steps and asthma severity score, in 13-22 year olds with asthma, exposed to environmental tobacco smoke (ETS-= not exposed; ETS+= exposed) and recruited to the BREATHE study
Table 7.3.4: Overall effect of *Merged GSTM1/GSTP1/GSTT1* genotype (wildtype (W/T) and mutant varieties) on mean % predicted lung function categorised by age and exposure to environmental tobacco smoke

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Exposed to environmental tobacco smoke</th>
<th>Not exposed to environmental tobacco smoke</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5-12</td>
<td>P Value</td>
</tr>
<tr>
<td><strong>Merged GSTM1/GSTP1/GSTT1</strong></td>
<td>Mutant</td>
<td>W/T</td>
</tr>
<tr>
<td>Mean ppPEFR (sd)</td>
<td>85.7 (17.6)</td>
<td>86.9 (17.7)</td>
</tr>
<tr>
<td>Total</td>
<td>113</td>
<td>46</td>
</tr>
<tr>
<td>Mean ppFEV1 (sd)</td>
<td>94.5 (15.6)</td>
<td>96.3 (17.2)</td>
</tr>
<tr>
<td>Mean ppFVC (sd)</td>
<td>94.2 (14.7)</td>
<td>94.5 (15.0)</td>
</tr>
<tr>
<td>FEV1/FVC (sd)</td>
<td>84.8 (9.2)</td>
<td>85.8 (9.5)</td>
</tr>
<tr>
<td>Total</td>
<td>113</td>
<td>48</td>
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</tbody>
</table>

Key: pp: % predicted; ppb: parts per billion; FEV1: Forced expiratory volume in 1 second; FVC: Forced vital capacity W/T: Wild type variants of GSTM1, GSTT1 and GSTP1 rs1695; Mutant: at least one mutant variant in the form of GSTM1 or GSTT1 null or GSTP1 rs1695 variant

P-values were calculated by univariate analysis of variance corrected for sex
We tested for an interaction between GST mutant variants and measures of pulmonary function, categorised by age. Bronchodilator reversibility and exhaled nitric oxide were excluded due to insufficient cases (Table 7.3.4). No significant effect was observed between the variant and any investigated measure of pulmonary function.
**GST mutant variants and atopy**

1222 individuals recruited to the BREATHE study and PAGES were tested for an association between the mutant variant and any reported allergy (Table 7.3.5; Figure 7.3.3). 355 individuals recruited to PAGES were tested for an association between the mutant variant and any confirmed sensitivity to a selection of tested allergens.

When compared to the wildtype variant, the mutant variant is associated with increased risk of reported allergy in children and young adults from both the BREATHE study and PAGES (p=0.020). The odds ratio for the mutant variant, when compared to the wildtype variant is 1.47, indicating that the mutant variant is a risk factor in allergy. We did not observe any significant association between mutant variant and confirmed sensitivity.

<table>
<thead>
<tr>
<th></th>
<th>Merged GSTM1/GSTP1/GSTT1</th>
<th>Mutant</th>
<th>W/T</th>
<th>OR (95% CI)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reported allergy</td>
<td>184/858 (21.5%)</td>
<td>58/364  (15.9%)</td>
<td>1.47 (1.06-2.04)</td>
<td>0.020*</td>
<td></td>
</tr>
<tr>
<td>Positive SPT result to any allergen</td>
<td>59/256 (23.0%)</td>
<td>22/99 (22.2%)</td>
<td>1.28 (0.70-2.34)</td>
<td>0.416</td>
<td></td>
</tr>
</tbody>
</table>

Table 7.3.5: Overall effect of **Merged GSTM1/GSTP1/GSTT1** genotype (wildtype (W/T) and mutant varieties) on participant reported allergy and skin prick test identified sensitivity

Key: *: p<0.05; OR: Odds ratio; CI: Confidence interval; SPT: Skin prick test

P-values and odds ratios were calculated by binary logistic regression corrected for age and sex
Figure 7.3.2 Illustration of Overall effect of GST mutant variants on modified treatment steps and reported allergy and SPT sensitivity in children and young adults with recruited to the BREATHE study and PAGES
**GST mutant variants and quality of life**

212 individuals recruited to PAGES were tested for an association between GST mutant variants and mean quality of life scores derived from the Paediatric Asthma Quality of Life Questionnaire (Table 7.3.6).

We did not observe any significant association between GST mutant variants and mean activity score, mean symptoms score, mean emotions score or mean overall score (Table 7.3.6). These results indicate there is no relationship between quality of life and this SNP.

<table>
<thead>
<tr>
<th></th>
<th>Merged GSTM1/GSTP1/GSTT1</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mutant (n=129)</td>
<td>W/T (n=56)</td>
<td>P value</td>
</tr>
<tr>
<td>Mean activity score (sd)</td>
<td>4.7 (1.7)</td>
<td>4.7 (1.8)</td>
<td>0.956</td>
</tr>
<tr>
<td>Mean symptoms score (sd)</td>
<td>5.1 (1.6)</td>
<td>5.1 (1.7)</td>
<td>0.543</td>
</tr>
<tr>
<td>Mean emotion score (sd)</td>
<td>5.5 (1.5)</td>
<td>5.1 (1.8)</td>
<td>0.834</td>
</tr>
<tr>
<td>Mean overall score (sd)</td>
<td>5.2 (1.5)</td>
<td>5.0 (1.7)</td>
<td>0.749</td>
</tr>
</tbody>
</table>

Table 7.3.6: Overall effect of Merged GSTM1/GSTP1/GSTT1 genotype (wildtype (W/T) and mutant varieties) on mean quality of life scores derived from the Paediatric Asthma Quality of Life Questionnaire.

P-values were calculated by univariate analysis of variance corrected for age and sex.
DISCUSSION

The next six chapters in this thesis present the discussion for each results chapter. Each discussion chapter is in a comparable format. In each chapter discusses each of the five hypotheses followed by a discussion of the relationship between the variant and asthma severity. Chapter 12 discusses the proxy markers tested in this thesis and Chapter 13 discusses the implications of the variants on asthma severity, concluding the thesis. As an aide memoir, the next two pages contain two summary tables, the first table summarises the findings reported in this thesis, the second table compares the sample sizes used in the analysis with sample sizes required to achieve 90% power.
<table>
<thead>
<tr>
<th>Variant</th>
<th>Asthma exacerbations</th>
<th>Modified treatment steps/Severity score</th>
<th>Pulmonary function</th>
<th>Asthma exacerbation + ETS</th>
<th>Modified treatment steps / Severity score +ETS</th>
<th>Pulmonary function + ETS</th>
<th>Asthma exacerbation + damp</th>
<th>Allergy</th>
<th>QOL</th>
</tr>
</thead>
<tbody>
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<td>&lt; &lt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&lt;&lt;</td>
<td>&lt;</td>
<td>&lt;</td>
</tr>
<tr>
<td>MMP9 rs17576</td>
<td>&lt;&lt; &lt;</td>
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<td>&lt;&lt; &lt;&lt;</td>
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<td>GST mutant</td>
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<td></td>
<td></td>
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</tr>
</tbody>
</table>

**Table D.1: Summary of statistically significant findings**

Key: >, Associated with increased risk of outcome measure; <, associated with a protective effect on outcome measure. Duplication of symbols represents the number of associations in a category, including age-categorised findings; ETS, Environmental tobacco smoke; QOL, Quality of Life
<table>
<thead>
<tr>
<th>Gene</th>
<th>Outcome</th>
<th>( m )</th>
<th>( \delta )</th>
<th>( \sigma )</th>
<th>( p0 )</th>
<th>( p1 )</th>
<th>( n ) for mutant variant at 90% power</th>
<th>Current smallest mutant n</th>
<th>Total n at 90% power</th>
<th>Current total n</th>
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<td>0.4</td>
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<td></td>
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<tr>
<td></td>
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<td>12</td>
<td>15</td>
<td>-</td>
<td>-</td>
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<td>25</td>
<td>15</td>
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<td>157</td>
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<td>-</td>
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<td>-</td>
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<td>56</td>
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Table D.2: Comparison of sample size required to achieve 90% power and sample size used for analysis.

Key: FVC, Forced vital capacity; BDR, Bronchodilator reversibility; HDM, House dust mite; QOL, Quality of life; SPT, Skin prick test; ETS, Environmental tobacco smoke; \( m \), ratio of distribution between two samples; \( \delta \), Minimum important difference; \( \sigma \), Standard deviation; \( p0 \), Probability of exposure in population 0; \( p1 \), Probability of exposure in population 1.
Discussion of the impact of *CHI3L1* (rs4950928) on asthma severity in children and young adults

*CHI3L1* (rs4950928)

**RESEARCH QUESTION:**
Does *CHI3L1* (rs4950928) influence the severity of asthma in children and young adults?

**Hypothesis 1** - *CHI3L1* (rs4950928) plays a role in the severity of asthma in children and young adults (Section 4.1)

*CHI3L1* SNP rs4950928 is significantly associated with asthma-related hospital admissions in children and young adults with asthma (Appendix 1). The odds ratio for the presence of the G allele (CG or GG) was 0.72, indicating that the minor G allele may confer protection against asthma-related exacerbations (Table 4.1.2; Figure 4.1.1). Our previously published data on a sample of 1071 participants also found an odds ratio of <1 (0.62) again indicating a protective effect on asthma-related exacerbations in the form of hospital admissions. There was a difference of 5.1% between the two groups, with 14.2% of the group with the G allele suffering from at least one exacerbation requiring a hospital admission in the previous six months. Although 5.1% is a relatively small difference, in terms of hospital admission even a relatively small reduction or increase in the number can have a large impact on patient lives and healthcare. No other measures of exacerbation were associated with the rs4950928 variant (Table 4.1.2).

We tested for an association between the rs4950928 variant and modified treatment steps and a novel asthma severity score. The modified treatment steps have been used successfully in previous publications as a proxy for measuring a gene-dosage effect\(^3\). The asthma severity score is derived from modified treatment steps, adjusted by bronchodilator use. No association was found between either measure, suggesting there is no gene-dosage effect and no drug associated asthma severity with this variant.
In 2007 Chupp et al\textsuperscript{100} associated increased quantities of CHI3L1 in the serum and the lungs with asthma severity in an adult sample. In 2008 Ober et al\textsuperscript{54} reported that \textit{CHI3L1} is a susceptibility gene for asthma and that CHI3L1 can be used as a biomarker for asthma and decline in lung function. This was derived from an adult sample of Hutterites and from the COAST cohort\textsuperscript{54}. Increased quantities of serum CHI3L1 has been observed in Chinese patients with asthma, and the level has been correlated with asthma exacerbations\textsuperscript{106}. Contrary to these findings, Wu et al have reported an absence of any association with three chitinases, from a cohort of 422 children recruited across the USA\textsuperscript{89}. The rs4950928 \textit{CHI3L1} polymorphism was not investigated by Wu et al. An association between CHI3L1 and atopy was identified in Korean children, but no association was found with asthma\textsuperscript{168}. Rathcke et al\textsuperscript{23} investigated the \textit{CHI3L1} polymorphisms in a sample of 6514 Danish adults. Of this sample, 540 had asthma, 300 of whom were defined as atopic asthma. In contrast to other studies, these researchers observed an association between the minor G allele, atopic asthma and self reported physician diagnosed asthma. \textit{CHI3L1} has not been identified in any genome wide association study as an asthma susceptibility risk. \textit{CHI3L1} SNP’s were however investigated through a genome wide association study of serum CHI3L1 levels, known to be elevated in patients with severe asthma\textsuperscript{107}.

This study was undertaken to replicate the initial findings of Ober et al\textsuperscript{30}. We did not investigate serum or lung concentrations of CHI3L1 as a marker for severity. The odds ratios for all measures of exacerbation were less than one, consistent with the previously reported observation of the protective role of rs4950928. There was no direct association between \textit{CHI3L1} and asthma severity in this sample. Our identification of an association between \textit{CHI3L1} and exacerbations, may explain the enhanced risk from \textit{CHI3L1} as hypothesised by Ober et al\textsuperscript{30}. No data regarding exacerbation triggers was collected in this study, though this data would be important in exploring the clinical and therapeutic impact of \textit{CHI3L1}.

We subsequently categorised by age, however no association was detected. We conducted a power calculation to obtain the sample size required to detect a 10% difference in overall asthma exacerbations at 90% power (Table D.2). The sample size required to detect a difference in the mutant group was 417 participants, we analysed
with a sample of 65 participants. As such, we are underpowered to detect any effect from an age stratified population with rs4950928 and further, more powered work is required to detect, or exclude an effect in this population.

In summary, the rs4950928 variant influences the severity of asthma in children and young adults. The rs4950928 dominant, minor G allele (CG/GG) exerts a protective effect on asthma severity when compared to the major C allele (CC). As such, homozygosity for the C allele is a risk factor for asthma exacerbations in the form of hospital admission.

**Hypothesis 2 - CHI3L1 (rs4950928) effects the severity of asthma in children and young adults through influencing airway remodelling and leukocyte infiltration into the lung (Section 4.2)**

*CHI3L1* SNP rs4950928 is associated with mean % predicted FVC (a proxy measure of obstruction). The dominant G allele (CG or GG) allele reduces mean % predicted FVC in children and young adults with asthma when compared to the homozygous CC sample by 1.7%. (Table 4.2.1; Figure 4.2.1). Further categorisation by age increased the effect indicating that in the 13-22 year age group, the minor -131G allele is associated with reduced mean % predicted FEV1 by 4.9% and FVC by 5.2% in our pulmonary function cohort of over 1000 participants (Table 4.2.2; Figure 4.2.2). Our previously published data on a pulmonary function cohort of approximately 500 participants failed to identify any association with pulmonary function.

Using mean change in % predicted FEV1 post bronchodilator as a proxy measure for smooth muscle hyperplasia, we tested for an association with the rs4950928 variant. However, we failed to identify any association between percentage change in lung function with bronchodilator and the presence of the rs4950928 variant (Table 4.2.1). The sample was subsequently classified by age, however there was no discernable association between the rs49509528 variant categorised by age and mean change in FEV1 post bronchodilator. (Table 4.2.2)

Exhaled nitric oxide was used as a proxy measure of pulmonary eosinophil infiltration, we tested for an association between the rs4950928 variant and mean exhaled nitric oxide (ppb) and further classified the exhaled nitric oxide levels into clinically elevated, elevated
and normal ranges. However, no association between exhaled nitric oxide and either variant was identified in this sample (Table 4.2.1). The sample was subsequently categorised by age, however there was no discernable association between the rs49509528 variant categorised by age and exhaled nitric oxide (Table 4.2.2).

In 2008 Ober et al\textsuperscript{30} reported that CHI3L1 can be used as a biomarker for decline in lung function in adults and that the C allele of the rs4950928 variant is a predictor of decreased FEV1 and FEV1/FVC.

In contrast to the findings by Ober et al and in a closer relationship with the findings of Rathcke et al\textsuperscript{55} we found that the -131G allele is associated with decline in FEV1 and FVC in a sample of 13-22 year olds with asthma, and is associated with a decline in FVC across children and young adults.

We did not collect any data on bronchial hyper-responsiveness in the form of methacholine challenge in this study, though this data would be important in exploring the clinical and therapeutic impact of CHI3L1. We did measure bronchial hyper-responsiveness by proxy through bronchodilator reversibility and exhaled nitric oxide, however, unlike Ober et al we found no association.

In summary, although this data is statistically significant, the effect is relatively small, and in both sub groups is above the clinically relevant threshold of 80%. Furthermore, the direction of effect is contrary to the pathophysiology and to data reported from other studies. Although the sample size of this cohort is sufficiently powered (Table D.2), the small effect size could indicate a type II error and further studies will be required to confirm or discount the impact of the rs4950928 on pulmonary function in children and young adults with asthma. Furthermore, all the mean lung function measures are in the clinically normal range, as such, in this cohort the rs4950928 variant does not appear to influence the severity of lung function in children and young adults with asthma, through airway remodelling and increased leukocyte migration leading to a hyper-responsive lung.

**Hypothesis 3- CHI3L1 (rs4950928) interacts with environmental tobacco smoke (ETS) exposure and exposure to damp to influence the severity of asthma (Section 4.3).**
This is the first study to associate the dominant G allele (CG/GG) with mean % predicted FEV1 and mean % predicted FVC in participants between 13-22 years of age, exposed to environmental tobacco smoke (Table 4.3.2; Figure 4.3.1). When exposed to environmental tobacco smoke, the dominant G allele (CG or GG) allele reduces mean % predicted FEV1 in children and young adults with asthma when compared to the homozygous CC sample by 4.4% and mean % predicted FVC by 5.6%. We first tested for associations with measures of asthma exacerbations, but there was no discernible association (Table 4.3.1), nor was there a detectable association with PEFR and FEV1/FVC (Table 4.3.2).

This is the first study to significantly associate CHI3L1 SNP rs4950928 with asthma-related exacerbations, asthma-related hospital admissions and asthma-related absence in children and young adults exposed to damp. The odds ratio for the presence of the -131G allele (CG or GG) in overall exacerbations was 0.13 (Difference of 42.4%), the odds ratio for the presence of the -131G allele (CG or GG) in hospital admissions was 0.08 (Difference of 23.3%), the odds ratio for the presence of the -131G allele (CG or GG) in absence due to asthma exacerbations was 0.20 (Difference of 38.7%) indicating that the minor allele may confer protection against asthma-related exacerbations (Table 4.3.3; Figure 4.3.2). There was no association with oral steroid use, however despite a lack of statistical significance, there is a difference of 14.9% between the two groups, and an odds ratio of 0.28. The sample size investigated was underpowered by a factor of four to investigate this interaction (Table D.2). As such, it is important that this interaction is reinvestigated with a larger sample size before the results are fully accepted or discounted. The results are however, biologically plausible. It is possible that a decrease in CHI3L1 would lead to reduced reactivity to CHI3L1 and therefore less asthma exacerbations. As such it is likely that these findings are true and with approximately 15% of the population exposed to household damp, could be an important therapeutic target.

In summary, the rs4950928 variant interacts with exposure to damp to influence the severity of asthma. When both samples are exposed to damp, the rs4950928 dominant, G allele (CG/GG) exerts an enhanced protective effect in comparison to the enhance risk effect exerted by the homozygous C allele (CC). As such, homozygosity for the C allele is a
risk factor for asthma exacerbations in the form of hospital admission, absence and overall exacerbations.

