EFFECT OF GENETIC VARIATION ON HEALTHCARE OUTCOMES IN CHILDREN AND ADULTS WITH ECZEMA AND ASTHMA

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Abstract

Background: Asthma and eczema are common chronic diseases. Filaggrin (FLG), Adrenoreceptor β_2 (ADRB2), and the Fc fragment of IgE receptor II (FCER2) gene have been associated with asthma and eczema susceptibility, clinical response to medication and asthma exacerbations. However, it is unclear whether these genotypes contribute to differences in healthcare outcomes such as prescribing and whether these lead to different healthcare costs.

Methods: A systematic review was undertaken to identify evidence on pharmacogenetic associations in asthma and to inform which genetic model to use for secondary analysis of BREATHE, a study of gene-environment associations with asthma severity. BREATHE data were collected on 1100 children and young adults with asthma, in Tayside and Fife, Scotland. A collaboration with the Health Informatics Centre, Dundee, enabled BREATHE to be linked to routine healthcare data over 9-years: A&E attendances, hospital admissions, and community prescribing. Data were analysed using generalised linear models with random effects. Public engagement activities were performed to understand parent's and children's opinion about personalised medicine in asthma and eczema.

Results: An association was found between the presence of FLG mutations and prescribing of emollients (IRR: 2.19, 95% CI: 1.36-3.52), treatment for severe eczema (IRR: 2.18, 95% CI: 1.22-3.91), prescribing of a combination of Long-acting β_2-agonists (LABA)/Inhaled Corticosteroid (ICS) (IRR: 3.29, 95% CI: 1.68-6.43), and asthma-related hospitalisations (IRR: 2.37, 95% CI: 1.51-3.71). An association was found between the Arg16Gly polymorphism and the prescribing of Leukotriene Receptor Antagonist (LTRA) (Gly/Gly vs. Arg/Arg $-$ IRR: 2.33, 95% CI: 1.06-5.13), and a combination of LABA/ICS (Gly/Arg vs. Arg/Arg $-$ IRR: 2.80, 95% CI: 1.35-5.81; Gly/Gly vs. Arg/Arg $-$ IRR: 3.15, 95% CI: 1.50-6.63). An association was found

between the Glu27Gln polymorphism and the prescribing of LTRA (Gln/Gln vs. Gln/Glu – IRR: 0.53, 95% CI: 0.29-0.98; Gln/Gln vs. Glu/Glu IRR: 0.43, 95% CI: 0.20-0.95) and a combination of LABA/ICS (Gln/Gln vs. Glu/Glu – IRR: 0.47, 95% CI: 0.23-0.98). FCER2 was associated with prescribing of LTRA (TT vs. CC – IRR: 3.85, 95% CI: 1.43-10.34; TC vs. CC IRR: 4.96, 95% CI: 1.77-13.86). Patients with FLG mutations or with Arg/Arg genotype had greater healthcare costs than patients without FLG mutations or with Gly/Arg or Gly/Gly genotype.

Conclusion: Genetic variations in FLG, Arg16Gly and FCER2 exert long-term influences on healthcare outcomes. The ability to define genetic subgroups requiring more long-term medication or those not responding to particular medications could help develop targeted management strategies, potentially reducing morbidity and treatment costs.

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Acronyms

A&E Accident & Emergency.

AAI Adrenaline Auto-Injector.

ACQ Asthma Control Questionnaire.

ACRN Asthma Clinical Research Network dataset.

ACT Asthma Control Test.

ADRB2 Adrenoreceptor β_2.

ALOX5 5-Lipoxygenase.

ALOX5AP Arachidonate 5-Lipoxygenase activating protein.

AQLQ(S) Asthma Quality of Life Questionnaires with standardised activities.

ARG1 Arginase 1 gene.

ASB3 Ankyrin repeat and SOCS box protein 3.

BCa Bias corrected and accelerated.

BDR Bronchodilator response.

BEEP Barrier Enhancement for Eczema Prevention.

BNF British National Formulary.

BSACI British Society for Allergy & Clinical Immunology.

BTS British Thoracic Society.

CA10 Carbonic anhydrase 10.

CAMP Childhood Asthma Management Program dataset.

CARE Childhood Asthma Research and Education network.

CHI Community Health Index.

CHI3L1 Chitinase 3 Like 1.

CI Confidence interval.

CLIC Characterizing the response to a LTRA and an ICS dataset.

COPD Chronic obstructive pulmonary disease.

CRHR-2 Corticotropin releasing hormone receptor 2.

CRHR-1 Corticotropin releasing hormone receptor 1.

CTLA4 Cytotoxic T-Lymphocyte Associated protein 4.

CTNNA3 Catenin α 3.

CYP3A4 Cytochrome P450 family 3 subfamily A member 4.

CysLTR1 Cysteinyl leukotriene receptor 1.

DNA Deoxyribonucleic acid.

DUSP1 Dual specificity phosphatase 1.

ER Emergency room.

FBXL7 F-Box and leucine rich repeat protein 7.

FCER2 Fc fragment of IgE receptor II.

FeNO Fractional exhaled nitric oxide.

FEV$_1$ Forced expiratory volume in one second.

FLG Filaggrin.

FVC Forced vital capacity.

GALA Genetics of Asthma in Latino Americans dataset.

GAS8 Growth arrest specific 8.

GLCCI1 Glucocorticoid induced 1.

GP General Practitioner.

GR Glucocorticoid receptor.

GSNOR Glutathione-dependent S-nitrosoglutathione reductase.

GWAS Genome-wide association studies.

HDAC1 Histone deacetylase 1.

HIC Health Informatics Centre.

HLA Human leukocyte antigen.

HR Hazard Ratio.

ICD International Classification of Disease.

ICS Inhaled Corticosteroid.

IgE Immunoglobulin E.

IKZF1 IKAROS family zinc finger 1.

IL-6 Interleukin 6.

IL-4Rα Interleukin 4 receptor α.

IL-4 Interleukin 4.

IL-13 Interleukin 13.

IQR Interquartile range.

IRR Incidence rate ratio.

KRT25 Keratin 25.

LABA Long-acting β_2-agonists.

LAMA Long-acting anti-muscarinic.

LOCCS Leukotriene modifier or corticosteroid salmeterol study.

LODO Effectiveness of low dose theophylline.

LTA4H Leukotriene A4 hydrolase.

LTC4S Leukotriene C4 synthase.

LTRA Leukotriene Receptor Antagonist.

MAF Minor Allele Frequency.

MEDLINE Medical literature analysis and retrieval system online.

MeSH Medical subject headings.

MMP12 Matrix Metalloproteinase 12.

NHS National Health Service.

NOS3 Nitric oxide synthase 3.

NR3C1 Nuclear Receptor subfamily 3 group C member 1.

OR Odds ratio.

ORMDL3 ORMDL sphingolipid biosynthesis regulator 3.

PAAP Personalised Asthma Action Plan.

PACMAN Pharmacogenetics of Asthma medication in Children: Medication with ANti-inflammatory effects.

PACT Personalised medicine for Asthma ConTrol.

PAGES Paediatric Asthma Gene Environment Study.

PASS Pediatric Asthma Severity Score.

PEF Peak expiratory flow.

PiCA Pharmacogenomics in Childhood Asthma.

PRISMA Preferred Reporting Items for Systematic Reviews and Meta-Analyses.

PTGDR Prostaglandin D2 receptor.

QALY Quality adjusted life years.

RCT Randomized Controlled Trials.

REF Research Excellence Framework.

RMST Rhabdomyosarcoma 2 associated transcript.

RPS15P6 Ribosomal protein S15 pseudogene 6.

RREB1 Ras responsive element binding protein 1.

SABA Short-acting β_2-agonists.

SAGE Study of African Americans, Asthma, Genes & Environments.

SD Standard deviation.

SIGN Scottish Intercollegiate Guidelines Network.

SLIC SaLmeterol Inhaled Corticosteroids trial.

SMR Scottish Morbidity Records.

SNPs Single nucleotide polymorphisms.

SOCS Suppressor of cytokine signaling.

ST13 Suppression of Tumorigenicity 13.

STEMM Science, Technology, Engineering, Mathematics and Medicine.

STREGA Strengthening the reporting of genetic association studies.

TBX21 T-box 21.

TBXA2R Thromboxane A2 receptor.

TEWL TransEpidermal Water Loss.

THRB Thyroid hormone receptor beta.

TNFα Tumor necrosis factor α.

UK United Kingdom.

USA United States of America.

VEGFA Vascular endothelial growth factor A.

WHO World Health Organization.

WWT Wet Wrap Therapy.

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Author Declaration

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 this or any other university for a degree, and does not incorporate any material already submitted for a degree.

Signed 

Dated 29/06/2018

Chapter 1

Introduction

This thesis will focus on the effect of genetic variation on healthcare outcomes, such as prescribing and hospitalisations, in children and adults with eczema and asthma. The introduction will be divided into several sections to provide a basic understanding of all the areas explored. Section 1.1 will describe eczema, its epidemiology and treatment, while section 1.2 will focus on asthma epidemiology and treatment. The clinical responsiveness to medication in both diseases is affected by genetic variants. Section 1.3 will explain the genetic function of the three genes studied that influence eczema and asthma severity: Filaggrin (FLG), Adrenoreceptor β_2 (ADRB2) and Fc fragment of IgE receptor II (FCER2). Section 1.4 will explore the role of FLG on eczema severity in more detail. The effect of genetic variation on healthcare outcomes was possible due to the data linkage between BREATHE and healthcare routine databases. Hence, section 4.1 will provide an explanation of data linkage and the advantages of using data linkage. Similarly, section 1.5 will describe pharmacoeconomics and the importance of this field to genetic association studies. Lastly, section 1.6 will characterise public engagement and how these activities should complement any scientific project.

1.1 Eczema

1.1.1 Epidemiology

Eczema, also called atopic dermatitis, is a chronic inflammatory skin disease. In 2003, the World Allergy Organization recommended the use of the term 'eczema' as preferable to atopic eczema/dermatitis syndrome.[1,2] Eczema can be subdivided into atopic and non-atopic. Children with atopic eczema have a hereditary predisposition to an excessive Immunoglobulin E (IgE) reaction, which leads to certain disease characteristics known as atopy, while children with the non-atopic disease may not exhibit these atopic features. Children with atopic eczema are more likely to have persistent eczema and are more likely to develop asthma later in life.[1]

Eczema results from a combination of genetic and environmental factors, with prevalence rates varying across the world. The differences in prevalence rates could be in part due to difficulties in disease definition, diagnosis, and differences in methods of measurement. They could also result from differences in genetic characteristics, and resultant differences in gene-environment interactions among different ethnic groups across the world. Different prevalence rates are also reported based on the age, the time period studied (atopic diseases have seasonal variation), and geography. A study in Brazil[3] reported eczema prevalence higher than 10%, while Civelek et al.[4] found an 8% and 5.8% prevalence in Turkey and Italy, respectively. However, Mediterranean countries have reported lower rates compared to the United States of America (USA), 17%, and Japan, 24%.[4] In the United Kingdom (UK), eczema affects 15 to 20% of schoolchildren and 2 to 10% of adults.[5,6]

Children with eczema will often develop food allergy, asthma and allergic rhinitis. This is often called the 'atopic march', which corresponds to the progression of atopic manifestations over time.[7,8] Eczema, asthma and allergic rhinitis are common chronic diseases. The 'atopic march' usually starts with eczema, with the child developing an allergic airway disease later during his or her childhood. Criticism has been made of this hypothesis since patients with asthma can develop eczema later in life, and not every patient with eczema will develop asthma and/or allergic rhinitis. Furthermore,

the mechanism behind this march is still unclear.[7,9] The current hypothesis for the 'atopic march' relies on an impaired skin barrier, leading to sensitisation, initiating the progression to further airway diseases. Figure 1.1 illustrates the current hypothesis underlining the 'atopic march'.

Looking specifically at the co-development of eczema, asthma and allergic rhinitis, a review study calculated the worldwide prevalence of the concurrent occurrence of these three diseases as 1.17%.[10] The number may seem low, but the authors estimated than it is 10 times higher than the expected prevalence if these diseases were to occur independently of each other, thus suggesting an interrelationship between these three diseases. Given geographical differences in prevalence across countries and the fact that the UK has a prevalence of these diseases higher than the one calculated worldwide, the prevalence of the occurrence of eczema, asthma and allergic rhinitis in these patients may be greater than 1.17% in the UK. A patient with eczema has a fourfold risk of reporting asthma and allergic rhinitis compared to a patient without eczema.[10] Other studies found that almost three-quarters of children with an early onset of eczema and a severe and persistent form of eczema will develop asthma in later life.[11,12]

In 65% of cases, the disease develops in the first few months of life, and 90% will present with eczema before 5 years old. The symptoms tend to disappear on reaching adulthood, but in some cases, flare-ups can occur during adolescence and adulthood. In a minority of patients, symptoms persist into adult life.[6]

The real burden of eczema is difficult to calculate since studies have mainly been focused on direct costs, such as General Practitioner (GP) costs, prescribing, and hospital admission. Indirect costs, such as quantification of pain, school and/or work absences, performance at school and/or work and impaired quality of life, are harder to quantify and measure. In the mid-nineties, it was estimated that the National Health Service (NHS) spent an annual cost of £125 million to treat eczema.[6] In 2002, it was estimated that the cost of community dispensed prescriptions only for topical corticosteroids was £11.6 million.

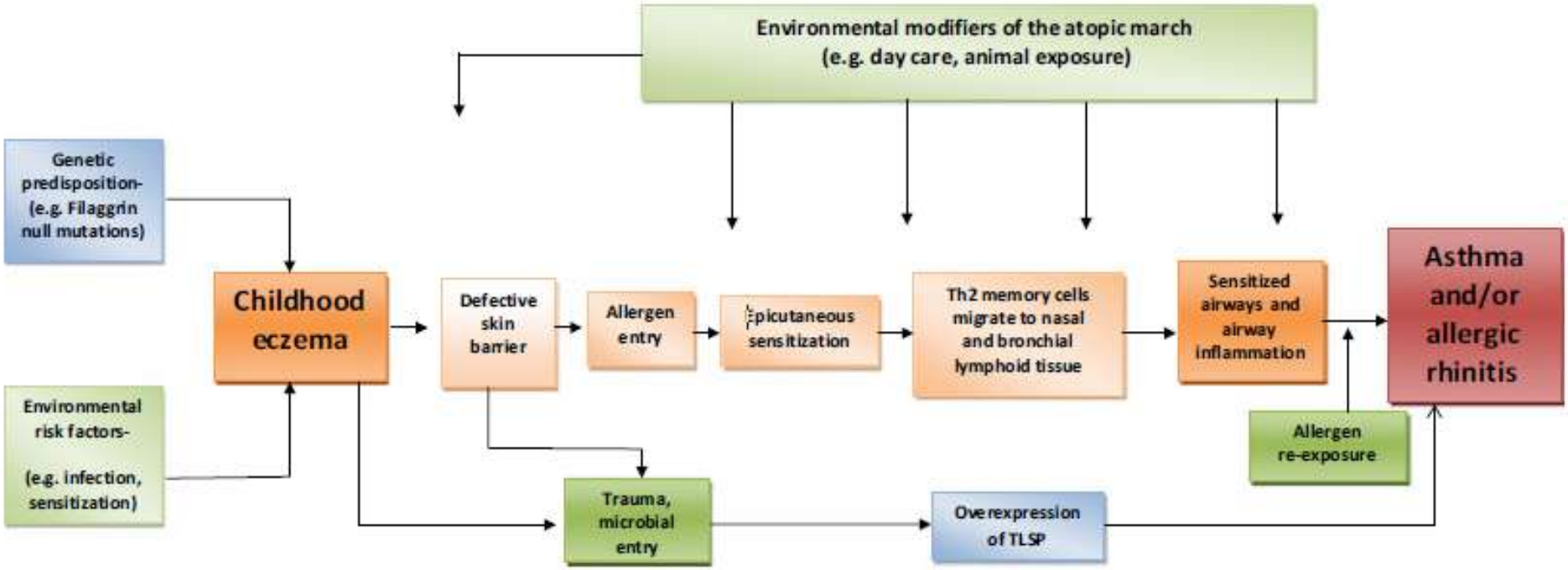

Figure 1.1: Current hypothesis explaining the 'atopic march'[9]

1.1.2 Clinical Symptoms and Predisposing factors

Eczema symptoms and the severity of these symptoms differ between individuals. However, most patients with eczema complain of itching. This symptom can be so intense that, in some cases, the patient will scratch his/her skin until it bleeds, which will make the rash worse leading to inflammation and more itching; this is known as the itch-scratch cycle. Itching leads to many problems, such as sleep disturbance and stress.[1] Usually, individuals with eczema also have dry and sensitive skin. Dry and inflamed patches may also be present on the skin.

Several risk factors have been associated with the development of eczema. Current evidence suggested that **fish intake** during childhood acts as a protective factor, reducing the risk of eczema and allergic rhinitis. However, no association was found between maternal fish intake during pregnancy and the development of atopic diseases.[13] A recent systematic review showed an association between individuals who are overweight or obese, and prevalence of severe eczema, although this effect was not significant in Europe. Nevertheless, it is still unclear whether **obesity** causes eczema or eczema leads to obesity.[14] Tsakok et al.[15] conducted a systematic review and meta-analysis to assess the impact of **antibiotic exposure**, *in utero* and during the first year of life, on eczema risk. They found a 40% increased risk of developing eczema for children exposed to antibiotics in their first year of life. They did not find an association between prenatal exposure to antibiotics and the development of eczema. Of note is the fact that atopic conditions can increase the patient's susceptibility to infections, leading to a greater use of antibiotics.[15–17] The effect of early life **pet exposure** on eczema is still a controversial issue due to the presence of conflicting results. Some studies[17–19] have found a protective effect for children exposed to dogs at birth. Interestingly, one study[20] has explored the interaction between having a pet during the first year of life and the presence of FLG mutations on the development of eczema. The authors found that the presence of a cat significantly increased the risk of eczema in children with FLG mutations. Two meta-analyses found no association between **Caesarian section** (C-section) and the development of eczema.[21,22] **Maternal food antigen avoidance**, such as milk, eggs and wheat avoidance, during pregnancy and breastfeeding, was found to confer no protection for the development

of eczema. The NHS does not recommend excluding foods during pregnancy and breastfeeding.[6,23] Evidence regarding the effect of **breastfeeding** has been reported as a protective factor for eczema development, but the evidence is conflicting.[1,6] The NHS strongly recommends breastfeeding on the basis of overall beneficial effect on maternal and child health, for at least three months.[6] In late 2017, results from a trial followed term infants during 16 years showed that breastfeeding reduced the risk of flexural dermatitis.[24] The **family history of atopic diseases** is a known risk factor; Wandalsen et al.[3] found a higher risk of eczema for someone with maternal history compared to paternal history of eczema.

Higher socio economic position[25] and elevated levels of personal hygiene, measured by frequency of washing[17] were associated with a higher risk of eczema or atopy, while day-care attendance was associated with a lower risk of developing eczema.[17] However, these risk factors have been debated for their significance, and more studies should be done to ascertain the relevance of these associations. A summary of the factors associated with eczema can be seen in table 1.1.

Predisposing factor	Association	References
Fish intake during childhood	Protective	13
Maternal fish intake	No association	13
Obesity	No association	14
Antibiotic exposure during the first year of life	Risk factor	15
Antibiotic exposure *in utero*	No association	15
Early life dog exposure	Protective	17–19
Early life cat exposure	Risk factor for carriers of FLG mutations	19,20
C-section	No association	21,22
Maternal food avoidance	No association	23
Breastfeeding	Protective	24
Family history of atopy	Risk factor	3
Higher socio economic position	Risk factor	25
High washing frequency	Risk factor	17
Day-care attendance	Protection	17

Table 1.1: Summary of factors associated with eczema onset

Flare-ups can occur at any time and can be caused by several factors: irritants, for which the recommendation is to avoid the product that may lead to flare-ups; house dust mite, although the benefits of cleaning the house are unclear; clothing, for which silver-coated textiles may reduce symptoms;.[6] Heat, sweating, anxiety and frustration are also triggers.[26] Individuals with eczema experience a lower quality of life, poorer work and/or school performance, and lower self-esteem.[26]

1.1.3 Diagnosis

For the majority of cases, an eczema diagnosis is made by a GP. However, the patient may be referred to secondary care if the GP is unsure, for example, in the case of severe eczema not responding to treatment, or in cases involving severe infection. Eczema diagnosis is not straightforward, since it is based on visual assessment and clinical history. The Scottish Intercollegiate Guidelines Network (SIGN) diagnostic criteria define that the patient should have an itchy condition during the previous year and three or more of the following conditions: an history of skin creases; personal and/or family history of atopic diseases; asthma, hay fever or food allergy; a history of dry skin during the past 12 months; onset under 2 years old; and/or flexural dermatitis.[6]

The physician will collect information about the family and personal history of atopy and eczema; the distribution and onset of the disease; and any aggravations due to pets and irritants. The physician will also discuss and enquire regarding the impact of the condition on quality of life, such as school absence, sleep disturbance; evidence of bacterial infection suggested by crusting or weeping; evidence of herpes infection suggested by vesicles and erosions; previous treatments and other medications taken; and the patient and family expectations from treatment.

The severity of the disease will help to decide the best course of therapy to follow. Eczema severity can be divided into mild, moderate or severe.[6] Mild eczema is characterised by some areas of dry skin and infrequent itching. Moderate eczema is characterised by areas of dry skin, frequent itching and redness, while severe eczema corresponds to multiple areas of dry skin, with incessant itching and redness. Severe eczema is associated with lower quality of life due to constant itching and lack

of sleep.[6]

The same eczema medicine can sometimes be prescribed for different severities: a lower concentration can be used for mild eczema and a stronger concentration for severe eczema. Different strengths are also used in various parts of the body, for instance, it is recommended to use lower concentrations for the face than the rest of the body.

1.1.4 Management

Although eczema has no cure, regular treatment can minimise the severity of flare-ups, improve the quality of life and prevent complications from infections.[26] According to Civelek et al.,[4] less than half of patients with current eczema use medication.

The main goal of eczema medication is to restore the function of the epidermal barrier and control the skin inflammation. Emollients and topical corticosteroids are used as first line treatment whereas topical calcineurin inhibitors and systemic immunosuppressants are used as second line treatment. Other treatment options are antihistamines and Wet Wrap Therapy (WWT). Antimicrobials should be used in patients with secondary bacterial infection, usually Staphylococcus aureus infection, or herpes virus infection. Besides medication, an important step in managing eczema is family and patient education. They should receive information about symptoms, potential triggers and the proper use of corticosteroids, to avoid over- or under-treatment.[1,26–28] The overuse of topical corticosteroids can eventually lead to skin atrophy.[1]

Emollients

Dry skin, which is common in eczema, favours the formation of cracks and can lead to infections and irritation from microbes and allergens if they enter the skin. Emollients create a protective barrier on the epidermis keeping the skin hydrated, reducing the symptoms and the itching, and increasing the efficacy of topical corticosteroids.[6]

Emollients are available as creams, lotions, ointments, and shower and bath products, and should be used daily to avoid the rupture of the epidermis and prevent

the need for corticosteroids.[1,27,28] Ointments are more effective than creams; however, ointments may be poorly tolerated by some patients. Creams are usually well tolerated during hot, humid days. A combination can be used to optimise adherence, and patient education will promote better choice by the patient.[6,26] Creams soak into the skin faster than ointments. Soap substitutes, containing emollient with mild emulsifiers, can be used to replace common soaps and detergents.[6]

During flare-ups, baths can be taken up to three times daily. Showers are not so effective at hydrating the skin as baths. Emollients can eventually become contaminated with bacteria if the patient uses his fingers to remove the emollient from the container. A clean spoon or a pump-dispenser should be used.[6,26]

In 2015, a Randomized Controlled Trials (RCT) found that the use of emollients was associated with fewer flare-ups, and the need of topical corticosteroids was also reduced.[27] However, data are limited regarding the efficacy of a specific emollient over others. Therefore, the selection of the emollient is usually made through trial-and-error and patient preference.[28,29]

Topical corticosteroids

Corticosteroids have an important role in treating eczema, reducing the inflammation, and are very efficient. However, special care should be taken due to their side effects.[26] Once again, patient and family education is crucial for successful treatment.

Topical corticosteroids should be used concurrently with emollients. The British Association of Dermatologists recommends the use of topical corticosteroids for up to a week for acute eczema and up to six weeks in chronic eczema. In the UK, topical corticosteroids are divided into mild, moderate, potent and very potent.[6,30–32] The British National Formulary (BNF) has information about the range of products and formulations available, with information about their potency. Treatment is prescribed following a stepwise approach, and the strength of the topical corticosteroids prescribed increases with the severity of eczema.

Topical corticosteroids can be formulated in creams, ointments and lotions. Ointments contain fewer preservatives and create a more occlusive barrier and are

therefore more potent. Lotions have more preservatives and create a weaker occlusive barrier compared to ointments. Lower potency should be prescribed for the face and genitalia since the skin is thinner and has a greater chance of absorption. For the remaining areas, the potency should be guided by the severity, distribution and age of the patient.[26,30–32]

Systemic corticosteroids should be avoided since their discontinuation usually results in severe flare-ups. Children with both eczema and asthma needing systemic corticosteroids should be treated earlier with potent topical corticosteroids to reduce the rebound inflammatory response.[26]

Topical calcineurin inhibitors

Tacrolimus ointment, and pimecrolimus cream are the two types of topical calcineurin inhibitors used in eczema. Tacrolimus is licensed for moderate to severe eczema and pimecrolimus for mild to moderate eczema. They are immunomodulating agents, whose function is to reduce skin inflammation. Topical calcineurin inhibitors are used as steroid-sparing agents for patients that require long-term anti-inflammatory treatment. Their efficacy has been proven, but long-term effects of these medicines are currently under debate. In that sense, topical calcineurin inhibitors should only be used as second line treatment for the treatment of moderate-to-severe eczema, in patients older than 2 years.[1,6,26]

Systemic immunosuppressants

Immunosuppressants are used in individuals with severe eczema with multiple flare-ups, or in those unresponsive to topical corticosteroids. Their function is to lower the immune response since in eczema the immune response is over-active, causing flare-ups. They are potent medicines and should be used only as second line treatment or last resort. Normally, immunosuppressants are prescribed by specialists for short-term use since the odds of an adverse reaction are high.

Some of the systemic immunosuppressants used are Ciclosporin, Azathioprine, Methotrexate and Mycophenolate Mofetil[1,6]

Antihistamines

As previously mentioned, some patients will go through the itch-scratch cycle. It is called a cycle because the itch causes patients to scratch, causing histamines to be released, which aggravate the symptoms, causing more itching and scratching. Antihistamines are prescribed to stop this cycle, for lesions to heal, and in patients with sleep disturbance and acute urticaria, common among those with eczema. The efficacy of these medicines is not clearly established in patients affected by eczema.[6,28]

Wet Wrap Therapy (WWT)

WWT has been proven to be an effective treatment for moderate-to-severe eczema. WWT consists of the patient receiving a soaking bath, followed by the application of emollients or topical corticosteroids to lesions, then wrapping the lesions with a bandage that has been squeezed after soaking in warm water. Another bandage is placed on top of the wet bandage for a few hours during the day or while the patient is sleeping.[6,26,28]

Occlusion of dry skin with dry dressing alone has not been proven effective. However, WWT with topical corticosteroids have been shown more efficient than emollients alone.[28] By providing a moist environment, the skin stays hydrated, and the epidermal barrier is restored. The itch-scratch cycle is also broken since the dressing provides a physical barrier for itching. WWT should be used before implementing systemic therapies for a maximum of two weeks. This could help by reducing the need for systemic therapies, thus avoiding the possible side effects of systemic therapies.[28]

Infection-related complications

The most frequent co-morbidities associated with eczema are Staphylococcus aureus infection, and eczema herpeticum, caused by herpes simplex virus (HSV-1). Around 90% of patients affected with eczema have skin lesions colonised with Staphylococcus aureus.[6] Patients with infected eczema should apply topical corticosteroids with antibacterials or antifungals. There is no evidence of any benefit from using topical corticosteroids with antibiotics for the treatment of eczema that is not infected.[33] In the case of eczema herpeticum, antiviral treatment should be

given.[26]

1.2 Asthma

1.2.1 Epidemiology

It is estimated that 334 million people worldwide have asthma,[34] a chronic inflammatory disease characterised by hypersensitive airways. Asthma has been recognised as a public health problem since 1970.[35] There is a high prevalence of morbidity. Three main processes are involved in the effect on the airways: inflammation of the airway tissue, which is always present even if the patient does not experience symptoms causing the airway to narrow; constriction of the muscles that surround the airway, decreasing airflow; and an increase in mucus secretion that further blocks the airways. This process is illustrated in figure 1.2; where A represents the location of the lungs and airways in the body, while B and C demonstrates a cross-section of a normal airway and of an airway during asthma symptoms respectively.

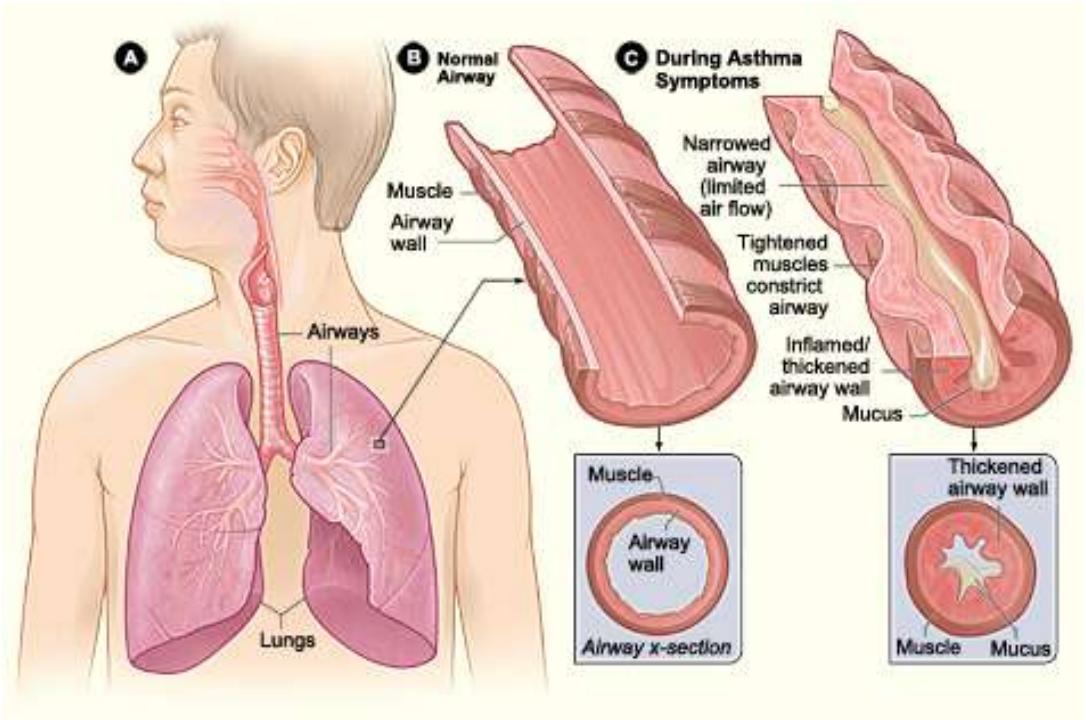

Figure 1.2: Overview of the asthma mechanism[36]

In all these processes the airways narrow causing a decrease in the airflow, leading to breathing difficulties and triggering coughing, wheezing, tightness in the chest and shortness of breath.[37] Asthma is also caused by a combination of genetic and environmental factors, and the prevalence varies between countries. In 2016, a study estimated that the prevalence of asthma with a physician diagnosis in the UK and Scotland was 16% and 14%, respectively.[38] In 2014, asthma was considered the 14th most important disorder globally, considering the extent and duration of the disease. Reports of misdiagnosis in asthma are common. In 2003, another study in the USA studied the impact of undiagnosed asthma.[39] He found that sleep disturbance, school absence, hospitalisations and activity limitations were higher in undiagnosed asthmatics compared to diagnosed asthmatics. Another study in 2016 found that one third of individuals with asthma-related symptoms were undiagnosed and 33% of children with a physician diagnosis of asthma did not receive any medication. Another problem is the rate of over-diagnosis since children are over-prescribed unnecessary medication. A study in the Netherlands found that 54% of the children were over-diagnosed, and only 16% had an asthma diagnosis confirmed by spirometry.[40] Another study found that 30% of adults with a physician diagnosis of asthma had no clinical and laboratory evidence of asthma and safely stopped the medication during one year of follow-up.[41] It is possible that these patients had a remission of their active asthma or they could have been misdiagnosed.

The majority of asthma patients, 85 to 95%, develop asthma before the age of 6 and more than half of these children experience an asthma attack every year. In some patients, the symptoms will disappear or significantly reduce after puberty. The reappearance of symptoms is associated with a more severe form of asthma. A population-based birth cohort in New Zealand, examined 26-year-old individuals that had childhood asthma and found that early onset of wheezing, airway hyperresponsiveness and allergy to house dust mites were associated with relapse after remission of symptoms.[42–44]

As mentioned before, children with eczema will often develop allergic diseases, such as food allergy, allergic rhinitis, also known as hay fever, and asthma, known as the 'atopic march'. Regardless of the existence or not of an 'atopic march', an asthmatic individual has a fivefold risk of reporting eczema and allergic rhinitis compared to a

person without asthma,[10] while a patient with allergic rhinitis is three-times more likely to develop asthma than someone without allergic rhinitis.[45] Asthma and allergic rhinitis are both inflammatory diseases with a similar pathophysiology. Allergic rhinitis is characterised by inflammation of the nasal mucous membranes, caused by exposure to allergens. Common symptoms are nasal itching, sneezing, nasal obstruction, and red and watery eyes.[46,47] Asthma is characterised by allergic symptoms of the lower airways and allergic rhinitis of the upper airways. The link between these two diseases has led to the concept of a 'united airway', suggesting similar inflammatory mechanisms.[8,45] Patients with allergic rhinitis present complete or partial nasal obstruction and breathe through the mouth, leading to an increased number of allergens entering the body.[47] The focus on this thesis is on eczema and asthma. However, due to the association between asthma and allergic rhinitis, and the idea of an 'united airway', allergic rhinitis will also be included in the analysis.

Asthma care costs the UK more than $\pounds$1 billion per year. A survey in the UK found that a large proportion of asthma deaths and asthma-related hospitalisations are avoidable, and found evidence of over- and under-prescribing and lack of proper medical supervision.[34] Patients with severe asthma are estimated to be responsible for more than half of the total asthma-related healthcare costs.[48] Severe asthma attacks may require emergency care and can be fatal. It is thus important to treat symptoms as soon as possible with the most appropriate medication, to reduce the severity of, and prevent, future attacks.

1.2.2 Clinical Symptoms and Predisposing factors

Some asthmatic individuals may experience symptoms rarely, while others may have mild, persistent or severe symptoms which limit their daily routine. Symptoms may include coughing, especially at night, wheezing, fatigue, tightness in the chest, shortness of breath, and trouble sleeping.

Prenatal and postnatal exposure to **smoke** has been associated with early wheezing and an increased risk of food allergies and asthma.[44,49,50] No association was found between the development of asthma and **fish intake** during childhood and pregnancy.[13] No protective effect was found between asthma development and

maternal exclusion of dairy food.[44] A review found that **prenatal intakes of vitamin E and zinc** were associated with lower risk of wheezing until 5-years of age. Several studies have found an association between **antibiotic exposure**, *in utero* and during the first years of life, and the development of asthma.[16,51] Complications during pregnancy were also associated with the development of asthma. Bager et. al[21] conducted a meta-analysis to access the association between **C-section** and allergic diseases. The meta-analysis found an association between birth by C-section and the risk of asthma and asthma-related hospitalisations. However, another review found that atopy was associated with emergency C-sections and not elective C-sections.[44] The role of **breastfeeding** in atopy and asthma is still debated. A review found a protective effect of breastfeeding, whereas some studies found that breastfeeding was associated with a higher risk of atopic asthma.[44,49] In 2017, results from a trial, comparing breastfeeding promotion with current clinical practice, followed term infants for 16 years and showed that breastfeeding had no effect on lung function.[24] The **gender** of the patient has also been associated with severe asthma and an increased number of asthma-related hospitalisations; however, the gender at risk seems to vary according to age. Males younger than 12-years old appear to be more at risk than females; however, during adolescence and young adulthood, females are more at risk than males. Remission is more frequent among males than females. The reasons for these differences are speculated to be due to puberty, airway responsiveness and interaction with allergens.[44] **Inhaled chemicals** and **air pollution** have also been associated with a higher risk of asthma.[49] An increased risk of wheezing and asthma was found for individuals **exposed to mould**.[49,50] The effect of being **exposed to a cat** is still unclear. Some studies found a higher risk of allergic sensitization, asthma and eczema, while others showed a decrease in the risk.[44,49,50,52]

Other triggers discussed in the literature are food and alcohol consumption, maternal infection,[53] lower socio economic position,[25,44,49,54] area deprivation,[25] stress during infancy, chlorinated swimming pools, exposure to paracetamol, and birth order.[44] A summary of the factors associated with asthma can be seen in table 1.2.

Predisposing factor	Association	References
Exposure to smoke	Risk factor	[44,49]
Fish intake during childhood	No association	[13]
Maternal fish intake	No association	[13]
Maternal avoidance of dairy food	No association	[44]
Prenatal intake of vitamin E and zinc	Protective	[44]
Antibiotic exposure during the first year of life	Risk factor	[16,51]
Antibiotic exposure *in utero*	Risk factor	[16,51]
Emergency C-section	Risk factor	[44]
Breastfeeding	Conflicting evidence	[24,44,49]
Air pollution	Risk factor	[49]
Exposure to mould	Risk factor	[49]
Early life cat exposure	Conflicting evidence	[44,49,50,52]
Lower socio economic position	Risk factor	[25,44,54]
Exposure to paracetamol	Risk factor	[44]

Table 1.2: Summary of factors associated with asthma onset

1.2.3 Diagnosis

Asthma diagnosis is difficult, especially in children, since there is no diagnostic test that can be used in all patients to confirm the diagnosis.[50,55]

To make a diagnosis, the physician should initially assess the probability of asthma, based on the symptoms (such as wheezing, breathlessness, chest tightness, cough), and family history of atopic diseases. The next step is to perform respiratory tests. Several diagnostic tests can be used; one of the most common of which is spirometry, which measures the quantity of air that can be exhaled, along with the velocity of the exhaled air. The spirometer returns two important measurements: the volume of air exhaled in the first second of exhalation - Forced expiratory volume in one second (FEV_1), - and the total amount of air exhaled - referred to as the Forced vital capacity (FVC). To understand if the airways are obstructed, the readings are compared with the average measurements of people with the same age, gender and height.[50] Another frequently used test is the Peak expiratory flow (PEF) that measures the maximum velocity of air exhaled in one breath. When properly done, a drop in the measurements reflects an obstruction in the airways. The device is used by the child to evaluate his or her lung function. Asthmatic individuals will have PEF readings lower than expected for their age, size, and gender, and their PEF readings

will be usually lower in the morning than in the evening. Although, false negatives are common. A diary should be kept, with several measurements at different times of the day. Typically, a symptomatic individual with asthma will have low and variable PEF readings over several days. PEF readings improve when the narrowed airways are opened with treatment. Regular PEF readings can be used to help assess how well treatment is working. However, the utilisation of the device requires practice, and if the child lacks practice, the measurements may be incorrect, leading to an incorrect diagnosis and treatment.

Allergy tests can be performed to confirm if asthma symptoms are associated with specific allergens. To check the airway inflammation or atopy, the doctor can take a sample of mucus, or measure the level of nitric oxide in the breath. A positive Fractional exhaled nitric oxide (FeNO) test may suggest eosinophilic inflammation and can be an indication of asthma; however, FeNO levels are increased in patients with allergic rhinitis and rhinovirus infection, and lower in children than adults. A skin-prick test, blood eosinophilia and allergen-specific IgE are other possible tests. One should be aware that both clinical assessment and lung function tests have variable specificity and sensitivity (specificity varies between 7% and 97% and sensitivity varies between 36% and 94%). The results of objective tests are more helpful in diagnosis asthma in individuals with an intermediate probability of asthma, in comparison to individuals with a low likelihood of asthma.[50]

The second step in asthma diagnosis is the assessment of asthma severity. Asthma can be intermittent or persistent (mild, moderate or severe). The classification is made according to the symptoms, number of times the patient wakes up at night due to asthma, the use of Short-acting β_2-agonists (SABA), interference with normal activity and the results of the lung function tests. Table 1.3 presents a summary of the classification of asthma severity. Asthma medication is prescribed according to such observed severity.

Components of Severity	Age (Years)	Classification of Asthma Severity			
		Intermittent	Persistent		
			Mild	Moderate	Severe
Symptoms	All	$\leq$ 2 days/week	$>$ 2 days/week but not daily	Daily	Throughout the day
Nighttime awakenings	0–4	0	1–2 per month	3–4 per month	$>$ 1 per week
	$\geq$ 5	$\leq$ 2 per month	3–4 per month	$>$ 1 per week but not nightly	Often 7 per week
SABA use for symptom control	All	$\leq$ 2 days/week	$>$ 2 days/week but not daily	Daily	Several times a day
Interference with normal activity	All	None	Minor limitation	Some limitation	Extremely limited
FEV_1 (predicted)	$\geq$ 5	Normal FEV_1 between exacerbations $>$ 80,%	$>$ 80, %	60–80 %	$<$ 60%
FEV_1/FVC	5–11	$>$ 85,%	$>$ 80,%	75–80,%	$<$ 60%
	$\geq$ 12	Normal	Normal	Reduced 5,%	Reduced $>$ 5%
Exacerbations requiring oral corticosteroids	0–4	$\leq$ 1 per year	$\geq$ 2 $\times$ in 6 months or $\geq$ 4 wheezing episodes/year lasting $>$ 1 day AND risk factors for persistent asthma		
	5–11		$\geq$ 2 per year		
	$\geq$ 12		Consider severity and interval since last exacerbation. Frequency and severity may fluctuate over time for patients in any severity category. Relative annual risk of exacerbations may be related to FEV_1.		

Table 1.3: Reference chart for the classification of asthma severity[56,57]

1.2.4 Management

UK guidelines, formulated by the British Thoracic Society (BTS) and the SIGN, have adopted a stepwise approach to management.[50,58,59] Asthma management includes a written Personalised Asthma Action Plan (PAAP), which should be given to the family by the healthcare professionals. Several benefits have been associated with the use of PAAPs, such as reduced number of symptoms, hospitalisations, and improvement in the quality of life.[50] In addition to treatment and the PAAP, patient education is crucial in asthma treatment. The patient should receive information about: the pathophysiology of the disease; the correct technique for using an inhaler; avoidance of specific triggers; and in some cases environmental manipulation (e.g. removing all soft furnishings to reduce house dust mite levels). Explaining the condition and treatment to the patient may increase the odds of the patient complying with the prescribed treatment, reducing the risk of future exacerbations.[60]

One of the goals of the asthma guidelines is to control asthma with the minimum amount of medication.[58] Therefore, asthma medication is given according to a stepped approach, where the dose and number of medicines are increased as necessary and decreased whenever possible. Several medications are available to treat asthma symptoms and prevent exacerbations. There are two types of asthma therapies: reliever medication and controller medication. Relievers are fast-acting, used for quick relief of symptoms or the prevention of exercise-induced bronchoconstriction. Regardless of asthma severity, all patients should have a rescue inhaler. Controller medicines are used on a daily basis for long-term control and provide long-acting bronchodilation and have an anti-inflammatory action.[61] Corticosteroids, Long-acting β_2-agonists (LABA), and Leukotriene Receptor Antagonist (LTRA) are usually prescribed as controller medication.[50,55]

Short-acting β_2-agonists (SABA)

SABA is the first-line of treatment for a patient with intermittent symptoms and should be used only as needed. SABA act within minutes relaxing the smooth bronchial muscles in the airway, causing a reduction in the airway narrowing and symptoms. This medication provides a rapid relief of symptoms since they act within 5 minutes

and relieve symptoms for 3 to 6 hours. However, this first line therapy does not control the inflammation in the airway and should not be used continuously.[50,57,60] The frequent use of the inhaler, more than twice a week is an indication that the asthma is not well controlled or that the inhaler may not be suitable for that particular individual and controller therapy should be added.[60] Inhaled ipratropium bromide, theophyllines and oral β_2-agonists can also be used as short-acting bronchodilators, but inhaled SABA are usually prescribed for patients with symptomatic asthma.[50]

Inhaled corticosteroids (ICS)

If reliever medication is needed frequently a "controller" or anti-inflammatory treatment should be added. Inhaled Corticosteroid (ICS) is the most effective controller medication for adults and children. Studies have found several benefits of ICS, such as reduction of symptoms and exacerbations, reduction of inflammation caused by several inflammatory mediators e.g. Tumor necrosis factor α (TNFα) and other cytokines, an improvement of lung function, and an improvement in the quality of life of the patient.[57,60]

Patients with mild to moderate asthma should take a regular low dose of ICS, twice daily, unless the patient has good asthma control, and use SABA as needed. ICS include beclomethasone, budesonide, flunisolide, fluticasone, mometasone and triamcinolone.

The BTS/SIGN recommends monitoring the growth of children on ICS due to possible side effects of medium or high doses of ICS. In that sense, the lowest dose of ICS should be used whenever possible.[50] Despite the effect on growth and other side effects, such as sore throat, dry mouth, etc., ICS are safe in low doses, and patients should be educated and reassured to improve compliance and achieve asthma control.[57,60]

Long-acting β_2-agonists (LABA)

Add-on therapy should be introduced when the patient is already on a high dose of ICS or not responding to medication. A combination of LABA and ICS leads to improvement in FEV_1, reduction of reliever medication use and improvement of symptoms and lung function.[50,60] However, LABA should not be used alone since it

masks the increase in inflammation, delaying the awareness that asthma was getting worse, and increasing the risk of exacerbations and deaths.[57,62] In 2008, NHS Scotland issued a statement discontinuing the use of salmeterol alone.[63] A report was published containing the results of a multicentre study, SMART, that showed a significant increase in the cases of asthma-related deaths in patients taking salmeterol without ICS.[64] From that point onward, BTS and SIGN recommended the use of LABA only in conjunction with ICS. The BTS/SIGN recommends the prescribing of a combined medication of LABA and ICS, rather than prescribing these medications individually, to minimise the risk that the patient takes LABA without the respective ICS, and to increase adherence.[50] The two most common LABAs are Salmeterol and Formoterol, and their effects last more than 12 hours.

Leukotriene receptor antagonist (LTRA)

Another controller medication is LTRA, used to block the binding of leukotrienes to proinflammatory cells in the airways. Patients using LTRAs have reported an increase in PEF and quality of life and a reduction in symptoms and asthma exacerbations.[57] The most frequently used medication is montelukast, an orally active medication. Studies on adults have found that the addition of LABA and ICS is more effective at reducing asthma attacks and improving the quality of life than adding LTRA as a first add-on with ICS.[50] However, in some situations - patients with high levels of leukotrienes in urine, cough-variant asthma, patients with allergic rhinitis, and children younger than 10 years old - the use of LTRA has shown a better response over the use of ICS.[57]

Oral corticosteroids

Patients with severe uncontrolled asthma, despite the use of ICS, LABA and/or LTRA, may need oral corticosteroids in low doses. Oral corticosteroids quickly reduces pulmonary swelling and inflammation that leads to asthma exacerbations.[57] Patients should use oral corticosteroids for a short period, and visit their physician afterwards to make sure the symptoms are controlled. Caution should be applied using oral corticosteroids due to their side effects. Short-term use is associated with nausea, vomiting, increased appetite, and changes in mood, while long-term use is associated with worse side effects, such as a growth suppression, hypertension, diabetes,

muscle weakness, and impairment of the immune system.[57] Prednisolone is widely used, and there is no evidence that other oral corticosteroids perform better.[50]

1.3 Genetics

1.3.1 Filaggrin (FLG)

FLG, derived from 'filament aggregating protein', is used to describe a class of protein isolated from the stratum corneum, the outermost layer of the epidermis. Approximately 10% of individuals with an European ancestry have one or more loss-of-function FLG mutations, which results in at least 50% reduction in protein expression[65,66]

Human profilaggrin is encoded by the FLG gene located on chromosome 1q21. The FLG gene has 3 exons and 2 introns. Exon 1 is non-coding, exon 2 initiates protein translation, and profilaggrin protein is encoded by exon 3, the largest exon in the FLG gene. FLG is characterised by a large repeat domain consisting of several proteins motifs arranged in tandem. The sequencing of the third exon of the FLG gene revealed several null mutations in a proportion of individuals. R501X occur in FLG repeat 1 on an 11 allele-repeat, and 2282del4 occur in FLG repeat 1 in a 10 allele-repeat. R501X and 2282del4 were the first FLG variants identified.[65] R501X is a nonsense mutation (mutation of arginine codon 501 to a stop codon); 2282del4 is a frame shift mutation (deletion of a 4-base-pair - bp - sequence at position 2282 in the filaggrin-coding DNA sequence). R2447X occur in repeat 7 and S3247X in repeat 9.[66,67] All the mentioned variants occur in individuals with European ancestry; the location of the variants can be seen in figure 1.3.

The profilaggrin protein ($>$400kDa) consists of 10 to 12 tandem FLG repeats, which are flanked on both sides by two partial FLG repeats and by N- (divided into an A and B domain) and C-terminal domains. All FLG repeats have 324 amino acids and contain a short linker region that is cleaved during the conversion of profilaggrin into monomeric FLG.[67]

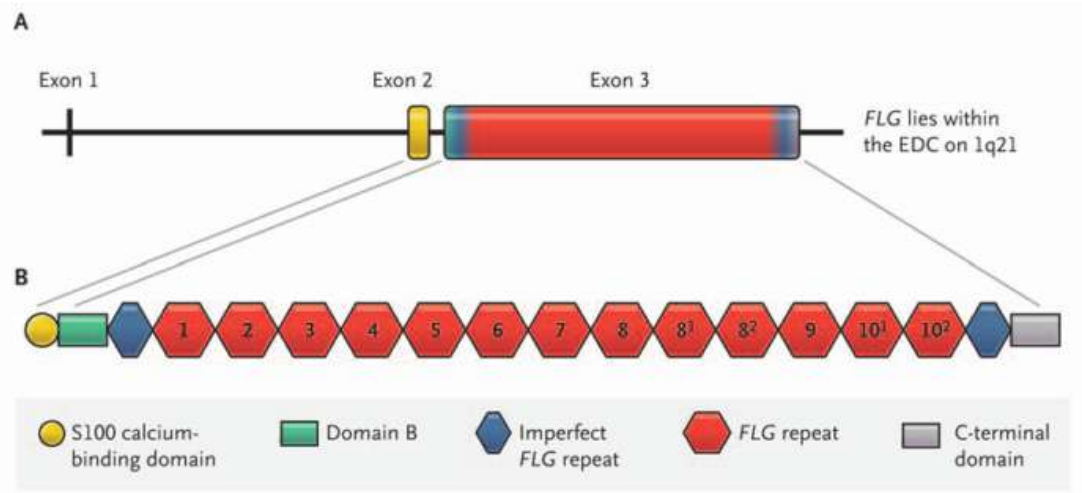

Figure 1.3: Scheme of the profilaggrin molecule with the FLG repeats[65]

The formation of the epidermal barrier results from the complex interplay between profilaggrin, FLG and amino-acids, all contributing to the creation of the protein-lipid matrix. During epidermal differentiation (see figure 1.4), which is responsible for the maturation of the human epidermis, profilaggrin is dephosphorylated and cleaved into FLG filaments. The function of these filaments is to initiate aggregation and collapse of keratin filaments; contributing to cellular compaction and cross-linking of keratin by transglutaminases forming an insoluble keratin matrix. This matrix acts as a scaffold for the attachment of cornified envelope proteins and lipids that together form the stratum corneum.[67]

FLG deficiency impairs the filament aggregation, which will lead to a defective matrix forming the stratum corneum. The pH of the skin is higher in individuals with an FLG mutation, and this increases protease activity, leading to inflammation and proliferation of Staphylococcus aureus.[68] Individuals carrying FLG mutations, either as homozygotes or as compound heterozygotes, fail to express any detectable FLG protein within their epidermis.[67]

Several studies have found an association between loss-of-function FLG mutations and the development of eczema.[69–71] FLG-related eczema typically presents as an early-onset, persistent disease and is associated with secondary allergic conditions, such as asthma.[67] Although the role of FLG in eczema has been demonstrated in multiple association studies, it is interesting that only 42% of FLG mutations carriers

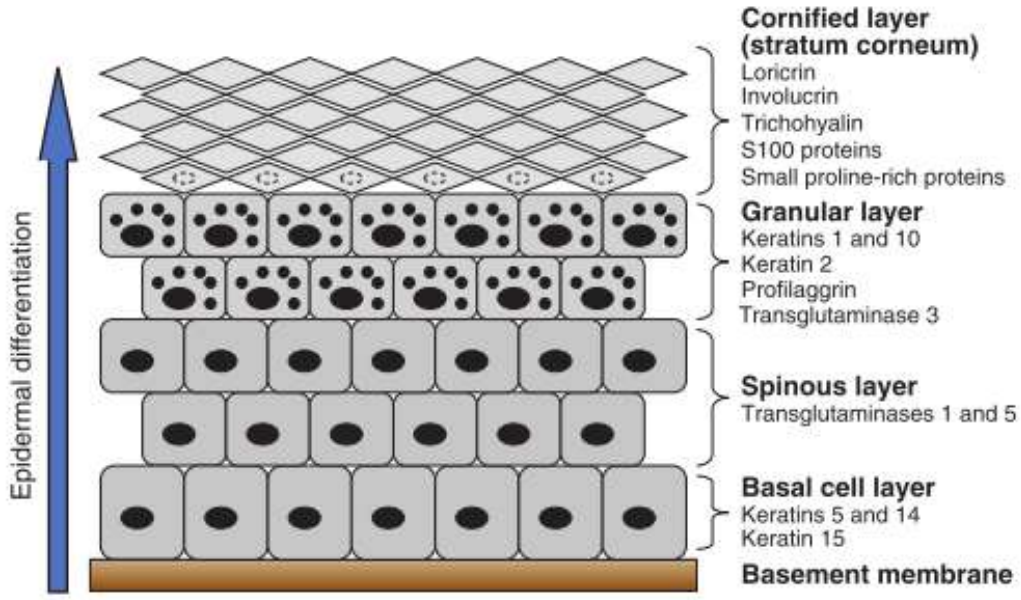

Figure 1.4: Illustration of the epidermal differentiation[67]

will develop eczema, suggesting other genetic and environmental factors regulate disease expression.[66]

Individuals with FLG loss-of-function mutations have a higher risk of developing asthma in the presence of eczema. The presence of FLG mutations is also associated with a more severe form of asthma and with a higher risk of asthma exacerbations in children.[72]

1.3.2 Adrenoreceptor β_2 (ADRB2)

ADRB2 is expressed on bronchial smooth muscle cells and mediates the physiologic response of the airways.[73] The primary function of the β_2-agonists is the relaxation of the airway smooth muscle. Therefore β_2-agonists are used as relievers or rescue treatment for asthma resulting in activation of the ADRB2 and bronchodilation.[74] Activation of the receptor leads to bronchodilation while down-regulation leads to diminished clinical response.[75,76]

The β adrenergic receptor is encoded by three genes: β_1 adrenergic receptor, ADRB2 and β_3 adrenergic receptor. ADRB2 is highly expressed in bronchial muscle cells and is also expressed on cardiac myocytes, vascular smooth muscle cells, inflammatory

cells and adipose tissue.[74] ADRB2 is a small intronless gene localised on chromosome 5q31-q32, a region linked with asthma phenotypes, which encodes 413 amino acids.[73,77] The allele frequency varies across different ethnic backgrounds.

More than 80 polymorphisms in the ADRB2 gene have been identified: some have been identified within the promoter region, influencing the expression of the gene, and others in the coding region of the gene, altering the function of the gene. Several polymorphisms within the gene are in Linkage Disequilibrium, and so far, 12 haplotypes have been described.[74,78] Five of these polymorphisms create non-synonymous changes in the amino acid sequence, glycine (Gly) to arginine (Arg) mutation at position 16, glutamine (Gln) to glutamic acid (Glu) at position 27, valine (Val) to methionine (Met) at position 134, threonine (Thr) to isoleucine (Ile) at position 164 and serine (Ser) to Cysteine (Cys) at position 220.[73,77]

No association was found between asthma development and ADRB2 polymorphisms. However, several studies have speculated that these polymorphisms may influence disease severity and clinical responsiveness to β_2-agonists.[73,77,79] Arg16Gly and Glu27Gln are two of the most studied polymorphisms. The Arg16Gly polymorphism has been associated with increased corticosteroid use, nocturnal asthma, a more severe form of asthma and clinical responsiveness to SABA and LABA. The Glu27Gln polymorphism has been associated with airway responsiveness. However, for both polymorphisms, different results have been reported in patients from different ethnic backgrounds.[73]

1.3.3 Fc fragment of IgE receptor II (FCER2)

The FCER2, also known as CD23, gene encodes the low affinity II receptor for IgE. Studies have proposed that FCER2 is involved in the regulation of IgE responses, growth and differentiation of T and B cells, cellular adherence and antigen presentation.[80–82] The activation of the receptor results in down-regulation of IgE-mediated immune responses.[81] FCER2 is located at chromosome 19p13.3 and consists of 11 exons with 321 amino acids in length.[80]

The immune response to allergic conditions is usually triggered by contact with an allergen, leading to the production of a large quantity of IgE, an antigen. The IgE then binds to the FCER receptors on the surface of mast cells releasing inflammatory mediators such as histamines, cytokines, leukotrienes and prostagladins to fight the invasion.[82] These chemicals are responsible for some of the symptoms patients with allergic conditions experience, such as airway constriction, local inflammation and mucus secretion. Hence the use of corticosteroids to reduce the inflammation, β_2-agonists for the airways, and antihistamines and anti-leukotrienes to antagonise histamines and leukotrienes.