**Hypothesis 4- The genetic variant rs4950928 is associated with sensitivity and/or allergy to chitin containing allergens (Section 4.3)**

We tested for an association between the rs4950928 variant, reported house dust mite allergy, and sensitivity to three chitin containing allergens (house dust mite, *A. alternans*, *A. fumigatus*). Our study corroborates the findings of Ober *et al.*, that *CHI3L1* SNP rs4950928 is not associated with increased risk of allergy or sensitivity (Table 4.3.4). This observation contrasts with the association with atopic asthma published by Rathcke *et al.* We performed a power calculation on sensitivity to house dust mite, which indicated that to obtain 90% power, a sample size of 148 in the CG/GG population would be required (Table D.2). However, the sample analysed contained only 31 participants in the CG/GG population and as such we are not in the position to discount the possibility of sensitivity to any of the investigated allergens in this population.

In summary, the rs4950928 variant does not appear to be associated with an increased risk of sensitivity and/or allergy to chitin containing allergens in this sample, however further work is required to confirm this finding.

**Hypothesis 5- *CHI3L1* (rs4950928) influences the quality of life of children and young adults with asthma (Section 4.3)**

We have found an association between asthma exacerbations in the form of hospital admissions, but no association in the form of drug associated asthma severity. Asthma
Exacerbations have been shown to have a detrimental impact on quality of life\textsuperscript{169}. The rs4950928 variant was tested for associations with overall quality of life scores, activity score, emotion score and symptom score in a cohort of approximately 200 participants, however we found no association between the rs4950928 variant and quality of life in this sample (Table 4.3.5). The sample we investigated was underpowered by an approximate factor of four for this investigation, as such we cannot exclude the possibility of an effect on quality of life (Table D.2).

In summary, the rs4950928 variant does not appear to impact upon the quality of life in children and young adults with asthma in this sample.

**Summary of the relationship between CHI3L1 (rs4950928) and asthma severity in a sample of UK children and young adults**

The chitinase family of hydrolys are associated with TH2 induced inflammation and airway hypersensitivity\textsuperscript{5}. CHI3L1 increases myofibroblast replication and differentiation through activation of MAP kinase and PI-3K signalling cascades in fibroblast, enhances production of the pro-inflammatory cytokines and chemokines, TNF\(\alpha\), IL-8, RANTES,
eotaxin and increases activity of NF-κB \cite{8,25}. An increase in secretion of these chemo attractants will lead to leukocyte migration.

The C allele of the rs4950928 variant of \textit{CHI3L1} increases the risk of hospital admissions due to asthma exacerbations in children and young adults when compared to the dominant G allele. The dominant G allele has been associated with a decrease in circulating serum levels of CHI3L1, and is exerting a protective effect on asthma exacerbations. As a sentinel molecule CHI3L1 binds to substrate and initiates a severe immune response\cite{96,101}. Cumulative adhesion of chitin and CHI3L1 substrates in the inflammation-active lung area may result in a rapid, severe immune response resulting in asthma exacerbation requiring hospital admission.

We tested for eosinophil infiltration into the lung through measurement of exhaled nitric oxide. Although there was no statistical significance detected. We know that through TNF-α, RANTES and Eotaxin up-regulation, CHI3L1 increases migration and infiltration of eosinophils, basophils and T-cell migration. We also know that CHI3L1 is expressed under inflammatory conditions, so it is plausible that there was no discernible difference due to the relative health of participants undertaking the study. However further studies are required on children and young adults currently experiencing exacerbations to determine if \textit{CHI3L1} has an influence on the extent of eosinophilic inflammation under inflammatory conditions.

The C allele of the rs4950928 variant increases CHI3L1 activity in the lung\cite{7}. The C allele may also increase inflammatory infiltration into the lung during an inflammatory active period. The G allele exerts a protective effect on exacerbation in the form of hospital admissions and when exposed to damp this effect if further amplified. It is plausible to suggest that a higher concentration of CHI3L1 molecules increases the reactivity of the chitin identifying ‘net’ within the lung. This would result in higher bronchial epithelial concentrations of CHI3L1-chitin complexes. Leukocyte infiltration into the lung is a key factor in asthma pathogenesis. The C allele may lead to increased leukocyte infiltration into the lung. An increase in chitin binding and presentation of chitin to antigen-presenting cells (APC) (macrophages and dendritic cells), leading to a proactive TH2 and IL-13 mediated immune response, may increase cellular damage from inflammatory cells.
Chitin is found on moulds and fungi, which are often found in household damp. Exposure of an airway with high concentrations of CHI3L1, to increased quantities of chitin could explain the heightened effect with exposure to damp. To investigate this further, studies need to be performed on larger populations and the presence of household damp needs to be confirmed, as does the presence of mould or fungi and the type of mould and fungi. This may help in the development of future interventions, whether these take the form of education or medicinal interventions is yet to be determined.

Although the C allele of the rs4950928 variant is associated with increased exacerbations, and as such increased asthma severity, the allele appears to have a protective effect on mean % predicted FEV1, and FVC in 13-22 year olds and FVC across the sample. Although this finding is statistically significant, the minimum mean statistically significant measure is a % predicted value of 93.1 and the greatest difference between statistically significant variants is 5.1%. According to BTS guidelines a clinically significant deterioration of lung function is <80% of mean predicted. The sample was sufficiently powered to detect a clinically relevant difference of 12% (Table D.2). As such, despite the statistically significant difference between the two variant samples our findings are not clinically relevant, as the differences are of the order of 3-5%. Furthermore, in accordance with the known and hypothesised pathogenesis of the variant, it is increasingly unlikely that this is a true effect, but instead may be a result of statistical chance, as such these results must be considered with caution. It is plausible that differences between the two populations may be more noticeable during a period of inflammatory activity, as such until this investigation is repeated and the results corroborated, or an investigation undertaken on children and young adults currently experiencing asthma exacerbations, these findings should be considered with a degree of scepticism.

Although lung function may unreliable in this sample, we know the rs4950928 variant of CHI3L1 leads to over expression of CHI3L1. This may cause increased fibroblast hyperplasia and increased secretion of TNFα, through NF-κB pathway up-regulation, leading to airway smooth muscle hypertrophy and inhibition of matrix metalloproteinases 1, 3 and 13\(^{(26)}\). There was no significant association with bronchodilator reversibility of FEV1 in either sample. The mean change in % predicted FEV1 post bronchodilator in the CG/GG sample was 6.19 and in the CC sample was 4.46. Neither result is statistically or
clinically significant. The sample was sufficiently powered to detect a difference of 12%, which is the minimum clinically important difference in the BTS guidelines (Table D.2). Although there was no significant difference between the two populations, the samples are indicative of the expected pathogenesis. It is possible, that with the C allele variant, there is increased airway smooth muscle hypertrophy. As such it is logical that children and young adults with this variant would be less reactive to bronchodilator, as is indicated by the mean change of 4.46%, in contrast to the G allele group (6.19%). Although the difference between the populations is small, it is informative and would benefit from further investigation, including cellular evaluation of remodelling. In conclusion the rs4950928 variant is a clinically significant risk factor for asthma exacerbations. The variant appears to strongly interact with damp increasing the risk of asthma exacerbations, although does not appear to have a strong effect on pulmonary function. Further work is required to confirm these findings and clarify the pathogenesis of the risk variant.
CHAPTER 9

Discussion of the impact of MMP9 (rs17576 and rs6073983) on asthma severity in children and young adults

MMP9 rs17576 & rs6073983

RESEARCH QUESTION:
Is there an association between the MMP9 gene variants, rs17576 and rs6073983, and severity of asthma in children and young adults?

Hypothesis 1- Genetic variants of MMP9 influence the severity of asthma in children and young adults (Section 5.1)

This is the first study to significantly associate the MMP9 rs17576 variant and the rs6073983 variant with asthma-related exacerbations and absence due to asthma exacerbations in children and young adults with asthma. In the rs17576 variant, the odds ratio for the sample homozygous for the G allele (GG) when compared to the dominant A allele (AG/GG) was 0.68 (Difference of 7.9%) for overall asthma exacerbations and was 0.63 (Difference of 9.2%) for absence due to asthma exacerbations, indicating that the minor allele may confer protection against asthma-related exacerbations (Table 5.1.3; Figure 5.1.1). In the rs6073983 variant, the odds ratio for the sample homozygous for the T allele (TT) when compared to the sample dominant for the A allele (AA/AT) was 0.53 (Difference of 13.0%) for overall asthma exacerbations and was 0.39 (Difference of 17.1%) for absence due to asthma exacerbations, indicating that the minor allele may confer protection against asthma-related exacerbations (Table 5.1.5; Figure 5.1.3). No other measures of exacerbation were associated with the rs17576 variant (Table 5.1.3; Figure 5.1.1), or with the rs6073983 variant (Table 5.1.5; Figure 5.1.3).

We tested for an association between both variants, modified treatment steps and the novel asthma severity score described earlier. For the rs17576 variant, we found no association between either measure, indicating a lack of gene-dosage effect and no
relationship with drug associated asthma severity (Table 5.1.3). We performed a power calculation for overall asthma exacerbations in children and young adults with the rs17576 variant (Table D.2). To achieve 90% power, 297 participants with the GG variant are required. The investigated sample was comprised of 193 children and young people with the GG variant, and as such was underpowered. The statistically significant difference for overall asthma exacerbations for the rs17576 variant is 7.9%. Although this does not reach the 10% minimum clinically important difference, the power calculation was based on. The difference may still be clinically significant. It is plausible that with increased sample size, the effect size may also increase, as such until the analysis is repeated the potential clinical impact is difficult to approximate. Approximately 80% of participants with the GG variant were moderate to severe and approximately 82% of the AA/AG variant were moderate to severe. As such, although we cannot exclude the possibility of an association, it is unlikely that the rs17576 variant has an effect on drug associated asthma severity. The rs6073983 variant was significantly associated with modified treatment steps and drug associated asthma severity in children and young adults with asthma. The odds ratio for the sample homozygous for the T allele (TT) when compared to the sample dominant A allele sample (AA/AT) was 0.48 (Difference of 12.7%) for modified treatment steps and was 0.49 (Difference of 12.0%) for asthma severity score, indicating that the minor allele may confer protection against drug associated asthma severity and use of preventer medication (Table 5.1.5; Figure 5.1.3).

We also performed a power calculation for overall asthma exacerbations in children and young adults with the rs6073983 variant (Table D.2). 77 of the participants investigated possessed the TT variant. To achieve 90% power, we required 274 participants, as such the investigation was underpowered. However, to obtain 274 participants with the TT variant, the overall sample size would need to consist of approximately 4847 children and young adults with asthma. As such, the findings identified within this population should be considered with some scepticism. However, although we cannot exclude the possibility that some findings may have been missed and that effect sizes may changes when the population size is increase until the findings are confirmed in a larger sample, the findings are biologically plausible and we have identified significant difference between he two populations which may be clinically important.
We subsequently categorised by age and repeated the analysis. We know that age has an effect on asthma and on genotype. This effect most likely takes the form of either a difference in disease phenotype or could be representative of cumulative environmental exposure or cellular damage. It is hypothesised that a reduction in MMP9, associated with the GG variant will lead to less inflammation and remodelling in the airways. The rs17576 variant was significantly associated with asthma-related exacerbations, absence due to asthma exacerbations and oral steroid intake due to asthma exacerbations in 13-22 year olds with asthma. The odds ratio for the sample homozygous for the G allele (GG) when compared to the sample dominant A allele sample (AA/AG) was 0.23 for overall asthma exacerbations, was 0.38 for absence due to asthma exacerbations and was 0.28 for oral steroid intake due to asthma exacerbations, indicating that the minor allele may confer protection against asthma-related exacerbations (Table 5.1.4; Figure 5.1.2). As alluded to above, the sample analysed was underpowered. When categorised by age, in the 13-22 year old category, 46 participants had the GG variant. As such, these results must be considered with some scepticism. However, there is a difference of greater than 10% between the two variants for absence due to asthma exacerbations, oral steroid intake due to asthma exacerbations and overall asthma exacerbations and the direction of effect is biologically plausible. There was a difference of 5.6% between the GG variant and the AA/AG variant for hospital admissions due to asthma exacerbations, with only 1 out of 46 participants in the GG group experiencing a hospital admission. In this age group the rs17576 variant could affect all measures of asthma exacerbations, as such there is an urgent need to investigate this variant in a larger population to exclude any type 1 error and obtain effect sizes which may be more generalisable to the population, particularly the 13-22 year old population.

The rs6073983 variant was associated with a decreased risk of higher modified treatment steps and reduction in severity in 13-22 year olds with asthma. The odds ratio for the sample homozygous for the T allele (TT) when compared to the dominant A allele sample (AA/AT) was 0.36 for modified treatment steps and was 0.36 for asthma severity score, indicating that the minor allele may confer protection against drug associated asthma severity and use of preventer medication (Table 5.1.6; Figure 5.1.5). We found no further association with the rs6073983 variant. We have previously alluded to the fact that this
analysis was underpowered, and was further underpowered when subcategorised by age (Table D.2). In the 13-22 year old category, 18 participants out of the required 274 possessed the TT variant. However, the effect is consistent in the general population and is further amplified in the 13-22 year old variant, in this sample. In the 3-4 and 5-12 year old categories, there was a difference of approximately 5% between the two variants. In the un-categorised analysis, there was a difference of approximately 12% between the variants with an odds ratio of approximately 0.5. In the 13-22 year old age category, there was a difference of approximately 23% between the variants with an odds ratio of 0.36. Therefore, as above, although these findings must be considered with some cynicism, they are biologically plausible and clinically significant findings which, with further work, could have a impact on the healthcare of children and young adults with asthma. It is apparent that MMP9 may play a more significant role in 13-22 year olds, and could perhaps play a role in the persistence of asthma into either adolescence, or adulthood.

Increased quantities of MMP9 are a marker of increased asthma severity\textsuperscript{83}, and pulmonary inflammatory cell hyperplasia\textsuperscript{170}. Inhaled steroids have been shown to decrease MMP9 expression through the increase of TIMP inhibitors\textsuperscript{171} and decreased MMP9 through excess TIMP1 expression has been associated with the airway wall thickening\textsuperscript{125}. Yasmin \textit{et al}\textsuperscript{172} reported decreased expression and activity of MMP9 with the rs17576 variant. Pinto \textit{et al}\textsuperscript{82} were the first to associate the rs17576 variant with increased risk of non atopic asthma and non atopic wheeze in German children aged 9-11 from ISAAC II. This is the only previous genetic study undertaken on variants of \textit{MMP9} in paediatric asthma.

This study was undertaken to expand upon the findings of Pinto \textit{et al} on a larger paediatric cohort, investigating the effect of the rs17576 variant on asthma severity. The rs6073983 variant, a previously untested variant was also investigated. We did not investigate concentrations of MMP9 as a marker for severity, or of variant functionality, nor did we differentiate between atopic and non-atopic asthma. The odds ratios for overall exacerbations and absence due to asthma exacerbations in both variants was <1. This indicates that both the G allele of the rs17576 variant, associated with decreased expression, and the T allele of the rs6073983 variant are protective against asthma exacerbations. The T allele of the rs6073983 variant is also associated with reduced risk of
drug associated asthma severity and use preventer medication. We know that use of inhaled steroids decreases MMP9, the protective effect exhibited by the rs6073983 variant against addition of preventer medication may indicate a reduction in MMP9 expression or activity through the rs6073983 variant. For both variants, effects were enhanced in the 13-22 year old age group, perhaps indicating a role of MMP9 in the persistence of asthma.

In summary, both the rs17576 and the rs6073983 variants influence the severity of asthma in children and young adults. The G allele of the rs17576 variant and to a greater extent the T allele of the rs6073983 variant are protective against asthma exacerbations. The T allele of the rs6073983 variant is also associated with reduced risk of drug associated asthma severity and use of preventer medication. As such, the dominant A allele in both variants is a risk factor for asthma severity.

**Hypothesis 2- Genetic variants of MMP9 influence pathological remodelling via structural changes in the lung (Section 5.2)**

In chapter 1, we outlined the current evidence for the relationship between remodelling and asthma severity. In order to measure the extent of basement membrane thickening using proximal measures of obstruction we tested both variants for an association with mean % predicted PEFR, FEV1, FVC and FEV1/FVC in children and young adults with asthma. We found no discernible association with either variant. The sample was further classified by age, however there was no apparent association for either variant. These findings are consistent with those reported by Pinto et al. We performed power calculations for both variants, based on a minimum clinically important difference of 12%. Both variants were sufficiently powered to detect a difference of 12% (Table D.2). As such, we can support the findings of Pinto et al, in saying it is likely that pulmonary function measures do not have an effect on lung function in children and young adults with controlled asthma. We cannot exclude any possible effects theses variants may have on cellular airway remodelling, nor can we exclude any effects that may be exhibited if the study participants has uncontrolled asthma.

As a proxy measure of smooth muscle hyperplasia we tested for an association between both variants and mean change in % predicted FEV1 post bronchodilator. However, no
association between percentage change in bronchodilator was identified (Table 5.2.1). We further classified by age, however there was no discernable association between either variant, categorised by age and mean change in FEV1 post bronchodilator (Table 5.2.2). We performed power calculations for both variants, based on the minimum clinically important difference outlines by the BTS of 12% (Table D.2). The rs17576 variant was sufficiently powered to detect a difference of 12% when analysed together and when categorised by age. The rs6073983 variant required 12 participants with the TT allele to achieve power. From the population analysed, four participants possessed the TT variant. When we further categorised by age, this reduced to two participants in the 5-12 year age group and five in the 13-22 year age group. As such, we cannot exclude the possibility of the rs6073983 variant having an effect on bronchodilator reversibility.