Tantisira et al.[83] genotyped 10 polymorphisms in the FCER2 gene and found a novel variant, T2206C, which was associated with asthma exacerbations and clinical responsiveness to ICS.[81] Sharma et al.[84] found several polymorphisms in the FCER2 related to mild to moderate increases in IgE levels. Beside high levels of IgE, FCER2 variants have also been associated with high levels of IgE, poor lung function, and increased levels of FeNO.[80,85]

1.4 How filaggrin (FLG) gene variation affects eczema severity

The role of FLG in the development of eczema is well established,[86] and many studies have found an association between FLG and eczema severity, although different effect sizes have been reported.[87–89] One of the causes for differences found in effect size can be the variation in the definition of eczema severity. Some studies used parental questionnaires to define severity of clinical symptoms.[86,87] However, the clinical symptoms used to define severity are often different between studies, and can vary over time. Another option to define eczema severity was using a published scoring system. Several systems are currently published, such as Severity Scoring of Atopic Dermatitis (SCORAD), Eczema Area and Severity Index (EASI) or the Patient-Oriented Eczema Measure (POEM).[88,90] All these scoring systems combine disease extent, severity and subjective symptoms. However, they have different scales. Other studies used a modified version of these published scoring systems,

sometimes without justification and/or a clear explanation for modification.[91]

Nevertheless, the association between FLG mutations and eczema severity has been assessed in several studies. A meta-analysis by Rodriguez et al.[89] found that decreased FLG expression is strongly associated with a severe form of eczema and a dermatologist-diagnosed disease, which may be a sign that eczema definition not supported by a dermatologist may be less accurate, or that severe cases are referred to a specialist. Other studies[87,88,92] also reported that individuals with an FLG mutation suffering from eczema were more likely to have a severe and persistent form of the disease. Only one study did not find an association.[86] Even though Ballardini et al.[86] did not find an association, the study reported that children with moderate-to-severe eczema used more moisturisers and corticosteroids than children with mild eczema.

A good epidermal barrier prevents water loss and the penetration of allergens and irritants. It has been shown that individuals suffering from eczema have higher TransEpidermal Water Loss (TEWL), clinically dry and thicker skin compared with individuals without eczema. FLG mutations were associated with higher TEWL, clinically dry and thicker skin even in individuals without eczema. Individuals with FLG mutations were also more likely to develop eczema by 3 months of age, earlier than individuals without FLG mutations.[93–95] The fact that TEWL is greater, and dry and thicker skin is more prevalent in individuals without eczema but with FLG mutations may suggest that impaired barrier precedes clinical eczema.[93–95]

A significant association was also found between FLG mutations and asthma susceptibility in individuals with asthma and eczema. However, FLG mutations were not associated with asthma susceptibility in individuals with asthma but without eczema.[2,71,89] Palmer et al.[72] explored the effect of FLG mutations on asthma severity and found an association regardless of the presence of eczema in asthmatic patients. The mechanism of the association between FLG mutations and asthma is not entirely known. FLG is expressed in the skin and outer layers of the oral and nasal mucosa, where it is also assumed to contribute to epithelial barrier function. Therefore, FLG-related asthma may be mediated by immunological mechanisms influenced by the impaired epithelial barrier. However, the development of asthma

and rhinitis is not restricted to those with allergic sensitization, suggesting that the hypothesis that allergen sensitization caused by barrier impairment leads to disease may be too simplistic and different endotypes may contribute to the disease.[89]

A systematic review exploring the role of genes on eczema severity was not undertaken. A preliminary search was done but no relevant study was found. Several genes have been associated with eczema susceptibility. Chromosome 11q13.5, the Major Histocompatibility Complex (MHC) loci, Interleukin 13 (IL-13) and Interleukin 4 (IL-4) have been associated with eczema susceptibility.[96] Serine Protease Inhibitor Kazal-type 5 (SPINK5) and FLG-2 have been associated with persistent eczema in Eastern-Asian and African-American populations, respectively.[97] More recently, in 2017, the Caspase recruitment domain-containing protein 11 (CARD11) gene has been associated with severe eczema in four individuals and their family members.[98] However, replication, larger sample sizes and population stratification is needed to understand the effect of these genes.[96,97] To date, FLG remains the gene most strongly associated with eczema susceptibility and severity. Hence, the association of FLG with healthcare outcomes, such as prescribing, was explored in this thesis. On the other hand, several Single nucleotide polymorphisms (SNPs) have been associated with asthma severity and clinical responsiveness. Therefore a systematic review was undertaken regarding this (see chapter 2).

1.5 Pharmacoeconomics

Healthcare and medicine are often evaluated based on safety, efficacy and effectiveness considerations. However, with the groundbreaking advances in healthcare technology and the ageing of the population, current resources are not sufficient to have unlimited access to healthcare, and governments rely on efficiency for decision-making to provide quality care at the cheapest cost.[99,100]

Decisions are made based on priorities, budgets, analysis of the evidence, and quality of life. Any innovation or new medicine will only be accepted if its clinical efficacy and cost-effectiveness have been proven. Health economics studies the efficiency, effectiveness, value, and behaviour in the consumption of the healthcare

system, while pharmacoeconomics can be viewed as a branch of health economics focusing on the efficiency of pharmaceutical therapies.[100]

Several analyses can be done to understand the value of each therapy. Cost minimization analysis is one of the simplest health economic tools, and it is used to compare the costs of two interventions with similar benefits, to ascertain the cheapest option. Cost-effectiveness analysis considers cost and outcome when the result of an intervention may vary, but it can be measured in the same unit. For instance, the cost of implementing a stroke unit may be high but may also avoid a large number of deaths. A cost-effectiveness analysis will consider both the cost of a stroke unit and the benefits of implementing such unit. A cost-utility analysis uses the Quality adjusted life years (QALY), which provides a measurement of health-related quality of life combined with the increased number of years lived. In every analysis, the different perspectives should be considered: patient, physician, society, and the national reimbursement authority, and whenever possible, indirect costs should also be taken into account. An example of an indirect cost could be the money spent by the patient to travel to the hospital and days off work or productivity lost due to illness.[99,100]

A pharmacoeconomics analysis can be conducted during clinical trials, using real life data, or theoretical models to estimate the cost benefit of an intervention. Pharmacoeconomics also plays a role in reviewing and/or setting the prices of medicines included in the formulary.[100] However, unlike other areas of research, pharmacoeconomics results are not easy to generalise across countries or extrapolate since the healthcare systems differ globally, and therapeutic approaches are influenced by culture. In addition, countries may follow different guidelines and the threshold for cost-effectiveness analysis depends on the country.[99] An example is given of a market analysis of asthma medication across the world. The USA alone was responsible for 64% of asthma sales, mainly due to the lack of generic medicines and higher prices.[101] Hence, it is crucial to undertake a pharmacoeconomic analysis whenever possible to understand the impact of the research, and to assist decision-making.

The emergence of personalised medicine, along with the ability to analyse genomic and genetic data, has brought new challenges for pharmacoeconomics. Some

studies have already been done to understand the cost-effectiveness of pharmacogenetics and genetic screening. Musci et al.[102] performed a cost-effectiveness analysis and found that screening pregnant woman for the fragile X mutation would be cost-effective. Veenstra et al.[103] looked at the cost-effectiveness of screening a known polymorphism associated with impaired hearing in patients affected with cystic fibrosis. However, in this case, the genetic testing was not found to be cost-effective.

Pharmacogenetics has been associated with saving money while improving the health of the patient. However, studies performing a cost-effectiveness analysis are scarce. Ideally, any pharmacogenetic study should be replicated and confirmed in RCT, which would include a cost-effectiveness analysis of the genetic testing approach. This pipeline could speed-up the transition from bench to bedside.[104–106] A theoretical model simulated a cohort of 10,000 patients with asthma and divided them into responders and non-responders. The authors used allelic prevalences reported for ADRB2 polymorphisms and derived the cost data using a database of health claims data.[105] Although the model was based on estimates and several assumptions, it shed light on the possible advantage of using pharmaceutical data to estimate the potential cost-effectiveness of pharmacogenetic approaches.

1.6 Communicating personalised medicine to children and parents

There has been an increase in both the need for involving and engaging the public in research in the past few years.[107] By engaging the public, scientists can help the public make informed decisions, and scientists can improve their research and learn from different perspectives.[108,109] A clear example of the importance of public engagement can be seen in climate change discussions. Studies in the USA have shown that only 13% of individuals understood the agreement of the scientific community regarding global warming causes. However, when presented with data of a scientific consensus regarding climate change, individuals were more likely to believe that human-caused climate change was real.[110,111]

The increasing interest in fields like pharmacogenetics is linked with a greater possibility of performing genetic testing on populations, and this leads to ethical concerns. Some individuals fear that mandatory genetic testing would lead to discrimination, for example, insurance companies may refuse healthcare coverage or increase the premium if the client has a genetic risk of developing a disease, or employers could screen potential employees on the basis of their risk of future debilitation. Another issue relates to personal privacy.[112] A recent review explored parent's attitudes regarding childhood genetic testing in two situations, when genetic testing led to a clinical benefit, and where it was not associated with any apparent clinical benefit.[113] Overall, parents had positive attitudes towards testing their child and were able to understand the potential advantages, such as the development of personalised medicine approaches. Even with the current lack of medical treatment for diseases such as autism, parents were able to find benefits in predictive genetic testing since it enabled tailored environmental modification strategies. However, some parents would not authorise genetic testing for diseases they did not perceive as having a genetic cause, such as deafness. Despite negative opinions regarding childhood genetic testing of the BRCA1/2, a genetic variant which increases the risk of developing breast and ovarian cancer, parents with low education would still test their child, whilst educated parents were less supportive of genetic testing as the benefit from testing was not clear. Disadvantages mentioned by the parents were employment and insurance discrimination, and an increase in anxiety. Several factors were associated with positive perceptions; in the case of genetic testing with clinical benefit, a higher socio-economic background and higher education level were associated with positive attitudes, while in the case of genetic testing without apparent clinical benefit, lower education level and perceiving benefits to outweigh the risks were associated with positive perceptions.

In this day and age, understanding the contribution of science to our lives, and being able to distinguish facts from opinions is crucial. Individuals should be capable of understanding their contribution to climate change, how to avoid non-genetic risk factors for diseases, (especially if they have a strong probability of developing the disease), and how their knowledge can encourage policy-makers to enforce change. Schools should empower students with critical thinking skills and the ability to

distinguish data from speculation. However, the responsibility should also be shared by scientists. Any researcher should be able to understand the main message of their work and convey the importance of the research to the public and not only academics. The ability to do so could help justify an increase in the financial support for the research and can improve the quality of the research in the university, since public engagement and its impact is included in the Research Excellence Framework (REF), which assesses the quality of research in the UK.[114]

However, public engagement does not come without its challenges. There is no formulated model for a public engagement project, since it depends on the research carried out, the public involved and the reasons to engage the public. No training is provided by universities on how to communicate with a broader audience, outside of academia, and how researchers should be aware of their own biases before starting a public engagement project.[107,108,115] One of the main misconceptions is the belief that the public is misinformed and uneducated about science.[108] Hence, researchers may hope to see a change of opinion when providing resources and education to the public. However, evidence shows that knowledge gained is not associated with a change of attitudes, but instead that a change of attitudes depends on interacting factors, such as social context, psychological and environmental factors.[108,116] To better understand this interplay of factors, one can imagine a hypothetical scenario. The doctor informs the patient about the benefits and risks of skipping his treatment and prescribes the patient ICS twice daily. However, the patient misses a few doses now and then. The missing doses may not lead to an immediate deterioration in symptoms hence leading to a false perception that the medication may not work properly or that high adherence is not necessary. Another issue regarding public engagement is related to the section of the public involved in public engagement. Many projects rely on the participation of volunteers, whether in an informal pub event, a festival, or a forum discussion. However, the researcher should be aware that volunteers are usually well educated and already understand the importance of science, and may not be representative of the general population.[108]

Nevertheless, the benefits of engaging with the public seem to surpass the difficulties. Scientists involved in public engagement increased their communication skills, became aware of ethical issues, improved the depth and quality of their results,

formulated new research questions, and have found the public friendly and eager to engage.[108,109]

To properly interact with the public, it is important to define the objectives, understand the intended impact and the values associated with public engagement, and understand whether or not the communication has been effective.[107,108] In other words, one should have a clear understanding of why one wants to promote public engagement within an area of research and also have an idea regarding when to do this.[115]

Chapter 2

How does genotype affect asthma severity and clinical response - a systematic review

2.1 Introduction

As previously discussed, asthma is caused by a combination of genetic and environmental factors with an estimated genetic contribution to asthma susceptibility varying from between 40 and 75%.[117–119] There is also wide variability in terms of response to treatment, with an estimated 30 to 40% of people with asthma having a poor response to treatment. In approximately 10% of poor responders asthma worsens, leading to severe asthma.[79,118,120] Evidence is growing, from advances in genomics, to suggest that a large portion of variability in both disease severity and response to treatment may be related to genetic diversity.[79,118,120]

The condition studied for this systematic review are asthmatic children and/or adults with childhood onset of asthma. The articles included consist of Genome-wide association studies (GWAS), candidate-gene studies and/or Randomized Controlled Trials (RCT) stratified by genotype. The outcomes considered are asthma exacerbations (defined by asthma-related hospitalisations and/or Emergency room (ER) visits, and oral corticosteroids use), asthma severity (defined by Forced expiratory volume in one second (FEV_1) or symptoms) and asthma control (scores calculated with the Asthma Control Test (ACT) or Asthma Control Questionnaire (ACQ) questionnaires).

The primary purpose of this review was to understand the current picture regarding the role of the genes associated with asthma severity and clinical responsiveness to medication in children. Genetic susceptibility to asthma was not explored. A secondary aim was to understand the methodological approaches of these studies, especially regarding adjustment of P-values for multiple testing and to inform which genetic model to use for analysis in this thesis.

2.2 Methods

Eligibility criteria

The population included in this systematic review were asthmatic children or adults with childhood onset of asthma, either as the primary cohort or as replication cohort. However, to avoid the exclusion of relevant studies, replication cohorts whose population were adults were kept as long as the population of the primary cohort were children, and vice-versa, studies where the population of the primary cohorts were adults were kept as long as the population of the replication cohorts were children. In GWAS primary studies are usually performed to find significant polymorphisms. Several GWAS included replications to assess the significance of polymorphisms found. GWAS, candidate-gene studies, RCT stratified by genotype, or RCTs on a specific genotype, were included in this review. The outcomes considered were asthma severity (defined by FEV_1 or symptoms), asthma exacerbations, or asthma control. Systematic reviews or review papers were not included in the review, although the bibliography of each article was screened to include additional articles that were not identified by the search terms. Meta-analyses were not included in the systematic review but results from individual studies in a paper reporting a meta-analysis were included in the review as independent results.

A limit was applied in the search to include only papers including humans and children, whether as a main cohort or replication cohort (Age Groups: All Infant birth to 23 months *or* All Child 0 to 18 years *or* Newborn Infant birth to 1 month *or* Infant 1 to 23 months *or* Preschool Child 2 to 5 years *or* Child 6 to 12 years *or* Adolescent 13 to 18 years). There was no restriction in the language of the articles searched.

Search strategy

Three databases were used for this review, Medical literature analysis and retrieval system online (MEDLINE), Cochrane and Embase. A search was performed using keywords, including Medical subject headings (MeSH) terms (represented in uppercase) and non-MeSH terms ((ASTHMA *or* asthma) AND (SINGLE NUCLEOTIDE POLYMORPHISM *or* DNA POLYMORPHISHM *or* GENETIC POLYMORPHISM *or* GENE MUTATION *or* MUTATION *or* GENOTYPE *or* geno* *or* genome-wide *or* variant* *or* polymorphism* *or* SNP *or* protein* *or* gene*) AND (PHARMACOGENETICS *or* PHARMACOGENOMICS *or* PERSONALIZED MEDICINE *or* INDIVIDUALIZED MEDICINE *or* pharmacogenomic* *or* pharmacogenetic* *or* individualized AND medicine *or* personalized AND medicine)). Databases were searched until July 2017. A total of 241 articles were found by this search, 142 in Cochrane, 56 in Embase and 43 in MEDLINE, of which 34 were duplicates. The appendix 'MEDLINE search' corresponds to the MEDLINE search. A similar search was made in Embase and the Cochrane Database of Systematic Reviews. Thirty articles were added based on reviewing identified references. Of the 237 articles screened, 60% were excluded based on the title and 13% after reading the abstract. Of the 64 eligible articles, 7 were excluded because they were not original articles but reviews. A total of 56 studies were included in the systematic review (see PRISMA flow figure 2.1). The protocol for the systematic review is registered in the International prospective register of systematic reviews (PROSPERO), the protocol identifier is CRD42017076593.

Data synthesis

Data extracted from each study were entered into a summary table (see table 2.2 at the end of the chapter) to enable comparisons of study characteristics including: type of study; gene/s studied; study population, containing information such as sample size and ethnicity of the participants; information about the replication/s cohort, when available; definition of the clinical outcome; and the statistical methods used, specifically the genetic model used and whether the P-value was adjusted for multiple testing.

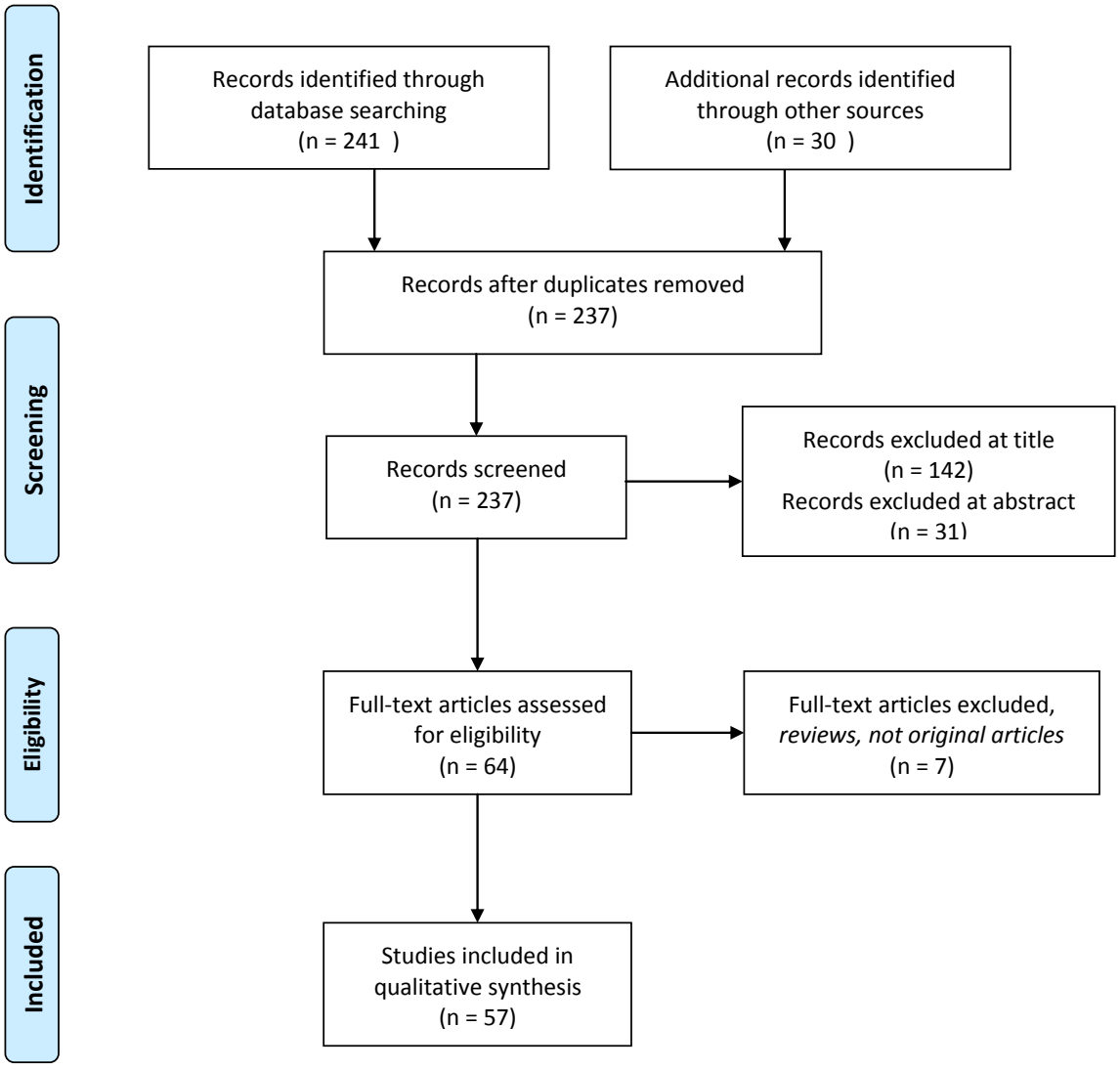

Figure 2.1: PRISMA flow diagram for the identification of pharmacogenetic studies in asthma

Quality assessment

Each study was also assessed for a number of factors. Genetic association studies are different from epidemiological studies. Hence the quality assessment in both these studies needs to be different. A limited number of papers have used a formal genetic score as part of quality assessment.[121–123] However, the score created by these three papers do not agree on every point. For this review, instead of choosing one score, an informal quality assessment was performed looking at the items the three papers agreed on. The checklist includes two different sections. Section 1 concerns the validity of the methods, while section 2 concerns the reliability of the

results. In the first section, methodological aspects of the studies will be evaluated, such as population stratification as ignoring population stratification may lead to incorrect associations. Hence, it is important that papers mention the ethnicity of the individuals studied and, if needed, correct for stratification. Another aspect to consider is whether individuals with and without mutations are properly represented and have similar demographic characteristics, where appropriate. The first section focuses on the definition of the outcome, the adequacy of the sample size, the appropriate identification of polymorphisms, whether with the accession number or flanked sequences, and the reporting of the minor allele frequency and the Hardy-Weinberg Equilibrium. The second section focuses on the reliability of the results, such as correction of P-values for multiple testing, the choice of the genetic model used, the report of any risk measures, and the magnitude and precision of the estimates. Appendix 2.1 presents the checklist created with an explanation of all the items, as well as how to score each item. Each study will be described as "adequately described" or "inadequately described".

2.3 Results

Study characteristics

The majority of studies (n=45) were published between 2006 and 2016. The outcomes measured were mainly Bronchodilator response (BDR) to a medication based on the % FEV_1 change, and asthma exacerbations on and off different classes of medication. Study populations worldwide were diverse with almost half of the studies (n=26) being performed in the United States of America (USA), which included individuals from Mexico and Puerto Rico such as Genetics of Asthma in Latino Americans dataset (GALA) dataset. In Europe, the majority of the studies were performed in Western Europe (n=15), Scotland (n=9), Northern Ireland (n=1), Denmark (n=1), and the Netherlands (n=4). Five studies were performed in Eastern Europe, 4 of them in Slovenia and one in Poland. Another study was performed in Southern Europe, Greece (n=1). Six studies were performed in South Korea, 1 in Australia, 1 in Turkey and another in Colombia. Study types were also diverse: 7

were GWAS, which examined Single nucleotide polymorphisms (SNPs) across parts of the genome between healthy individuals and individuals with a disease, 48 were candidate-gene studies, and 2 were RCTs. Sample size varied considerably within and between study types, RCTs varied between n=12 and n=110, candidate-gene studies varied between n=82 and n=1182, and GWAS varied between n=110 and n=1644.

Adrenoreceptor β_2 (ADRB2) was the most studied candidate gene, with 7 papers detailing asthma severity and/or the clinical responsiveness to β_2-agonists according to ADRB2 genotype, with some conflicting results. It is hypothesised that decrease in the function or expression of Corticotropin releasing hormone receptor 1 (CRHR-1) may lead to diminished natural steroid levels. Hence, the role of CRHR-1 on asthma severity and the clinical responsiveness to Inhaled Corticosteroid (ICS) was studied in 6 papers. Leukotriene C4 synthase (LTC4S), which is involved in the production of leukotrienes, has been described in 4 papers regarding its effect on asthma severity and Leukotriene Receptor Antagonist (LTRA) responsiveness. Outcomes related to ORMDL sphingolipid biosynthesis regulator 3 (ORMDL3), which has been linked to asthma susceptibility, Fc fragment of IgE receptor II (FCER2), Interleukin 13 (IL-13), which induces matrix metalloproteinase in the airways and has anti-inflammatory properties, and Glucocorticoid induced 1 (GLCCI1), whose expression is induced by glucocorticoids, were also reported on in three different papers.

Quality assessment

For each study, several characteristics were considered to assess the overall quality of the study. The checklist used can be found in the Appendix 6.8. Table 2.1 provides a summary of the quality assessment of the studies included in the systematic review. The header of the table corresponds to each item assessed. Column 1.1 refers to population stratification, 1.2 refers to a comparison between individuals who have the mutation and individuals who do not have the mutation, while column 1.3 refers to the study outcome and 1.4 to the sample size. Column 1.5 refers to the identification of the polymorphism, 1.6 to the minor allele frequency and 1.7 to the Hardy-Weinberg Equilibrium. The column 2.1 verify whether the study was adjusted for multiple testing and 2.2 and 2.3 verify whether the genetic model used was stated and the reason for

using the particular model. Columns 2.3 and 2.4 verify whether any risk measure was used, and its magnitude and precision. Overall, the studies were summarised as "adequately described" and "inadequately described".

Asthma severity

Several genes were studied regarding their effect on asthma severity (see table 2.2 at the end of the chapter). The majority of these studies used asthma exacerbations as the outcome measure.[124–131] One study classified individuals with difficult-to-treat asthma, based on ongoing asthma symptoms despite medication.[120] Other studies defined bronchial hyperreactivity using the concentration of methacholine that induced a 20% reduction in FEV_1.[132,133]

The Arg16 variant of the ADRB2 gene was associated with an increased odds of exacerbations[124,126,130] but not with difficult-to-treat asthma.[120] These four studies were adequately described. Turner et al.[126] performed a meta-analysis on five studies, and found an 11% increase in the odds of asthma exacerbations for each copy of the Arg allele. ORMDL3 gene was also associated with an increase in the odds of exacerbation,[128,129] individuals with two copies of the minor allele had double the odds of having an exacerbation than individuals with two copies of the common allele. Although the minor allele differs among these studies, both studies found an increase of asthma exacerbations for carriers of the T allele, and were adequately described.

Column 1.1 refers to population stratification, 1.2 refers to a comparison between individuals who have the mutation and individuals who do not have the mutation, while column 1.3 refers to the study outcome and 1.4 to the sample size. Column 1.5 refers to the identification of the polymorphism, 1.6 to the minor allele frequency and 1.7 to the Hardy-Weinberg Equilibrium. The column 2.1 verify whether the study was adjusted for multiple testing and 2.2 and 2.3 verify whether the genetic model used was stated and the reason for using the particular model. Columns 2.3 and 2.4 verify whether any risk measure was used, and its magnitude and precision. Overall, the studies were summarised as "adequately described" and "inadequately described".

Study	1.1	1.2	1.3	1.4	1.5	1.6	1.7	2.1	2.2	2.3	2.4	2.5	Summary
Martinez 1997[134]	Unclear	Limited	Yes	No	Yes	Yes	No	No	No	NA	Yes	No	Inadequately described
Whelan 2003[135]	**Unclear**	**Limited**	**Yes**	**Yes**	**Yes**	**Yes**	**NA**	NA	No	No	Yes	Unclear	Inadequately described
Tantisira 2004[136]	**Yes**	**No**	**Yes**	**No**	**Yes**	**Yes**	**Yes**	Unclear	Yes	No	Yes	Unclear	**Adequately described**
Tantisira 2004[137]	**Yes**	**No**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	Unclear	No	NA	Yes	Unclear	**Adequately described**
Cho 2005[138]	No	No	Yes	No	No	Yes	No	No	No	NA	Yes	Unclear	Inadequately described
Choudhry 2005[139]	**Yes**	**No**	**Yes**	**Yes**	**No**	**No**	**Yes**	No	Yes	No	Yes	No	Inadequately described
Martin 2006[127]	Unclear	No	Yes	No	No	Yes	Yes	Unclear	No	NA	Yes	No	Inadequately described
Palmer 2006[130]	**No**	**Limited**	**Yes**	**Yes**	**Yes**	**Yes**	**NA**	**NA**	**Yes**	**No**	**Yes**	**Yes**	**Adequately described**
Hunninghake 2007[140]	**Yes**	**No**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	Unclear	Yes	Yes	No	No	**Adequately described**
Lee 2007[141]	No	Limited	Yes	No	Yes	No	No	No	No	No	No	No	Inadequately described
Tantisira 2007[83]	**Yes**	**No**	**Yes**	**No**	**Yes**	**Yes**	**Yes**	**No**	**Yes**	**Yes**	**Yes**	**Yes**	**Adequately described**
Dijkstra 2008[142]	No	No	Yes	No	Yes	No	No	No	No	NA	Yes	Yes	Inadequately described
Kang 2008[143]	**No**	**Limited**	**Yes**	**No**	**Yes**	**Yes**	**Yes**	No	Yes	No	Yes	Unclear	Inadequately described
Kim 2008[144]	**No**	**No**	**Yes**	**Yes**	**Yes**	**Yes**	**No**	**Yes**	**No**	**NA**	**Yes**	**Yes**	**Adequately described**
Litonjua 2008[145]	**Yes**	**No**	**Yes**	**No**	**Yes**	**No**	**Yes**	Yes	Yes	No	No	No	Inadequately described
Poon 2008[146]	**Yes**	**No**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	No	Yes	No	Yes	No	**Adequately described**
Szczepankiewicz 2008[147]	**Yes**	**No**	**Yes**	**No**	**Yes**	**Yes**	**Yes**	No	No	NA	Yes	No	**Adequately described**
Tavendale 2008[128]	**No**	**No**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**No**	**Yes**	**No**	**Yes**	**Yes**	**Adequately described**
Basu 2009[124]	**Yes**	**Limited**	**Yes**	**Yes**	**No**	**Yes**	**No**	**No**	**Yes**	**No**	**Yes**	**Yes**	**Adequately described**

Continues overleaf.

Table 2.1 – continued from previous page

Column 1.1 refers to population stratification, 1.2 refers to a comparison between individuals who have the mutation and individuals who do not have the mutation, while column 1.3 refers to the study outcome and 1.4 to the sample size. Column 1.5 refers to the identification of the polymorphism, 1.6 to the minor allele frequency and 1.7 to the Hardy-Weinberg Equilibrium. The column 2.1 verify whether the study was adjusted for multiple testing and 2.2 and 2.3 verify whether the genetic model used was stated and the reason for using the particular model. Columns 2.3 and 2.4 verify whether any risk measure was used, and its magnitude and precision. Overall, the studies were summarised as "adequately described" and "inadequately described".

Study	1.1	1.2	1.3	1.4	1.5	1.6	1.7	2.1	2.2	2.3	2.4	2.5	Summary
Bisgaard 2009[129]	**No**	**Limited**	**Yes**	**No**	**Yes**	**Yes**	**Yes**	**No**	**Yes**	**No**	**Yes**	**Yes**	**Adequately described**
Corvol 2009[148]	**Yes**	**No**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**No**	**Yes**	**Yes**	**Adequately described**
Moore 2009[149]	Unclear	No	Yes	No	Yes	No	Yes	Unclear	NA	NA	Yes	No	Inadequately described
Rogers 2009[150]	Unclear	No	Yes	No	Yes	Yes	No	**No**	**Yes**	**No**	**Yes**	**Yes**	Inadequately described
Berce 2010[151]	**No**	**Limited**	**Yes**	**No**	**Yes**	**Yes**	**Yes**	NA	No	NA	Yes	Unclear	Inadequately described
Mukhopadhyay 2010[125]	**No**	**Limited**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Unclear**	**Yes**	**Yes**	**Yes**	**Yes**	**Adequately described**
Tcheurekdjian 2010[152]	**Yes**	**No**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**No**	**NA**	**Yes**	**Yes**	**Adequately described**
Cunningham 2011[131]	**No**	**No**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**NA**	**No**	**NA**	**Yes**	**Yes**	**Adequately described**
Jin 2011[153]	**Yes**	**Limited**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	Unclear	Yes	No	No	No	**Adequately described**
Kang 2011[154]	**No**	**Limited**	**Yes**	**No**	**Yes**	**Yes**	**Yes**	No	Yes	No	Yes	Unclear	Inadequately described
Koster 2011[81]	**No**	**No**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**NA**	**Yes**	**No**	**Yes**	**Yes**	**Adequately described**
Perin 2011[133]	**No**	**No**	**Yes**	**No**	**Yes**	**Yes**	**Yes**	NA	Yes	No	Yes	No	**Adequately described**
Tantisira 2011[155]	**Yes**	**No**	**Yes**	**No**	**Yes**	**Yes**	**Yes**	Yes	Yes	No	No	No	**Adequately described**
Balantic 2012[156]	**No**	**Limited**	**Yes**	**No**	**Yes**	**Yes**	**Yes**	No	Yes	No	Yes	No	Inadequately described
Iordanidou 2012[157]	**Yes**	**No**	**Yes**	**No**	**No**	**Yes**	**Yes**	Yes	No	NA	Yes	No	**Adequately described**
Isaza 2012[118]	**No**	**Limited**	**Yes**	**No**	**Yes**	**Yes**	**Yes**	No	No	NA	Yes	No	Inadequately described
Almomani 2013[120]	**Yes**	**Limited**	**Yes**	**No**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**No**	**Yes**	**Yes**	**Adequately described**
Berce 2013[158]	**Yes**	**No**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	NA	Yes	No	Yes	No	**Adequately described**

Continues overleaf.

Table 2.1 – continued from previous page

Column 1.1 refers to population stratification, 1.2 refers to a comparison between individuals who have the mutation and individuals who do not have the mutation, while column 1.3 refers to the study outcome and 1.4 to the sample size. Column 1.5 refers to the identification of the polymorphism, 1.6 to the minor allele frequency and 1.7 to the Hardy-Weinberg Equilibrium. The column 2.1 verify whether the study was adjusted for multiple testing and 2.2 and 2.3 verify whether the genetic model used was stated and the reason for using the particular model. Columns 2.3 and 2.4 verify whether any risk measure was used, and its magnitude and precision. Overall, the studies were summarised as "adequately described" and "inadequately described".

Study	1.1	1.2	1.3	1.4	1.5	1.6	1.7	2.1	2.2	2.3	2.4	2.5	Summary
Duan 2013[159]	**Yes**	**No**	**Yes**	**No**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**No**	**Yes**	**Yes**	**Adequately described**
Kim 2013[160]	No	No	Yes	No	Yes	No	Yes	**Yes**	**Yes**	**No**	**Yes**	**No**	Inadequately described
Lipworth 2013[161]	**Yes**	**Yes**	**Yes**	**No**	**No**	**NA**	**NA**	**NA**	**NA**	**NA**	**Yes**	**Yes**	**Adequately described**
Mougey 2013[162]	**Yes**	**Yes**	**Yes**	**No**	**Yes**	**Yes**	**No**	**NA**	**Yes**	**No**	**Yes**	**Yes**	**Adequately described**
Stockmann 2013[163]	Unclear	No	Yes	No	Yes	Yes	Unclear	Yes	No	NA	Yes	No	Inadequately described
Zuurhout 2013[164]	**No**	**Limited**	**Yes**	**Yes**	**Yes**	**Yes**	**Unclear**	NA	Yes	No	Yes	No	**Adequately described**
Park 2014[165]	**Yes**	**Limited**	**Yes**	**No**	**Yes**	**Yes**	**Unclear**	No	Yes	No	Yes	No	Inadequately described
Perin 2014[166]	**No**	**No**	**Yes**	**No**	**Yes**	**Yes**	**Yes**	Unclear	Yes	No	No	No	Inadequately described
Vijverberg 2014[167]	**Yes**	**No**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**NA**	**Yes**	**No**	**Yes**	**Yes**	**Adequately described**
Dahlin 2015[168]	Unclear	No	Yes	No	Yes	No	Yes	**Yes**	**Yes**	**No**	**Yes**	**No**	Inadequately described
Israel 2015[169]	Yes	No	Yes	No	Yes	No	No	**Yes**	**Yes**	**No**	**Yes**	**No**	Inadequately described
Vijverberg 2015[170]	**Yes**	**No**	**Yes**	**Yes**	**Yes**	**No**	**Yes**	**Yes**	**Yes**	**No**	**Yes**	**Yes**	**Adequately described**
Keskin 2016[171]	No	No	Yes	No	Yes	Yes	No	**Yes**	**No**	**NA**	**Yes**	**Yes**	Inadequately described
Leusink 2016[172]	**Unclear**	**No**	**Yes**	**No**	**Yes**	**Yes**	**Yes**	Yes	No	NA	Yes	No	Inadequately described
Turner 2016[126]	**Unclear**	**Limited**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**NA**	**Yes**	**No**	**Yes**	**Yes**	**Adequately described**

Table 2.1: Summary of the quality assessment. NR: Not reported, NA: Not applicable.

The following polymorphisms were associated with difficult-to-treat asthma: rs2660845 − Leukotriene A4 hydrolase (LTA4H),[120] rs1805011, rs1805015 and rs1801275 − Interleukin 4 receptor α (IL-4Rα), which binds Interleukin 4 (IL-4) and IL-13 to regulate Immunoglobulin E (IgE) antibody production,[120] and rs1800630 and rs1800629 − Tumor necrosis factor α (TNFα), which is involved in the regulation of immune cells.[120] Carriers of two copies of the minor allele for the LTA4H variant had a three-fold increased odds of difficult-to-treat asthma compared to carriers of at least one copy of the common allele.[120] Carriers of two copies of the minor allele for the IL-4Rα and TNFα variants had a two-fold increased odds of difficult-to-treat asthma compared to carriers of at least one copy of the common allele.[120] The study was adequately described.

The following polymorphisms were associated with asthma exacerbations: rs652438 − Matrix Metalloproteinase 12 (MMP12), which controls the infiltration of eosinophils and macrophages to the airways,[125] and rs4950928 − Chitinase 3 Like 1 (CHI3L1), which increases bronchial smooth muscle proliferation.[131] Carriers of one or two copies of the serine variant, MMP12, had two-fold increased odds of asthma exacerbations compared to carriers of none.[125] Children carrying the minor allele for the CHI3L1 variant were 40% less likely to have asthma-related hospitalisations than children with two copies of the common allele.[131] Both studies were adequately described.

Both CD14 and CC16 are involved in immunological and inflammatory pathways. Two studies explored the role of CD14 (rs2569190) gene variation on asthma severity and found different results. Martin et al.[127] found an increase in asthma attacks for carriers of one or two C alleles, compared to carriers of two T alleles, and Perin et al.[133] found increased bronchial hyperreactivity for carriers of one or two C alleles, compared to carriers of two T alleles. Regarding the CC16 gene (A38G), Martin et al.[127] found increased odds of asthma attacks for individual carriers of the common allele compared to carriers of one or two copies of the minor allele. However, using the quality assessment developed, Martin et al. inadequately described the methods and results, and Perin et al. inadequately described the results.

Another polymorphism associated with bronchial responsiveness was rs1741981 − Histone deacetylase 1 (HDAC1), whose conditional deletion resulted in airway inflammation and increased T helper 2 cytokine production.[160] Carriers of two copies of the minor allele for the HDAC1 variant had lower FEV_1 increase compared to carriers of at least one copy of the common allele (14% vs. 19%).[160] However, using the quality assessment developed, this study was inadequately described.

Clinical responsiveness to β_2-agonists

The majority of studies exploring the relationship between genes and clinical responsiveness to β_2-agonists (see table 2.2 at the end of the chapter) studied the % change in FEV_1 after the use of a Short-acting β_2-agonists (SABA), commonly salbutamol, or Long-acting β_2-agonists (LABA), salmeterol.[118,134,138,139,145,146,148,159,159,169] Other studies used asthma exacerbations as an outcome measure.[124,130]

The association between β_2-agonists and the Arg16 variant of the ADRB2 gene has been widely explored, but with conflicting results. Palmer et al.[130] found that individuals with two copies of the Arg allele treated with salmeterol had a three-fold increase in the risk of exacerbations compared to individuals with two copies of the Gly allele treated with salmeterol. Basu et al.[124] found those with one or two copies of the Arg allele and taking salbutamol or salmeterol daily, had a 65% increase in the odds of asthma exacerbation compared to individuals with Gly/Gly genotype taking salbutamol or salmeterol daily. On the other hand, Martinez et al.[134] found that children with Arg/Arg genotype had a five-fold increase in % change FEV_1 after the use of salbutamol compared to children with Gly/Gly genotype. However the sample size for the Martinez study was smaller (n=269) than the sample size for the Palmer et al. (n=546) and the Basu et al. (n=1182) studies, and Palmer et al. and Basu et al. were adequately described, unlike Martinez et al. Choudhry et al.[139] also found better outcomes for carriers of the Arg allele. Carriers of at least one copy of the Arg allele had a 3% increase in BDR compared with Gly/Gly carriers. However, this association was only found in Puerto Ricans and not in Mexicans. Three studies did not find an association between the use of β_2-agonists and the presence of the Arg16 variant.[118,138,149] However, using the quality assessment developed, these studies

were inadequately described. No association was found between the Glu27 variant and clinical responsiveness to β_2-agonists.[118,124,130,134,138,139,149]

Thyroid hormone receptor beta (THRB) gene was associated with greater BDR[159] and the study was adequately described. Individuals with at least one copy of the minor allele for the THRB variant are 33% more likely to improve BDR after the use of salbutamol than individuals with two copies of the common allele.

The polymorphisms rs1800796 and rs13306435 $-$ Interleukin 6 (IL-6), an anti-inflammatory cytokine,[148] were associated with reduced BDR, rs255100 and rs2267715 $-$ Corticotropin releasing hormone receptor 2 (CRHR-2), which is involved in the smooth muscle relaxation,[146] rs2781659, rs2781663 and rs2781665 $-$ Arginase 1 gene (ARG1), a gene in the β-adrenergic pathway,[145] and rs350729, rs1840321, rs1384918 and rs1319797 $-$ Ankyrin repeat and SOCS box protein 3 (ASB3), involved in muscle development.[169] Mexicans with one or two copies of the minor allele for the IL-6 variant were associated with a 40% decrease in BDR compared to Mexicans with two copies of the common allele. No association was found in Puerto Ricans and the study was adequately described.[148] Carriers of one or two minor alleles for the CRHR-2 gene had a reduced BDR compared to carriers of two copies of the common allele. However, the result involving rs255100 and rs2267715 were not replicated in the adult cohorts, and the study inadequately described the results, using the quality assessment developed.[146] Individuals with at least one copy of the minor allele for the ARG1 variants had significantly lower BDR than individuals with two copies of the common allele but the results were inadequately described, using the quality assessment developed.[145] Individuals with at least one copy of the minor allele for the ASB3 variant had a 20% reduction in the degree of BDR compared to individuals with two copies of the common allele, however, using the quality assessment developed, the study was inadequately described in terms of methodology.[169]

Clinical responsiveness to Inhaled Corticosteroid (ICS)

Several polymorphisms have been studied regarding their effect on clinical responsiveness to ICS. The outcome measures studied were the % change in FEV_1 after ICS use[136,137,142,150,151,156,158,166,171,172] and occurrence of asthma

exacerbations.[81,83,140,167,170]

Rogers et al.[150] found a 80% reduction in BDR for individuals with the minor allele of the FCER2 rs28364072 variant, compared to individuals with one or two copies of the common allele. Individuals homozygous for the minor allele had a two-fold increased odds of asthma exacerbations and uncontrolled asthma than individuals with at least one copy of the common allele.[81,83,150] Although Rogers et al. inadequately described the methodology, Tantisira et al. and Koster et al. were adequately described, using the quality assessment developed.

Vijverberg et al.[167] found no association between the GLCCI1 variant and asthma exacerbations and the study was adequately described. However, using the quality assessment developed, two studies inadequately described reported different results. One study[155] (n=118), inadequately described the results, found a two-fold decreased BDR for carriers of two minor alleles compared with carriers of one or two common alleles, while another study (meta-analysis of three studies: n=1037, n=323, n=431), inadequately described the methodology, found no association.[171] Another polymorphism with varying results is the rs242941 SNPs in the CRHR-1 gene was described in some papers. Tantisira et al.[136] (n=415) found an increased BDR for individuals with at least one minor allele compared to individuals with two copies of the common allele (% FEV_1 change: 18% vs. 8%). Using the same dataset, but with a different length of follow-up, Rogers et al.[150] (n=202) found a decreased BDR for individuals with the minor allele, compared with individuals with two copies of the common allele. Two other studies examined the same SNPs but found no association.[142,171] However, using the quality assessment developed, these four studies inadequately described the methodology and the results.

The rs2240017 SNPs in the T-box 21 (TBX21), responsible for the induction of T helper cells, was found associated with increased bronchial hyperreactivity[137] although another study was unable to find an association.[171] However, using the quality assessment developed, both studies inadequately described the results and methodology, respectively.

The following polymorphisms were associated with increased BDR: rs2872507 – ORMDL3,[158] Nuclear Receptor subfamily 3 group C member 1 (NR3C1) gene, which

encodes the glucocorticoid receptor,[171] rs1741981 − HDAC1 gene,[160] rs967676 − Carbonic anhydrase 10 (CA10),[166] rs2146323 − Vascular endothelial growth factor A (VEGFA), which increases matrix metalloproteinase activity,[156] rs35742417 nearby Ras responsive element binding protein 1 (RREB1);[172] rs11953266 nearby Ribosomal protein S15 pseudogene 6 (RPS15P6);[172] and Cytotoxic T-Lymphocyte Associated protein 4 (CTLA4) gene.[151] Carriers of the minor allele for ORMDL3 variant had better BDR than carriers of the common allele (median FEV_1 change: 10% vs. 6%).[158] Children with two copies of the minor allele for the NR3C1 variant had a greater increase in FEV_1 compared to children with one or two copies of the common allele (% FEV_1: 24 vs. 8).[171] Children with two copies of the minor allele for the HDAC1 variant had lower % FEV_1 increases than children with one or two copies of the common allele (14% vs. 19%).[160] Individuals with at least one copy of the minor allele for the CA10 variant had higher BDR than wild-type individuals (% FEV_1: 8 vs. 4).[166] Individuals with two minor alleles for the VEGFA variant had a better BDR than individuals with at least one copy of the common allele (% change in FEV_1: 9 vs. 5).[156] However, using the quality assessment developed, all of these studies inadequately described the methodology[160,171] or results.[156,158,166,172]

Other polymorphisms were associated with decreased BDR: rs1786929 − Catenin α 3 (CTNNA3),[166] rs117053233 nearby Growth arrest specific 8 (GAS8);[172] rs72821893 nearby Keratin 25 (KRT25);[172] rs10484568 nearby Human leukocyte antigen (HLA);[172] and rs1456896 nearby IKAROS family zinc finger 1 (IKZF1);[172] and four SNPs in the Dual specificity phosphatase 1 (DUSP1) gene.[153] Children with two copies of the minor allele for the CTNNA3 variant had lower improvement than children with one or two copies of the common allele.[166] Using the quality assessment developed, these studies inadequately described the results.

The following polymorphisms were associated with asthma exacerbations: rs1800925 −IL-13,[140] and rs138335 and rs138337 − Suppression of Tumorigenicity 13 (ST13), which is involved in the maturation of the corticosteroid receptor.[170] Individuals with at least one minor allele for the IL-13 variant had more asthma exacerbations than individuals with two copies of the common allele. This was only observable in Caucasian children, but not Costa Rican children.[140] However, using the quality assessment developed, the study inadequately described the results. Individuals with

at least one minor allele for the ST13 variant had a 35% increased odds of asthma exacerbations. These results were found in three different cohorts but not replicated in three further different cohorts. The study was adequately described.[170]

Stockman et al.[163] explored the effect of the rs35599367 polymorphism in the Cytochrome P450 family 3 subfamily A member 4 (CYP3A4) gene on the variation in asthma control for children treated with ICS. The presence of a copy of the minor allele was associated with a decrease of two points in asthma control, compared with the presence of two copies of the common allele. However, using the quality assessment developed, the methods and results were inadequately described. Individuals with two copies of the minor allele in rs1558726 in the Rhabdomyosarcoma 2 associated transcript (RMST) gene and rs2388639 in the LOC728792 gene showed lower symptoms scores compared with individuals with at least one common allele, while rs10044254 in the F-Box and leucine rich repeat protein 7 (FBXL7) gene had an improvement in asthma symptoms scores after ICS treatment.[165] However, using the quality assessment developed, the study inadequately described the results.

Clinical responsiveness to combined Long-acting β_2-agonists (LABA) and Inhaled Corticosteroid (ICS)

The majority of studies exploring the relationship between genes and clinical responsiveness to combined LABA and ICS focused on the Arg16 variant in the ADRB2 gene (see table 2.2). The majority of these studies used asthma exacerbations as an outcome measure.[126,161,164]

Turner et al.[126] analysed data from six cohorts and found an increase in asthma exacerbations for carriers of the Arg allele in two cohorts. In the remaining three cohorts, no association was found. The authors pooled the data and presented a meta-analysis and found that individuals with at least one copy of the Arg allele was associated with a 50% (95% Confidence interval (CI): 17%,99%) increase in the odds of asthma exacerbations. The study was adequately described. Zuurhout et al.[164] reported a higher Odds ratio (OR) (OR: 12.13) for asthma exacerbations for individuals with Arg/Arg genotype compared to individuals with Gly/Gly genotype, however, despite the study being adequately described, the CI was wider (95% CI:

2.18,67.60). An RCT by Lipworth et al.,[161] adequately described, on children with the Arg/Arg genotype, found that children treated with combined LABA and ICS had more school absences, a higher daily use of salbutamol and lower quality life scores than children treated with montelukast.

A polymorphism in the Nitric oxide synthase 3 (NOS3) gene, which is hypothesised to be involved in airway inflammation, was associated with differences in BDR. Individuals with two copies of the minor allele had higher % FEV_1 change compared to individuals with two copies of the common allele (22 vs. 4).[157] However, using the quality assessment developed, the study was inadequately described.

Clinical responsiveness to Leukotriene Receptor Antagonist (LTRA)

The number of studies exploring clinical responsiveness to LTRA is smaller than the number of studies on β_2-agonists and ICS (see table 2.2 at the end of the chapter). Most studies on clinical responsiveness to LTRA used as outcome the % change in predicted FEV_1.[141,152,154,156,162,168]

Polymorphic variation in the LTA4H gene was associated with difficult-to-treat asthma in one study.[120] Another study found that children with one or two copies of the minor allele at rs2540491 were associated with higher BDR compared with children with two copies of the common allele, while heterozygous at rs2540487 were associated with higher BDR compared with children with two copies of the minor and common allele.[152] The study was adequately described.

5-Lipoxygenase (ALOX5) gene, which is involved in the production of leukotrienes,[162] was associated with decreased BDR. Carriers of the minor allele for ALOX5 variant had significantly lower BDR (% change FEV_1: 84 vs. 91) than carriers of at least one common allele.[162] The study was adequately described. Prostaglandin D2 receptor (PTGDR) gene[154] was also associated with decreased BDR, however, using the quality assessment developed, the study inadequately described the results. Carriers of the minor allele for the PTGDR variant had decreased BDR compared with carriers of two copies of the common allele.[154]

Individuals with a copy of the minor allele of rs730012 $-$ LTC4S had higher Fractional exhaled nitric oxide (FeNO) levels than individuals with two copies of the common

allele.[135] However, two other studies explored the role of LTC4S on BDR and found no association.[141,154] However, using the quality assessment developed, these studies inadequately described their results. The rs1800925 polymorphism in the IL-13 gene was previously reported to be associated with increased odds of asthma exacerbations while on ICS, and Kang et al.[143] found that for the same variant individuals on LTRA with at least one copy of minor allele were associated with decreased BDR compared to individuals with two copies of the common allele. However, using the quality assessment developed, the study inadequately described the results.

The polymorphisms MLLT3 gene[168] and rs833058 in the VEGFA gene[156] were associated with increased BDR. Carriers of two copies of the minor allele for the MLLT3 variant showed increased BDR, compared with carriers of the common allele.[168] However, using the quality assessment developed, the study inadequately described the methods. Carriers of two copies of the minor allele for VEGFA variant had a better BDR than carriers with at least one common allele (% change in FEV_1: 9 vs. 1).[156] Using the quality assessment developed, the study inadequately described the results.

rs2146323 in the VEGFA gene was associated with a better BDR on ICS, but there was no improvement for patients on LTRA. Interestingly, despite individuals with two copies of the minor allele improving with ICS, individuals with two copies of the minor allele on LTRA were 90% less likely to have controlled asthma than individuals with at least one common allele.[156] Using the quality assessment developed, the study inadequately described the results.

Statistical approach

The secondary aim of this systematic review was to understand which statistical methods are usually used to analyse genetic data, with special consideration for the genetic model used and adjustment of P-values.

The majority of the papers (n=22) adopted an additive genetic model, which assumes a linear dose-response. Ten papers adopted a dominant genetic model, comparing individuals with two copies of the common allele with individuals with one or two copies of the minor allele. Seven papers opted for a genotypic model which does not

assume the mode of inheritance and instead compares genotypes with each other. A recessive model was adopted in three papers, individuals with two copies of the minor allele were compared with individual with one or two copies of the common allele. The papers that did not specify a genetic model did not provide any explanation regarding the choice of the model. In 14 articles, the genetic model adopted was not mentioned in the methodology section. However, analysing the results section it was possible to understand the genetic model the authors had selected. In 3 articles the information presented was not sufficient to confidently specify which genetic model was adopted. Several studies (n=12) tried different genetic models and selected the model based on statistics, such as Akaike Information Criteria (AIC) and Bayesian Information Criterion (BIC), instead of biological mechanisms.

Regarding adjustment of P-values, a third of the studies included in the systematic review considered the issue of multiple testing. The remaining studies did not consider the problem of multiple comparison, nor provide a reason for not adjusting the P-value. Bonferroni was the most common correction used (n=14), followed by permutation tests (n=2), the Nyholt correction (n=1), and the Holm correction (n=1). Another paper mentioned P-value adjustment but did not mention which method was used.

Overall, the quality of reporting the results was low, with only 35% of the papers "adequately described". Using the quality assessment developed, the majority of the papers were "inadequately described", and for 74% the issue was the reliability of the results. Less than 60% of the studies included in the systematic review reporting the effect size or difference in means found with the respective CI. Only 29 studies presented a multivariate analysis with the effect size and the CI. A t-test, a test to compare proportions, or a correlation test was also a common analysis. However, only 4 studies reported the mean difference found with the respective CI. Other studies (n=15) reported the effect size or the difference in means but did not report the associated CI, despite mentioning in the methods that the CI was calculated. In 9 studies, only the P-value was reported without any information about the difference in means, the effect size, or the precision found.

Study	Type of study	Gene (SNPs)	Study population	MAF	Replication	Definition of response	Study outcome
Martinez et al. 1997[134]	Candidate-gene study. 2 SNPs	ADRB2 (Arg16Gly, Glu27Gln)	269 children. 188 had Caucasian parents, 41 had Hispanic parents, 40 had one Caucasian and one Hispanic parent	Arg16Gly 0.38 (Arg) Gln27Glu 0.36 (Glu)	-	100 x (FEV_1 after two puffs of salbutamol − FEV_1 before salbutamol)	Arg was associated with a positive BDR (ArgArg vs GlyGly OR:5.3, 95% CI: 1.6-17.7; ArgGly vs GlyGly OR:2.3, 95% CI: 1.3-4.2), while no association was found for Gln27Glu.
Whelan et al. 2003[135]	RCT	LTC4S (rs730012)	12 children randomised to montelukast or placebo. 5 Caucasian, 7 African-American	0.17 (C)	-	100 x (FeNO montelukast FeNO placebo) / FeNO baseline	Montelukast reduced FeNO over time in heterozygotes.
Tantisira et al. 2004[136]	**Candidate-gene study. 131 SNPs in 14 genes**	**CRHR-1 (rs242941)**	**415 adults from The Adult Study. Caucasian**	**0.30 (allele NR)**	**201 children from CAMP and 224 adults from ACRN**	**100 x (FEV_1 on ICS − FEV_1 before ICS) / FEV_1 before ICS**	**Children homozygous for the minor allele has increased FEV_1 compared with homozygous for the wild-type allele (% mean FEV_1 change: 17.80 vs 7.57, respectively).**

Table 2.2 – continued from previous page

Study	Type of study	Gene (SNPs)	Study population	MAF	Replication	Definition of response	Study outcome
Tantisira et al. 2004[137]	**Candidate-gene study. 49 SNPs**	**TBX21 (rs2240017)**	**139 children randomised to ICS and 377 randomised to placebo from CAMP. Caucasian**	**0.04 (G)**	**-**	**100 x (FEV_1 at the end of a period $-$ FEV_1 at randomization) $/$ FEV_1 at randomization**	**Individuals homozygous for the minor allele had increased airway responsiveness compared with homozygous for the wild-type allele (OR: 3.5)**
Cho et al. 2005[138]	Candidate-gene study. 2 SNPs in 1 gene	ADRB2 (Arg16Gly, Glu27Gln)	195 unrelated children. (Korea)	Arg16Gly 0.48 (Gly) Gln27Glu (0.09 (Glu)	-	100 x (FEV_1 after salbutamol $-$ FEV_1 after inhalation of methacholine) $/$ (baseline FEV_1 $-$ FEV_1 after inhalation of methacholine)	Arg was associated with increased BDR (% change FEV_1: 62.8 vs 46.7). No association was found for the Gln27Glu variant.
Choudhry et al. 2005[139]	Candidate-gene study. 8 SNPs in 1 gene.	ADRB2 (Arg16Gly)	667 children. (Puerto Ricans and Mexicans)	NR	-	100 x (FEV_1 after salbutamol $-$ FEV_1 before salbutamol)	Each Arg allele was associated with a 3% increase in FEV_1 in Puerto Ricans.