To determine the extent of eosinophilic infiltration we tested for an association between both variants and mean exhaled nitric oxide (ppb) (as a proxy marker) and further classified into clinically elevated, elevated and normal ranges. However, we failed to identify any association between exhaled nitric oxide and either variant (Table 5.2.1). The sample was subsequently classified by age, however there was no discernable association (Table 5.2.2). Power calculations were performed for both variants, based on a minimum clinically important of 25 ppb as outlined by the BTS (Table D.2). Neither variant was sufficiently powered in either, the general analysis or in the age categorised analysis. Therefore, we cannot exclude the possibility of either variant having an effect on exhaled nitric oxide. In the general analysis, there was a difference of approximately 10ppb between groups in both variants. When this was subcategorised by age in the rs17576 variant, there was a difference of 4.4ppb in the 5-12 year olds and a difference of 16.4ppb in the 13-22 year olds. In the rs6073983 variant, there was a difference of 9.8ppb in the 5-12 year olds and 2.75 in the 13-22 year olds although there was only one participant with the TT variant. Given the size of variation in the 13-22 year olds with the rs17576 variant and the 5-12 year olds with the rs6073983 variant, MMP9 variance in MMP9 could have an effect on exhaled nitric oxide. However, in both variants there was a large standard deviation, ranging from 17.3 to 38.2. It would be biologically plausible for variation in concentrations of MMP9 to influence eosinophil inflammation. However, the proportions of the different inflammatory cells attracted by MMP9 are unknown. Therefore, variants
of \textit{MMP9} could play a role on the concentration of inflammatory cells in the asthmatic lung and this would not be measurable through nitric oxide. Likewise, it is also possible that during heightened inflammatory activity, for instance exacerbations, the effect of the any variation would be more apparent.

In summary, neither variant appears to influence the severity of asthma in children and young adults through airway remodelling and increased leukocyte migration leading to a hyper-responsive lung. Although both variants require further investigation in larger populations.

\textbf{Hypothesis 3- Environmental tobacco smoke (ETS) interacts with genetic variants of \textit{MMP9} to influence the severity of asthma (Section 5.3)}

This is the first study to significantly associate the \textit{MMP9} rs17576 variant and the rs6073983 variant with oral steroid intake due to asthma exacerbations in children and young adults exposed to environmental tobacco smoke with asthma. In the rs17576 variant, the odds ratio for the sample homozygous for the G allele (GG) when compared to the dominant A allele (AA/AG) was 0.37 for oral steroid intake due to asthma exacerbations, with a difference of approximately 13% between the two groups, indicating that the minor allele may confer protection against asthma-related exacerbations (Table 5.3.1; Figure 5.3.1). In the rs6073983 variant, the odds ratio for the sample homozygous for the T allele (TT) when compared to the dominant A allele (AA/AT) was 0.29 for oral steroid intake due to asthma exacerbations, with a difference of 15.5% between the two groups, indicating that the minor allele may confer protection against asthma-related exacerbations (Table 5.3.3; Figure 5.3.2). No other measures of exacerbation were associated with the rs17576 variant (Table 5.3.1; Figure 5.3.1), or with the rs6073983 variant (Table 5.3.3; Figure 5.3.2).

We tested both variants for an association with mean % predicted PEFR, FEV1, FVC and FEV1/FVC in children and young adults exposed to environmental tobacco smoke with asthma, categorised by age. However, we found no discernible association with either variant (Table 5.3.2; Table 5.3.4).
However, despite a lack of statistical significance, the sample size investigated was underpowered to investigate this interaction (Table D.2). Although interactions were identified between environmental tobacco smoke and oral steroids, there is the possibility that interactions have been missed due to insufficient power. To obtain power for investigation asthma exacerbation a total sample of 4532 is required. To obtain power for lung function, a total sample of 1181 is required. As such, it is important that this interaction be reinvestigated with a larger sample size before the results are fully accepted or discounted.

In summary, both variants interact with exposure to environmental tobacco smoke to influence the severity of asthma. When both samples are exposed to environmental tobacco smoke, the mutant alleles of both variants exert an enhanced protective effect on asthma exacerbations when compared to the dominant A allele sample. As such, the dominant A allele is a risk factor for asthma exacerbations in the form of hospital admission, absence and overall exacerbations.

Hypothesis 4- Genetic variants of **MMP9** are associated with allergy and atopic sensitivity in children and young adults with asthma (Section 5.3)

We tested for an association between both variants, reported allergy, and positive skin prick test. Neither variant is associated with increased risk of allergy or sensitivity (Table 5.3.6; 5.3.5).

To obtain 90% power on sensitivity to house dust mite for the rs17576 variant, a sample size of 1203 participants is required (Table D.2). The sample analysed contained only 329 participants. To obtain 90% power for the rs6073983 variant, a sample size of 2558 participants is required. The sample analysed consisted of only 414 participants. As such, we are not in the position to discount the possibility of sensitivity to any of the investigated allergens in this population.

In summary, neither variant appears to be associated with an increased risk of sensitivity and/or allergy to chitin containing allergens in this sample, however further work is necessary due to insufficient power.
Hypothesis 5- Genetic variants of MMP9 influence the quality of life of children and young adults with asthma (Section 5.3)

We have found an association between asthma exacerbations in the rs17576 variant and in the form asthma exacerbations and drug associated asthma severity in the rs6073983 variant. Both exacerbations and drug associated asthma severity have been shown to have a detrimental impact on quality of life. We tested for associations with overall quality of life scores, activity score, emotion score and symptom score in a cohort of approximately 200 participants, however we found no association between either variant and quality of life in this sample (Table 5.3.7; 5.3.8).

To achieve 90% power for the rs17576 variant a sample size of 951 participants is required, and for the rs6073983 variant, 2017 participants are required (Table D.2). Therefore, the sample we investigated was considerably underpowered by an approximate factor of four for the rs17576 variant and a factor of 10 for the rs6073983 variant. As such we cannot exclude the possibility of an effect on quality of life. In summary, neither variant appears to impact upon the quality of life in children and young adults with asthma in this sample.

Summary of the relationship between MMP9 gene variants, rs17576 and rs6073983, and asthma severity in a sample of UK children and young adults

An increased expression of MMP9 has been detected in the lungs of individuals with asthma during periods of exacerbation. Through the targeting of tight junctions, MMP9 plays a role in anoikosis and macrophage differentiation. Through barrier loss, MMP9 leads to increased leukocyte activity and migration, variation in MMP9 could play a role in the development of the hyper-responsive lung leading to increased cell damage and death. Thus resulting in remodelling, through repeated formation of a temporary epithelial barrier\(^\text{20}\), ultimately increasing the severity of asthma in children and young adults.

Both The rs17576 variant and the rs6073983 variant decrease expression and activity of MMP9 in children and young adults with asthma. The recessive mutant alleles of both
variants are protective against asthma exacerbations, particularly exacerbations resulting in absence in children and young adults.

In its normal function MMP9 controls inflammatory egression of macrophages, eosinophils and neutrophils\textsuperscript{125,173,174}, and leads to inflammatory hyperplasia post asthma exacerbation\textsuperscript{170}. MMP9 is involved in eosinophil and T-cell recruitment to the lung following allergen challenge in mice\textsuperscript{175}. Inflammatory hyperplasia in the lung results in a rapid, severe immune response following allergen challenge resulting in asthma exacerbation. Both investigated variants have a protective effect on exacerbations, plausibly due to decreased MMP9 expression and activity, therefore reducing the pulmonary inflammatory hyper-responsiveness previously observed in individuals with asthma. The effect is further amplified in 13-22 year olds with the rs17576 variant. It is possible that as the lungs mature, the expression of the rs17576 variant remains constant, but becomes proportionally diffuse in conjunction with the overall increase in pulmonary surface area, heightening the protective effect. We proximally tested for eosinophil infiltration into the lung through measurement of exhaled nitric oxide, however no statistical signal was detected. Interestingly, there is a difference in mean exhaled nitric oxide between the allele groups in both variants. Further work is required in a larger sample, and in a sample of children and young adults undergoing exacerbation to determine if either variant has an effect on exhaled nitric oxide. However, although the observed differences do not exceed the minimum clinically important difference, the direction of the difference is biologically plausible and may, with further investigation, providing further evidence for a decrease in activity and expression associated with the mutant alleles of both variants.

The rs6073983 variant also has a protective effect on progression to preventative medication and drug associated asthma severity. This effect is further amplified in the 13-22 year old age group. Both variants have a protective effect on oral steroid intake due to asthma exacerbations when exposed to environmental tobacco smoke. The rs6073983 variant also has a protective effect on overall asthma exacerbations.

If the rs17576 and rs6073983 variants decrease the expression or activity of MMP9, it is conceivable that there is a reduction in inflammatory infiltration and therefore a
reduction in requirement of inhaled steroids. The observed protective effect could also be due to a balance between MMP9 and TIMP1, nullifying the need for addition of inhaled steroids. Substantive reduction in MMP9 due to TIMP1 expression has been associated with airway wall thickening. It is plausible to suggest that despite pathways leading to increased MMP9 expression in the lungs of individuals with asthma, both the rs17576 variant and the rs6073983 variant reduce the expression of MMP9 sufficiently to maintain a relatively healthy pulmonary environment. This could also explain the reduction in risk of asthma exacerbations. In contrast the dominant samples of both variants, may have comparably increased expression and activity, which may result in an increase in pulmonary inflammation eventually leading to asthma exacerbations and a requirement for preventer medication for management through steroidal increased expression of TIMP's, which inhibit the MMP family.

In conclusion, both the rs17576 and rs6073983 variants are associated with asthma severity. Both variants are clinically significant risk factors for asthma exacerbations and interact with environmental tobacco smoke to increase the risk for asthma exacerbations requiring intake of oral steroids. The rs6073983 variant also, influences severity measured by the asthma severity score and requirement of preventer medication.
Discussion of the impact of *MMP12* (rs2276109 and rs652438) on asthma severity in children and young adults

*MMP12* rs652438 & rs2276109

**RESEARCH QUESTION:**
Is there an association between the *MMP12* gene variants, rs2276109 and rs652438, and severity of asthma in children and young adults?

**Hypothesis 1- Genetic variants of *MMP12* influence the severity of asthma in children and young adults (Section 6.1)**

The *MMP12* rs652438 variant is associated with asthma-related exacerbations in children and young adults with asthma. The odds ratio for the dominant G allele (AG/GG) when compared to the homozygous A allele (AA) was 1.51 (Difference of 9.1%) for overall asthma exacerbations, indicating the common G variant may be a risk factor for asthma-related exacerbations (Table 6.1.4; Figure 6.1.1). The individual measures of exacerbation were, however, not associated with the rs652438 variant (Table 6.1.4; Figure 6.1.1), and no association was found with the rs2276109 variant and individual or overall measures of exacerbation (Table 6.1.3).

We tested for an association between both variants and modified asthma treatment steps (described in Section 3.7). We also tested the association between the variants and a novel asthma severity score (described in Section 3.7). The modified treatment steps have been used successfully in previous publications as a proxy for measuring a gene-dosage effect. The asthma severity score is derived from modified treatment steps, adjusted by bronchodilator use. For the rs2276109 variant, no association was found between either measure, indicating a lack of gene-dosage effect and no relationship with drug associated asthma severity (Table 6.1.5). The rs652438 variant was significantly associated with modified treatment steps and drug associated asthma severity in children.
and young adults with asthma. The odds ratio for the sample with the common G allele (AG/GG) when compared to the homozygous A allele (AA) was 1.83 for modified treatment steps (Difference of 7.8%) and was 1.74 for asthma severity score (Difference of 7.1%), indicating that the common dominant G allele may be a risk factor for increased drug associated asthma severity and use of preventer medication (Table 6.1.6; Figure 6.1.2).

We subsequently performed power calculations for both variants on the overall asthma exacerbation variable (Table D.2). For the rs2276109 variant, a sample size of 1487 participants was required to achieve 90% power, requiring 334 participants with the AG/GG variant. The sample analysed consisted of 1440 participants, with 319 with the AG/GG variant and as such, although the sample did not achieve 90% power was sufficient to conduct this analysis. For the rs652438 variant a sample size of 2900 participants, with 287 participants with the AG/GG variant was required to achieve 90% power. There were 1422 participants in the sample analysed, and 137 of these participants possessed the AG/GG variant. As such the rs652438 variant was underpowered for the analysis undertaken and further associations may be identified if the sample was repeated in a population approximately twice the size of the current cohort.

This study was undertaken to repeat and expand on previous work undertaken on MMP12 by Mukhopadhyay et al. The previous study was conducted on 1017 participants in the BREATHE study. We have expanded the study to 1440 participants and include pulmonary function and quality of life data from approximately 220 participants recruited to PAGES. The previous study undertaken by Mukhopadhyay et al associated the rs652438 variant with increased risk of higher asthma treatment steps, increased risk of bronchodilator use, increased risk of exacerbations and higher asthma treatment steps in children over the age of 6 years. Hunninghake et al associated the rs2276109 variant with increased FEV1 in children with asthma and in adults with asthma exposed to ETS.

In summary, the rs652438 variant influences the severity of asthma in children and young adults. The dominant G allele of the rs652438 variant is a risk factor for overall exacerbations, modified treatment steps and drug associated asthma severity.
Hypothesis 2 - Genetic variants of MMP12 play a role in pathological remodelling via structural changes in the lung (Section 6.2)

We measured the extent of remodelling through proximal measures of obstruction. We tested both variants for an association with mean % predicted PEFR, FEV1, FVC and FEV1/FVC in children and young adults with asthma. There was no discernible association with the rs2276109 variant, in contrast to the findings of Hunninghake et al (Table 6.2.1). This is the first study to associate the common dominant G allele (AG/GG) of the rs652438 variant with increased PEFR (Difference of 4.4%), FEV1 (Difference of 3.0%) and FVC (Difference of 3.0%) in children and young adults with asthma (Table 6.2.2; Figure 6.2.1). We further classified by age, there was no apparent association for the rs2276109 variant, however the G allele of rs652438 variant was significantly associated with increased PEFR (Difference of 7.0%), FEV1 (Difference of 6.1%) and FVC (Difference of 6.9%) in the 13-22 year old age group. We subsequently undertook a power calculation for both variants and both variants were sufficiently powered to detect a difference of 12% (Table D.2). Although the rs652438 variant was statistically associated with PEFR, FEV1 and FVC, in both the general analysis and the sub-categorised analysis the difference between variants is relatively small. Furthermore, the direction of effect is contrary to what would be expected and what would be considered biologically plausible considering the direction of severity as measured by asthma exacerbations and drug associated severity is in opposite. Additionally the mean values all exceed 80%, which is the threshold for clinical significance as outlined in the BTS guidelines. Other than error, one explanation for the direction of effects could be that the population on higher medication steps are receiving adequate management for their asthma and as such have marginally improved pulmonary function.

To estimate smooth muscle hyperplasia we tested for an association between both variants and mean change in % predicted FEV1 post bronchodilator as a proxy marker. However, we failed to identify any association between percentage change in bronchodilator in either variant (Table 6.2.1; Table 6.2.2). We further classified by age, however there was no discernible association between the rs2276109 variant, categorised by age and mean change in FEV1 post bronchodilator (Table 6.2.3). Both variants were sufficiently powered to conduct this analysis.
Using exhaled nitric oxide as a proxy measure of pulmonary eosinophil infiltration, we tested for an association between both variants and mean exhaled nitric oxide (ppb) and further classified the exhaled nitric oxide levels into clinically elevated, elevated and normal ranges. However, we failed to identify any association between exhaled nitric oxide and either variant (Table 6.2.1; Table 6.2.2). We further classified the rs2276109 variant by age. In the 5-12 year age group and the G allele (Difference 15ppb) was significantly associated with increased Nitric oxide (Table 6.2.3). The rs2276109 variant has previously been associated with pulmonary function, however in this sample we failed to identify any associations with any measures of pulmonary function other than exhaled nitric oxide. The rs2276109 variant appears to play a role on eosinophil infiltration in 5-12 year olds with asthma. It is surprising that this effect is singular, and although the difference in variants is relatively large, it does not span the minimum clinically important difference of 25ppb. If the rs2276109 variant does play a role on increasing inflammation in the airway, it would be expected that the children with inflamed airways would suffer from more reactive asthma and as such exacerbations, increased medication and poorer lung function. However, none of these effects have been detected. Therefore, this finding should be considered with scepticism and requires functional investigation to confirm if the variant truly has an effect on airway inflammation.

We undertook a power calculation for both variants (Table D.2). For the uncategorised analysis both variants exceeded 90% power. When we categorised by age, the 5-12 year old group of the rs2276109 variant was sufficiently powered, however the 13-22 year old group was underpowered by 8 participants out of 20 require participants. For the rs652438 variant 17 participants with the AG/GG variant were required to achieve 90% power. In the 5-12 year old group, 12 participants had the AG/GG variant and in the 13-22 age group, 6 participants. As such, we cannot exclude the possibility that either variant could play a role on exhaled nitric oxide.

In summary, the rs652438 variant appears to influence PEFR, FEV1, FVC. However, this effect should be considered with caution. The effect is contrary to biological plausibility and should be subject to further scrutiny to either discover the functional reasoning for
this effect or exclude the effect as a statistical error. The rs2276109 variant appears to influence exhaled nitric oxide in the 5-12 year old age group.

**Hypothesis 3- Environmental tobacco smoke (ETS) interacts with genetic variants of MMP12 to influence the severity of asthma (Section 6.3)**

We tested for an interaction between environmental tobacco smoke with both variants, asthma exacerbation and asthma severity score. However we found no association with either variant. We subsequently categorised by age and repeated the analysis. This is the first study to significantly associate the MMP12 rs652438 variant with absence due to asthma exacerbations in 13-22 year olds exposed to environmental tobacco smoke with asthma. The odds ratio for the G allele (AG/GG) was 5.67 (Difference 25.8%), indicating that the G may be a risk factor for absence due to asthma exacerbations (Table 6.3.4; Figure 6.3.1). No other measures of exacerbation were statistically associated with either variant. However, in the 13-22 year old age category exposed to environmental tobacco smoke the odds ratio of overall exacerbations was significant (3.15 (SD; 1.04-9.53)), indicating that the G allele may also be a risk factor for overall exacerbations. Furthermore, again in the participants exposed to environmental tobacco smoke across the 5-12 year old and 13-22 year old categories the differences in exacerbations between the populations would also be considered to be clinically significant, with differences between populations exposed to environmental tobacco smoke ranging from approximately 5% to 25% across all measures of exacerbation.

We tested both variants for an association with mean % predicted PEFR, FEV1, FVC and FEV1/FVC in children and young adults exposed to environmental tobacco smoke with asthma, categorised by age. In the 13-22 year old age group with the rs652438 variant and exposed to environmental tobacco smoke the G allele is positively associated with PEFR (Difference of 12.3%), FEV1 (Difference of 10.9%) and FVC (Difference of 14.8%) (Table 6.3.5; Table 6.3.2). As in the earlier analysis, the rs652438 variant was statistically associated with PEFR, FEV1 and FVC, and in this interaction the difference between variants exceeds the minimum clinically important difference in PEFR and FVC. However, as previously discussed the direction of effect is contrary to what would be expected and what would be considered biologically plausible considering the direction of severity as
measured by asthma exacerbations and drug associated severity. This sample was underpowered and was not corrected for multiple testing. As such, type 2 error could be a possibility for this effect. However, as the effect is present across three separate measures in the same age category, it is also equally plausible that this effect is not due to statistical error. A possible explanation for the direction of effects could be that the population on higher medication steps are receiving adequate management for their asthma and as such have marginally improved pulmonary function.

We performed a power calculation for environmental tobacco smoke exposure and concluded that the sample size investigated was underpowered to investigate this interaction (Table D.2). To obtain power for investigation asthma exacerbation a total sample of 4532 is required. To obtain power for lung function, a total sample of 1181 is required. As such it is important that this interaction is reinvestigated with a larger sample size before the results are fully accepted or discounted.

In summary, the rs652438 variant interacts with exposure to environmental tobacco smoke to influence the severity of asthma in 13-22 year olds. When both samples are exposed to environmental tobacco smoke, the G allele is a risk factor for absence due to asthma exacerbations when compared to the dominant A allele sample. The G allele also appears to be associated with comparatively improved lung function, however this result should be considered with caution and requires further investigation.

**Hypothesis 4- Genetic variants of MMP12 play a role in the development of allergy and atopic sensitivity in children and young adults with asthma (Section 6.3)**

We tested for an association between both variants, reported allergy, and positive skin prick test. Neither variant is associated with increased risk of allergy or sensitivity (Table 6.3.7).

We subsequently undertook a power calculation for both variants (Table D.2). In order to obtain 90% power for the rs22761096 variant, a sample size of 780 participants was required. The sample analysed contained only 329 participants. To obtain 90% power for the rs652438 variant, a sample size of 1528 participants was required. The sample
analysed consisted of only 424 participants. Therefore, we are not in the position to
discount the possibility of sensitivity to any of the investigated allergens in this
population.

In summary, neither variant appears to be associated with an increased risk of sensitivity
and/or reported allergy in this sample.

**Hypothesis 5- Genetic variants of MMP12 influence the quality of life of children and
young adults with asthma (Section 6.3)**

We have found an association between asthma exacerbations and drug associated
asthma severity in the rs652438 variant. Both exacerbations and drug associated asthma
severity have been shown to have a detrimental impact on quality of life. We tested for
associations with overall quality of life scores, activity score, emotion score and symptom
score in a cohort of approximately 200 participants, however we found no association
between either variant and quality of life in this sample (Table 6.3.8; 6.3.9).

We subsequently undertook a power calculation at 90% power for both variants (Table
D.2). For the rs22761096 variant, a sample size of 619 participants was required. The
sample analysed contained only 214 participants. For the rs652438 variant, a sample size
of 1208 participants is required. The sample analysed consisted of only 205 participants.
Consequently, we are not in the position to discount the possibility of sensitivity to any of
the investigated allergens in this population.

In summary, neither variant appears to impact upon the quality of life in children and
young adults with asthma in this sample.
Summary of the relationship between *MMP12* gene variants, rs2276109 and rs652438, and asthma severity in a sample of UK children and young adults

*MMP12* is synthesised under inflammatory conditions, through macrophage activation, but also from epithelial cells, smooth muscle cells and endothelial cells in the alveolar walls. *MMP12* plays a role in matrix turnover through the inhibition of elastin breakdown. This subsequently results in remodelling, through repeated thickening of the epithelial barrier\(^\text{20}\), which may increase the severity of asthma in children and young adults. *MMP12* has been associated with macrophage dependant inflammatory diseases, including asthma and reduced lung function as a result of smoking related lung injury. ETS causes damage to the lung through exposure to free radical oxidants, leading to more inflammation and ultimately remodelling in the lung. *MMP12* is elevated in the lung during inflammation and the interaction with ETS may result in increased severity through airway inflammation and remodelling.

In children and young adults, the G allele is associated with increased risk of asthma exacerbations and drug associated asthma severity. The rs652438 variant is a missense transition substitution SNP which occurs in the promoter region, affecting the haemopexin domain of *MMP12*. The SNP alters expression and substrate binding through alteration of the flexibility of the *MMP12* binding site. The A allele is approximately 3 times as active as the G allele. As such, the A allele increases the rate and quantity of macrophage migration into the lung in comparison to the G allele\(^\text{143}\). Reduced activity of *MMP12* will reduce *MMP12* mediated activation of *MMP1*, *MMP2* and subsequently *MMP1* and *MMP9*. A reduction in MMP concentrations within the lung will reduce the breakdown and turnover of the ECM, leading to gradual airway thickening and remodelling. Although macrophage migration may be inhibited, other inflammatory cell migration may be enhanced. Elastin degradation may be reduced, due to reduced effectiveness of *MMP12*. The increased risk of preventer medication dependence with this variant would suggest inflammatory cell infiltration. The rs2276109 variant was associated with increased nitric oxide in 5-12 year olds, although, the effect size was relatively small, and does not appear to associate with any other measures of asthma.
severity. Further functional investigation as well as investigations to observe variation during exacerbation are required to determine if this effect is true and the clinical impact of this finding.

The rs652438 variant is associated with improved PEFR, FEV1 and FVC. This effect is enhanced in the 13-22 year old sample. However, although this finding is statistically significant, the effect is contrary to biological plausibility, and therefore should be considered with scepticism and would benefit from further investigation.

We could not test bronchodilator reversibility as a surrogate marker for smooth muscle hyperplasia due to insufficient case numbers.

If a reduction in MMP concentrations within the lung leads to increased inflammatory cell infiltration. It is likely that the reduction in MMP concentrations leads to cellular remodelling. Our findings suggest some variation in pulmonary function, however further investigations are required to determine if remodelling in increased in association with either variant. Smooth muscle hyperplasia may be measured by methacholine challenge. BAL fluid or induced sputum may confirm increased inflammatory cell infiltration with this variant, and pulmonary biopsies are required to determined the extent of pulmonary remodelling.

In conclusion, the rs652438 variant but not the rs22761089 variant is a clinically significant risk factor for asthma exacerbations and drug associated asthma severity. The variants appear to have some effect on pulmonary function, however before any conclusions can be drawn, further investigations are required.
CHAPTER 11

Discussion of the impact of \textit{GST} mutant variants on asthma severity in children and young adults

\textit{GSTM-1 null & GSTP-1 rs1695 & GSTT-1 null}

\textit{RESEARCH QUESTION:}
Do \textit{GST} mutant variants influence the severity of asthma in children and young adults?

\textit{Hypothesis 1-} \textit{GST} mutant variants increase the severity of asthma in children and young adults (Section 7.1)

This is the first study to associate the \textit{GST} mutant variants with hospital admission due to asthma exacerbations in children and young adults with asthma. The odds ratio for the mutant variant when compared to the wildtype variant was 1.51 (Difference of 6.4%) indicating that the mutant variant is a risk factor in asthma exacerbations (Table 7.1.2; Figure 7.1.1). No other measure of exacerbation was associated with the mutant variant. We also tested for an association between the risk variant, modified treatment steps and a novel asthma severity score. We found no association between either measure, indicating a lack of gene-dosage effect and no relationship with drug associated asthma severity. We subsequently repeated the analysis, categorising the mutant variant by age. This is the first study to report an association between the mutant variants and hospital admission in 5-12 year olds with asthma. The odds ratio for the mutant variant when compared to the wildtype variant is 1.88 (Difference of 8.4%), indicating that the mutant variant is a risk factor in asthma exacerbations in the 5-12 years age group (Table 7.1.3; Figure 7.1.2). A power analysis was undertaken to estimate the sample size required to achieve 90% power based on the minimum clinically important difference for overall asthma exacerbations of 10% (Table D.2). A sample size of 736 participants with the mutant variant was required to achieve 90% power. None of the samples analysed achieved 90% power. The combined analysis consisted of 607 participants with the
mutant variant, this was subsequently categorised by age, reducing the sample size to 101 participants with the mutant variant in the 3-4 year old group, 377 in the 5-12 year old group and 124 in the 13-22 year old group. Consequently, it is plausible that associations have been missed in this analysis and therefore the analysis should be repeated in a larger cohort. In the 3-4 year old category, the direction of effect in all measures of asthma exacerbation, other than oral steroid intake due to asthma exacerbation, is contrary to known biological plausibility, this in addition to the small sample indicates that this data may be erroneous. This is also true in the 13-22 year old group. To achieve sufficient power to investigate all sub-categories, including age, then a sample size of 3402 participants is required.

This study provides a novel insight, repeating and expanding on previous work undertaken on the GSTM1, GSTP1 and GSTT1 by Palmer et al. The previous study was conducted on 504 participants in the BREATHE study. The study has been expanded the study to 867 participants and include pulmonary function and quality of life data from approximately 220 participants recruited to PAGES. Children who possess the GST mutant variants and have been exposed to ETS in utero have an increased risk of early onset asthma, asthma with current symptoms, persistent asthma, lifetime wheezing, wheezing with exercise, wheezing requiring medication and hospital admissions. A meta-analysis undertaken by Minelli et al reported approximately 20 studies across the three mutant variants which have shown an association between the mutant variants, asthma risk and wheeze. Hanene et al 2007 reported an association between all three mutant variants and risk of asthma in Tunisian children. Tamer et al 2004 reported an association between all three mutant variants and atopic asthma.

We have merged the mutant variants of GSTM1, GSTP1 and GSTT1 into one mutant variant for the purpose of the analysis, this is the first study to undertake this. In summary, the mutant variant increases the severity of asthma in children and young adults. The mutant variant is a risk factor for hospital admissions due to asthma exacerbations, particularly in children between 5-12 years of age. We found no association between the mutant variants and drug associated asthma severity.
**Hypothesis 2** - **GST mutant variants increase the severity of asthma in children and young adults through airway remodelling and increased leukocyte infiltration into the lung (Section 7.2)**

The extent of remodelling was measured through proximal measures of obstruction, we tested the mutant variant for an association with mean % predicted PEFR, FEV1, FVC and FEV1/FVC in children and young adults with asthma. We found no discernible association with the mutant variant. We further classified by age however, there was no apparent association for the mutant variant, contrary to the previous study undertaken on the BREATHE study by Palmer *et al*[^45].

We tested for an association between the both variants and mean change in % predicted FEV1 post bronchodilator. However, we failed to identify any association between percentage change in bronchodilator and the mutant variant (Table 7.2.1).

We tested for an association between both variants and mean exhaled nitric oxide (ppb) and further classified the exhaled nitric oxide levels into clinically elevated, elevated and normal ranges. However, we failed to identify any association between exhaled nitric oxide and the mutant variant (Table 7.2.1; Table 7.2.2). We further classified the mutant variant by age, however there was no discernible association (Table 7.2.3). As such, it is unlikely there is a relationship between the mutant variant and pulmonary eosinophil infiltration.

With the exception of nitric oxide, power was achieved in all age categories for all measures of pulmonary function (Table D.2). The sample size required to achieve 90% power for nitric oxide was 55 participants. This was achieved in the combined analysis, and in the 5-12 year old group, but was not achieved in the 13-22 year old group. The sample was analysed on 21 participants with the mutant variant in the 13-22 year old group.

In summary, the mutant variant does not appear to influence the severity of asthma through airway remodelling and increased leukocyte migration in children and young adults through airway.
Hypothesis 3- GST mutant variants interact with environmental tobacco smoke (ETS) exposure to increase the severity of asthma (Section 7.3)

We tested for an interaction between environmental tobacco smoke with the mutant variant, asthma exacerbations and asthma severity score. However, we found no association (Table 7.3.1). We subsequently categorised by age and repeated the analysis. However, we found no association (Table 7.3.2). As with the previous genes, for investigating an interaction with environmental tobacco smoke this sample was underpowered and therefore any findings should be regarded with caution and the sample repeated (Table D.2). Although it should be noted that in 5-12 year olds exposed to environmental tobacco smoke there is a difference of 17.2% between the two populations experiencing hospital admissions due to asthma exacerbations, with a significant odds ratio of 3.53, although the interaction is not statistically significant.

The mutant variant interacts with environmental tobacco smoke in 13-22 year olds to increase the risk for requirement of preventer medication and drug associated asthma severity. The odds ratio for addition of preventer medication is 5.45 (Difference of 21.3%) and for drug associated asthma severity is 6.22 (Difference of 23.3%), indicating that the mutant variant may be a risk factor in drug associated asthma severity (Table 7.3.3; Figure 7.3.1). The GST risk variant may be a clinically vital tool in predicting the need for higher medication in 13-22 year olds exposed to environmental tobacco smoke. Further work is required to determine is a particular exposure level increases risk, or whether exposure over a certain time frame increases risk. Work also needs to be done on evaluating the most effective means of employing this finding in clinical practice. A potential future pragmatic, randomised single blind trial, could be an educational based intervention investigating the impact of informing parents and children of the increased risk posed with exposure to environmental tobacco smoke. A salivary cotinine sample could be taken from the child at base line and group A, informed of the genotype and group B not informed. Both groups would be informed of the risks of tobacco smoke exposure. A secondary salivary cotinine could be collected 3 months later, and the difference in exposure compared. This would primarily determine the effect of informing the parent of the child’s at risk genotype on their day to day behaviour, which is applicable for the majority of genotype, educational based interventions.
We tested the mutant variant for an association with mean % predicted PEFR, FEV1, FVC and FEV1/FVC in children and young adults exposed to environmental tobacco smoke with asthma, categorised by age. However, contrary to previously published work, no association was discernible (Table 7.3.4). This sample was sufficiently powered for this analysis (Table D.2).

In summary, the mutant variant interacts with exposure to environmental tobacco smoke to influence the severity of asthma. This effect is variable with age, increasing the risk drug associated asthma severity and preventer medication requirement in 13-22 year olds.

**Hypothesis 4- GST mutant variants infer allergy and atopic sensitivity in children and young adults with asthma (Section 7.3)**

We tested for an association between the mutant variant, reported allergy, and positive skin prick test. The mutant variant is associated with increased risk of allergy, measured by reported allergy, the odds ratio for the mutant variant is 1.47 (Table 7.3.5; Figure 7.3.2). This is in concordance with the findings of Tamer et al 2004.

The sample analysed was underpowered to investigate an association between the variant and positivity sensitivity to skin prick test. In order to obtain 90% power, 612 participants are required (Table D.2). The sample analysed consisted of 355 participants. Although the sample for reported allergy is sufficiently powered, consisting of 1222 participants. However, the direction of effect is consistent. It is known that reported allergy can be an unreliable method and ideally we would use formal allergy testing to ascertain this result.

In summary, in an enhanced asthma cohort, the mutant variant appears to infer an increased risk of allergy or atopic asthma in this sample.
Hypothesis 5- GST mutant variants impair the quality of life of children and young adults with asthma (Section 7.3)

We have found an association between asthma exacerbations and drug associated asthma severity in the mutant variant. Both exacerbations and drug associated asthma severity have been shown to have a detrimental impact on quality of life. We tested for associations with overall quality of life scores, activity score, emotion score and symptom score in a cohort of approximately 200 participants, however we found no association between either variant and quality of life in this sample (Table 7.3.6).

As with the previous variants, the sample analysed was underpowered, containing only 212 participants (Table D.2). To achieve 90% power a sample of 460 participants was required. Therefore, we are not in the position to discount the possibility of sensitivity to any of the investigated allergens in this population.

In summary, the mutant variant does not appear to impact upon the quality of life in children and young adults with asthma in this sample.
Summary of the relationship between GST mutant variants and asthma severity in a sample of UK children and young adults

The GST mutant variant is associated with increased risk of hospital admission, particularly in children between 5-12 years of age, drug associated asthma severity and use of preventer medication when exposed to environmental tobacco smoke and with allergy.

The Glutathione S-transferase (GST) gene family codes for the GST enzymes, a group of phase II detoxification enzymes. The GST enzymes are a fundamental component of the cell damage defence system. GSTs are expressed in the bronchial wall. GSTs function as an antioxidant defence through reactive oxygen species metabolism. They repair damaged reactive oxygen species and play a role in detoxification of xenobiotics, for instance tobacco smoke carcinogens. Detoxification occurs through the secretion of Glutathione (GSH). GSH is a tripeptide thiol that is induced by GSTs. GSH functions to reduce the organic peroxides, protecting the airway from lipid peroxidation. GSH homeostasis is altered in children with severe asthma. The loss of homeostatic balance between oxidative forces and antioxidant defence is believed to be the cause of forced oxidative injury, attributing to the severity of asthma. The increase in reactive oxidative species in asthma is reflected in the increase in activity of eosinophils and macrophages in peripheral blood.

The GST mutant variant is associated with increased risk of asthma severity. The mutant variant increases the risk of hospital admissions. An effect amplified in the 5-12 year age group. The mutant variant also interacts with environmental tobacco smoke to increase the risk of increased drug associated asthma severity and requirement of preventer medication requirement in 13-22 year olds. The mutant variant also increases this risk of reported allergy.

The GST mutant variant reduces enzymatic activity and expression of the GST enzymes, disrupting the homeostatic balance through their absence. An increase of macrophages can attribute to the association between the mutant variant and atopy.
Forced oxidative injury leads to inflammatory cell hyperplasia and pulmonary remodelling, this effect would be heightened by the introduction of further oxidants in the form of environmental tobacco smoke, which may explain the increased drug associated asthma severity and preventer medication requirement in 13-22 year olds.

The merged mutant variant provides a unique tool which enables the identification of an at risk group. To gain a true insight into the role of the mutant variant on asthma further investigations into environmental interactions such as dietary oxidants and ozone are required. The risk group could benefit from post diagnostic screening, which may enable clinicians to ascertain a more accurate representation of the risk group within their encatchment area and take appropriate action. Whether through, frequent clinical monitoring or increased treatment steps or through parental education remains unknown and requires further investigation.