Table 2.2 – continued from previous page

Study	Type of study	Gene (SNPs)	Study population	MAF	Replication	Definition of response	Study outcome
Martin et al. 2006[127]	Candidate-gene study. 2 SNPs	CD14 (rs2569190), CC16 (A38G)	148 children. 92% Caucasian	CD14 0.45 (C) CC16 0.32 (A)	-	Severity of acute asthma attacks defined by a validated scoring system 5-15; mild asthma 5-7; moderate asthma 8-11; severe asthma 12-15	Children homozygous for the minor allele were more likely to have higher asthma severity scores (OR: 3.7, 95% CI:1.04-13.2). No association was found between CC16 and asthma severity scores.
Palmer et al. 2006[130]	**Candidate-gene study. 2 SNPs**	**ADRB2 (Arg16Gly, Glu27Gln)**	**164 children taking regular inhaled salmeterol 50μg twice daily and 382 children not taking salmeterol. Caucasian (Scotland)**	**Arg16Gly 0.38 (Arg) Gln27Glu 0.43 (Glu)**	**-**	**Asthma exacerbations defined as asthma-related school absence, use of oral corticosteroids, and/or asthma-related hospital admissions**	**Children homozygous for the Arg allele had a higher risk of asthma exacerbations compared with homozygous for the wild-type allele (OR:3.40, 95% CI: 1.19-9.40)**.

Table 2.2 – continued from previous page

Study	Type of study	Gene (SNPs)	Study population	MAF	Replication	Definition of response	Study outcome
Hunninghake et al. 2007[140]	**Candidate-gene study. 2 SNPs**	**IL-13 (rs1800925, rs20541)**	**417 children in Costa Rica (Hispanic) and 503 children from CAMP (Caucasian)**	**rs1800925 Costa-Rican 0.19 (T) CAMP 0.21 (T) rs20541 Costa-Rican 0.32 (A) CAMP 0.21 (A)**	**-**	**Concentration of methacholine that induced a 20% reduction in FEV_1, and asthma exacerbations defined as any asthma-related hospitalisation during the first 4 years**	**Increased risk of asthma exacerbations in white children.**
Lee et al. 2007[141]	Candidate-gene study. 2 SNPs in 2 genes	LTC4S (rs730012), CysLTR1 (T1927C)	100 asthmatic children with exercise-induced bronchoconstriction. (Korea)	NR	-	100 x (maximal % fall in FEV_1 before montelukast $-$ maximal % fall in FEV_1 after montelukast) $/$ maximal % fall in FEV_1 before montelukast	No association was found.

Table 2.2 – continued from previous page

Study	Type of study	Gene (SNPs)	Study population	MAF	Replication	Definition of response	Study outcome
Tantisira et al. 2007[83]	**Candidate-gene study. 10 SNPs in 1 gene**	**FCER2 (rs28364072)**	**148 children on ICS with one or more exacerbations over 4 years and 53 children on ICS without exacerbations over 4 years, from CAMP. Caucasian**	**0.26 (C)**	**22 children on ICS with one or more exacerbations over 4 years and 22 children on ICS without exacerbations over 4 years, from CAMP. African-American**	**Asthma exacerbation: emergency department visit and hospitalizations**	**Children homozygous for the minor allele had increased risk of asthma exacerbations that children with at least one wild-type allele (OR:3.70, 95% CI:1.99-6.91)**
Dijkstra et al. 2008[142]	Candidate-gene study. 3 SNPs	CRHR-1 (rs1876828, rs242939, rs242941)	98 adults with childhood asthma (Netherlands)	NR	-	100 x (FEV_1 on ICS $-$ FEV_1 before ICS), and rate of decline in FEV_1	No association was found.

Table 2.2 – continued from previous page

Study	Type of study	Gene (SNPs)	Study population	MAF	Replication	Definition of response	Study outcome
Kang et al. 2008[143]	Candidate-gene study. 3 SNPs	IL-13 (rs1800925, rs20541, -1512 A/C)	109 asthmatic children without exercise-induced bronchoconstriction, 265 asthmatic children with exercise-induced bronchoconstriction and 242 controls. (Korea)	rs1800925 0.28 (C) rs20541 0.20 (T) -1512 A/C 0.35 (A)	-	100 x (maximum % fall in FEV_1 before LTRA $-$ maximum % fall in FEV_1 after LTRA) / maximum % fall in FEV_1 before LTRA	Among individuals homozygous, 77% were responders against 52% of individuals with at least one copy of the wild-type allele.
Kim et al. 2008[144]	**Candidate-gene study. 2 SNPs**	**TBXA2R (+795T>C, +924T>C)**	**551 children with atopic asthma, 144 children with non-atopic asthma, and 159 controls. (Korean)**	**+795T>C 0.40 (C) +924T>C 0.21 (C)**	**-**	**Concentration of methacholine that induced a 20% reduction in FEV_1**	**No association was found.**
Litonjua et al. 2008[145]	Candidate-gene study. 844 SNPs in 111 genes	ARG1 (rs2781659, rs2781663, rs2781665), CRHR-2 (rs7793837, rs2267716)	209 children from CAMP. Caucasian	NR	432 adults from the Asthma trial, 166 adults from the LOCCS trial, 155 adults from the LODO trial	100 x (FEV_1 after salbutamol use $-$ FEV_1 before salbutamol use) / FEV_1 before salbutamol use	The polymorphisms were not significant in the childrens database.

Table 2.2 – continued from previous page

Study	Type of study	Gene (SNPs)	Study population	MAF	Replication	Definition of response	Study outcome
Poon et al. 2008[146]	Candidate-gene study. 28 SNPs in 1 gene	CRHR-2 (rs255100, rs7793837)	607 children from CAMP. Caucasian	rs255100 0.39 (A) rs7793837 0.24 (T)	427 adults from sepracor and 152 adults from LODO. Caucasian	100 x (FEV_1 after 2 puffs of salbutamol $-$ % FEV_1 before salbutamol)	The minor allele was associaed with reduced BDR compared with two copies of the wild-type allele.
Szczepankiewicz et al. 2008[147]	Candidate-gene study. 3 SNPs	GR (rs6190, rs41423247, rs6195, rs10052957)	113 asthmatic children and 123 controls, and 54 children with severe asthma on high doses of ICS. Caucasian (Poland)	rs6190 0.03 (A) rs41423247 0.36 (G) rs6195 0.07 (G) rs10052957 0.39 (T)	-	Poor response defined necessity to take $>$800mcg of budesonide and $>$500mcg of fluticasone propionate	No association was found.
Tavendale et al. 2008[128]	Candidate-gene study. 3 SNPs in 3 genes	ORMDL3 (rs7216389)	1054 asthmatic children and 1465 control children. (Scotland)	0.44 (C)	-	Asthma exacerbations defined as asthma-related hospitalisation, asthma-related absence from school and/or use of oral corticosteroids	Children with a wild-type allele were more likely to have an asthma exacerbation compared to children with at least one copy of the minor allele (OR:1.30, 95% CI: 1.07-1.59).

Table 2.2 – continued from previous page

Study	Type of study	Gene (SNPs)	Study population	MAF	Replication	Definition of response	Study outcome
Basu et al. 2009[124]	Candidate-gene study. 2 SNPs	ADRB2 (Arg16Gly, Glu27Gln)	1182 children and young adults from BREATHE. Caucasian (Scotland)	Arg16Gly 0.37 (Arg)	-	Asthma exacerbations defined as asthma-related school absence, use of oral corticosteroids, and/or asthma-related hospital admissions	Children with the minor allele had an increased risk of asthma exacerbations on β_2-agonists (OR:1.64, 95% CI: 1.22-2.20)
Bisgaard et al. 2009[129]	GWAS. 561466 SNPs	ORMDL3 (rs7216389)	376 children followed during the first 6 years of life, 66 developed asthma	0.53 (C)	-	Age of onset and asthma exacerbations defined by oral corticosteroids, high-dose of ICS, and/or acute asthma-related hospitalization	Children with the minor allele had an increased risk of asthma exacerbations compared with children with the wild-type allele (Hazard Ratio (HR):2.66, 95% CI:1.58-4.48)

Table 2.2 – continued from previous page

Study	Type of study	Gene (SNPs)	Study population	MAF	Replication	Definition of response	Study outcome
Corvol et al. 2009[148]	**Candidate-gene study. 5 SNPs in 2 genes**	**IL-6 (rs1800796, rs13306435)**	**301 Mexicans, 399 Puerto Ricans and 267 African-Americans from GALA**	**rs1800796 Mexicans 0.34 (C) Puerto Ricans 0.13 (C) African-Americans 0.10 (C), rs13306435 Mexicans 0.11 (A) Puerto Ricans and African-Americans $<$0.05)**	**-**	**100 x (FEV_1 $-$ after salbutamol $-$ FEV_1 before salbutamol) $/$ FEV_1 before salbutamol**	**The minor allele of rs1800796 was associated with increased BDR in Mexicans (OR:1.40, 95% CI:1.00-1.96), while the minor allele of rs13306435 was associated with reduced BDR in Mexicans (OR:0.57, 95% CI:0.36-0.90).**
Moore et al. 2009[149]	Candidate-gene study. 9 SNPs in 5 genes	ADRB2 (Arg16Gly, Glu27Gln), GSNOR (rs1154400)	196 children enrolled after an asthma exacerbation. 89 Caucasian and 107 African American	NR	-	Patients requiring continuous salbutamol for more than 5 hours were classified as less responsive	GSNOR was associated with reduced BDR

Table 2.2 – continued from previous page

Study	Type of study	Gene (SNPs)	Study population	MAF	Replication	Definition of response	Study outcome
Rogers et al. 2009[150]	Candidate-gene study. 2SNPs in 2 genes	CRHR-1 (rs242941), FCER2 (rs28364072)	66 children with recurrent exacerbations (two or more exacerbations during the first 2 years and two or more during the last 2 years) and 136 children with rare exacerbations ($0-1$ exacerbations over 4 years), randomised to ICS, from CAMP. 65% Caucasian, 16% African-American, 10% Hispanic, 9% other	rs242941 0.27 (T), rs28364072 0.33 (C)	-	Asthma exacerbation: any Emergency Department visit, hospitalization and/or oral corticosteroids burst. Patients who never improved by 7.5% predicted from baseline FEV_1 were classified as poor lung function responders	The minor allele of rs242941 and rs28364072 were associated with reduced BDR (OR:1.6, 95% CI:1-2.7 and OR:2.1, 95% CI:1.2-3.5, respectively).
Berce et al. 2010[151]	Candidate-gene study. 1 SNPs	CTLA4 (rs3087243)	102 children with mild or moderate persistent asthma and 84 controls. (Slovenia)	0.44 (A)	-	100 x (FEV_1 on ICS $-$ FEV_1 before ICS) / FEV_1 before ICS	Carriers of the minor allele had increased FEV_1 on ICS than carriers of the wild-type allele (mean FEV_1: 12% vs 8%)

Table 2.2 – continued from previous page

Study	Type of study	Gene (SNPs)	Study population	MAF	Replication	Definition of response	Study outcome
Mukhopadhyay et al. 2010[125]	Candidate-gene study. 2 SNPs	MMP12 (rs652438)	1017 children (Scotland)	0.05 (G)	989 adults with COPD (Scotland)	Asthma exacerbations defined by asthma-related hospitalisation, oral corticosteroids use and/or absence from school	Children carriers of the minor allele had increased risk for asthma exacerbations compared with children carriers of the wild-type allele (OR:1.90, 95% CI:1.19-3.04)
Tcheurekdjian et al. 2010[152]	Candidate-gene study. 6 SNPs in 2 genes	LTA4H (rs2540487, rs2540491), ALOX5AP (rs10507391, rs9551963)	546 children using LTRA and 103 children not using LTRA from GALA. 293 Mexican and 356 Puerto Ricans	rs2540487 Mexicans 0.15 (A) Puerto Ricans 0.24 (A), rs2540491 Mexicans 0.08 (A) Puerto Ricans 0.08 (A) rs10507391 Mexicans 0.44 (A) Puerto Ricans 0.45 (A), rs9551963 Mexicans 0.42 (A) Puerto Ricans 0.52 (A)	-	100 x (FEV_1 after LTRA $-$ FEV_1 before LTRA) $/$ FEV_1 before LTRA	Carriers of the minor allele for rs2540491 and heterozygotes for rs2540487 had increased FEV_1 after LTRA compared with carriers of the wild-type allele and homozygotes, respectively. Puerto Rican carriers of the wild-type allele for ALOX5AP had increased FEV_1 compared with Puerto Rican carriers of the minor allele.

Table 2.2 – continued from previous page

Study	Type of study	Gene (SNPs)	Study population	MAF	Replication	Definition of response	Study outcome
Cunningham et al. 2011[131]	Candidate-gene study. 1 SNPs	CHI3L1 (rs4950928)	1071 children (Scotland)	0.22 (G)	-	Asthma exacerbations defined by asthma-related hospitalisation, oral corticosteroids use and/or absence from school	Children homozygous for the minor allele had reduced risk of hospital admissions compared with children with at least one wild-type allele (OR:0.62, 95% CI:0.41-0.92)
Jin et al. 2011[153]	Candidate-gene study. 107 SNPs in 1 gene	DUSP1 (rs881152, rs34507926, rs7702178, rs3805476)	646 children and adults from GALA. 291 Mexican and 355 Puerto Ricans	rs881152 Mexicans and Puerto Ricans 0.14 (A), rs34507926 Mexicans 0.15 (G) Puerto Ricans 0.08 (G), rs7702178 Mexicans 0.24 (C) Puerto Ricans 0.18 (C), rs3805476 Mexicans 0.25 (A) Puerto Ricans 0.18 (A)	264 children and adults from SAPPHIRE. Africans	100 x (FEV_1 on ICS $-$ FEV_1 before ICS)	These SNPs were associated with clinical responsiveness to ICS in Puerto Ricans

Table 2.2 – continued from previous page

Study	Type of study	Gene (SNPs)	Study population	MAF	Replication	Definition of response	Study outcome
Kang et al. 2011[154]	Candidate-gene study. 3 SNPs in 2 genes	PTGDR (rs803010), LTC4S (rs730012)	69 children responders to montelukast and 23 children non-responders to montelukast (Korean)	rs730012 0.18 (C), rs803010 0.25 (C)	-	Improvement was calculated: 100 x (max % fall in FEV_1 before treatment $-$ max % fall in FEV_1 after treatment) $/$ max % fall in FEV_1 before treatment. Individuals showing a $\geqslant$10% post-treatment improvement were classified as responders while individuals with $<$0% improvement were classified as non-responders	The presence of the minor allele of the PTGDR polymorphism was associated with poor response to LTRA.

Table 2.2 – continued from previous page

Study	Type of study	Gene (SNPs)	Study population	MAF	Replication	Definition of response	Study outcome
Koster et al. 2011[81]	Candidate-gene study. 1 SNPs	FCER2 (rs28364072)	386 children from PACMAN	0.28 (C)	Meta-analysis	Asthma exacerbations defined as asthma-related emergency room visits and/or asthma-related hospitalisation, and oral corticosteroids use	FCER2 was not associated with asthma exacerbations. Children homozygous for the minor allele had an increased risk of uncontrolled asthma (OR:2.64, 95% CI:1.00-6.98), wheeze (OR:3.43, 95% CI:1.39-8.44), shortness of breath (OR:2.64, 95% CI:1.07-6.53), and asthma-related sleep disturbances (OR:2.96, 95% CI:1.19-7.38).

Table 2.2 – continued from previous page

Study	Type of study	Gene (SNPs)	Study population	MAF	Replication	Definition of response	Study outcome
Koster et al. 2011[81]	Candidate-gene study. 1 SNPs	FCER2 (rs28364072)	939 children from BREATHE	0.27 (C)	Meta-analysis	Asthma exacerbations defined as asthma-related emergency room visits and/or asthma-related hospitalisation, and oral corticosteroids use	FCER2 was not associated with asthma exacerbations. Children homozygous for the minor allele had increased daily dose of ICS compared to children with at least one wild-type allele (OR:2.46, 95% CI:1.38-4.39)
Perin et al. 2011[133]	Candidate-gene study. 1 SNPs	CD14 (rs2569190)	247 asthmatic children and 158 control children. (Slovenia)	0.46 (T)	-	Concentration of methacholine that induced a 20% reduction in FEV_1	Non-atopic asthmatics with a wild-type allele have increased bronchial hyperactivity compared with non-atopic asthmatics with a minor allele (0.41mg/ml vs 1.50mg/ml)

Table 2.2 – continued from previous page

Study	Type of study	Gene (SNPs)	Study population	MAF	Replication	Definition of response	Study outcome
Tantisira et al. 2011[155]	**GWAS of lung function response to ICS. 547645 SNPs**	**GLCCI1 rs37972**	**118 children from CAMP. Caucasian**	**0.40 (T)**	**264 adults from SOCS, 385 adults from SLIC, 185 adults from LOCCS and 101 children from CARE. Caucasian**	**100 x (FEV_1 on ICS $-$ FEV_1 at baseline)**	**Children homozygous for the minor allele had reduced change in FEV_1 compared with children homozygous for the wild-type allele.**
Balantic et al. 2012[156]	Candidate-gene study. 2 SNPs	VEGFA (rs2146323, rs833058)	40 children receiving daily ICS, 47 children receiving LTRA on a regular basis and 44 children receiving LTRA episodically. (Slovenia)	rs2146323 0.38 (A), rs833058 0.34 (T)	-	100 x (FEV_1 on medication $-$ FEV_1 before medication) and asthma control using the ACT scores	Children homozygous for the minor allele of rs2146323 had an increased FEV_1 change after ICS compared with children with at least one wild-type allele. Children homozygous for the minor allele of rs833058 had an increased FEV_1 change after LTRA compared with children with at least one wild-type allele.

Table 2.2 – continued from previous page

Study	Type of study	Gene (SNPs)	Study population	MAF	Replication	Definition of response	Study outcome
Iordanidou et al. 2012[157]	**Candidate-gene study. 2 SNPs in 1 gene**	**NOS3 (G894T)**	**81 asthmatic children and 96 healthy controls. Caucasian (Greece)**	**0.31 (T)**	**-**	**100 x (FEV_1 on combined LABA and ICS $-$ FEV_1 before combined LABA and ICS) $/$ FEV_1 before combined LABA and ICS. Patients who never improved by 7.5% predicted from baseline FEV_1 were classified as poor lung function responders**	**Children homozygous for the minor allele had increased FEV_1 change, after LABA with ICS, compared with children homozygous for the wild-type allele (21.9 vs 1.6)**
Isaza et al. 2012[118]	Candidate-gene study. 2 SNPs	ADRB2 (Arg16Gly, Glu27Gln)	109 asthmatic children and 137 controls (Colombia)	Arg16Gly 0.45 (Arg), Glu27Gln 0.17 (Glu)	-	Lung function change before and after treatment	No association was found.

Table 2.2 – continued from previous page

Study	Type of study	Gene (SNPs)	Study population	MAF	Replication	Definition of response	Study outcome
Almomani et al. 2013[120]	Candidate-gene study. 19 SNPs in 9 genes	LTA4H (rs2660845), IL-13 (rs1800925), IL-4Rα (rs1801275), TNFα (rs1800629)	112 children with difficult to treat asthma (DTA) and 68 with mild/moderate (MM) asthma. Caucasian (North Ireland)	rs2660845 0.27 (A), rs1800925 0.17 (T), rs1801275 0.18 (A), rs1800629 0.24 (G)	-	DTA defined on ongoing symptoms despite treatment of at least 800mg of ICS (400mg for children <5 year old). MM was used for children with controlled asthma on low doses of ICS	The minor allele of LTA4H, IL-4Rα and TNFα had increased risk of difficult asthma compared with carriers of the wild-type allele (OR:3.0, 95% CI:1.60-5.70, OR:2.4, 95% CI:1.30-4.60, and OR:2.20, 95% CI:1.10-4.10, respectively).
Berce et al. 2013[158]	Candidate-gene study. 1 SNPs	ORMDL3 (rs2872507)	300 children with mild or moderate persistent asthma and 251 controls. Caucasian (Slovenia)	0.39 (A)	-	100 x (FEV_1 on ICS $-$ FEV_1 before ICS) / FEV_1 before ICS	Children carriers of the minor allele had an increased FEV_1 change after ICS use, compared with children carriers of the G allele (8.5 vs 5.5).
Duan et al. 2013[159]	Candidate-gene study. 1116 SNPs in 98 genes	THRB (rs892940)	403 children from CAMP. Caucasian	0.42 (NR)	435 adults from Sepracor, 159 adults from LOCCS and 155 adults from LODO	100 x (FEV_1 after 2 puffs of salbutamol $-$ FEV_1 before salbutamol) / FEV_1 before salbutamol	The minor allele was associated with increased BDR compared with the wild-type allele (OR:1.44, 95% CI:1.08-1.92)

Table 2.2 – continued from previous page

Study	Type of study	Gene (SNPs)	Study population	MAF	Replication	Definition of response	Study outcome
Kim et al. 2013[160]	Candidate-gene study. 19 SNPs in 2 genes	HDAC1 (rs1741981)	477 adults (Korea)	NR	35 adults on systemic corticosteroids and 70 children on ICS	100 x (FEV_1 after ICS $-$ FEV_1 at baseline) / FEV_1 at baseline X 100	Children homozygous for the minor allele had reduced FEV_1 changes in response to ICS, compared with children with at least one wild-type allele (14.1 vs 19.4)
Lipworth et al. 2013[161]	**RCT**	**ADRB2 (Arg16Gly)**	**62 children homozygous for Arg16Gly randomized into: flixotide + montelukast OR seretide + placebo for montelukast. Caucasian (Scotland)**	**NA**	**-**	**Primary outcome based on school absence over one year. Secondary outcome based on asthma-related hospitalizations, use of oral corticosteroids, asthma exacerbations, use of inhaled bronchodilator as reliever and daily asthma symptoms self-reported**	**Children who received montelukast had a reduced number of school absences and lower use of salbutamol compared with children who received salmeterol (difference in score:$-$0.40, 95% CI:$-$0.22;$-$0.58, and difference in score:$-$0.47, 95% CI:$-$0.16;$-$0.79, respectively)**

Table 2.2 – continued from previous page

Study	Type of study	Gene (SNPs)	Study population	MAF	Replication	Definition of response	Study outcome
Mougey et al. 2013[162]	**Candidate-gene study. 1 SNPs**	**ALOX5 (rs59439148)**	**270 children. 135 African America and 103 Caucasian**	**African-Americans 0.48 (5) White 0.18**	**-**	**100 x (FEV_1 after LTRA $-$ FEV_1 before LTRA) and asthma control was assessed using the 7-item ACQ**	**Children with the X/X genotype had reduced lung function compared with children with X/5 or 5/5 genotype (mean% FEV_1:84 vs 91)**
Stockmann et al. 2013[163]	Candidate-gene study. 9 SNPs in 3 genes	CYP3A4 (rs35599367)	268 children treated daily with fluticasone propionate	0.04 (T)	-	Asthma control defined by a questionnaire	No association was found.
Zuurhout et al. 2013[164]	**Candidate-gene study. 1 SNPs**	**ADRB2 (Arg16Gly)**	**468 children using only ICS and 129 children using combined LABA and ICS from PACMAN (Netherlands)**	**0.42 (Arg)**	**-**	**Asthma exacerbations defined as asthma-related hospitalisation and/or oral corticosteroids use, and asthma control assessed by the ACQ**	**Children homozygous for the minor allele had an increased risk of oral corticosteroids and ER visits compared with children homozygous for the wild-type allele (OR:14.9, 95 % CI:1.59-140.1, and OR:11.9, 95% CI:1.22-115.8, respectively)**

Table 2.2 – continued from previous page

Study	Type of study	Gene (SNPs)	Study population	MAF	Replication	Definition of response	Study outcome
Park et al. 2014[165]	GWAS of lung function re-sponse to ICS. 440862 SNPs	FBXL7 (rs10044254), RMST (rs1558726), LOC728792 (rs2388639)	124 children from CAMP. Caucasian	rs10044254 0.18 (NR), rs1558726 0.09 (NR), rs2388639 0.19 (NR)	77 children from CARE, 110 adults from ACRN and 110 adults from LOCCS. Caucasian	Average symptom score of the last week on ICS treatment − Average symptom score of 1 week before ICS treatment	Children homozygous for the minor allele of these SNPs had increased asthma symptom scores compared with children with a wild-type allele (median: 1.14 vs -0.28)
Perin et al. 2014[166]	Candidate-gene study. 6 SNPs in 6 genes	CTNNA3 (rs1786929)	288 children and 276 controls	0.33 (C)	-	100 x (FEV_1 after ICS − FEV_1 before ICS)	Children homozygous for the minor allele for rs1786929 had a decreased FEV_1 response compared with children with the wild-type allele.
Vijverberg et al. 2014[167]	**Candidate-gene study. 1 SNPs**	**GLCCI1 (rs37972)**	**1037 children from BREATHE treated with ICS. Caucasian (Scotland)**	**0.45 (T)**	**Meta-analysis of three studies**	**Asthma exacerbations defined as asthma-related emergency room visits, asthma-related hospitalisation, and/or oral corticosteroids use**	**No association was found**

Table 2.2 – continued from previous page

Study	Type of study	Gene (SNPs)	Study population	MAF	Replication	Definition of response	Study outcome
Vijverberg et al. 2014[167]	Candidate-gene study. 1 SNPs	GLCCI1 (rs37972)	323 children from PAGES treated with ICS. Caucasian (Scotland)	0.41 (T)	Meta-analysis of three studies	Asthma exacerbations defined as asthma-related emergency room visits, asthma-related hospitalisation, and/or oral corticosteroids use	No association was found.
Vijverberg et al. 2014[167]	Candidate-gene study. 1 SNPs	GLCCI1 (rs37972)	431 children from PACMAN treated with ICS. Caucasian (Scotland)	0.44 (T)	Meta-analysis of three studies	Asthma exacerbations defined as asthma-related emergency room visits, asthma-related hospitalisation, and/or oral corticosteroids use	No association was found.

Table 2.2 – continued from previous page

Study	Type of study	Gene (SNPs)	Study population	MAF	Replication	Definition of response	Study outcome
Dahlin et al. 2015[168]	GWAS of lung function response to montelukast. 532264 SNPs	MLLT3 (rs6475448)	64 adults from LOCCS (64.3% European, 8.3% African and 27.4% Asian) and 69 adults from CLIC (68.3% European, 7.3% African and 24.4% Asian) on montelukast	$>$0.05 (A)	126 children from CLIC (53.7% European, 20.2% African and 26.1% Asian) and 58 children from Pediatric Asthma Controller Trial (56.7% European, 13.3% African and 30% Asian) on montelukast	FEV_1/Forced vital capacity (FVC) ratio, and Concentration of methacholine that induced a 20% reduction in FEV_1	Children homozygous for the minor allele had increased FEV_1 change compared with children with the wild-type allele.
Israel et al. 2015[169]	GWAS of acute BDR to SABA. 444088 SNPss	ASB3 (rs350729, rs1840321, rs1384918, rs1319797)	306 children from CAMP and 157 children from CARE (Caucasian)	NR	439 adults with moderate to severe asthma	100 x (FEV_1 after two puffs of salbutamol $-$ FEV_1 before salbutamol) $/$ FEV_1 before salbutamol	The presence of the minor alleles reduced the BDR by 20%.

Table 2.2 – continued from previous page

Study	Type of study	Gene (SNPs)	Study population	MAF	Replication	Definition of response	Study outcome
Vijverberg et al. 2015[170]	Candidate-gene study. 38 SNPs in 12 genes	ST13 (rs138335, rs138337)	820 children from BREATHE (Scotland)	NR	172 children from CAMP (Caucasian), 391 children from PASS and 745 children from GALA (Hispanic). Meta-analysis of six studies	Asthma-related hospitalisation and oral corticosteroids use	The minor allele was associated with an increased risk of asthma hospital admissions and oral corticosteroids use compared with the wild-type allele (rs138335 – OR:1.32, 95% CI:1.00-1.75, and OR:1.30, 95% CI:1.03-1.64, respectively).
Vijverberg et al. 2015[170]	Candidate-gene study. 38 SNPs in 12 genes	ST13 (rs138335, rs138337)	391 children from PAGES (Scotland)	NR	172 children from CAMP (Caucasian), 391 children from PASS and 745 children from GALA (Hispanic). Meta-analysis of six studies	Asthma-related hospitalisation and oral corticosteroids use	The minor allele was associated with an increased oral corticosteroids use compared with the wild-type allele (rs138335 – OR:1.51, 95% CI:1.08-2.11).

Table 2.2 – continued from previous page

Study	Type of study	Gene (SNPs)	Study population	MAF	Replication	Definition of response	Study outcome
Vijverberg et al. 2015[170]	**Candidate-gene study. 38 SNPs in 12 genes**	**ST13 (rs138335, rs138337)**	**357 children from PACMAN (Netherlands)**	**NR**	**172 children from CAMP (Caucasian), 391 children from PASS and 745 children from GALA (Hispanic). Meta-analysis of six studies**	**Asthma-related hospitalisation and oral corticosteroids use**	**The minor allele was associated with an increased risk of asthma hospital admissions compared with the wild-type allele (rs138337 – OR:1.88, 95% CI:1.00-3.52).**
Keskin et al. 2016[171]	Candidate-gene study. 8 SNPss in 5 genes	NR3C1 (rs41423247), CRHR-1 (rs242941), TBX21 (rs2240017)	82 children with moderate to severe asthma exacerbations	NR3C1 0.20 (C), CRHR-1 0.27 (T), TBX21 0.04 (Gln)	-	Improvement in FEV_1 at 4 hours of treatment with fluticasone	Children homozygous for the wild-type allele had increased FEV_1 change compared to children with a minor allele (% change: 24.2 vs 7.9).
Leusink et al. 2016[172]	RCT and GWAS. 36519 SNPs	rs10484568 (nearby HLA), rs72821893 (nearby KRT25)	110 children using ICS (European ancestry)	rs10484568 0.04 (NR), rs72821893 0.03 (NR)	-	Changes in the % predicted FEV_1 and concentration of methacholine that induced a 20% reduction in FEV_1	rs10484568 and rs72821893 were associated with FEV_1 change.