In conclusion, the mutant variant is a clinically significant risk factor for asthma exacerbations, drug associated asthma severity and allergy. The analysis supports the use of exacerbations, modified treatment steps and asthma severity score as a surrogate marker for asthma severity, and requirement of preventer medication and exhaled nitric oxide as a surrogate marker for leukocyte infiltration, all of which may be clinically and scientifically beneficial for non-invasively investigating asthma pathogenesis.
CHAPTER 12

Discussion of proxy markers

The data gathered in this thesis enabled us to test the applications of proxy markers of variables associated with asthma severity pathogenesis. Asthma exacerbations, modified treatment steps and the asthma severity score are all clinically relevant measures of asthma severity, and have produced multiple clinically pertinent findings throughout this thesis. We have not recorded the date when medication was last updated by a clinician in the BREATHE study, and as such we have not taken into account any time required for preventative treatment steps to take effect in the asthma severity score. We tested PEFR, FEV1, FVC and FEV1/FVC as surrogate markers of obstruction, a proxy of basement membrane thickening, associated with asthma severity. In this cohort, all mean measures of pulmonary function are above the clinically significant range and the variation between variants is relatively small, and often contrary to biological plausibility. It is interesting that the majority of statistical associations were contrary to biological plausibility. It is possible that patients with the risk variants are on higher medication steps and the risk effect is only apparent during exacerbations of asthma, although this will require further investigation. We were primarily interested in asthma severity, hence the investigation of basement membrane thickening and smooth muscle hyperplasia, though, as an alternative measure of remodelling we could have taken bronchial biopsies, although for the purpose of this research that would be deemed un-ethical. We also tried to use bronchodilator reversibility to measure the degree of smooth muscle hyperplasia, also associated with asthma severity, however no findings were clinically significant. We failed to find any statistically significant finding with exhaled nitric oxide. The mean measures of exhaled nitric oxide had broad standard deviations which may result in false positives. The sample would benefit from increased population size to reduce deviation from the true mean and may be more clinically pertinent when categorised by clinically elevated, elevated and normal. As alluded to earlier, one possible reason for clinically normal ranges and a lack of statistical significance could be because at the time of measurement all participants were well controlled. To investigate the effect of the variants on
pulmonary function, investigations should be undertaken on a cohort currently undergoing exacerbations of asthma to determine if the effect of the risk variant becomes more apparent. To further investigation the pathogenesis of each variant cellular investigation of remodelling, categorised by variant is required.

The data presented in this thesis provides supporting evidence for a phenotypic difference between asthma in children and young adults. Although we were unable to investigate cellular variation, the variants identified in this thesis as age-specific may be of clinical and scientific benefit in discerning phenotypic difference based on genotype between these phenotypically different conditions.

This purpose of this thesis was to investigate the impact of airway remodelling genes on asthma severity and to evaluate proxy measures of asthma severity pathogenesis. This thesis has identified twenty-six pertinent findings using proxy measures of asthma severity. The merger of the lung function measures from the BREATHE study and PAGES created a large lung function cohort. Unfortunately, we were unable to identify any clinically relevant findings from this cohort, although this may be due to the clinical normality of the participants at the time of collection.

This study may have benefited from merging the paediatric asthma questionnaire from the BREATHE study and PAGES. If the PAGES questionnaire was completed by clinically trained research staff, rather than parentally/participant reported, as with the BREATHE questionnaire, then this investigation could have been undertaken on a sample of approximately 2000 participants. As allergy was parentally/participant reported in both questionnaires, we were able to merge this data, creating a powerful allergy cohort. Our skin prick test cohort consisted of approximately 400 participants, and as such was insufficient for detecting variation.

We found no significant findings with QOL and any variant. The QOL of questionnaire is well validated, although it only captures a two-week period of the participants life. In the case of asthma exacerbations over six months, this time frame may be insufficient to capture a true picture of the participants QOL. Despite this, our study investigated the effect of variants on approximately 200 participants with QOL. The sample size was
insufficient to identify any genetic effect on QOL, although we are aware QOL should be viewed in conjunction with other measures of asthma severity to ascertain a true picture of asthma severity. It would be worthwhile to investigate genetic influence on QOL in a larger cohort, and perhaps in other conditions to determine if QOL has any potential as a marker of severity.

The statistical analysis undertaken in this thesis was driven by the research questions and individual hypotheses described in Chapter 2 and in each discussion chapter. No adjustments for multiple testing were made because of the complications of multiple outcome measures. We were conscience of the risk of Type I error, but were also wary of making adjustments which may have resulted in Type II error. This may mean there is an increased risk of incorrectly identifying an association where in fact there is none\textsuperscript{167}. 
CHAPTER 13

Concluding statement

The impact of variation in airway remodelling genes and their role on asthma severity in children and young adults

OUTCOME

The role of eight genetic variants of four genes implicated in airway remodelling have been investigated for hypotheses relating to asthma pathogenesis, to determine the role of each variant on asthma severity. We tested eighteen outcome measures individually, twelve measures were subsequently categorised by age and tested for interaction with environmental risk factors. Seven of the investigated genetic variants play a role on asthma severity in children and young adults with asthma. Across all seven variants, 26 findings are clinically pertinent, of the 24 findings, six signals are detectable when the sample is categorised by age and eight are as a result of an interaction with either environmental tobacco smoke or damp (Table 8.1.1).

The rs4950928 variant of CHI3L1 is associated with reduced risk of hospital admission due to asthma exacerbations. This effect is amplified by exposure to damp, reducing the risk of asthma exacerbations overall, hospital admissions due to asthma exacerbations and absence due to asthma exacerbations.

The rs17576 variant of MMP9 is associated with reduced risk of overall asthma exacerbations and absence due to asthma exacerbations. This effect is amplified in 13-22 year olds, reducing the risk of asthma exacerbations overall, absence due to asthma exacerbations oral steroid intake due to asthma exacerbations. The effect is also present when participants are exposed to environmental tobacco smoke, reducing the risk of oral steroid intake due to asthma exacerbations.
The rs6073983 variant of \textit{MMP9} is associated with reduced risk of overall asthma exacerbations and absence due to asthma exacerbations. The variant also reduces the risk of drug associated asthma severity and of use of preventer medications. This effect is amplified in 13-22 year olds. The variant reduces the risk of oral steroid intake due to asthma exacerbations when participants are exposed to environmental tobacco smoke.

The rs2276109 variant of \textit{MMP12} is associated with increased risk of elevated exhaled nitric oxide.

The rs652438 variant of \textit{MM12} is associated with increased risk of overall asthma exacerbations. The effect is also present in 13-22 year old participants exposed to environmental tobacco smoke, increasing the risk of absence due to asthma exacerbations. The variant also increases the risk of drug associated asthma severity and of use of preventer medications.

The \textit{GST} mutant variant is associated with increased risk of hospital admission, particularly in children between 5-12 years of age, drug associated asthma severity and use of preventer medication in 13-22 year olds exposed to environmental tobacco smoke and increased risk of reported allergy.
Asthma costs the UK approximately £996 million per year\textsuperscript{176}, and the US approximately $37.2 billion per year\textsuperscript{177}. Approximately 48\% of this cost is attributed to hospital admission alone\textsuperscript{178}. The cost of care of asthma requiring hospital admission is around three fold greater than the cost of asthma management in the community. The annual average cost per patient in European children between the ages 0-4 was €789, equating to €37,486,179 for admissions alone\textsuperscript{178}. 12.7 million work days are lost per year due to asthma exacerbation, accounting to approximately £1.2 million\textsuperscript{2}. There are 1.1 million children in the UK affected by asthma. Asthma prescription attributes to approximately £659 million per year.

Identification of children and young adults positive for risk or protective variants through genetic screening could reduce the healthcare costs of children and young adults with asthma. Post diagnostic screening of children and young adults with asthma could enable healthcare workers to categorise patient by risk. For instance, a patient could be classified by risk of persistence into adolescence or adulthood, risk of asthma exacerbations, responsiveness to treatment and risk of exacerbations with certain exposures. This could lead to personalised medicine, rather than trial and error prescription. Hopefully, leading to a reduction in medication use, in favour of specific, effective treatment regimes, improving the patients daily life and significantly reducing the costs attributed to asthma maintenance.

The implementation of post diagnostic screening for identification of risk genotypes in asthmatic children and young adults has many ethical and economic implications,\textsuperscript{179} which would need to be weighed against the benefit to healthcare systems and patient benefit. However, post diagnostic screening is several years off, there are a series of studies and trials which need to be undertaken to identify the best approach to characterising patients by asthma phenotypes and genotypes, the optimum methods of characterising the patients, the most effective means of treating the patients and the most efficient means of managing the patients. To comprehensively categorise the patient, prospective longitudinal cohort studies are required. These studies will characterise the patients genotype and phenotype, enabling sub-categorisation into individual sub-asthma syndromes and identify the optimum means of characterising the
patient. The model of the comprehensive cohort could then be used to undertake pragmatic trials, based on the identified characteristics, identifying the most effective treatments. As an alternative study, the BREATHE cohort can be used to identify genotypes which pose a risk to persistence of asthma symptoms into adolescence and adulthood, this will enable clinicians to determine if and when a child or young person will grow out of asthma. This can be performed through the means of a second cross sectional questionnaire at an appropriate time point in the child or young person’s future. Further work needs to be undertaken on the underpowered investigations in this thesis, for instance a study needs to be performed to evaluate the impact of different asthma exacerbations and the frequency of asthma exacerbations on quality of life. Finally, future quantitative, heath economic and clinical trials, determining the ethical considerations, health economic factors and outcome of monitoring and treatment variation on the genetic variants, may help us to weigh this benefit. The identification of asthma severity markers, such as those identified in this thesis, and investigations into the effects of multiple variants on clinical outcome may enable discrimination between different asthma phenotypes by genotype. For instance, Figure 2.4.1 of this thesis illustrates interactions between the molecules investigated in this study. As a hypothetical model of pathogenesis, if all risk variants investigated in this thesis were present in one model, then through CHI3L1 rs4950928, inflammatory cell infiltration through NF-κB, TNF-α, RANTES, eotaxin and IL-8 mediated pathways and fibroblast mitosis through P1-3K and TNF-α would lead to fibrosis, and an increase in harmful free radicals, leading to hypertrophy and basement membrane thickening. The absence of the GST family would enhance this risk further, providing no protection against free radical mediated cell damage, produced internally or introduced to the body system. Increased MMP9 activity through either variant would lead to increased infiltration of inflammatory cells and matrix breakdown, particularly eosinophil and macrophage infiltration and IL-13 mediated T-cell infiltration. Finally, a reduction in MMP12 activity will lead to thickening of the ECM through a reduction in elastin degradation. Overall, the lungs would be prone to inflammatory cell mediated cell damage, and as a result thickening of the basement membrane and smooth muscle hyperplasia. We were unable to investigate cellular models in this study and we were unable to investigate gene-gene interactions in this
study due to the reduction in participant number. To investigate the effect of gene-gene interactions a study of at least 10,000-20,000 participants is required.

The novel, clinically pertinent findings of this thesis make a substantive contribution to scientific knowledge. The identification of seven risk variants, and the use of common non-invasive techniques as proxy markers of remodelling and severity is innovative. There may be application in other allergic and pulmonary conditions such as allergy, rhinitis and COPD. The findings presented in this thesis will contribute to the progression of research into the development of personalised treatment strategies, which will substantially benefit patients and healthcare systems across the UK, Europe and Internationally.
Appendix 1

BREATHE Questionnaire
BREATHE STUDY
(Children’s Genotype Project)

Name________________________ dob/CHI__/__/___________

Address________________________ Occupation__________________
________________________ Telephone Number__________________
________________________ Mobile Number__________________

Name of Parent________________________ E-Mail__________________

Mother’s dob________________________ Father’s dob__________________

GP________________________ Health Centre__________________

Height (cms)________________________ Weight (kgs)__________________

Date & Source________________________ Date Data Entered__________________

Ethnicity________________________

History

Does the child have: Eczema ☐ Perennial Rhinitis ☐ Seasonal Rhinitis ☐

Does anyone else in the family have:

Mother
Father
Siblings
Other
None

Asthma ☐ ☐ ☐ ☐ ☐

Eczema ☐ ☐ ☐ ☐ ☐

Allergic Rhinitis ☐ ☐ ☐ ☐ ☐

Does the child have any allergies?

<table>
<thead>
<tr>
<th>Agent</th>
<th>Reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Known triggers for asthma?

________________________________________

Does the child / young adult smoke? Yes ☐ No ☐

If yes how many per day?

__________

Is the child exposed to smoking?

At home ☐ With Relatives ☐ With Childminder ☐ Other ☐ Not exposed ☐

Version 3; 18 Aug 08
Is the child exposed to animals?

- Cat
- Dog
- Horse
- Bird
- Other (Specify)
- Not exposed

Has the child ever had an allergy test? Yes (SPT / RAST) No

Results: ____________________________________________

Current Medication

<table>
<thead>
<tr>
<th>Drug</th>
<th>Device</th>
<th>Dose per puff</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Inhaler Technique

- Good
- Poor
- Not Applicable

Bronchodilator use in the last 6 months

- None
- Occasional
- Daily
- Excessive

Comments: ____________________________________________

Courses of oral steroids in the last 6 months: ____________________________________________

Number of hospital admissions in the last 6 months: ____________________________________________

Absence from school/nursery/college/work?

- None
- 1-2 days
- Up to 1 week
- > 1 week

Lung Function

<table>
<thead>
<tr>
<th>PEFR</th>
<th>FEV₁</th>
<th>FVC</th>
<th>FEV₁/FVC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Predicted</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Actual</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% Predicted</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Reversibility</th>
<th>PEFR</th>
<th>FEV₁</th>
<th>FVC</th>
<th>FEV₁/FVC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Actual (post β₂)</td>
<td>L/min</td>
<td></td>
<td>L</td>
<td></td>
</tr>
<tr>
<td>% Change</td>
<td>%</td>
<td></td>
<td>%</td>
<td></td>
</tr>
</tbody>
</table>

Other Information: ____________________________________________

________________________________________

Version 3; 18 Aug 08
Appendix 2

PAGES Asthma questionnaire
Asthma questionnaire version 2 07/05/08

Dear Parent,

Thank you for taking time to complete this questionnaire. We would suggest that you ask your child to help answer the questions if you think they are old enough to do so. We will get valuable information about your child’s asthma and their environment from the answers. You can answer many of the questions by ticking a box or circling an answer. For some answers you will need to write your answer in.

Once you have completed the questionnaire, please return this and the other questionnaires and the signed consent form in the pre paid envelope.

Many thanks for your time

Dr Steve Turner (Consultant Paediatrician) on behalf of the Scottish Paediatric Respiratory Interest Group Asthma Database.
Asthma questionnaire version 2 07/05/08

Please write your child’s full name and date of birth below here:
(name)___________________________ (date of birth)_____________________

Please put today’s date here

What is your child’s school called?__________ Which town(city is your school in?___)

Was your child born early (ie before 36 weeks)? (please circle one answer) Yes/No

About your child’s asthma
1. How old (in years) was your child when diagnosed with asthma?__________

2. How many times has your child been admitted to hospital for asthma.....

          ever?__________

          in last year?_______

          in the last 6 months?_______

3 How many times has your child been absent from school/nursery with asthma....

          in the past six months?_______

          in the past year?_______

4 What is the longest time (in days) that your child has ever been off school/nursery for an
   asthma attack?__________

5 How many courses of steroid tablets (red tablets) has your child had.....

          in the past six months?_______

          in the past year?_______

6. Which of the following trigger your child’s asthma (please circle as many answers as you
   need)?

   A cold    Dust    Pets or animals    Exercise
   Change in the weather    Cigarette smoke    Emotions
   Other (please describe this)________________________________________
Asthma questionnaire version 2 07/05/08

7. Does your child only wheeze when they have a cold? (please circle one answer) Y/N

About your child’s asthma control

If your child is aged 12 or over please go to question 8, if your child is aged 4-11 please go to question 11

8. During the last 4 weeks, how many days did your child have any daytime asthma symptoms?

- Not at all
- 1-3 days
- 4-10 days
- 11-18 days
- 19-24 days
- Everyday

9. During the last 4 weeks, how many days did your child wheeze during the day because of asthma?

- Not at all
- 1-3 days
- 4-10 days
- 11-18 days
- 19-24 days
- Everyday

10. During the last 4 weeks, how many days did your child wake up during the night because of asthma?

- Not at all
- 1-3 days
- 4-10 days
- 11-18 days
- 19-24 days
- Everyday

Please go to question 16 now

11. In the past 4 weeks, how much of the time did your asthma keep your child from getting as much done at school?

- All of the time
- Most of the time
- Some of the time
- Not at all

12. During the past 4 weeks, how often has your child had shortness of breath?

- More than once a day
- Once a day
- 3 to 6 times a week

13. During the past 4 weeks, how often did your child’s asthma symptoms (wheezing, coughing, shortness of breath, chest tightness or pain) wake them up at night or earlier than usual in the morning?

- 4 or more nights a week
- 2 or 3 nights a week
- Once a week
- Not at all
Asthma questionnaire version 2 07/05/08

14. During the past 4 weeks, how often has your child used their blue (reliever) inhaler?

☐ 3 or more times per day  ☐ 1 or 2 times per day  ☐ 2 or 3 times per week  ☐ 4 times per week  ☐ 5 times per week  ☐ 6 times per week  ☐ 7 times per week  ☐ 8 times per week  ☐ 9 times per week  ☐ 10 times per week  ☐ 11 times per week  ☐ 12 times per week

15. How would you rate your asthma control during the past 4 weeks?

☐ Not controlled at all  ☐ Poorly controlled  ☐ Somewhat controlled  ☐ Well controlled  ☐ Completely controlled

16. Over the 6 months, on average how frequently does your child use their bronchodilator (blue inhaler)? (please tick only one answer)

☐ Never  ☐ Occasionally  ☐ Once a day  ☐ More than once a day

About your child's asthma treatment

Is your child prescribed any of these medications to be taken every day?

17. Inhaled steroids. Please tick only one answer

☐ Becotide (a brown inhaler also called Clenil, Qvar, Beclomethasone or Beclometasone)  ☐ Flutotide (an orange inhaler also called Fluticasone)  ☐ Pulmicort (a brown inhaler also called Budesonide)  ☐ Seretide (a purple inhaler)  ☐ Symbicort (a white inhaler with a red bottom)

What is the dose* of the inhaled steroid? ________________

(*this will be written on the side of the inhaler. If it is a "pump" type of inhaler, the dose will be written on the canister inside the plastic part of the inhaler)

At the moment, how many puffs of the inhaled steroid does your child take a day when they are well? Please tick only one answer

☐
Asthma questionnaire version 2 07/05/08

One puff twice a day
☐ Two puffs twice a day
☐ Other (please say what this is) ____________________________

18 Oral steroids (prednisolone), these are red tablets (please circle one answer) Yes/No

19 Please tick against the following medications if your child is currently taking them (they may be on more than one of these)

☐ Singulair (a pink chewy tablet also called Mone/lukasi)
☐ Stophyllin (a tablet also called Theophylline)
☐ Serevent (a green inhaler also called salmeterol)
☐ Oxis (a white inhaler with a blue/green bottom also called formoterol)
☐ Antihistamines (e.g. Piriton, Benadryl)
☐ Cyclosporin (a tablet used in a few children with severe asthma)
☐ Monthly steroid injections (called Kenalog or triamcinolone)
☐ Immuno globulin injections each two weeks (called Omalizumab, a monoclonal Anti-IgE antibody)
Asthma questionnaire version 2 07/05/08

About other conditions linked to asthma

20. Has your child ever had eczema? (please circle one answer)
Yes/No

21. If yes, do they still have eczema? (please circle one answer)
Yes/No

If you circled “No” in question 20, please go on to question 26

22. Has your child ever been admitted to hospital with eczema?

23. Do they have any of the following steroid creams (there may be more than one)?

- Hydrocortisone
- Betnovate (also called diprobax or betamethasone)
- Dermovate (also called clobetasol)
- Eumovate (also called Trimovate or clobetasone)
- Cutivate (also called flatacason)
- Elecon (also called mometasone)
- Aureocort (also called triamcinolone)

24. Are they prescribed any of the following creams? (again there may be more than one)

- Aqueous cream
- Paraffin
- E45
- Diprobax
- Olateum
- Epadern
- Ultrabase

25. Is your child prescribed Tacrolimus cream “Protopic” (please circle one answer) Yes/No

26. Has your child ever had rhinitis (sneezing or a runny/blocked nose in the absence of a cold)? (please circle one answer) Yes/No (if no, please go on to question 29)

27. If yes to question 26, do they still have these symptoms Yes/No

28. If yes to question 26, are the symptoms present all year round or just in the spring/summer months? All years round


Asthma questionnaire version 2 07/05/08

[ ] Only spring/summer months

29. Has your child ever had a food allergy? Yes/No (if no please go on to question 32)

30. What food(s) are they allergic to?

31. If yes to question 29, how did they react? (there may be more than one reaction they had)
   - [ ] Swollen lips
   - [ ] Swollen tongue
   - [ ] Itchy skin
   - [ ] Wheezy
   - [ ] Difficulty breathing
   - [ ] Other (please state)

About asthma and related conditions in your family

32. Is there anyone in your child’s family with asthma? Yes/No
   (if no please go on to question 34)

33. If yes to question 32, what relation(s) are they to your child? (you may need to tick more than one box here)
   - [ ] Brother
   - [ ] Sister
   - [ ] Mother
   - [ ] Father
   - [ ] Aunt
   - [ ] Uncle
   - [ ] Cousin
   - [ ] Grandparent
   - [ ] Other relative

34. Is there anyone in your child’s family with eczema? Yes/No
   (if no please go on to question 36)

35. If yes to question 34, what relation(s) are they to your child? (you may need to tick more than one box here)
   - [ ] Brother
   - [ ] Sister
   - [ ] Mother
   - [ ] Father
   - [ ] Aunt
   - [ ] Uncle
   - [ ] Cousin
   - [ ] Grandparent
   - [ ] Other relative

36. Is there anyone in your child’s family with rhinitis (runny nose and sneezing when they don’t have a cold)? Yes/No
   (if No please go on to question 38)

37. If yes to question 36, what relation(s) are they to your child? (you may need to tick more than one box here)
Asthma questionnaire version 2 07/05/08

☐ Brother     ☐ Sister     ☐ Mother
☐ Father      ☐ Aunt       ☐ Uncle
☐ Cousin      ☐ Grandparent ☐ Other relative

About your child’s environment

The following questions relate to the house where your child lives for most of the time

38. What type of accommodation do you live in? (please tick just one)
   ☐ House        ☐ Bungalow      ☐ None of these
   ☐ Flat         ☐ Maisonette

39. Do you own or rent the accommodation you live in? (please circle one answer) Own/rent

40. Have you lived in this house since your child was born? Yes/No (if yes please go to question 43)

41. How many years have you lived in your house?

42. If no to question 40, please list the addresses your child has lived at since birth

43. Do you have any of the following in your home? (you can tick more than one answer here)
   ☐ Gas hob        ☐ Gas cooker
   ☐ Open solid fuel fire ☐ None of these

44. Is there visible damp within the house? (please circle one answer) Yes/No

45. If yes to question 44, is it in:
   ☐ Kitchen        ☐ Bathroom
   ☐ Child’s bedroom ☐ Other living areas

46. What flooring does your child have in their bedroom
   ☐ Carpet        ☐ Laminate
   ☐ Laminate and rug ☐ Other

In some children, asthma may be related to dust brought home from parent’s work place. To allow us to study this, it would be helpful to know some details about your job

47. What is your child’s mothers’s job title (e.g. teacher)?

What duties does she have at work (e.g. teach 8 year olds)?

What does the organisation she works for mainly make or do (e.g. education)?
Asthma questionnaire version 2 07/05/08

48. What is your child’s father’s job title? ___________________________

       What duties does he have at work? ___________________________

       What does the organisation he works for mainly make or do? ________________

49. Is your child exposed to smoking? (please circle one answer) Yes/No

50. How many people in your household smoke tobacco? ________________

51. How many visitors to your house smoke tobacco regularly (i.e. at least once per week)? _______

52. Is your child exposed to animals? (please circle one answer) Yes / No

53. If yes to question 52, which of the following animals? (please tick as many as necessary)

       □ Cat    □ Rat    □ Rabbit
       □ Dog    □ Gerbil □ Hamster
       □ Bird   □ Fish   □ Cows
       □ Sheep  □ Pigs   □ Horses □ Other ________________

54. What is your child’s ethnic group? Please tick the box the appropriate box to indicate your child’s cultural background.

       □ White British □ Indian □ Pakistani
       □ Bangladeshi □ Chinese □ Caribbean
       □ African □ Any mixed background
       □ Other ________________
Appendix 3

PAGES Quality of life questionnaire
QUALITY OF LIFE QUESTIONNAIRE FOR CHILDREN

The aim of this questionnaire is to identify the things that bother your child about their asthma.

Your child’s name __________________________ Your child’s date of birth _______________________

Because of their asthma, your child may have found some activities difficult or not so much fun. Please have a look at the following list and put a tick next to those activities which have been affected in the past week:

- Basketball
- Studying
- Playing at break time
- Doing craft or hobbies
- Riding a bicycle
- Rollerblading
- Shopping
- Skiing
- Walking
- Talking
- Laughing
- Football
- Singing
- Playing with friends
- Gymnastics
- Skipping rope
- Athletics
- Swimming
- Climbing
- Walking upstairs
- Dancing
- Doing household chores
- Playing with pets
- Shouting
- Running
- Skateboarding
- Sleeping
- Ice skating
- Walking uphill
- Getting up in the morning

1. Of those that you have ticked, please write down here the three that bother your child most:

The next section is a bit of a quiz where an adult will ask a number of questions and a child with asthma gives the answers. Please can you give the second sheet of paper (page 3, the one with the answers) to your child?

Here is a copy of the answers to questions 2-8 for you. You can write down the whole answer to each of the above questions, for example “Quite bothered” or just the number, for example “3”.

1. Extremely bothered
2. Very bothered
3. Quite bothered
4. Somewhat bothered
5. Hardly bothered at all
6. Not bothered

2. In the last week, how much have you been bothered by your asthma in each of the three activities listed in question 1?
   answer to first activity ______________________
   answer to second activity ______________________
   answer to third activity ______________________

3. How much did coughing bother you in the past week? ______________________

4. How much did asthma attacks bother you during the past week? ______________________
5. How much did wheezing bother you in the past week during the past week? __________________

6. How much did tightness in your chest bother you during the past week? __________________

7. How much did shortness of breath bother you during the past week? __________________

8. Think about all the activities you did during the past week. How much were you bothered by your asthma doing these activities? __________________

Here is a copy of the answers to questions 9-22 for you. Again, you can either write down the whole answer or just put the number down after each question.

1. All of the time
2. Most of the time
3. A good bit of the time
4. Some of the time
5. A little of the time
6. Hardly any of the time
7. None of the time

9. How often did your asthma make you feel frustrated during the past week? __________________

10. How often did your asthma make you feel tired during the past week? __________________

11. How often did you feel worried, concerned or troubled because of your asthma during the past week? __________________

12. How often did your asthma make you feel angry during the past week? __________________

13. How often did you feel irritable (cranky) during the past week? __________________

14. How often did you feel different or left out because of your asthma during the past week? __________________

15. How often did you feel frustrated because you couldn’t keep up with others during the past week? __________________

16. How often did your asthma wake you up during the night during the past week? __________________

17. How often did you feel uncomfortable because of your asthma during the past week? __________________

18. How often did you feel out of breath during the past week? __________________

19. How often did you feel you couldn’t keep up with others because of your asthma during the past week? __________________

20. How often did you have trouble sleeping at night because of your asthma during the last week? __________________

21. How often did you feel frightened by an asthma attack during the past week? __________________

22. How often did you have difficulty taking a deep breath during the past week? __________________
Answers

Here are the answers for your child to have whilst you ask the questions. (Your child can either say the whole answer, for example “Quite bothered” or say the number, for example “three”)

Answers to questions 2-8

1. Extremely bothered
2. Very bothered
3. Quite bothered
4. Somewhat bothered
5. Bothered a bit
6. Hardly bothered at all
7. Not bothered

Answers to questions 9-22

1. All of the time
2. Most of the time
3. A good bit of the time
4. Some of the time
5. A little of the time
6. Hardly any of the time
7. None of the time
Appendix 4


A methodology to establish a database to study gene environment interactions for childhood asthma

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Abstract

Background: Gene-environment interactions are likely to explain some of the heterogeneity in childhood asthma. Here, we describe the methodology and experiences in establishing a database for childhood asthma designed to study gene-environment interactions (PAGES - Paediatric Asthma Gene Environment Study).

Methods: Children with asthma and under the care of a respiratory paediatrician are being recruited from 15 hospitals between 2008 and 2011. An asthma questionnaire is completed and returned by post. At a routine clinic visit saliva is collected for DNA extraction. Detailed phenotyping in a proportion of children includes spirometry, bronchodilator response (BDR), skin prick reactivity, exhaled nitric oxide and salivary cotinine. Dietary and quality of life questionnaires are completed. Data are entered onto a purpose-built database.

Results: To date 1045 children have been invited to participate and data collected in 501 (48%). The mean age (SD) of participants is 8.6 (3.9) years, 57% male. DNA has been collected in 436 children. Spirometry has been obtained in 172 children, mean % predicted (SD) FEV1 97% (15) and median (IQR) BDR is 5% (2, 9). There were differences in age, socioeconomic status, severity and %FEV1 between the different centres (p ≤ 0.024). Reasons for non-participation included parents not having time to take part, children not attending clinics and, in a small proportion, refusal to take part.

Conclusions: It is feasible to establish a national database to study gene-environment interactions within an asthmatic paediatric population; there are barriers to participation and some different characteristics in individuals recruited from different centres. Recruitment to our study continues and is anticipated to extend current understanding of asthma heterogeneity.

Background

Asthma is a common condition diagnosed in as many as 25% of Scottish children by the age of 11 years[1]. Childhood asthma is heterogeneous in terms of severity [2], natural history [2] and response to treatment[3] and mechanisms for the disease heterogeneity among children with asthma is not well-understood but genetic and environment factors and, crucially, combinations thereof are thought to be relevant.

Asthma heterogeneity is most commonly classified by severity. Several genetic variations have been associated with asthma severity in children, including those within genes coding for filaggrin [4], macrophage inhibitory factor [5], interleukin-4 [6] and the beta 2 adrenoceptor [7]. Additionally, regulatory genes for ORMDL3 [8] and IL12B [9] have been associated with asthma severity. Environmental exposures have also been associated with asthma severity and include tobacco smoke[10] and
outdoor air pollution[11]; there is also evidence of increased asthma severity in association with obesity[12] and reduced dietary antioxidants[13]. Whilst gene-environment interactions are described for asthma causation in children [14,15] there is also the potential for such interactions to explain why asthma severity varies within a population[16]. A proof-of-concept study[17] found that children with more severe asthma were more likely to carry at least one G allele for the CD14 gene but this is seen only among those exposed to tobacco smoke.

Large databases, for example BioBank UK http://www.ukbiobank.ac.uk and the Avon Longitudinal Study of Parents and Children (ALSPAC), have the potential to study gene-environment interactions in the context of asthma causation. However, understanding the relevance of gene-environment interactions to asthma heterogeneity will require a relatively large study population of children with asthma to provide enough statistical power to observe common interactions with a small effect and less frequent interactions which have a greater effect. Following a successful pilot of the methodology in Aberdeen, we have commenced recruiting patients under the care of consultant paediatricians with a special interest in asthma across Scotland. The aims of the Paediatric Asthma Gene Environment Study (PAGES) are:

1. To recruit children with asthma attending secondary care clinics
2. To ascertain the children’s environmental exposures.
3. To obtain saliva samples consented for DNA preparation and genetic analysis.
4. To create a dataset sufficient to elucidate hypothesis-driven gene-environment interactions using a nested case-control design.

Initial gene-environment interactions of primary interest are between (i) exposure to tobacco smoke and dietary oxidants and genetic variations of the Glutathione S-transferase (GST) gene family and (ii) exposure to tobacco smoke and variations in the gene coding for filagrin. With the advent of rapidly advancing technologies and the reduction in sequencing costs, we aim to combine the present population with the BREATHE cohort [4,18] and apply whole genome technologies to a population which is expected to number in excess of 2500 children and young adults with asthma.

The aims of the present report are to describe the methodology, demonstrate the feasibility of recruitment, describe our experience in establishing the database and report the characteristics of the children initially recruited.

Methods

Study design

This is a cross-sectional study of asthmatic children attending hospital clinics across Scotland (figure 1) and Brighton, England (included as a validation centre). An asthma questionnaire is completed and returned by post. Children are invited to attend a clinical assessment that includes: spirometry, bronchodilator response, skin prick reactivity, exhaled nitric oxide measurement and saliva collection for cotinine assay and DNA extraction. Dietary and quality of life questionnaires are also completed during or after the clinical assessment. A sample of saliva for DNA analysis can be collected by parents and returned by post in cases when clinical attendance is refused.

Researchers

Researchers are based in five centres: Aberdeen (recruiting in Aberdeen, Inverness, Elgin, Perth and Dundee), Edinburgh (Edinburgh, Kirkcaldy, Melrose and Stirling), Glasgow (Glasgow, Wishaw and Paisley), Kilmarnock (Kilmarnock, Paisley and Dumfries) and Brighton. Researchers dispatch questionnaires, collect DNA and undertake the assessments in accordance with standard operating procedures and using identical apparatus.

Eligibility criteria

Asthma is defined as a recurrent wheezing condition diagnosed as asthma by a consultant paediatrician who is a member of the Scottish Paediatric Respiratory Interest Group. All asthmatic children aged 2-16 years attending a clinic between March 2008 and November 2011 will be eligible. Exclusion criteria include children with coexisting respiratory morbidity, for example cystic fibrosis, bronchopulmonary dysplasia (BPD), and the following significant non-respiratory problems, cerebral palsy, Down’s syndrome, gastro-oesophageal reflux (prescribed medications) and marked developmental delay. Children born prematurely but who did not have BPD are eligible; premature delivery is noted in the questionnaire.

Enrolment

Our pilot study indicated that recruitment will not exceed 60% and therefore the gender, age and postcode of all children invited to participate are recorded to demonstrate how representative participants are of all the children invited to enroll. A deprivation index is derived from the postcode using 2009 Scottish Index of Multiple Deprivation data[19] (SIMD). Written consent is obtained from the parent when the asthma questionnaire is completed and verbal assent from the child at the time of the clinical assessment. This study has been approved by the Cornwall and Plymouth Research Ethics Committee.

Questionnaires

The asthma questionnaire (available at http://www.asthma-pages.com/participants/what/) included the
respiratory questions validated in the BREATHE study [6], questions relating to asthma control (the Child Asthma Control Test®, used with permission) and environmental exposures (from Biobank). The Paediatric Asthma Quality of Life Questionnaire [7] and food frequency questionnaires (Scottish Collaborative Group semi-quantitative food frequency questionnaire version C1 http://www.foodfrequency.org) were also completed by parents.

Clinical assessment
The assessment took place in conjunction with a scheduled clinic appointment or at a dedicated research clinic. The detailed assessment included (in this order) exhaled nitric oxide (FENO), spirometry, skin prick reactivity and bronchodilator response. The assessment of children aged under 5 years included only skin prick reactivity since FENO and reliable spirometry are often not obtained in this younger age group[20,21].

Exhaled NO
Exhaled NO is measured using a portable NO analyser (NIOX MINO®, Aerocrine, Solna Sweden) in accordance with international recommendations[22]. Measurements from this device have been validated against a gold standard[20] and can be obtained in >90% in those aged over 7 years[20].

Spirometry and bronchodilator response
These are measured in accordance with standard guidelines[23] using a portable spirometer (ML3500, MicroLab), calibrated before each assessment. Values are expressed as percentage of predicted according to normative data[24]. The bronchodilator response is defined as the change in FEV₁ 15 minutes after inhalation of 200 micrograms salbutamol, delivered from a pressurised metered dose inhaler via large volume spacer device (Volumatic®, GlaxoSmithKline, UK). Parents and children are asked to withhold short acting beta agonists

Figure 1 A map of Scotland identifying the recruitment centres. 1 = Inverness, 2 = Elgin, 3 = Aberdeen, 4 = Dundee, 5 = Perth, 6 = Stirling, 7 = Kirkcaldy, 8 = Paisley, 9/10 = Glasgow, 11 = Wishaw, 12 = Edinburgh, 13 = Kilmarnock, 14 = Melrose, 15 = Dumfries.
for 6 hours prior to testing and long acting beta agonists for 12 hours.