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Study	Type of study	Gene (SNPs)	Study population	MAF	Replication	Definition of response	Study outcome
Turner et al. 2016[126]	Candidate-gene study. 1 SNPs	ADRB2 (Arg16Gly)	1210 children from BREATHE (Scotland)	0.36 (Arg)	Meta-analysis of five studies	Asthma exacerbations was defined by asthma-related hospitalisation, oral corticosteroids use, and/or absence from school	No association was found.
Turner et al. 2016[126]	Candidate-gene study. 1 SNPs	ADRB2 (Arg16Gly)	1171 children from GALA (100% Hispanic)	0.45 (Arg)	Meta-analysis of five studies	Asthma exacerbations was defined by asthma-related hospitalisation, oral corticosteroids use, and/or absence from school.	Children with a minor allele had an increased risk of asthma exacerbations compared with children with a wild-type allele (OR:2.07, 95% CI:1.03-4.16).
Turner et al. 2016[126]	Candidate-gene study. 1 SNPs	ADRB2 (Arg16Gly)	760 children from PACMAN (90% Caucasian, 8.6% Other, 1% African, 0.4% Hispanic)	0.41 (Arg)	Meta-analysis of five studies	Asthma exacerbations was defined by asthma-related hospitalisation, oral corticosteroids use, and/or absence from school	Children with a minor allele had an increased risk of asthma exacerbations compared with children with a wild-type allele (OR:2.54, 95% CI:1.06-6.06).

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Study	Type of study	Gene (SNPs)	Study population	MAF	Replication	Definition of response	Study outcome
Turner et al. 2016[126]	**Candidate-gene study. 1 SNPs**	**ADRB2 (Arg16Gly)**	**695 children from PAGES**	**0.37 (Arg)**	**Meta-analysis of five studies**	**Asthma exacerbations was defined by asthma-related hospitalisation, oral corticosteroids use, and/or absence from school**	**No association was found.**
Turner et al. 2016[126]	**Candidate-gene study. 1 SNPs**	**ADRB2 (Arg16Gly)**	**390 children from PASS (99% Caucasian and 1% Other)**	**0.37 (Arg)**	**Meta-analysis of five studies**	**Asthma exacerbations was defined by asthma-related hospitalisation, oral corticosteroids use, and/or absence from school**	**No association was found.**

Table 2.2: Characteristics of the asthma pharmacogenetic studies. Studies on bold corresponds to adequately described studies. NR - Not reported, NA- Not applicable.

2.4 Discussion

Summary

Over the last decade, an increasing number of studies have explored the role of candidate genes in both asthma severity and response to asthma treatment. Interestingly, most studies were performed in the USA, Western Europe and South Korea. The fact that several SNPs were associated with different clinical responses may therefore be due to differences in allele frequencies related to ethnicity. Of the discussed genes, the most promising gene showing a consistent effect on clinical responsiveness to ICS is the FCER2,[81,83,150] and the most promising showing a consistent effect on clinical responsiveness to β_2-agonists, in Caucasians, is the ADRB2 gene. Studies with different ethnic groups and bigger sample size are needed to ascertain the real effect of these genes in each population.

Limitations of the included studies

As with gene association studies in many areas, there are several factors that may contribute to the observed heterogeneity in findings. An important issue is the low sample size of most of the studies, likely giving insufficient statistical power to detect associations. More than half of the studies in this review (n=32) had a sample size lower than 500 individuals and only 9 studies had a sample size greater than 1000, of which 7 of these 9 studies used the BREATHE dataset. Regarding small sample size, the effect of CD14 polymorphism was explored – Perin et al.[133] and Martin et al.[127] The minor allele in the Perin study[133] is the C allele while in the Martin study[127] the minor allele is the T allele. Although the results point to an increase in asthma exacerbations for carriers of the C allele, it is possible that the different allelic frequencies are due to ethnicity. However, it is also possible that the differences in Minor Allele Frequency (MAF) were not representative of the population, but instead a consequence of a small sample size, the Perin study had 247 asthmatic children from Slovenia while the Martin study had 148 asthmatic children from Australia. Nevertheless, both studies had issues since both inadequately described the results, using the quality assessment developed. A further study by Tantisira et al.[155] (n=118) involving the GLCCI1 gene found an association between GLCCI1 polymorphism and

BDR, while another study by Keskin et al.[171] (n=82) found no such association. The sample size for both studies is small, which could explain the different results. In addition, the studies have different study populations. Keskin et al.[171] studied children who had moderate-to-severe asthma exacerbations. It is likely that these children have a more severe status of asthma than Tantisira et al.[155] It is important to note that using the quality assessment developed both studies were inadequately described. Tantisira et al. inadequately described the results, while Keskin et al. inadequately described the methods. An equally important issue related to the outcome measured in the studies. Most studies used the % change in FEV_1 to define the BDR to medication. However, BDR varies between and within patients, depending on the measurement time at baseline and environmental factors. For instance, some studies included a wash-out period to reduce the possibility that BDR is affected by medication, while others did not, and this could affect the relationship found between the clinical responsiveness to the medication and the genotype. Another common outcome under study is asthma exacerbation. Some studies categorised this variable into presence or absence of asthma exacerbations, while others categorised participants into recurrent or rare exacerbators, based on the number of asthma exacerbations in a determined time-frame. The time frame studied was also variable, ranging between 4 weeks and 4 years. Thus, longer time periods may have higher numbers of asthma exacerbations than shorter time periods. The definition of asthma exacerbations was also variable. Some studies defined asthma exacerbations as asthma-related hospitalisations, emergency visits, and asthma-related absence from school, while others defined asthma exacerbations as emergency visits only. This difference could increase the number of asthma exacerbations in the first group compared to the latter. Another important consideration relates to the time span of the study. Some studies that used asthma exacerbations as an outcome only used information regarding the past 6 months. However, asthma exacerbations are seasonal, being more common after the beginning of the school year, around early autumn, and during the spring, due to the allergens.[173] In that sense, using the last 6 months of data may not be sufficient to capture the variability in the number of exacerbations over a year.

Another important aspect relates to the reporting of ethnicity. The importance of describing the ethnicity of each patient can be seen in studies involving the Arg16Gly polymorphism and response to salbutamol. Two studies[124,130] found that carriers of the Arg allele had an increased odds of asthma exacerbations, while two studies[134,139] found that carriers of two Arg alleles had a greater improvement in FEV_1 in response to salbutamol. Three studies[118,138,149] were unable to find any positive or negative association between the presence of the Arg allele and asthma-related outcomes. However, it is important to note the differences in allelic frequencies in the mentioned studies. Studies of Caucasians[124,130,134] reported a MAF of 37-39 %, studies with Hispanic individuals[118,139] reported a MAF of 45%, and another study from South Korea[138] reported a MAF of 51% . Moore et al. reported a MAF of 39%, however 55% of this study population was African-American, which have a higher frequency of the Arg allele, 49%[145] and the remaining 45% were European Americans. Curiously, the studies that found a higher risk of asthma exacerbations for carriers of the Arg allele were adequately described while the remaining studies were inadequately described, using the quality assessment developed. It is thus important to explore the effect of the Arg allele in other populations. Another example of ethnic differences can be found in the Corvol study.[148] Corvol et al. found an association between IL-6 polymorphisms and BDR. However, these results were only significant in Mexicans, not in Puerto Ricans or African-Americans. The polymorphisms included in the study were more common in the Mexican population than in Puerto Ricans and African-Americans (rs1800795 $MAF_{Mexicans}$ 0.34, $MAF_{Puerto\ Ricans}$ 0.13, $MAF_{African\text{-}Americans}$ 0.10; rs13306435 $MAF_{Mexicans}$ 0.11, $MAF_{Puerto\ Ricans\ and\ African\text{-}Americans}$ <0.05). The Corvol study was adequately described, highlighting the importance to report the ethnicity of each patient. As clinical responsiveness to medication has been found to be variable across ethnicities it was surprising that several studies did not describe the ethnicity of the patients. In several studies, Mexicans and Puerto Ricans are both classified as Hispanics, however, the MAF in several SNPs studied was different between these groups and different effects were found. Although these differences have not been studied in Caucasian populations, the MAF in some SNPs was different between British, Germans, and Italians, which could indicate different effects for individuals from different countries in Europe.

Future considerations

Problems may occur when using the same dataset several times. Using the same dataset repeatedly is useful, due to the difficulty in recruiting large number of patients with a low allele frequency, but one must be aware of multiple comparison problems associated with this. For instance, data from the Childhood Asthma Management Program dataset (CAMP) was used in 15 studies. CAMP is a longitudinal study and FEV_1 measurements were taken at several time points. However, few authors take advantage of the longitudinal aspect of the dataset. As a result, the CAMP dataset is analysed differently in various studies with some authors comparing the FEV_1 measurement at baseline with the FEV_1 measurement after 4 years, while others use the FEV_1 measurement after 6 or 8 weeks. BREATHE was also included in several studies, as a result of contributing to the Pharmacogenomics in Childhood Asthma (PiCA) consortium. Pediatric Asthma Severity Score (PASS), GALA, Pharmacogenetics of Asthma medication in Children: Medication with ANti-inflammatory effects (PACMAN) and Paediatric Asthma Gene Environment Study (PAGES) are also part of the PiCA consortium. Although larger studies and collaborations are encouraging, authors should be aware of ethnic differences and the possibility of false positives, especially without the support of a valid biological explanation. The problem of multiple comparisons can be overcome by adjusting P-values. However, no article considered previous studies using the same dataset as part of the hypothesis tested. Regarding methods to correct the P-value, Bonferroni was one of the most common corrections used across studies (n=13). However, Bonferroni correction is extremely conservative and increases the risk of type II error. There is no consensus among the statistical community about when it is appropriate to use the Bonferroni correction, and many experts recommend using the false discovery rate instead.

Another important consideration is the choice of the genetic model. Several studies (n=12) tried different genetic models, such as additive, dominant and recessive, and end up choosing the model based on a statistical output. This led to some problems as it increased the number of tests performed and increased the likelihood of "cherry-picking" the most convenient or significant result for publication. The genetic model should be a representation of the biological mechanism of the gene and not

guided by statistical significance. However, the biological function of the gene is often unknown. In these cases, the author needed to assume a model to perform the analysis and should state the genetic model used in the article. However, several studies did not state which genetic model was used. Any reader should be able to understand the steps of the research, such as planning, analysis, reporting of results and conclusions. However, 17 articles did not mention the genetic model used, and although in most cases it is possible to understand which model was used by studying the results, it was unclear how or why that particular model was chosen.

Two final aspects that may account for the heterogeneity between studies relates to the method used to measure clinical responsiveness. Patients that do not improve on ICS are usually said to not respond to ICS. However, one should be aware that more severe patients may need to be stepped up and have added a combination of LABA or an LTRA. Poor response to ICS could be an indication of poor response due to the genotype. It is also possible that patients with the genotype in question are partially responding to the medicine in question but have a more severe form of the disease and need more treatment for a more complete response. Most studies relied upon self-reported medication use. Hence, it is also possible that patients reported the prescribed use of medication and not the medication that the patient is actually taken, invalidating some associations found.

Limitations of the systematic review

Although 57 articles were identified in this systematic review, not every article studying the clinical responsiveness to medication in asthma was identified, since additional papers were found by screening the reference list of review papers. The keyword search was done with the help of a librarian; however, not every author used keywords such as ’pharmacogenetics’ or ’pharmacogenomics’. Removing these keywords from the search would have substantially increased the number of publications found since it would have included every paper associating genes with asthma susceptibility, which was not the aim of this review. This situation also highlights the importance of choosing keywords when submitting articles, and the current difficulties in finding all pharmacogenetic studies. Furthemore, this systematic review was also restricted to children. Some studies consider child and adult onset of

asthma as different subtypes of asthma. Hence, adult cohorts should report the onset age of asthma to distinguish the effects of different genotypes between children and adults. Finally, although a quality assessment was developed for the purpose of this review it was not a standardised one, and was not validated. It is important to note that although some studies were inadequately described, using the quality assessment developed, quality of reporting and risk of bias are different. Hence, the importance of increasing the quality of the articles will increase transparency and the potential for replication. Another point is that all items included in the checklist were assigned the same weight, however, a study with several minor flaws could be considered inadequate while a study with one major flaw would be considered adequate. For this reason, the quality of the studies should be interpreted with caution.

Recommendations for future

This systematic review highlights several issues in genetic association studies. One of them is the importance of reporting. Guidelines like Strengthening the reporting of genetic association studies (STREGA) should be used when writing the article which reports the study results. These guidelines will help the reader to understand and replicate the methods. Few articles have followed these guidelines, and lack essential information for anyone to replicate the methods. Another important point relates to the sample size. Larger sample sizes are needed to translate pharmacogenetic results into clinical practice. Since clinical responsiveness to medication has been found to be variable across ethnicities, it is important to report the ethnicity of the patients, and perhaps even the ethnicity of the parents. Table 2.3 presents a summary of the systematic review. Although several pharmacogenetic studies were published, a proportion of the adequately described studies lack replication. Other studies defined as "inadequately described", using the quality assessment developed, reported only P-values with insufficient information to translate results into clinical practice. ADRB2 and FCER2 were the most promising genes and are mentioned as a pharmacogenetic predictor. CRHR-1, LTC4S, TBX21, IL-13 and CRHR-2 may also be pharmacogenetic predictors; however studies related to these genes were defined as "inadequately described", using the quality assessment developed, and had variable results. None of the studies mentioned or performed a

cost-effectiveness analysis; hence this area was explored in more detail in this thesis.

Following analysis of the results obtained in this systematic review, the association between Filaggrin (FLG), ADRB2 and FCER2 and healthcare outcomes, such as prescribing and asthma exacerbations, were explored in this thesis. Other genes, such as CRHR-1, CRHR-2 and IL-13 were also found to be of interest in the systematic review but were not further explored due to lack of genotype data.

Gene - SNPs	Summary	Adequacy of evidence	Gaps	Next steps
ADRB2 - Arg16Gly	Three studies found an increased BDR on β_2-agonists for individuals with the Arg allele,[134,138,139] while four studies found a higher risk of asthma exacerbations, on β_2-agonists for individuals with the Arg allele.[124,126,130,164] Two studies found no association between Arg16Gly and clinical responsiveness to β_2-agonists.[118,149]	The studies that associated the Arg allele to higher risk of asthma exacerbations were adequately described.[124,126,130] However, the remaining studies were not adequately described, whether the studies inadequately described the results,[118,139,164] or inadequately described the methods and results.[134,138,149]	Although there is difference in the adequacy of the articles, there are also ethnic differences among the studies, so it would be important to further explore the effect of the Arg allele in the clinical responsiveness to β_2-agonists. Among the studies that have described Arg16Gly as a risk factor and have proposed a personalised approach in children with the Arg allele, none explored the possible cost-effectiveness of such intervention.	RCT are already recruiting patients to understand the benefit of adopting a personalised medicine approach, by given LTRA to children with the Arg allele, while children with the Gly/Gly genotype take a combination of LABA and ICS. Cost-effectiveness analyses are also planned on the the Personalised medicine for Asthma ConTrol (PACT) trial.
FCER2	Children homozygous for the minor allele, on ICS, had increased risk of asthma exacerbations,[83] poor lung function,[150] compared to children with at least one wild-type allele, on ICS. Another study did not find an significant association between asthma exacerbation and FCER2, but found worse asthma symptoms and increased ICS use for children homozygous for the minor allele compared with children with at least one wild-type allele.[81]	Two study were adequately described,[81,83] and one study inadequately described the methods.[150]	Evidence suggests that children on ICS may have more asthma exacerbations. However, despite being pointed out as a pharmacogenetic predictor no RCT have been performed, and it is unclear whether any personalised medicine approach would be cost-effective.	RCT should be planned to compare ICS with omalizumab or LTRA to understand whether children with two minor alleles have better outcomes on montelukast or omazilumab compared to ICS.

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Table 2.3 – continued from previous page

Gene - SNPs	Summary	Adequacy of evidence	Gaps	Next steps
ORMDL3 rs7216389, rs2872507	Children with a wild-type allele for rs7216389 were more likely to have an asthma exacerbation compared to children with at least one copy of the minor allele.[128] Children carriers of the minor allele had an increased FEV_1 change after ICS use, compared with children carriers of the G allele.[158]	One study was adequately described,[128] while one study inadequately described the results.[158]	The authors of the adequately described study caution regarding the found association and recommend replication as the association is not significant when adjusting the P-value for multiple testing.[128] Another limitation of these studies is the difference in the allelic frequency and further studies should address this gap.	Replication studies should be performed in different ethnic groups to understand the effect of this SNPs on the clinical responsiveness to ICS.
CD14 rs2569190	Children homozygous with the C allele had increased asthma severity scores and increased bronchial hyperactivity compared with the T allele.[127,133]	One study inadequately described the results,[133] and one study inadequately described the methods and results.[127]	Although both studies pointed on the same direction, the minor allele differed between studies	Replication studies are needed, properly designed, to understand possible allelic frequencies differences between populations.
IL-6 rs1800796, rs13306435	The minor allele of rs1800796 was associated with increased BDR in Mexicans, while the minor allele of rs13306435 was associated with reduced BDR in Mexicans[148]	The study was adequately described.[148]	Although the study was adequately described, it is the only study on Hispanic children and should be replicated	The study should be replicated and performed on other ethnic groups.
MMP12 rs652438	Children carriers of the minor allele had increased risk for asthma exacerbations compared with children carriers of the wild-type allele[125]	The study was adequately described.	Although the study was adequately described, it is the only study on Caucasian children and should be replicated	The study should be replicated and performed on other ethnic groups.

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Table 2.3 – continued from previous page

Gene - SNPs	Summary	Adequacy of evidence	Gaps	Next steps
GSNOR rs1154400	African-Americans homozygous for the minor allele had decreased BDR compared with the other genotypes.[149]	The study inadequately described the methods and results.	Since the evidence of this study is not adequate, further studies are needed to replicate these findings.	Replication studies should be performed and explored in other ethnic groups. An haplotype analysis should be planned as the authors found an association between this gene and the ADRB2 gene.
LTA4H rs2540487, rs2540491, rs2660845	Children carriers of the minor allele for rs2540491 and heterozygotes for rs2540487 had increased FEV_1 after LTRA compared with carriers of the wild-type allele and homozygotes, respectively.[152] The minor allele was associated with difficult asthma.[120]	Both studies were adequately described.	At the moment it is unclear whether children with the minor allele are at risk for severe asthma or whether the minor allele is associated with better clinical response to LTRA compared with the wild-type allele.	Replication studies should be performed, ideally exploring how LTRA influences asthma severity in carriers of the minor allele.
ALOX5AP rs10507391, rs9551963	Puerto Rican children carriers of the wild-type allele had increased FEV_1 compared with Puerto Rican carriers of the minor allele.[152]	The study was adequately described.	Although the study was adequately described, it is the only study on Caucasian children and should be replicated	The study should be replicated and performed on other ethnic groups.
ALOX5 rs59439148	Children with the X/X genotype had reduced lung function compared with children with X/5 or 5/5 genotype.[162]	The study was adequately described.	Although the study was adequately described, it is the only study on Caucasian children and should be replicated	The study should be replicated and performed on other ethnic groups.
CHI3L1 rs4950928	Children homozygous for the minor allele had reduced risk of hospital admissions compared with children with at least one wild-type allele.[131]	The study was adequately described	Although the study was adequately described, it is the only study on Caucasian children and should be replicated	The study should be replicated and performed on other ethnic groups.

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Table 2.3 – continued from previous page

Gene - SNPs	Summary	Adequacy of evidence	Gaps	Next steps
IL-4Rα rs1801275	The minor allele was associated with difficult asthma.[120]	The study was adequately described.	Although the study was adequately described, it is the only study on Caucasian children and should be replicated	The study should be replicated and performed on other ethnic groups.
TNFα rs1800629	The minor allele was associated with difficult asthma.[120]	The study was adequately described.	Although the study was adequately described, it is the only study on Caucasian children and should be replicated	The study should be replicated and performed on other ethnic groups.
ST13 rs138335, rs138337	The minor allele was associated with an increased risk of asthma hospital admissions and oral corticosteroids use compared with the wild-type allele.[170]	The study was adequately described.	Although the study was adequately described, it is the only study on Caucasian children and should be replicated	The study should be replicated and performed on other ethnic groups.
THRB rs892940	The minor allele was associated with increased BDR compared with the wild-type allele.[159]	The study was adequately described.	Although the study was adequately described, it is the only study on Caucasian children and should be replicated	The study should be replicated and performed on other ethnic groups.

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Table 2.3 – continued from previous page

Gene - SNPs	Summary	Adequacy of evidence	Gaps	Next steps
IL-13 rs1800925	White children homozygous on ICS for the minor allele had an increased risk of asthma exacerbations compared to children with the wild-type allele.[140] Among individuals homozygous, 77% were LTRA responders against 52% of individuals with at least one copy of the wild-type allele.[143] No association between IL-13 and difficult-to-treat asthma.[120]	The study that did not find an association was adequately described.[120] The two other studies inadequately described the results.[140,143]	The current evidence is unclear regarding the true effect of IL-13.	Replication studies should be performed and the results adequately described.
GLCCI1 rs37972	Children homozygous for the minor allele had reduced change in FEV_1 compared with children homozygous for the wild-type allele.[155] Two studies found no association.[167,171]	One study that did not find an association was adequately described,[167] one study inadequately described the methods[171] and the other study inadequately described the results.[155]	The studies were only performed in White populations	This association should be tested in different ethnic groups.

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Table 2.3 – continued from previous page

Gene - SNPs	Summary	Adequacy of evidence	Gaps	Next steps
LTC4S - rs730012	One study found that montelukast reduced FeNO levels for heterozygotes compared with homozygous for the wild-type allele.[135] Two other studies found no association between the polymorphism and the clinical responsiveness to LTRA[141,154]	The three studies were inadequately described. Two studies inadequately described the results,[135,154] while one study inadequately described the methods and results.[141]	Given the low quality of the studies involved, it is still unclear the true association between LTC4S and clinical responsiveness to LTRA.	Replication studies in different ethnic groups are needed to understand the effect of LTC4S.
CRHR-1 - rs242941	One study found that children homozygous for the minor allele had increased FEV_1,[136] while another study found that children homozygous for the minor allele had reduced BDR.[150] Two studies found no association.[142,171]	Two studies inadequately described the methods,[150,171] one study inadequately described the results,[136] while another study inadequately described the results and methods.[142]	Beside the lack of adequate studies, these articles reported varying results regarding the clinical response to ICS according to CRHR-1.	Replication studies in different ethnic groups are needed to understand the effect of CRHR-1.
TBX21 rs2240017	Individuals homozygous for the minor allele had increased airway responsiveness compared with homozygous for the wild-type allele.[137] One study did not find an association[171]	One study inadequately described the results[137] and one study inadequately described the methods.[171]	Results should be replicated due to the lack of agreement and poor reporting of results.	Replication studies in different ethnic groups are needed to understand the effect of TBX21.

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Table 2.3 – continued from previous page

Gene - SNPs	Summary	Adequacy of evidence	Gaps	Next steps
CRHR-2 rs255100, rs7793837	Children with at least one copy of the minor allele were associated with reduced BDR compared to children with two copies of the wild-type allele.[146] One study did not find an association.[145]	Both studies inadequately described the results.[145,146]	Results should be replicated due to the lack of agreement and poor reporting of results.	Replication studies in different ethnic groups are needed to understand the effect of CRHR-2.
CTLA4 rs3087243	Children carriers of the minor allele had reduced FEV_1 compared with children carriers of the wild-type allele.[151]	The study inadequately described the results.	This study did not adequately described the results and was not replicated, hence it is still unclear the effect of this SNPs on the clinical responsiveness to ICS	Replication studies should be performed, including different ethnic groups.
DUSP1 rs881152, rs34507926, rs7702178, rs3805476	These SNPs were associated with clinical responsiveness to ICS in Puerto Ricans.[153]	The study inadequately described the results.	This study did not adequately described the results and was not replicated, hence it is still unclear the effect of this SNPs on the clinical responsiveness to ICS	Replication studies should be performed, including different ethnic groups.
PTGDR rs803010	The presence of the minor allele was associated with poor response to LTRA.[154]	The study inadequately described the results.	This study did not adequately described the results and was not replicated, hence it is still unclear the effect of this SNPs on the clinical responsiveness to ICS	Replication studies should be performed, including different ethnic groups.

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Table 2.3 – continued from previous page

Gene - SNPs	Summary	Adequacy of evidence	Gaps	Next steps
NOS3 G894T	Children homozygous for the minor allele had increased FEV_1 change, after LABA with ICS, compared with children homozygous for the wild-type allele.[157]	The study inadequately described the results.	This study did not adequately described the results and was not replicated, hence it is still unclear the effect of this SNPs on the clinical responsiveness to ICS	Replication studies should be performed, including different ethnic groups.
HDAC1 rs1741981	Children homozygous for the minor allele had reduced FEV_1 changes in response to ICS, compared with children with at least one wild-type allele.[160]	The study inadequately described the methods.	This study did not adequately described the results and was not replicated, hence it is still unclear the effect of this SNPs on the clinical responsiveness to ICS	Replication studies should be performed, including different ethnic groups.
FBXL7 rs10044254	Children homozygous for the minor allele of these SNPs had increased asthma symptom scores compared with children with a wild-type allele.[165]	The study inadequately described the results.	This study did not adequately described the results and was not replicated, hence it is still unclear the effect of this SNPs on the clinical responsiveness to ICS	Replication studies should be performed, including different ethnic groups.
RMST rs1558726	Children homozygous for the minor allele of these SNPs had increased asthma symptom scores compared with children with a wild-type allele.[165]	The study inadequately described the results.	This study did not adequately described the results and was not replicated, hence it is still unclear the effect of this SNPs on the clinical responsiveness to ICS	Replication studies should be performed, including different ethnic groups.

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Table 2.3 – continued from previous page

Gene - SNPs	Summary	Adequacy of evidence	Gaps	Next steps
LOC728792 rs2388639	Children homozygous for the minor allele of these SNPs had increased asthma symptom scores compared with children with a wild-type allele.[165]	The study inadequately described the results.	This study did not adequately described the results and was not replicated, hence it is still unclear the effect of this SNPs on the clinical responsiveness to ICS	Replication studies should be performed, including different ethnic groups.
CTNNA3 rs1786929	Children homozygous for the minor allele had a decreased FEV_1 response compared with children with the wild-type allele.[166]	The study inadequately described the results.	This study did not adequately described the results and was not replicated, hence it is still unclear the effect of this SNPs on the clinical responsiveness to ICS	Replication studies should be performed, including different ethnic groups.
MLLT3 rs6475448	Children homozygous for the minor allele had increased FEV_1 change compared with children with the wild-type allele.[168]	The study inadequately described the methods.	This study did not adequately described the methods and was not replicated, hence it is still unclear the effect of this SNPs on the clinical responsiveness to LTRA	Replication studies should be performed, including different ethnic groups.
ASB3 rs350729, rs1840321, rs1384918, rs1319797	The presence of the minor alleles reduced the BDR by 20%.[169]	The study inadequately described the methods	This study did not adequately described the methods and was not replicated, hence it is still unclear the effect of this SNPs on the clinical responsiveness to LTRA	Replication studies should be performed, including different ethnic groups.

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Table 2.3 – continued from previous page

Gene - SNPs	Summary	Adequacy of evidence	Gaps	Next steps
NR3C1 rs41423247	Children homozygous for the wild-type allele had increased FEV_1 change compared to children with a minor allele[171]	The study inadequately described the methods.	This study did not adequately described the methods and was not replicated, hence it is still unclear the effect of this SNPs on the clinical responsiveness to ICS	Replication studies should be performed, including different ethnic groups.
rs10484568 (nearby HLA)	The polymorphism was associated with FEV_1 changes.[172]	The study inadequately described the results.	This study did not adequately described the results and was not replicated, hence it is still unclear the effect of this SNPs on the clinical responsiveness to ICS	Replication studies should be performed, including different ethnic groups.
rs72821893 (nearby KRT25)	The polymorphism was associated with FEV_1 changes.[172]	The study inadequately described the results.	This study did not adequately described the results and was not replicated, hence it is still unclear the effect of this SNPs on the clinical responsiveness to ICS	Replication studies should be performed, including different ethnic groups.

Table 2.3: Summary of the genetic associations found

Chapter 3

Hypotheses and Aims

Filaggrin (FLG) study:

As mentioned in section 1.4, Filaggrin (FLG) mutations have been associated with a higher risk of developing eczema, allergic sensitization, allergic rhinitis and asthma.[94,174,175] However, the role of FLG mutations on healthcare outcomes is unclear. The hypothesis tested in section 5.1 is that individuals with FLG mutations were dispensed more prescriptions for eczema, asthma, allergic reactions and allergic rhinitis than individuals without these mutations, translating into a higher cost for the National Health Service (NHS). Therefore, the aims of this study were:

1. To investigate whether patients carrying FLG mutations were dispensed more eczema-related prescriptions than patients without FLG mutations;
2. To determine if patients carrying FLG mutations were dispensed more asthma-related prescriptions and had more asthma exacerbations than patients without FLG mutations;
3. To examine whether patients carrying FLG mutations were dispensed more Adrenaline Auto-Injector (AAI) devices and allergic rhinitis prescriptions than patients without FLG mutations;
4. To explore whether differences in prescribing for patients with and without FLG mutations were associated with different healthcare costs.