**Skin prick reactivity**
The standard methodology[25] is used to determine skin prick reactivity to eight common environmental allergens including: *Dermatophagoides pteronyssinus*, cat dander, dog dander, whole egg, *Alternaria alternans*, *Aspergilus fumigatus*, peanut and grass (ALK, Northampton). The positive control is histamine 10 mg/ml and the negative control 0.9% saline. A positive skin test is defined as a weal ≥3 mm in longest diameter or, in cases of dermatographism, greater than the negative control. Testing is withheld in individuals who have taken anti histamines within the previous 72 hours. Testing for peanut is withheld in children with a history of peanut anaphylaxis. Children with a positive reaction to peanut but no history of reaction after ingestion of peanuts are offered referral for peanut challenge.

**DNA collection, storage and analysis**
Oragene sampling kits (DNA Genotek Inc, Ottowa, Canada), were used to obtain DNA from a saliva specimen. Five swabs (DNA Genotek Inc, Ottowa, Canada) are used to collect saliva for DNA analysis in young children who cannot actively provide a saliva sample. Saliva is either obtained by the researcher during a hospital visit or occasionally by the parent at home and then mailed to the researcher. Saliva samples are immediately labeled using preprinted barcode labels which are also attached to the case report form. This provides an immediate and robust de-identification system to retain the privacy of the study subjects, but retaining the ability to link genotype to phenotype within the study.

**Salivary Cotinine**
Saliva is collected for cotinine assay using a sterile absorbent cotton wool swab (Salivette®, Sarstedt Ltd, Leicester, UK) which is placed between teeth and buccal mucosal for three minutes before being inserted into a plastic case. Specimens are spun down and saliva frozen for analysis by ELISA (ABS laboratories, Welwyn Garden City, UK). Cotinine exposure is analysed as a continuous variable and values of ≥15 ng/ml assumed to indicate active smoking by the child. Values below the limit of detection (0.1 ng/ml) are assigned a value of 0.05 ng/ml.

**Establishment and population of the database**
The experience gained from establishing and validating the UK CF database[26] has been applied to the PAGES database. The database is currently a stand alone (non-networked) system (figure 2). There is the option for future remote, web based access to the database now that the fields are fully agreed and the methodology is stable. The advantage of using a database system for development (rather than working with a spreadsheet or statistics package) is that database systems naturally facilitate the inclusion and preparation for the analysis of longitudinal data. Standard validation checks have been incorporated as described in detail elsewhere [26,27] and include restricted fields, e.g. age is a numeric field between 2 and 16, gender is a categorical field either “male” or “female”.

**Quality control**
To ensure a standardised methodology all researchers work to the same standard operating procedure and use identical equipment. Researchers have a certificate from Association for Respiratory Technology and Physiology/ British Thoracic Society; this is a national qualification for those practitioners who complete the Spirometry assessment. All researchers attend a monthly minuted teleconference where methodological issues that arise are discussed and resolved. Finally, all data are entered onto one central database by an individual. All queries or errors identified at the verification stage are notified back to the researcher within 2 weeks of receipt; this approach is proven to result in data entry of high quality[26]. An audit of data is undertaken in the course of the present analysis.

**Power calculation**
Power calculations are based on the pilot study. The sample size needed to detect the interaction of a genetic factor with an exposure depends on the prevalence of exposure and genotype, the relative risk for exposure and genotype alone, magnitude of the gene-environment interaction, the case-control ratio and the type I and type II error [28]. For example to explore the relationship between tobacco smoke exposure (assuming 50% prevalence) and GSTT null genotype (assuming 50% prevalence) and the presence of atopic illness as an outcome among an asthmatic population, assuming relative risks of interaction (Ri) of 2 and 5, studies including 414 and 103 non-atopic asthmatics and second hand smoke exposure (assuming 50% prevalence) and the presence of filaggrin variants (assuming 10% prevalence) with an exposure depends on the prevalence between filaggrin variants (assuming 10% prevalence) and the presence of atopic illness as an outcome among an asthmatic population, assuming relative risks of interaction (Ri) of 2 and 5, studies including 199 and 105 asthmatics with asthma severity, assuming relative risks of interaction of 2 and 5, studies including 414 and 103 non-atopic asthmatics respectively will have power of 80% to detect an interaction at 5% level of significance [29]. To relate an interaction between filaggrin variants (assuming 10% prevalence) and second hand smoke exposure (assuming 50% prevalence) with asthma severity, assuming relative risks of interaction of 2 and 5, studies including 199 and 105 asthmatics on British Thoracic Society (BTS) treatment steps 4 and 5 respectively will have power of 80% at 5% level of significance[29].

**Statistical approach to analyzing the final dataset**
Statistical analysis will be conducted using logistic regression which permits adjustment for confounders,
and both crude and adjusted odds ratios will be presented. To test for gene-environmental interaction, an interaction variable will be created based on the combination of factors investigated[30]. First the model will be fitted with both variables as separate term, then with an interaction term. The statistical significance of the interaction will be obtained from likelihood ratio test of change in deviance between the second model and the first.

**Statistical approach to present report**

Differences between centres were compared using chi square, Mann Whitney-U test and Kruskal Wallis test as appropriate. Significance is assumed at p = 0.05. Standard statistical software is used (SPSS version 17.0.0)

**Results**

**Recruitment**

At the time of writing 1045 children have been invited to participate (figure 3), of whom a DNA sample has been obtained in 436 (42%) and asthma questionnaire in 382 (37%); the discrepancy in numbers where DNA and questionnaire were obtained is in part explained by many of the latter currently being entered onto the database. Recruitment has been underway for more than 12 months in seven centres (listed in table 1), for more than six months in nine centres and for more than one month in fourteen centres. An audit of the database found several discrepancies between treatment and BTS/SIGN treatment step; the error is not at the level of data entry rather at the level of the researcher coding the treatment step. The audit found no outliers and other than BTS/SIGN step, data were ready for analysis.

**Reasons for non-participation**

These were prospectively ascertained as part of the pilot study where 91 children were invited to participate of whom 57 (62%) were enrolled. Of the 34 children who were not enrolled, the parents of 17 did not have time to participate during the clinic visit, 10 did not attend the scheduled clinic appointment, five refused to take part and two children did not have asthma. When the centres recruiting for less than a year were combined
into an eighth group, inter centre recruitment rates varied between 38% and 72%, \( p = 0.004 \), table 1.

**Factors associated with participation**

For the purpose of the present study, participation is defined as at least a DNA sample or asthma questionnaire being obtained (\( n = 501 \)). The mean age (standard deviation) of those enrolled is 8.6 (3.9) years and 9.2 (4.0) years for those who did not enroll (T test \( p = 0.022 \)). There were 288 boys recruited (57% of all enrolled) and 359 boys (64%) among those who did not enroll \( (\chi^2 = 4.71, p = 0.030) \). The median (range) SIMD decile was the same (6 (1, 10)) for those who did and did not enroll (Mann Whitney U test \( p = 0.135 \)).

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**Figure 3 A Consort diagram demonstrating the number of individuals where data are available.** Recruitment is ongoing and these numbers will increase before the end of the study.
Differences between centres

These details are presented in table 1. The centres where recruitment has been under way for less than a year were combined to form an eighth group. There were significant inter-centre differences in the age of children invited to take part (p = 0.002), SIMD (p < 0.001), BTS treatment step (p = 0.002), % predicted FEV1 (p = 0.024) but not gender or FENO.

Participant details

The mean age (SD) was 8.6 years (3.9) and 57% were boys. There were 118 children aged under five years including 24 aged between two and three years and 55 aged three to four years. The proportions of children on each British Thoracic Society treatment step were: 29% step 2, 60% step 3, 10% step 4 and 1% step 5. Saliva was collected for DNA analysis in 436 and for cotinine assay in 225. Spirometry was measured in 172 children, mean (SD) % predicted FEV1, FVC and FEF25-75 were 97% (15), 106% (13) and 79% (27) respectively. Bronchodilator response was measured in 154 children and the median (interquartile range) was 5% (2, 9). Exhaled NO was measured in 185, median (IQR) value 18 (12, 50) parts per billion. Skin prick reactivity was measured in 120 children including 99 who were reactive. Reasons for missing pulmonary function data include young age, refusal to provide measurements and inability to provide measurements of adequate quality. Reasons for missing skin prick reactivity data include recent receipt of antihistamines, previous peanut anaphylaxis and refusal to have testing. There were no significant associations between BTS treatment step (severity) and physiological outcomes including spirometry, BDR or FE\textsubscript{NO}.

Salivary cotinine validation of tobacco smoke exposure

Salivary cotinine concentration was determined in 139 children and was in excess of 15 ng/ml in six individuals (median age 14.5 years). The median (SEM) cotinine for children with no resident smokers was 0.5 ng/ml (0.04), with one resident smoker was 0.58 ng/ml (0.16) and with two resident smokers was 1.39 (0.95), Kruskal Wallace test p < 0.001.

Different administrative requirements between centres

Although the study is categorised site-specific exempt by the ethics committee, three centres still requested Site Specific Assessment documents. One centre requested a materials transfer agreement with the host university. Some centres, but not all, required visiting researchers to have honorary contracts. In some centres, approval documents are sent initially to Research and Development departments whereas in others these documents go directly to the local Research Ethics Committee.

Discussion

Here we describe the methodology and feasibility for recruiting children with asthma on a national basis. To our knowledge this is the first study specifically designed

<table>
<thead>
<tr>
<th>Centre</th>
<th>Number of children Invited</th>
<th>Participated Mean age (years)</th>
<th>Median SIMD decile</th>
<th>Mean % FEV\textsubscript{1} (SEM), ppb</th>
<th>Median FE\textsubscript{NO} (SEM), ppb</th>
<th>Median BTS treatment step</th>
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<tbody>
<tr>
<td>Aberdeen</td>
<td>167</td>
<td>93</td>
<td>8.8 (4.1)</td>
<td>9.1 (4.3)</td>
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<tr>
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<td>91</td>
<td>8.1 (4.2)</td>
<td>9.0 (4.4)</td>
<td>6</td>
<td>5</td>
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<tr>
<td>Edinburgh</td>
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<td>8.5 (4.1)</td>
<td>9.8 (4.3)</td>
<td>7</td>
<td>5</td>
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<tr>
<td>Elgin</td>
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<td>8.9 (4.1)</td>
<td>9.8 (4.3)</td>
<td>6.5</td>
<td>5</td>
</tr>
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<tr>
<td>Other</td>
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<td>8.3 (4.3)</td>
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<td>4</td>
</tr>
<tr>
<td>Overall</td>
<td>1045</td>
<td>501</td>
<td>8.6 (3.9)</td>
<td>9.2 (4.0)</td>
<td>6 †</td>
<td>6</td>
</tr>
</tbody>
</table>

SIMD = Scottish Index of Multiple Deprivation, SEM = Standard Error of Mean, BTS = British Thoracic Society.*p = 0.022 for comparison between participants and non participants. †p value for trend tests for %FEV\textsubscript{1}, SIMD decile and BTS severity between centres <0.05.
to study asthma heterogeneity in children rather than asthma causation which has been the focus of much research interest. In addition to describing our methodology, we provide novel findings including barriers to participation in asthma research, details of passive smoke exposure in children with asthma and differences in the characteristics of children with asthma recruited from centres across the nation. These experiences may be relevant to colleagues interested establishing national and international single-disease databases in other countries.

The potential for national single-disease databases to study respiratory disease has already been demonstrated in cystic fibrosis. Cystic fibrosis (CF) databases have been established in several countries including the UK [31] and have lead to a better understanding of disease heterogeneity [32,33] and also added to our understanding of genetic mechanisms[34]. Asthma is more prevalent than CF and an asthma diagnosis is made on a solely clinical basis but notwithstanding these important differences between asthma and CF, we believe that the known benefits of national CF databases will be applicable to the present asthma database.

We are not aware of pre-existing large single-disease asthma databases from which the relationship between asthma heterogeneity and gene-environment interactions can be studied, but cross-sectional and longitudinal studies of asthmatic populations in Europe [18,35], United States[36] and Australia[2] have provided important insights into the heterogeneity of the natural history of asthma. A number of publications from the Childhood Asthma Management Programme[36] (a randomised-controlled trial including 1041 asthmatic children with follow up for over four years) have demonstrated associations between genetic variations and heterogeneity in response to asthma treatment [37-39]. A study of the 5244 asthmatic children who took part in the whole-population NHANES III survey in the US also found evidence for asthma heterogeneity in terms of both risk factors and severity [40]. These studies[2,18,35-40] give some insight into the heterogeneity of childhood asthma and the present study seeks to explore the variability described.

One challenge to our study is the absence of gold standard for measuring asthma heterogeneity outcomes. Severity is most commonly outcome used and in adult populations, indices of asthma severity have been developed and validated for use in large databases[41]. Based on our prior experiences [4,18] we will use BTS treatment step as our primary heterogeneity outcome. Once the dataset is complete we will explore the utility of other severity outcomes, for example asthma control, FEV₁, FE,No, hospitalisations and the number of courses of oral steroids over the past year. Additionally, we will explore heterogeneities in treatment response, i.e. bronchodilator response, and symptom triggers in the context of plausible gene-environment interactions.

Our methodology has a number of strengths, weaknesses and limitations. A key strength is that we have demonstrated that our methodology captures a heterogeneous population in terms of severity, as evidenced by BTS treatment, skin prick reactivity, exhaled nitric oxide and spirometry. A second strength is that phenotyping is carried out by researchers who use standardised methodology in all centres; this approach to quality control minimises the risk of heterogeneity arising between centres due to methodological differences. A third strength is that we have validated second hand smoke exposure, one of our principle exposures of interest, with a biomarker. A weakness of the study is the low recruitment rate, which at present is less than 50% and, as evidenced by our pilot study, is not expected to exceed 60%. We have described the barriers to participation and these are not previously described in the context of asthma and include pressure on parent’s time but uncommonly outright refusal to participate. An intervention study of behaviour problems in children has also reported time demands explaining 50% of non-participation[42]. Despite this relatively low recruitment rate, our study is likely to be adequately powered; for example we have already recruited more than half of the 105 children on BTS treatment steps 4 and 5 required to explore interactions between filaggrin gene variants and ETS. A second weakness is that spirometry, exhaled NO and skin prick reactivity data have not been obtained in individuals where exposure and genetic date are available; reasons for non-collection of clinical data include young children not being able to provide spirometry and exhaled NO data and skin reactivity data missing in those with peanut anaphylaxis. One limitation to this study is its cross sectional nature and we will seek funding to follow up those recruited to study disease remission and persistence over time. We acknowledge that the present study is hospital-based and the results may not be relevant to all children with asthma in the community. A further potential limitation to the study is inclusion of children as young as two years since asthma and viral induced wheeze are prevalent in preschool children and distinguishing between the two conditions can be difficult. The proportion of preschool children with recurrent wheeze is greater than in older children [43] but in the present study, preschool children were in the minority which might suggests that our inclusion criteria may be excluding children with non-asthmatic preschool wheeze; in our final analysis we will consider the whole dataset and then exclude the preschool group to address this limitation.
Assessment of environmental exposures is not without difficulties in such studies as this. Our methodology includes salivary cotinine as an objective index of exposure to second hand tobacco smoke (SHS) and we have observed higher exposure among those with greater reported exposure. The concentrations of salivary cotinine are lower than those in an earlier study undertaken in Dundee [44] where the average concentration in asthmatic children where two adults smoked is 4.02 ng/ml and 1.62 ng/ml where only mother smoked. This apparent fall in salivary cotinine concentrations may represent a genuine reduction in exposure of asthmatic children to SHS between 1994 and the present time or may be due to parents in the present study reducing their child’s exposure to SHS prior to the assessment knowing that cotinine is to be measured. Questionnaire information on factors such as smoking, parental smoking, use of gas fires and gas hobs and open fires is usually reasonably robust and memory of past exposures by the parents will be reasonable as the time scale is relatively short in comparison with studies of historical exposures in adults. The child’s home address will provide the ability to model outdoor air pollution exposure and future secondary studies could directly assess of indoor air quality, for example concentrations of particulate matter (PM$_{10}$ and PM$_{2.5}$), relevant gases (notably oxides of nitrogen) and volatile organic compounds. Exposure to other factors such as chemicals in the home known to be associated with respiratory symptoms[45] is less clear but the development of this database would allow additional studies to be added to the core concept (much as has been done in ALSPAC[45]) to allow specific issues to be addressed with more specific measurement of any relevant environmental exposures.

In summary, our study aims to provide a dataset from which to explore the influence of gene-environment interactions in childhood asthma. The gene-environment interactions associated with asthma causation will not necessarily explain asthma heterogeneity and we hope to report novel insights into genetic variations within an asthmatic population. Although the primary purpose of the study is to further understanding of asthma heterogeneity, there are other potential applications of the present study and these are listed in table 2.

### Conclusions

We conclude that it is feasible to study gene environment interactions in children with asthma by recruiting from multiple centres. There are differences in participation rates and patient characteristics between centres. The main reasons for non-participation in such a study were lack of time on parent’s behalf and failure to attend clinic appointments.

### Acknowledgements

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### Author details


### Authors’ contributions

SWT, JGA, TVM, AM, SM and CNAP conceived of the study, and participated in its design and coordination. SWT, SM, SC, TA, KA, DC, AD, GH, UM, JMcC, AM, AW and SW administered the study at each site thereby allowing data acquisition. CB, RL, DM and EWH undertook recruitment and assessment. SWtL undertook the pilot study. All authors read and approved the final manuscript.

### Table 2 Potential applications of a national asthma database

<table>
<thead>
<tr>
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<th>Resource</th>
</tr>
</thead>
<tbody>
<tr>
<td>Identification of genetically susceptible individuals for pharmacogenetic studies</td>
<td>DNA</td>
</tr>
<tr>
<td>Identification of genetically susceptible individuals for environmental modification studies</td>
<td>DNA</td>
</tr>
<tr>
<td>Characterisation of patterns of inhaled environmental hazards in children with asthma</td>
<td>Questionnaire and longitudinal component</td>
</tr>
<tr>
<td>Validate a system to score asthma severity</td>
<td>All data</td>
</tr>
<tr>
<td>Explore gene-environment interactions for subgroups, eg severe asthma, non-atopic asthma</td>
<td>Subgroups</td>
</tr>
<tr>
<td>Audit standard of care between centres</td>
<td>Management + CACQ</td>
</tr>
<tr>
<td>Identify associations between gene-environment interactions and natural history</td>
<td>Longitudinal study</td>
</tr>
<tr>
<td>Confirm associations seen in secondary care study</td>
<td>Primary care study</td>
</tr>
</tbody>
</table>
Competing interests

The authors declare that they have no competing interests.