Adrenoreceptor β_2 (ADRB2) study:

Chapter 2 provided an overview of the Single nucleotide polymorphisms (SNPs) associated with cinical responsiveness and asthma severity. The Arg16 and Glu27 variants of the Adrenoreceptor β_2 (ADRB2) gene have been shown in some studies to play a role in the clinical response to Short-acting β_2-agonists (SABA) and Long-acting β_2-agonists (LABA). However, the role of these two variants on asthma prescribing has not been described previously. The hypothesis tested in section 5.2 is that individuals with one or more copies of the Arg allele and the Gln allele were dispensed more asthma-related prescriptions than individuals with one or more copies of the Gly allele and the Glu allele, translating into a higher cost for the NHS. The aims of this study were:

1. To investigate whether carriers of the Arg allele were dispensed more long-term asthma-related prescriptions and had more asthma exacerbations than carriers of one or two copies of the Gly allele;
2. To determine whether carriers for the Gln allele were dispensed more long-term asthma-related prescriptions and had more asthma exacerbations than carriers of one or two copies of the Glu allele;
3. To explore whether differences in prescribing for individuals with and without mutations were associated with different healthcare costs.

Fc fragment of IgE receptor II (FCER2) study:

In chapter 2, the minor allele in the Fc fragment of IgE receptor II (FCER2) variant was shown to be associated with asthma exacerbations and clinical response to Inhaled Corticosteroid (ICS).[81,83] However, despite the role of FCER2 on atopy, the role of FCER2 gene mutations on healthcare outcomes is unclear. The hypothesis tested in section 5.3 is that individuals with one or more copies of the C allele were dispensed more prescriptions for eczema, asthma, allergic reactions and allergic rhinitis than individuals with one or more copies of the T allele, translating into a higher cost for the NHS. Hence, the aims of this part of the study were:

1. To investigate whether carriers of the C allele of the FCER2 variant were dispensed more prescriptions for eczema than carriers of the T allele;

2. To determine whether carriers of the C allele of the FCER2 variant were dispensed more prescriptions for asthma and had more asthma exacerbations than carriers of the T allele;
3. To examine whether carriers of the C allele of the FCER2 variant were dispensed more prescriptions for allergic reactions and allergic rhinitis than carriers of the T allele;
4. To explore whether differences in prescribing for individuals with and without these mutations were associated with different healthcare costs.

Public Engagement:

The final aim of this thesis relates to the communication of this research to the public. The aims of the public engagement activities were:

1. to convey to children and parents the concept of personalised medicine and how it may translate into clinical practice with particular relevance to this research;
2. to understand the parents attitudes regarding genetic testing and their willingness to allow their child to be tested.

Section 5.5 will provide the results obtained during the public engagement activities.

Chapter 4

Methodology

4.1 Data Linkage

For this thesis, the focus is on data linkage in health to generate population-based longitudinal data. Data linkage is the task of finding records, of a single individual, across different databases. Through data linkage, it is possible to augment the findings from a cross-sectional database, like BREATHE, into a longitudinal study, as demonstrated in figure 4.1.

Dundee is one of the four Centres of Excellence in research linking electronic health data in the United Kingdom (UK).[176] The Health Informatics Centre (HIC) is a research unit within the University of Dundee collaborating with National Health Service (NHS) Tayside and NHS Fife. HIC collects and manages high-quality data for the population of Tayside and Fife, approximately 16% of the Scottish population, covering community-dispensed prescriptions, hospital stays, diagnosis and interventions, laboratory tests and deaths. The linkage is done using mainly the Community Health Index (CHI) number, which is an identifier similar to the NHS number in England. The CHI number has 10 digits, the first 6 digits correspond to the date of birth of the individual, followed by 2 random digits, a digit indicating the gender of the person and a further digit corresponding to an internal check by HIC. To preserve the confidentiality and security of the data, HIC performs several steps. HIC act as a trusted third party by linking the data and/or databases required by the researchers, giving the researchers access to Safe Haven where the data is securely

stored. Each dataset is anonymised using a random identifier to replace the identifiable CHI number, to enable the researcher to link datasets and analyse the data. All the other individual identifiers such as name, address, birthdate, and postcode are removed from the datasets. HIC further ensures the confidentiality of each patient by reviewing each output produced by the researcher, ensuring confidential information is not exported from the system.[177]

Records are linked through two different methods: deterministic - where the data can be matched or unmatched based on unique identifiers, like the CHI number; and probabilistic when unique identifiers are not available, and records are linked based on a combination of variables, such as name, gender, birthdate and/or address. A combination of these two methods is usually used and was used by HIC to link BREATHE to routine healthcare databases.[177,178]

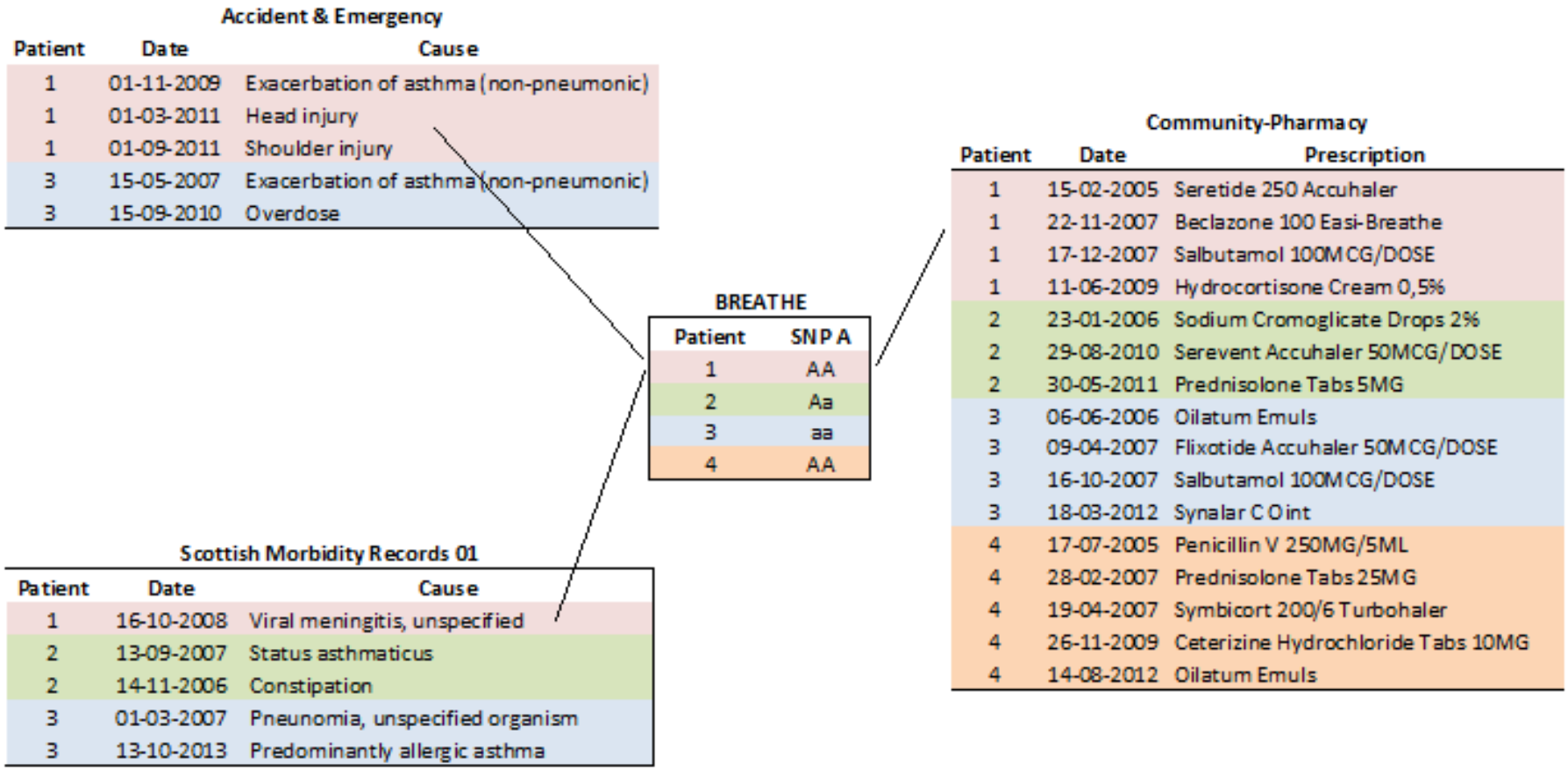

Figure 4.1: An example of the second step of the data linkage process

4.2 BREATHE

BREATHE was the name given to a cross-sectional study looking at gene-environment associations with asthma severity in Tayside and Fife, Scotland, between 2003 and 2005.[71,179] BREATHE contains clinical and genetic information on 1100 children and young adults, aged between 3 and 22 years of age, with a physician diagnosis of asthma. More specifically, the database contains demographic information, such as the CHI number, birthdate, gender, postcode address, height and weight of the children, family history of asthma, self-report of rhinitis and eczema, exposure to smoke, presence of household animals, and information about allergies and asthma triggers. Results of lung function tests were also recorded where available. Information about medicine used at the time of the study was also recorded. The database also captured asthma-related school absence and adequacy of inhaler technique. Participants provided a saliva sample so that genotyping could be performed. The genes genotyped for BREATHE included the Glutathione S-Transferase Mu 1 (GSTM1) and Glutathione S-Transferase θ 1 (GSTT1),[180] Fc fragment of IgE receptor II (FCER2),[81] Suppression of Tumorigenicity 13 (ST13), Nuclear Receptor subfamily 3 group C member 1 (NR3C1), Heat Shock 70 kDa Protein 4 (HSPA4), FK506 Binding Protein 4 (FKBP4), Serpin Family A member 6 (SERPINA6), CREB Binding Protein (CREBBP), TATA-Box Binding Protein (TBP), Nuclear Receptor Coactivator 3 (NCOA3), SMAD Family Member 3 (SMAD3), Arginase 1 gene (ARG1), Interleukin 2 Receptor Subunit β (IL-2Rβ), Interleukin 18 Receptor 1 (IL-18R1) and Phosphodiesterase 4D (PDE4D),[170] Adrenoreceptor β_2 (ADRB2),[130] Filaggrin (FLG),[72] Glucocorticoid induced 1 (GLCCI1),[167] Chitinase 3 Like 1 (CHI3L1),[131] Matrix Metalloproteinase 12 (MMP12),[125] ORMDL sphingolipid biosynthesis regulator 3 (ORMDL3),[128] and Matrix Metalloproteinase 9 (MMP9), Matrix Metalloproteinase 3 (MMP3), Transmembrane Protein 79 (TMEM79), Cadherin Related Family Member 3 (CDHR3), Nuclear Factor Erythroid 2 Like 2 (NRF2) and Vascular endothelial growth factor A (VEGFA).

For this PhD, a collaboration with the HIC in Dundee, Scotland, was developed offering the opportunity of linking, for the first time, the BREATHE database with

several routine healthcare databases from Accident & Emergency (A&E) attendances, community-pharmacy and the Scottish Morbidity Records (SMR)-01, containing details of acute hospital admissions. HIC performed the first step of the data linkage. Each participant in BREATHE had a CHI number and was matched to the mentioned databases. If the CHI number was missing, HIC tried to identify the corresponding CHI number using a set of unique identifiers such as name, gender, birthdate and postcode. For each database, HIC created a file containing only the information about the participants in BREATHE with a random identifier (study number). Confidential information such as the CHI number, birthdate, name, and postcode, were removed from the databases before HIC gave the researcher access to the databases.

4.2.1 Ethics

Written informed consent was obtained from the patient and/or parent/guardian in the BREATHE study, as relevant, in compliance with the Helsinki accord.[181] The BREATHE study and subsequent analysis were approved by the Tayside Committee on Medical Research and Ethics.[181] Duncan Heather, HIC Finance and Governance Manager, confirmed that ethics was not needed for the data linkage since all the databases provided by HIC were anonymised and permission for data linkage had been obtained. Duncan Heather has also received local NHS Research & Development approval for the data linkage as an HIC sponsored study.

4.2.2 Accident & Emergency (AE) databases

BREATHE was linked to two A&E databases, one from Tayside and Fife and another one from Tayside only. The Tayside and Fife database contains the cause of admission as related by the parents, and the Tayside database contains the final diagnosis made by a physician. To improve the quality of the analyses, only the database with the final diagnosis was considered.

The A&E Tayside database has information on 776 of the 1100 children in the BREATHE study, between 2005 and 2013. Most individuals did not attend A&E due

to asthma. A small proportion of these patients (n=158, 20%) had an asthma exacerbation leading to an A&E visit. The database contains information about the reason to visit A&E and the final diagnosis made during the A&E admission. The arrival and discharge dates were also recorded.

The analyses in this thesis, with respect to the A&E databases, will be focused on asthma-related admissions. The causes considered were described by "Exacerbation of asthma (non-pneumonic)", whether as the primary cause or not. "Exacerbation of asthma (non-pneumonic)" correspond to the term used in the database by HIC.

4.2.3 Scottish Morbidity Records (SMR)-01

The SMR-01 database has hospital inpatient admission data on 941 of the 1100 children in the BREATHE study between 2005 and 2013. SMR-01 has information about the hospital speciality the patient attended, the pattern of bed usage, the admission and discharge date and the type of admission - for instance, an emergency or a routine admission. The main medical condition during the patient stay was also provided. This condition is coded according to the International Classification of Disease (ICD)-10 or -9 depending on the year of admission; the ICD is a system of coding created by the World Health Organization (WHO) to classify diseases, symptoms and causes of injury. Besides the main condition, the database also has information about other conditions that co-existed or developed during the patient stay in hospital. If operations were performed the date and operation(s) were also registered.

The analyses in this thesis, with respect to the SMR-01 database, will be focused on asthma-related admissions. The causes considered were described as: "Other and unspecified asthma" (ICD: J45.9), "Predominantly allergic asthma" (ICD: J45.909) and "Status asthmaticus" (ICD: J45.902), whether as the primary cause for hospital admission or not. All of the terms in the database correspond to ICD codes, so these terms should capture the majority of asthma-related hospitalisations.

4.2.4 Community prescribing

The community prescribing database corresponds to the pharmacy records of all of the 1100 children and young adults in BREATHE, from Tayside and Fife. This database contains information on community-dispensed prescriptions, prescriptions issued by a General Practitioner (GP), and prescriptions written in the hospital that were dispensed in the community. Prescriptions made by the hospital staff that were dispensed within the hospital are not included in this database.

The prescription database contains the pharmaceutical and generic name of the medication prescribed, as well as the date. However, the doctor recommendation concerning the number of puffs and the daily frequency was not available.

Between 1993 and 2004, HIC had been collecting paper prescriptions, mainly from British National Formulary (BNF) categories of interest (Asthma was one of them but eczema was not) and entering them into the system manually. However, in 2003/2004 a backup failure caused a loss of information and while data for asthma may be completed before that time period, the continuous period of complete data for any disease started in 2005. For that reason, data were analysed longitudinally between 2005 and 2013. Since 2005, the prescription data was obtained electronically from the Practitioner Services Division, which was responsible for the processing and pricing of prescriptions across Scotland.

4.2.5 Deaths

BREATHE was also linked to two death registrars, the CHI death database, and the General Registration Office death database. The CHI database is maintained by HIC and contains demographic information about Tayside patients, including the date of death of individuals, but not the cause. This database is complemented by the General Registration Office database which contains the cause of death, but not always the date of death. In case of a mismatch in the date of deaths between the databases, the CHI date of death was used as per advice, due to the higher quality of the database. Both databases were used since the cause of death is only present in the General Registration Office death database. The records show that between

2005 and 2013, 8 children and young adults in BREATHE died, aged between 10 and 21 years. Two died from car collisions, two from asthma attacks (status asthmaticus), and one died from epilepsy. The cause of death for one BREATHE participant was described as "other-ill defined and unspecified cause of mortality". Two children were not present on the General Registration Office death database but were on the CHI database, which has no cause of death, hence the cause of death for these two patients was unknown. Of the 8 children and young adults who died, 3 were male and 5 were female, 6 had self-report eczema and one had self-report rhinitis. All of these individuals had one or more family members with asthma, 5 had one or more family members with eczema and 3 had one or more family members with rhinitis. Children who died are included in the analysis until the time of their death.

4.3 Statistical Analysis

4.3.1 Genetic associations

The main purpose of genetic associations studies lies in understanding whether one or more genotypes or alleles is associated with the clinical outcome of interest. An individual can have one of three possible genotypes: (i) wild-type - has two copies of the specified common allele, often termed AA, (ii) heterozygous - has one copy of the common allele and another of the minor allele Aa, or (iii) and homozygous - has two copies of the minor allele, termed aa. An analysis of genetic associations will explore the existence of an association between a clinical outcome and the presence of one or more genetic polymorphisms.

There are several types of genetic association studies. Genome-wide association studies (GWAS) examine Single nucleotide polymorphisms (SNPs) across the genome between healthy individuals and individuals with a disease, while candidate-gene studies are focused on genes previously connected to a disease due to biological plausibility of them being involved. BREATHE is a candidate-gene study, the researchers have genotyped several SNPs of interest, known to be associated with asthma or predicted to be associated with asthma clinical responsiveness due to

the biological function of the SNPs.

Candidate-gene studies often involve polymorphisms in Linkage Disequilibrium, since this reduces the number of SNPs genotyped and the associated cost. Linkage Disequilibrium corresponds to the non-random association of alleles between loci, i.e. the allele at one SNP is dependent on the allele at another SNP; this can occur even for SNPs on different chromosomes.

One of the first steps in analysing genetic associations is to perform a quality control of the genotype. Errors can occur during genotyping, and ignoring these errors may lead to decreased power in the statistical analysis and an increase in the number of false positive associations. First, one needs to understand the difference between "missing" and "no call". "Missing" would refer to an individual who did not supply a Deoxyribonucleic acid (DNA) sample to the study while "no call" will refer to the inability to ascertain the genotype for the sample. A high proportion of "no calls" suggests a problem with the samples, most likely due to low DNA concentrations. The first check relates to the performance of individual samples in the study. The second check relates to the ability of individual samples to be genotyped across several assays.

In this thesis, all samples with missing values or ’no call’ were excluded from the final analysis. Four FLG polymorphisms, common in a Caucasian population, R501X (rs61816761), 2281del4 (rs41370446), S3247X (rs150597413), R2447X (rs138726443), two polymorphisms in the ADRB2 gene Arg16Gly (rs1042713), Glu27Gln (rs1042714), and one variant in the FCER2 gene (rs28364072) were considered. Figure 4.2 displays the number of individuals removed.

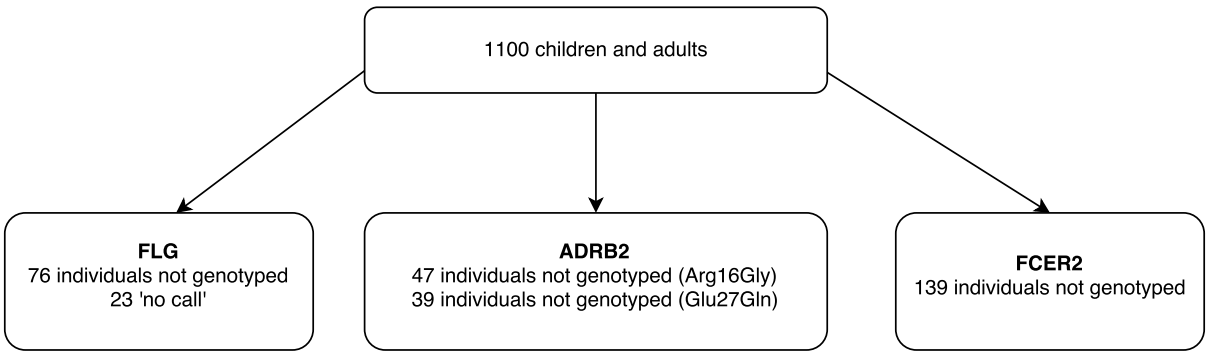

Figure 4.2: Flowchart of the genetic quality control

Each polymorphism considered had a genotype call rate of 99%.

Before starting the analysis, the researcher calculates the Minor Allele Frequency (MAF), i.e. the frequency of the rarest/minor allele, of each polymorphism, as defined by formula 4.1. A cut-off should be defined a priori, which is usually 5% or 1%. In this case, since FLG is a rare variant, every polymorphism analysed should have a MAF greater than or equal to 1%.

$$\text{MAF} = \frac{p_{aa} * 2 + p_{Aa}}{(p_{AA} + p_{Aa} + p_{aa}) * 2} \tag{4.1}$$

The MAF was calculated for every polymorphism analysed. R501X had a MAF of 5%, 2281del4 a MAF of 3%, and S3247X and R2447X a MAF of 1%. FLG polymorphisms are rare variants, therefore it is not surprising that the MAF was low. A meta-analysis by Baurecht et al.[182] estimated that allele frequencies for R501X ranged between 0.8% to 4.1% and for 2281del4 ranged between 0.5% and 6.6%. The frequency of combined FLG mutations, in this cohort, was 22.4% in individuals with eczema and asthma. Baurecht et al.[182] found a combined frequency of FLG mutations between 15.8% and 55.8%, with the highest frequencies in Ireland and the UK. The MAF of the Arg16Gly polymorphism was 37%, while for the Glu27Gln it was 44%. Litonjua et al.[76] reported the MAF for Arg16Gly to be around 39% in White Americans, 49% in Black Americans and 51% in Chinese. For the Glu27Gln variant, Litonjua et al.[76] reported the MAF to be around 25% in White Americans, 19% in Black Americans, and 9% in Chinese. The MAF for the FCER2 variant was 27%.

The final step of the quality control is the calculation of the Hardy−Weinberg equilibrium. The Hardy−Weinberg equilibrium theory states that the genotype frequency will remain constant across generations in the absence of evolutionary forces, which corresponds to the formula 4.2.

$$p_{aa} + p_{Aa} + p_{AA} = 1 \tag{4.2}$$

A deviation could be a sign of unstable allele frequency, which can be an indication of non-random mating, natural selection, or even problems in the genotyping. One of the most popular ways to test the Hardy−Weinberg equilibrium is to compare the observed genotype counts with values expected under the Hardy−Weinberg equilibrium and use a χ^2. The test statistic is the following:

$$\chi^2 = \sum \frac{(\text{Observed} - \text{Expected})^2}{\text{Expected}} \tag{4.3}$$

Observed	Expected
N_{AA}	$N(\frac{N_{Aa}+2N_{AA}}{2N})^2$
N_{Aa}	$N2(\frac{N_{Aa}+2N_{AA}}{2N})(1 - \frac{N_{Aa}+2N_{AA}}{2N})$
N_{aa}	$N(1 - (\frac{N_{Aa}+2N_{AA}}{2N})^2)$

The Hardy−Weinberg equilibrium was tested for each polymorphism analysed. All polymorphisms analysed in this thesis were in the Hardy−Weinberg equilibrium, except for R501X and S3247X FLG polymorphisms. However, variants associated with the occurrence of the disease will be overrepresented in patients, and may be expected to show a deviance from Hardy−Weinberg equilibrium. Therefore, these polymorphisms were not excluded from the analysis .

After the quality control, the data can be analysed. There are several modes of inheritance to consider. A dominant genetic model occurs when only one copy of the minor allele is required to cause an effect on the outcome, AA=0 vs. Aa+aa=1. A recessive genetic model occurs when two copies of the minor allele are required to cause an effect on the outcome, AA+Aa=0 vs. aa=1. An additive genetic model implies an additive effect on the outcome, i.e. it assumes a linear dose-response. The genetic model chosen will influence the approach to the analysis.[183,184] Another

option is to perform an analysis without making assumptions about the genetic model, also called a genotypic model, where the 3 genotypes AA, Aa and aa are treated as a factor with three levels. In most scenarios, the exact function and mechanism of the gene is not fully known. The huge number of polymorphisms in the human genome makes it difficult to understand all pathophysiological mechanisms influenced by these SNPs.[184] Therefore, the most common approaches are to assume an additive model or a model without assumptions, when exploring the role of SNPs. The additive genetic model performs well if the true mode of inheritance is dominant or additive, but it is underpowered if the true mode of inheritance is recessive.[184,185]

Several authors suggest testing the different inheritance modes to see which one fits the data better. However, there are several issues with this approach. One of them is multiple testing, which will be discussed in section 4.3.4. Another issue is relying on a statistical output to choose a true representation of the biological mechanism behind the possible phenotype.

Of the 978 patients with asthma included in the FLG analyses, only one individual was homozygous for the S3247X, six individuals were homozygous for the R501X, and two individuals were homozygous for the 2281del4. No individual was homozygous for more than one FLG polymorphism. Some individuals (n=10) were heterozygous for two polymorphisms. Hence, the four polymorphisms were combined into two categories: no FLG mutation vs. one or more FLG mutations. In the case of the ADRB2 variants and the FCER2 variant and for reasons described above, the data was analysed without assuming an inheritance model, i.e. a genotypic model.

4.3.2 Longitudinal studies

Data from a longitudinal study can be collected prospectively, following subjects forward in time, or retrospectively, by extracting multiple measurements on each person from historical records. Longitudinal data is characterised by the existence of between and within person variation, existence of correlation and dependency between observations from the same individual.

The outcomes will be discussed in section 4.3.5, and corresponds to the number of dispensed prescriptions and asthma-related hospitalisations. The methods available to analyse clustered count data are Generalised Estimated Equations and Generalised Linear Mixed Models. The Poisson model is the reference for modelling count data; however, the model has some assumptions, such as the equality of the mean and variance, which in real data is difficult to achieve since data are frequently over-dispersed and often have a large amount of zeros (so-called zero inflation). In the case of over-dispersion, when the variance exceeds the mean, a negative binomial distribution may be used. In the case of over-dispersion and a large number of zeros, Zero Inflated Models can be used. For this thesis, different statistical models were fitted to the data and diagnostics were assessed to choose the most adequate model. Time was added as a fixed variable into the model with a random effect, allowing each person to change over time at different rates.

A negative binomial model with a random effect for the patient was the most adequate model to assess the association between mutations and prescribing for eczema, asthma and allergic reactions and rhinitis, and asthma exacerbations. Incidence rate ratio (IRR) were estimated for all variables. Each of the models was adjusted for age, gender,[86,186–188] variant of interest, smoking status[44,49,50] or cat ownership.[20,44,49,50,52]

4.3.3 Model Diagnostics

Several measures were used to assess the quality of the model, such as the Variance Inflation Factor (multicollinearity) and residual analysis.

Multicollinearity corresponds to two or more predictor variables that are nearly perfectly correlated, leading to unstable estimates and inflated standard errors. The residual analysis provides information about the quality of adjustment of the model, the residual being the difference between the observed value and the estimated value.

4.3.4 Multiple testing

Several adjustments are available for multiple testing which will reduce the α level to produce the necessary protection against type I error, i.e., incorrectly rejecting a true null hypothesis (false positive). Some of the most common α adjustments are the Bonferroni and False Discovery Rate. However, these adjustments are mainly for fishing expeditions, which are very common in genomics. If theory and biological hypothesis are guiding the statistical methods, α correction may not be necessary. The adjustment reduces the statistical power since it will reduce the chances of finding a true effect, increasing type II error, i.e., failing to reject a false null hypothesis (false negative).[189,190]

In this thesis, no adjustments were made to P-values. All analyses were hypothesis-driven and the main focus was the magnitude of the effect size and the precision. Associations were categorised into weak-to-moderate and strong based on the IRR. An IRR between 0.5 and 2 was considered a weak-to-moderate association (excluding 1 of no association) and an IRR smaller than 0.5 or higher than 2 was considered a strong association.[191,192] The results were written guided by the Strengthening the reporting of genetic association studies (STREGA) statement and interpreted bearing in mind the Bradford Hill Criteria.[193,194]

4.3.5 Outcomes

One measure of assessing healthcare utilisation and costs is through dispensed prescriptions, which refers to a prescription issued by the physician and collected by the patient from a pharmacist. Prescriptions dispensed are a direct measure of the amount of medication collected for a patient and can track long-term healthcare dispensing.[195–197] HIC has tracked dispensed prescriptions in Tayside for several decades, thus providing a unique opportunity to explore differences in prescription utilisation according to the patient genotype for both eczema and asthma.

The outcomes analysed in this thesis correspond to the number of prescriptions dispensed per patient for each year. The analyses concerning individuals with eczema were performed on individuals with eczema and asthma, and the analyses

concerning individuals with asthma, acute allergic reactions and allergic rhinitis were performed on the entire cohort. Allergic rhinitis was examined in the entire cohort due to the discrepancy between self-reported rhinitis and the number of dispensed prescriptions for allergic rhinitis, while acute allergic reactions were possible within the entire cohort.