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References


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Appendix 5

INTRODUCTION

Asthma exacerbations are the commonest cause of medical admissions in childhood and have significant effects on quality of life\(^{(1)}\). Asthma-related hospital admissions are a major financial burden on the healthcare systems in the developed countries\(^{(2;4)}\). Exacerbations represent periods of enhanced airway inflammation and remodelling\(^{(5)}\) and are a marker of pulmonary decline\(^{(6)}\). In humans, chitinase and chitinase-like proteins are highly expressed in lungs of patients with asthma but not in healthy lungs\(^{(7)}\). The gene CHI3L1 codes for the protein Chitinase 3-Like-1 (CHI3L1). CHI3L1 is a 40 kDa protein and expressed in the bronchial smooth muscle cells, sub-mucosa, sub-epithelium, epithelium, alveolar macrophages, neutrophils and other non-respiratory cells\(^{(8;9)}\). The CHI3L1 gene is 10 exons long and is located on chromosome 1q31-q32.

CHI3L1 binds with high affinity to chitin and chitin-oligosaccharides through a preserved hydrophobic substrate binding cleft\(^{(10)}\). Chitin is the second most abundant polysaccharide in nature is found in most living organisms, including bacteria, insects and fungi\(^{(11)}\). CHI3L1 also interacts with glycosamines like heparin and hyaluronan, and binds to collagen types one, two and three\(^{(8;12)}\). CHI3L1 is only produced under inflammatory conditions and may be correlated to an increase in C-reactive protein\(^{(8)}\). When expressed, CHI3L1 is capable of increasing the rate of mitosis through activation of MAP kinase and PI-3K signalling cascades in fibroblasts, and by working synergistically with insulin like growth factor-1. CHI3L1 also plays a role in matrix preservation through inhibition of cytokine stimulated release of MMP1, MMP3 and MMP13, in CHI3L1 stimulated arterial chondrocytes and skin fibroblasts\(^{(8)}\). Due to these exacerbating responses CHI3L1 has been described as a sentinel molecule, responsible for picking up and binding to chitin, and triggering an immune mediated response\(^{(11)}\).

In adults the \textit{CHI3L1} -131C→G SNP rs4950928 is associated with increased CHI3L1 levels, increased susceptibility and severity of asthma, bronchial hyperresponsiveness and reduced pulmonary function\(^{(5)}\) whilst the minor −131G allele confers protection against exacerbations of asthma\(^{(5)}\).
However, the role of these polymorphisms on asthma exacerbations and, in particular, asthma-related hospital admissions in children, has not been explored.

Scotland has one of the highest risks of exacerbation in the UK. In 2002 five hospital admissions a day were due to childhood asthma exacerbation\textsuperscript{(13)}. Asthma UK report that Tayside has the highest risk of hospital admission with the exception of Shetland in Scotland. Children in Tayside are 2.5 times more likely to have an exacerbation than children in the borders\textsuperscript{(14)}. Making this population ideal for the investigation of polymorphisms associated with childhood asthma exacerbations.
METHODS

We recruited children and young adults (age 3-22 years) with physician-diagnosed asthma in Tayside and Dumfries, Scotland between June 2003 and October 2008. The dataset includes information about demographic, anthropometric and clinical details from 1071 individuals attending clinics in 29 primary care practices and three secondary care asthma clinics. The study was approved by the Tayside Committee on Medical Research and Ethics. Informed written consent was obtained from the patient and/or parent/guardian as relevant.

The methods have previously been described in detail\(^{(15;16)}\). In brief the patients were seen in the asthma clinic setting, where a detailed history, clinical examination and reviewal of medical notes were conducted. Information was collected on school absences, use of oral steroids and asthma-related hospital admissions over the previous 6 months. In children with asthma, school absences, use of short courses of oral steroids and asthma related hospital admissions represent well validated measures of asthma exacerbations. We have previously developed a combined exacerbation score, involving yes/no responses for any of these three measures of exacerbations over a 6 month period of reporting, which have been validated through previous publications.\(^{(15;18)}\). The asthma prescribing level was determined in accordance with the British Thoracic Society (BTS)\(^{(19)}\) guidelines for physician-led management of asthma, as follows: step 0, no use of inhaled albuterol on demand within the past month; step 1, inhaled albuterol on demand; step 2, regular inhaled steroids plus inhaled albuterol on demand; step 3a, regular inhaled long-acting b-agonists (salmeterol or formoterol) plus inhaled steroids with inhaled albuterol on demand; step 4, regular inhaled long-acting b-agonists plus inhaled steroids plus oral montelukast with inhaled albuterol on demand. From this data a global index of asthma severity was derived through construction of a composite variable. The use of inhaled short-acting b-agonists (bronchodilators) was categorized as follows: 0, rarely or never required; 1, required few times a week but less than once daily; 2, required daily; 3, multiple doses over a 24-hour period on a regular basis.
Genotyping for SNP rs4950928 was performed using a Taqman SNP genotyping assay c_27832042_10 (Applied Biosystems Europe, Warrington, UK). Allelic discrimination was assessed using an Applied Biosystems 7700 sequence detection system.

All statistical analyses were performed by using SPSS for Windows version 16 (SPSS Inc., Chicago, IL, USA). Binary logistic regression was used to calculate odds ratios (ORs) and p-values for measures of asthma exacerbations. To calculate the ORs for comparison of overall risk, asthma-related hospital admission, school absences and oral steroid intake were grouped as present (minimum once over the previous 6 months) or absent. The total asthma exacerbation response was calculated as any of these measures during the same period of time and grouped as present or absent.

Gender, presence of eczema and exposure to tobacco smoke were included as covariates.

The frequency of exacerbation related hospital admissions, school absences, short course of oral steroids and overall asthma exacerbations during the previous 6 months were compared between the CC genotype and the -131G allele.
RESULTS

The population characteristics of 1071 young Caucasian individuals with physician diagnosed asthma derived from both primary and secondary care are described in Table I. Individuals range from 3-22 years of age (mean 10.4 years, SD4.0). 59.1% are male. 33.3% of participants were exposed to tobacco smoke in their environment over the previous six months.

Over the previous six months 37.3% of the 1071 participants had at least one exacerbation, either requiring a hospital admission, a short course of oral steroids, a school absence or a combination two or more of these. 12.4% of participants had at least one hospital admission in the previous 6 months. 21.1% had been prescribed a short course of oral steroids. 31.7% reported a school absence due to their asthma exacerbation.

The 1071 recruited participants were genotyped for the SNP rs4950928. The overall effect of the rs4950928 genotype on exacerbations in individuals with asthma is described in Table II. The CC genotype was present in 60.2% (n=645) of participants, heterozygous mutation (CG) in 35.2% (n=377) and homozygous mutation (GG) in 4.6% (n=49) participants. The genotype distributions of all SNPs were in accordance with Hardy-Weinberg equilibrium (P> 0.05) in each set of samples.

60.2% of our population are homozygous for the risk allele. 39.69% of this risk group reported exacerbation in the previous six months (table 1). 79.3% of the participants were on regular inhaled corticosteroids with or without long-acting beta agonists and leukotriene receptor antagonist, and 81.87% of the population have reported using their bronchodilator short acting beta agonist occasionally over the previous six months (table1) From a population of children and young adults with mild to moderate asthma 14.57% reported exacerbations, requiring hospital admission.
Among the participants with the CC genotype, 39.69% reported at least one measure for asthma exacerbations in the previous six months: 14.57% reported hospital admission, 33.3% school absence and 22.79% use of oral steroids. Among the participants with the -131G allele, 33.80% reported an exacerbation in the previous six months. 9.15% reported hospital admission, 29.11% reported a school absence and 18.55% reported use of oral steroids due to exacerbation of asthma.

The -131C allele (CC) was associated with hospital admissions due to exacerbations (p=0.02) when compared to the minor -131G allele (CG or GG). The odds ratio for CG/GG was 0.62, indicating that the minor allele may have a protective role reducing the need for asthma-related hospital admissions. The CC genotype was significantly associated with total exacerbations (p=0.05).

We did not observe any significant association between the risk (-131C) SNP and oral steroid intake due to asthma exacerbations (OR 0.77 (95%CI 0.57-1.05); p=0.11), or asthma related school absences (OR 0.82 (95%CI 0.63-1.07); p=0.16) over the previous six months. We tested for the interaction between the risk allele and individual measures of exacerbation. We did not observe any significant interactions between the risk genotype, and requirement of oral steroids or school absences due to exacerbations as well as overall exacerbations. We also tested the hypothesis that there is an interaction between the genotype and asthma medication use. We did not observe any significant interactions between the risk genotype and inhaled steroid dosage, or increased use of short acting beta agonist as and when required.

We tested the gene-dosage effect for the risk allele for each treatment step for long-term control of asthma. However, no significant effect was observed indicating that there is not a gene-dose relationship with this SNP.
DISCUSSION

Our study is the first to show that CHI3L1 SNP rs4950928 is significantly associated with asthma related hospital admissions in children and young adults with asthma. The odds ratio for the presence of one or two -131G allele (CG or GG) was <1, indicating that the minor allele may confer protection against asthma-related exacerbations.

Chupp et al\(^{(9)}\) associated increased quantities of CHI3L1 in the serum and the lungs with severity of asthma in an adult population. Ober et al\(^{(5)}\) later reported that CHI3L1 is a susceptibility gene for asthma and that CHI3L1 can be used as a biomarker for asthma and decline in lung function. This was derived from an adult population of Hutterites and from the COAST cohort\(^{(5)}\). Increased quantities of serum CHI3L1 has been observed in Chinese patients with asthma, and the level has been correlated with asthma exacerbations\(^{20}\). Contrary to these findings Wu et al have reported an absence of any association with three chitinases, from a cohort of 422 children recruited across the USA\(^{(21)}\). The rs4950928 CHI3L1 polymorphism was not investigated by Wu et al. An association between CHI3L1 and atopy was identified in Korean children, but no association was found with asthma\(^{22}\). Rathcke et al\(^{23}\) investigated the CHI3L1 polymorphisms in a population of 6514 Danish adults. Of this population 540 had asthma, 300 of whom were defined as atopic asthma. In contrast to other studies, these researchers observed an association between the minor G allele, atopic asthma and self reported physician diagnosed asthma. CHI3L1 has not been identified in any genome wide association study as an asthma susceptibility risk. CHI3L1 SNP’s were however investigated through a genome wide association study of serum CHI3L1 levels, known to be elevated in patients with severe asthma\(^{24}\). This study is an attempt at replicating the initial findings of Ober et al\(^{(5)}\). We did not investigate serum or lung concentrations of CHI3L1 as a marker for severity. The odds ratios for all measures of exacerbation were less than one, in conjunction with the previously reported observation of the protective role of rs4950928. We did not find any direct association between CHI3L1 and asthma severity. Unlike the adult populations investigated in both these previous studies, we found no association
between lung function and CHI3L1. This correlates with the COAST cohort. A likely explanation for this is the characteristic reversibility of lung function in childhood asthma. Our identification of an association between CHI3L1 and exacerbations, may explain the exertion on asthma risk from CHI3L1 as hypothesised by Ober et al\(^{(5)}\). We did not collect any data regarding exacerbation triggers in this study, though this data would be important in exploring the clinical and therapeutic impact of CHI3L1.

The chitinase family of hydrolases are associated with TH2 induced inflammation and airway hypersensitivity\(^{(5)}\). CHI3L1 increases myofibroblast replication and differentiation through activation of MAP kinase and PI-3K signalling cascades in fibroblast, enhances production of the pro-inflammatory cytokines and chemokines, TNF\(\alpha\), IL-8, RANTES, eotaxin and increases activity of NF-\(\kappa\)B (figure I)(8;25). An increase in secretion of these chemo attractants will lead to leukocyte migration. Over expression of CHI3L1 may cause increased fibroblast hyperplasia and increased secretion of TNF\(\alpha\) leading to airway smooth muscle hypertrophy and a possible increase in membrane thickening. Membrane thickening could also be aided by inhibition of matrix metalloproteinases 1, 3 and 13.

As a sentinel molecule CHI3L1 binds to substrate and initiates a severe immune response\(^{(8;11)}\). Cumulative adhesion of chitin and CHI3L1 substrates in this inflammatory active area may result in a rapid, severe immune response resulting in asthma exacerbation requiring hospital admission. It is possible that the CHI3L1 SNP rs4950928, which is expressed in the inflammatory lung\(^{(7)}\), has increased affinity for chitin, thus resulting in higher bronchial epithelial concentrations of chitin. This could increase the exposure of antigen-presenting cells (APC) (macrophages and dendritic cells) to chitin, thus mediating a larger TH2 and IL13 mediated immune response associated with asthma severity and airway remodelling.

Through NF\(\kappa\)B pathway up-regulation, CHI3L1 increases leukocyte migration (figure I)(25). The rs4950928 polymorphism may up-regulate the efficiency of this pathway, which would result in greater quantities of leukocyte migration. As a bi-product of this effect
TNF-α secretion would be increased. This could possibly lead to significant levels of airway smooth muscle hypertrophy\(^{(26)}\).

It is possible that for a CHI3L1 elicited exacerbation of asthma (figure I), a minimal threshold concentration of CHI3L1–substrate complex be exceeded. Individuals with the \textit{CHI3L1} SNP have higher concentrations of CHI3L1\(^{(5)}\). Higher concentrations of CHI3L1 may lead to accelerated build up of CHI3L1-complex, which may lead to increased APC exposure, thus heightened immune education. Steroid based inhalers suppress leukocyte concentrations in the lung, through NFκβ inhibition.\(^{(26,27)}\) It might thus be possible that carriers of the risk genotype exacerbate more often due to immune education and hyper reactivity overcoming the general immune suppression caused by steroid based treatment\(^{(27)}\).

Asthma costs the UK approximately £996 million per year\(^{(4)}\), and the US approximately $37.2 billion per year\(^{(2)}\). Approximately 48% of this cost is attributed to hospital admission alone\(^{(3)}\). The cost of care of asthma requiring hospital admission is around three fold greater than the cost of asthma management in the community. The annual average cost per patient in European children between the ages 0-4 was €789, equating to €37,486,179 for admissions alone\(^{(3)}\). Identification of children and young adults positive for the risk genotype through genetic screening could improve the quality of life and reduce the healthcare costs of children and young adults with asthma. Post diagnostic screening of children and young adults with asthma could alert healthcare workers to high risk patients. These high risk patients would firstly have an empirical incentive to avoid sensitised triggers. Regular clinics and assessment of condition, including unique, regularly reviewed treatment regimes. It is also plausible that the risk genotype carriers would benefit from alternate treatment regimes like introduction of mometasone furoate.\(^{(28)}\) The regular monitoring of these high risk patients therefore is likely to dramatically improve their quality of life and significantly reduce the costs attributed to emergency secondary care.
The implementation of post diagnostic screening for identification of risk genotypes in asthmatic children and young adults has many ethical and economic implications, which need to be weighed against the benefit to healthcare systems and patient benefit. Future clinical trials, determining the outcome of monitoring and treatment variation on the CHI3L1 risk group, may help us to weigh this benefit. Using genotyping as a tool for quantifying risk of asthma-related hospital admissions and the understanding of the relationship between CHI3L1 and asthma thus might focus primary prevention strategies to reduce the burden on emergency care. 60.2% of the recruited population were positive for the risk genotype. 23.9% of the cohort were positive for the risk genotype and reported an exacerbation in the previous six months. A relatively small reduction in asthma-related hospital admissions may therefore still outweigh screening costs, thus reducing the financial burden on over-stretched health
<table>
<thead>
<tr>
<th>Table I: Characteristics of BREATHE study participants with asthma (n=1071)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong></td>
</tr>
<tr>
<td><strong>Sex (males: females)</strong></td>
</tr>
<tr>
<td>CHI3L1 functional promoter SNP rs4950928 (%) (CC: CG:GG)</td>
</tr>
<tr>
<td><strong>Exposure to smoke (yes/no) (n=1071)</strong></td>
</tr>
<tr>
<td><strong>Mean percent predicted FEV1 (SD)</strong></td>
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<tr>
<td><strong>Mean percent predicted FVC (SD)</strong></td>
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<td><strong>Mean FEV1/ FVC (SD)</strong></td>
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<td>*<em>BTS asthma treatment steps <em>(n=1069)</em></em></td>
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<tr>
<td><strong>Inhaled bronchodilator use † (n=1070)</strong></td>
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<tr>
<td><strong>Median inhaled corticosteroid dose (beclomaetasone dipropionate equivalent)</strong></td>
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<tr>
<td><strong>Hospital admissions (yes/ no) over previous 6 months due to exacerbations (n=1071)</strong></td>
</tr>
<tr>
<td><strong>Oral steroid intake (yes/ no) over previous 6 months due to exacerbations (n=1071)</strong></td>
</tr>
<tr>
<td><strong>School absence (yes/ no) over previous 6 months due to exacerbations (n=1071)</strong></td>
</tr>
<tr>
<td><strong>Overall exacerbations (yes/ no) over previous 6 months ‡ (n=1071)</strong></td>
</tr>
</tbody>
</table>

**Keys:**

*Step 1= no/occasional use of inhaled albuterol; Step2= step1+ inhaled steroids; step 3= step2+ inhaled long acting β2 agonists with or without montelukast

†Inhaled bronchodilator use: 0= none, 1= occasional, 2= daily, 3= excessive use

‡Defined as any one of the following in previous 6 months: school absences, courses of oral steroids, or hospital admissions
Table II: Overall effect of CHI3L1 functional promoter SNP rs4950928 genotype (codominant and mutant varieties) on hospital admissions, oral steroid intake and school absences due to asthma exacerbations, and overall asthma exacerbations, in children and young adults

<table>
<thead>
<tr>
<th>CHI3L1 functional promoter SNP rs4950928</th>
<th>CG/GG</th>
<th>CC</th>
<th>Total</th>
<th>OR (95% CI)</th>
<th>P value</th>
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<td>Total</td>
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<td>645</td>
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<td>School absence due to asthma exacerbations</td>
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<td>Overall asthma exacerbations</td>
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<td>256</td>
<td>400</td>
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<tr>
<td></td>
<td>Total</td>
<td>426</td>
<td>645</td>
<td>1071</td>
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</table>

Key: OR: Odds ratio; CI: Confidence interval

P values were calculated by binary logistic regression corrected for age, sex, personal eczema and exposure to tobacco smoke.
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