Eczema-related prescriptions

Table 4.1 presents the names of the medicines commonly prescribed for eczema and used in section 5.1.1 and 5.3.1. Prescriptions were divided with the help of a dermatologist and the BNF guidelines. The medicines were divided into emollients, antihistamines and prescriptions for mild, moderate and severe eczema. Treatment for mild eczema includes topical corticosteroids with mild strength with and without antibacterial or antifungal. Treatment for moderate eczema includes topical corticosteroids with moderate strength with and without antibacterial or antifungal, topical calcineurin inhibitors and antibacterials. Treatment for severe eczema also includes topical corticosteroids, with potent strength, with and without antibacterial or antifungal, systemic immunosuppressants and bandages.

Pharmaceutical names	**Formulation**
Antihistamines	
Alimemazine Tartrate	Oral
Cetirizine	Oral
Chlorphenamine Maleate	Oral
Desloratadine	Oral
Hydroxyzine Hydrochloride	Oral
Levocetirizine Dihydrochloride	Oral
Loratadine	Oral
Promethazine Hydrochloride	Oral
Emollients	
Emollients and Barrier Preparations	Topical
Liquid paraffin	Topical
Urea	Topical

Continued on next page

Table 4.1 – continued from previous page

Pharmaceutical name	Formulation
Zinc and Castor Oil	Topical
Treatment associated with mild eczema	
Topical corticosteroids	
Hydrocortisone	Topical
Topical corticosteroids with antibacterial/antifungal	
Hydrocortisone Nystatin Benzalkonium and Dimeticone	Topical
Hydrocortisone Nystatin and Chlorhexidine	Topical
Hydrocortisone with Clioquinol	Topical
Hydrocortisone with Fusidic acid	Topical
Hydrocortisone with Miconazole	Topical
Treatment associated with moderate eczema	
Antibacterial	
Fusidic Acid	Topical
Topical corticosteroids	
Betamethasone Esters	Topical
Clobetasone Butyrate	Topical
Fludroxycortide	Topical
Hydrocortisone with Urea	Topical
Hydrocortisone with Urea and Lactic Acid	Topical
Topical corticosteroids with antibacterial/antifungal	
Clobetasone with Oxytetracycline and Nystatin	Topical
Topical calcineurin inhibitors	
Pimecrolimus	Topical
Tacrolimus	Topical
Treatment associated with severe eczema	
Bandages	
Bandages	
Stockinette	
Topical corticosteroids	
Beclometasone Dipropionate	Topical
Betamethasone	Topical

Continued on next page

Table 4.1 – continued from previous page

Pharmaceutical name	Formulation
Betamethasone with Salicylic Acid	Topical
Clobetasol Propionate	Topical
Fluocinolone Acetonide	Topical
Fluticasone Propionate	Topical
Hydrocortisone Butyrate	Topical
Mometasone Furoate	Topical
with antibacterial/antifungal	
Betamethasone Esters	Topical
Betamethasone with Clioquinol	Topical
Betamethasone with Fusidic Acid	Topical
Betamethasone with Neomycin	Topical
Clobetasol with Neomycin and Nystatin	Topical
Fluocinolone Acetonide with Clioquinol	Topical
Fluocinolone Acetonide with Neomycin	Topical
Systemic Immunosuppressants	
Azathioprine	Oral
Ciclosporin	Oral
Methotrexate	Oral

Table 4.1: List of all eczema-related medicines

Six different analyses were performed, with the outcome being the total number of eczema-related prescriptions, the number of emollients, the number of antihistamines, the number of prescriptions for mild eczema, the number of prescriptions for moderate eczema, and the number of prescriptions for severe eczema. Models were adjusted for gender (Male vs Female), age (in years) and cat ownership (No vs Yes).

Infected eczema prescriptions

As mentioned in section 1.1.4, patients with severe eczema may improve with antibiotics. Hence, the association between FLG mutations, FCER2 genetic variation

and prescribing of antibiotics and antivirals for skin infection was explored.

The antivirals considered were Aciclovir (oral and topical), Penciclovir (topical) and Valaciclovir (oral), and the antibiotics considered for bacterial and fungal infections, including the medicines with an antibacterial or anti-fungal action are mentioned in table 4.1. Flucloxacillin (oral) and mupirocin (topical) were also added to the analysis since they are usually prescribed by dermatologists in case of infected eczema.

A model where the outcome was the number of antivirals dispensed for the skin was adjusted for age, gender and cat ownership. Another model where the outcome was the number of anti-bacterial antibiotics dispensed for the skin was adjusted for gender, age and cat ownership.

Asthma-related prescriptions

Table 4.2 presents the names of the medicines in the section 5.1.2, 5.2.1 and 5.3.2. Prescriptions were classified with the help of a respiratory physician and the BNF guidelines. The medicines were divided into relievers, which includes Short-acting β_2-agonists (SABA), and ipratropium bromide, Inhaled Corticosteroid (ICS), Long-acting β_2-agonists (LABA), combined LABA and ICS and Leukotriene Receptor Antagonist (LTRA). Long-acting anti-muscarinic (LAMA) controller medicine is used only in adults, therefore, the number of LAMA prescriptions in this cohort is small. Since LAMA controller prescriptions were dispensed only to 5 individuals, this group of medicine was not analysed.

In these analyses, six different analyses were conducted, with the outcome being the total number of asthma-related prescriptions, the number of reliever prescriptions, the number of ICS prescriptions, the number of LABA prescriptions, the number of combined LABA with ICS prescriptions, and the number of LTRA prescriptions. Models were adjusted for gender, age, smoking status (No vs Yes) and cat ownership.

Although the NHS recommends the prescribing of combined LABA and ICS, sometimes the physician prescribes these medications individually. To account for concurrent use of LABA and ICS, individual prescriptions of LABA and ICS dispensed in the same calendar year were treated as combined prescriptions of LABA and ICS. LABA prescriptions dispensed without a concurrent ICS prescription in a given year

Pharmaceutical name	Formulation
Relievers	
Atomisers Hand Operated	Inhaler
Ipratropium Bromide	Inhaler/Oral
Salbutamol	Inhaler/Oral
Terbutaline Sulfate	Inhaler
Long-acting anti-muscarinic	
Aclidinium Bromide	Inhaler
Tiotropium	Inhaler
Inhaled Corticosteroid	
Beclometasone Dipropionate	Inhaler
Budesonide	Inhaler
Ciclesonide	Inhaler
Fluticasone Propionate	Inhaler
Long-acting β_2-agonists	
Formoterol Fumarate	Inhaler
Salmeterol	Inhaler
Combination of LABA and ICS	
Flutiform (Fluticasone Propionate and Formoterol Fumarate)	Inhaler
Fostair (Beclometasone Dipropionate and Formoterol Fumarate)	Inhaler
Seretide (Salmeterol with Fluticasone Propionate)	Inhaler
Symbicort (Budesonide with Formoterol Fumarate)	Inhaler
Leukotriene Receptor Antagonist	
Montelukast	Oral
Zafirlukast	Oral

Table 4.2: List of all asthma-related medicines

were treated as separate LABA. ICS prescriptions without a concurrent LABA were treated as separate ICS.

Asthma-related A&E visits/admissions

The causes of hospital admissions considered are described in section 4.2.2 and 4.2.3. Inpatient hospital admissions and A&E admissions were grouped, analysed and treated as A&E visits/admissions for now on. Although it would be interesting to look at these two outcomes separately, the number of children that attended A&E was

less than 10%, while the number of hospital admissions was around 25%. It would still be possible to analyse them separately, but longitudinally the numbers would be low . The best solution was to aggregate them and analyse them together to improve the power of the analysis.

The number of asthma-related A&E visits/admissions was adjusted for age, gender, smoking status and presence of a cat.

Oral corticosteroids prescriptions

Acute asthma attacks are treated with short courses of oral prednisolone to reduce inflammation of the airways. The number of oral prednisolone prescriptions dispensed was adjusted for age, gender, smoking status and presence of a cat.

Asthma exacerbations

In this thesis, asthma exacerbations as per the European Task Force definition were utilised as an outcome measure. The number of oral prednisolone prescriptions dispensed per year was analysed with the number of hospitalisations and A&E visits per year.

Adrenaline Auto-Injector (AAI) and allergic rhinitis prescriptions

An individual with FLG-related eczema is more likely to develop allergic sensitization, allergic rhinitis and asthma.[94,174,175] Individuals with high levels of Immunoglobulin E (IgE) have an increased risk of developing allergies and it is known that FCER2 plays a role in IgE regulation. Thus, the association between FLG, FCER2 mutations and prescriptions for acute allergic reactions was explored. Prescribing for children with severe acute allergic reactions characteristically includes the use of Adrenaline Auto-Injector (AAI) (Anapen, Epipen, Jext and Minijet adrenaline). Prescribing for allergic rhinitis includes nasal antihistamines (Azelastine hydrochloride), nasal corticosteroids (such as budesonide, mometasone furoate, betamethasone, beclometasone dipropionate, fluticasone propionate, fluticasone furoate and triamcinolone acetonide), nasal ipratropium bromide and nasal sodium cromoglicate.

Section 5.1.3 and 5.3.3 explored the association between FLG and FCER2 and the number of AAI and allergic rhinitis prescriptions.

4.3.6 Variables

Each variable included in the model has been previously associated with asthma and/or eczema susceptibility or severity, as discussed in the introduction 1.1.2 and 1.2.2. An exploratory analysis was performed with several variables, including information about breastfeeding, birthweight and mode of delivery. However, since the percentage of missing data was higher than 90% these variables were excluded from subsequent analyses. Family history of eczema and asthma was not included in the model because there was no visual difference between the number of prescriptions dispensed in those with a family history of the disease and those who did not.

Age

Due to confidentiality issues, HIC removed the birthdate from the BREATHE file. However, the file still contained the date when the patient was interviewed and the age at that point in time. Therefore, through these two variables it was possible to calculate the age of the patient throughout the period of the study. Age was included in the analyses as a continuous variable.

Eczema and asthma are predominantly childhood diseases and although the disease can manifest during adulthood, the number of affected individuals usually decreases over the years. The association between age and the number of prescriptions was explored, to test the hypothesis that the number of prescriptions per year decreases as the child gets older.

Gender

When analysing the number of eczema-related prescriptions gender was included in the model. Gender was included in the FLG analyses to study whether gender was associated with prescribing for asthma, acute allergic reactions and allergic rhinitis.

Cat

Cat ownership was only reported during data collection, between 2003 and 2005. No follow-up was made to check whether the patient continued to live with a cat or not. The variable cat ownership was included in the longitudinal analysis, assuming that

individuals who own a cat between 2003 and 2005 were more likely to have experienced the effects of cat exposure. Obviously, this has limitations which will be considered in the discussion.

Smoking status

Individuals who smoke or are exposed to smoke have been widely reported to have an higher risk for several diseases, such as asthma and allergies.

This variable may change over time, however, an assumption was made that individuals who smoked and/or were exposed to smoke during data collection were more likely to continue to be exposed to such an environment. The limitations of this assumption will be considered in the discussion.

4.3.7 Loss to follow-up

Although routine healthcare databases were not built for the purpose of research, they can provide information about the history of a disease and they reduce the loss to follow-up, recall and non-response bias.

Nevertheless, patients can be lost to follow-up if they move from one country to another. HIC observes patients across different health boards in Scotland. To estimate the amount of time an individual remained in Tayside, HIC created a residency database with information about individuals leaving Tayside or evidence of appearance in other health boards. However, HIC does not have information about individuals who move outside Scotland.

4.4 Pharmacoeconomics

The analysis performed in chapter 5 describes different prescribing patterns according to the genotype of the patient. Prescribing was not influenced by the GP's knowledge of the genotype since the physicians were unaware of the genetic profile of the patient. Therefore, a cost minimization analysis was performed to understand whether individuals with mutations cost more to the healthcare system than

individuals without mutations.

A cost analysis was performed on the prescribed medication based on Scottish (Information Services Division) prices obtained from 2005 to 2013, expressed in 2014 pound sterling. LABA and ICS prescriptions dispensed in separate inhalers were not grouped for this analysis since inhalers of different formulations have different prices and it is not possible to ascertain which inhaler was prescribed in combination and which was prescribed in separate. For that reason, prices of separate inhalers for ICS and LABA were calculated separately, which represents a limitation of this analysis, since it can overestimate the cost for separate LABA and separate ICS, and underestimate the cost for combined LABA and ICS. Costs for hospital admission were only obtained for Fife in 2015. An assumption was made that prices between Tayside and Fife would not vary significantly over the 9 year period. To estimate the difference in costs for asthma exacerbation, only individuals admitted to the hospital with an asthma-related event as the main cause were considered. Unfortunately, access to a linked GP database was outside the scope of the PhD. Nevertheless, an assumption was made that each course of oral corticosteroids was associated with a GP consultation, to get an estimate of primary care emergency costs. However, the Information Services Division does not release data on the average price of a GP consultation in Scotland. For that reason, the cost of an emergency GP consultation was based on a published national average based on an 11.7 minute consultation.[198] Although, the price may differ from reality, it still brings insight into potential differences in the emergency GP consultations. A Bias corrected and accelerated (BCa) bootstrapped t-test was used to obtain a BCa Confidence interval (CI) for the means.[199]

Appendix A presents the costs of the prescriptions over the years and the hospitalisation costs. Table 1 presents the cost of the eczema-related prescriptions used in the analyses, Table 1 contains the prices for the corticosteroids with antibacterials/antifungals and Table 2 contains the prices for the remaining antivirals for the skin and bacterial skin antibiotics. Table 3 presents the cost of the asthma-related prescriptions. Table 4 contains the prices for the AAI and table 5 contains the prices for the allergic rhinitis prescriptions dispensed. Table 6 contains the price of the oral prednisolone used in the analyses and Table 7 describes the

costs of hospital admissions.

All statistical analysis were performed using Stata 14[200] and R 3.2.2.[201]

4.5 Communicating personalised medicine to children and parents

Planned activities

Two public engagement sessions were performed during the time frame of this PhD. The first session was incorporated into the 2016 Brighton Fringe festival and run by Dr. Christina Jones and Prof. Somnath Mukhopadhyay. Medical students at the Brighton & Sussex Medical School and junior doctors in Brighton & Sussex University Hospitals NHS Trust developed and delivered the activities to children and parents. The second session was conducted in two primary schools, in a rural village in Bajouca, Portugal. A number of adults and primary school teachers helped to set up the activities and engage with children. Parents signed a consent form authorising pictures and videos to be taken during the activities. The consent forms are presented in Appendix 6.8.

In both sessions, the aim was to teach children and their parents about personalised medicine, specifically about the association between allergens and eczema and the clinical responsiveness to β_2-agonists.[71,124,126,130,179] For this purpose, two activities were set-up. In one activity, called 'healing babies', children acted as doctors and treated dolls. Two dolls were presented with red marks indicating rash made from two different substances (lipstick and nail polish). Two 'medicines' were available to heal the dolls; medicine A was nail polish remover, which removed nail polish but not lipstick, and medicine B corresponding to makeup remover, which removed lipstick but not nail polish. The aim of this activity was to show children that individuals with what looks like the same or very similar disease may not respond to the same medication, and that individual A may respond to medication that is different from individual B, despite both A and B suffering from an apparently similar disease. Figure 4.3 A demonstrates the outline of the 'healing babies' activity. The aim of the

second activity, called 'skin barriers', was to illustrate the role of genetics in the development of eczema, allergy and asthma. This refers, more specifically, to the role of FLG mutations as described more fully in the Introduction section of this thesis. Figure 4.3 B shows the outline of the 'skin barriers' activity. In this activity, a sieve acted as the model for skin. Two sieves were available for the demonstration, one of them covered with plastic. In one, the sand would go through the sieve, and in the covered one, the sand would not go through the sieve. The sieve would represent the skin of children with or without barrier defects and the sand would represent allergic and other triggers penetrating the epidermal barrier. The covered sieve would represent the effect of normal skin that has effective barrier function or the skin of children with FLG defects that has been treated with emollients. In the latter situation, the skin is impermeable thus preventing the sand (representing the allergic and other triggers) from entering the skin. These two activities were developed by Dr. Christina Jones and Prof. Somnath Mukhopadhyay. The activities were developed for the 2016 Brighton Fringe festival outside the scope of this PhD, but were replicated in Portugal in similar conditions.

Figure 4.3: Outline of the activities performed in Brighton and Bajouca. **A**, 'healing babies' activity. **B**, 'skin barriers' activity.

Two further activities were performed in Portugal. The reasons for including these activities were mainly cultural. Primary school children, in Portugal, have a rather basic knowledge of science. Therefore, it was assumed that the children would have little to no knowledge of genetics. Hence, there was a need to convey basic ideas

about genetics. An introduction was made prior to the activities, where it was explained how children can inherit physical traits from their parents and some diseases, such as asthma. In that sense, one of the activities performed was a "genetic tree", with examples of inherited physical traits, such as the ability to curl the tongue, the presence of freckles and/or curly hair. The aim of this activity was to show the children that we inherit some diseases and visible traits, from our parents. At the end of the activity, the teachers kept the "genetic tree" of each group. Bearing in mind that asthma prevalence is lower in Portugal compared to the UK (7%[202] vs 15%) , there was a need to explain what asthma is and how the disease impacts the life of individuals with asthma individuals. To provide a better understanding of "breathing difficulty", a PowerLung, a device that restricts the amount of air exhaled and inhaled, was used and adjusted to simulate an asthma attack. Hence the second activity performed was "asthma inhalers", with placebo asthma inhalers and a PowerLung. A balloon was attached to the device, so children could measure the amount of air exhaled.

The other activity was a 'genetic tree', with examples of inherited physical traits, such as the ability to curl the tongue, the presence of freckles and/or curly hair. The aim of this activity was to show the children that we inherit some diseases and visible traits, from our parents. Figure 4.4 A demonstrates the outline of the 'genetic tree' activity. The second activity performed was 'asthma inhalers', with placebo asthma inhalers and a PowerLung, a device that restricts the amount of air exhaled and inhaled, simulating an asthma attack. A balloon was attached to the device, so children measure the amount of air exhaled. Figure 4.4 B shows the outline of the 'asthma inhalers' activity.

Evaluating feedback

At the end of the Brighton session, children and parents who consented to be videotaped were interviewed about the activities. Medical students who helped in the activities were also interviewed to understand their points of view about public engagement. In Bajouca, all children and parents were invited to the activities and to sign a consent form allowing pictures to be taken. The activities took place after school time, as an extra-curricular activity. At the end of the session, children were

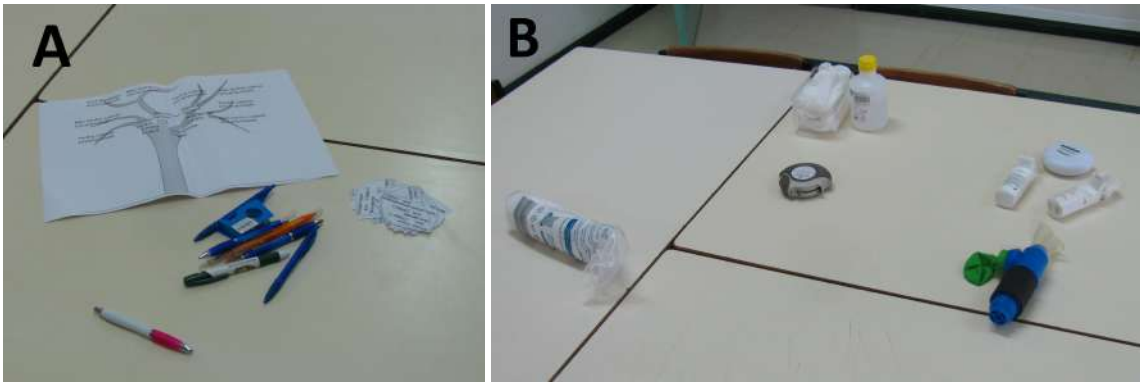

Figure 4.4: Outline of the activities performed in Bajouca. **A**, 'genetic tree' activity. **B**, 'asthma inhalers' activity.

interviewed and parents and teachers filled in a feedback questionnaire (see appendix 6.8). These public engagement interviews and questionnaires were not deemed to be research but as a tool to communicate the notion of personalised medicine with particular relevance to the work described in this thesis, and understand parents' and children's points of views about personalised medicine and genetic testing.

Chapter 5

Results

5.1 Understanding the association between filaggrin (FLG) gene variation and healthcare utilisation

Demographics

Of the 1100 children and young adults in BREATHE, 23 individuals were excluded due to genotyping failure and 99 were excluded due to missing information on one or more of the clinical variables considered. The final dataset for Filaggrin (FLG) analyses consists of 978 children and adults with asthma. Five hundred and thirty participants (54.2%) reported that they had eczema (see figure 5.1 for a flowchart of the sample size).

Of the 530 children with reported eczema and asthma, 43 (8.1%) had not had an eczema-related prescription during the 9-year period of study. Conversely, it is possible that some children reported not to have eczema at the time of their initial research consultation could have developed eczema over the period of follow-up. For this reason, the pattern of dispensed prescriptions was also analysed for participants who had not been reported to have eczema. Over the 9-year period, 2 individuals were most likely misclassified. In these individuals, while the carers had not reported eczema, the participants had a large number of dispensed treatments for eczema. One of the two patients had an FLG mutation and was dispensed 607 eczema-related

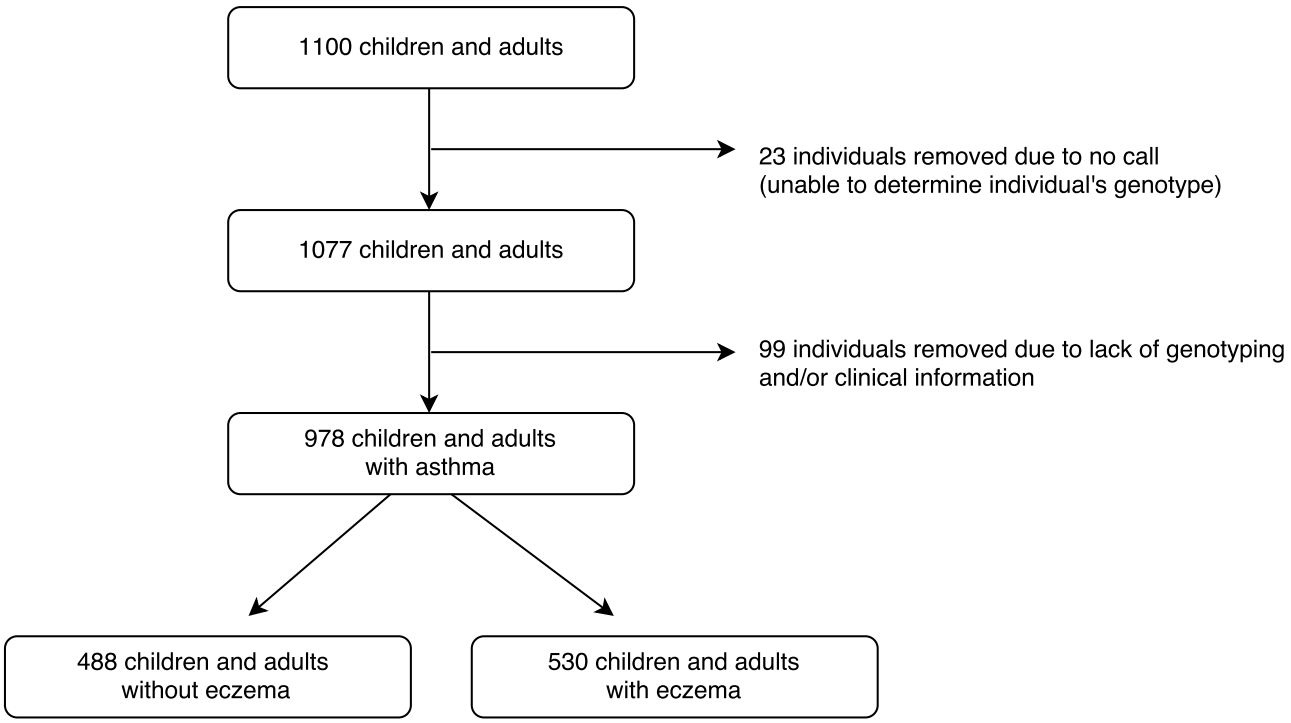

Figure 5.1: Flow diagram of the final sample included in the FLG analysis.

prescriptions (106 antihistamines, 215 emollients, 50 prescriptions for mild eczema, 148 prescriptions for moderate eczema, and 88 prescriptions for severe eczema) and the other patient did not have FLG mutations and was dispensed 149 eczema-related prescriptions (52 antihistamines, 17 emollients, 8 prescriptions for mild eczema, 11 prescriptions for moderate eczema and 61 prescriptions for severe eczema). In addition, among the participants with carers not reporting eczema, 12 other individuals could have had a mild form of eczema; between these individuals a mean of 11 antihistamines, 12 emollients, 6 prescriptions for mild eczema, 2 prescriptions for moderate eczema and 5 prescriptions for severe eczema were dispensed. Table 5.1 presents the characteristics of the study population.

The study followed children and adults between 2005 and 2013. However, not all participants were followed for the entire 9-year period as some individuals moved to Tayside and Fife after 2005, others moved out of Tayside before 2013. Eight children died before 2013, two of them due to asthma.

Variable	BREATHE N=978	Patients with eczema N=530	Patients without eczema N=448
Age, at data collection			
Median (IQR)	10 (7-13)	10 (7-13)	10 (7-13)
Number of males (%)	584 (59.7%)	323 (60.9%)	261 (58.2%)
Number of individuals with FLG mutations (%)	163 (16.7%)	119 (22.4%)	44 (9.8%)
Number of cat owners (%)	253 (25.9%)	124 (23.4%)	129 (28.8%)
Number of children and young adults with family members with eczema (%)	405 (41.4%)	321 (60.6%)	84 (18.7%)
Number of children and young adults with family members with asthma (%)	607 (62.1%)	338 (63.8%)	269 (60%)

Table 5.1: Characteristics of the dataset used in the FLG analyses. Interquartile range (IQR).

5.1.1 Association between filaggrin (FLG) gene variation and eczema-related prescribing

Figure 5.2 displays the number of individuals per year and how the proportion of children and adults varies over the years. Anyone under 18 was classified as a child and anyone 18 years old or older was classified as an adult. Although the number of individuals was different over the years, the proportion of individuals with FLG mutations remained constant.

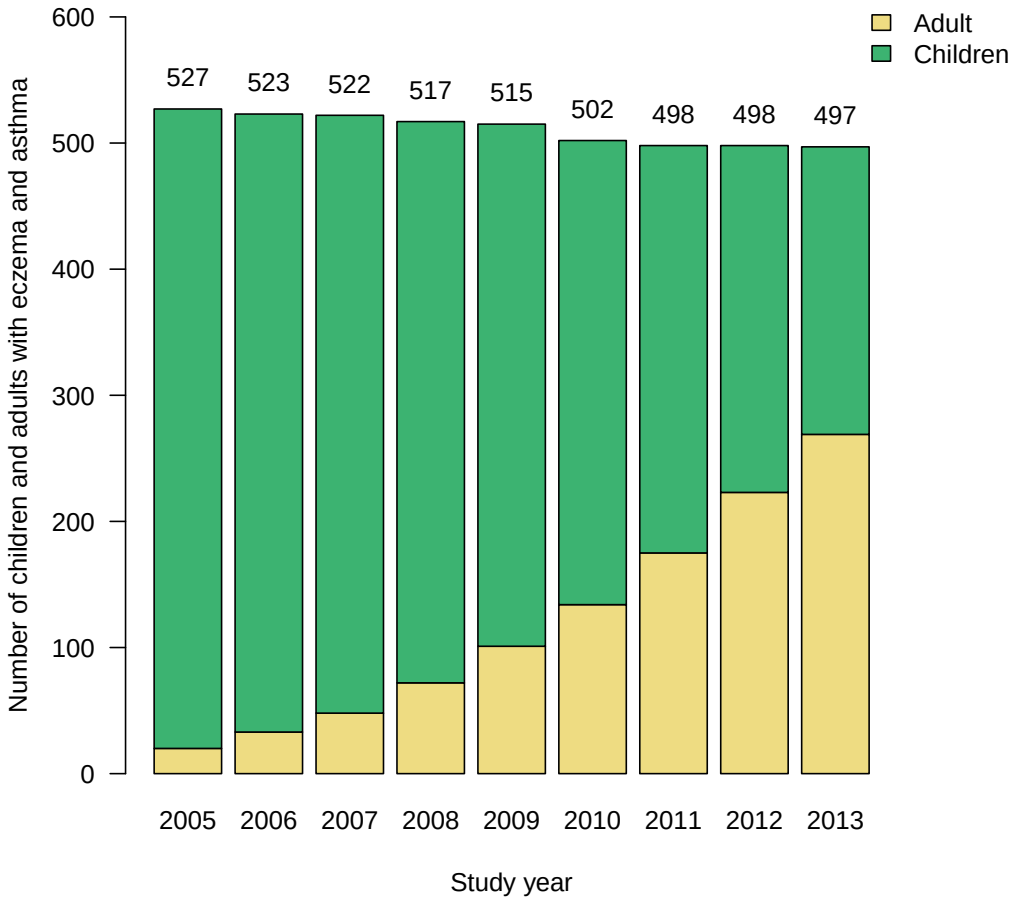

Figure 5.2: Number of children and adults with both eczema and asthma per year, in the FLG analyses.

Over 9-years, the median number of eczema-related prescriptions dispensed per patient was 11.5. Patients with FLG mutations were dispensed a median of 15

prescriptions, whereas patients without FLG mutations were dispensed a median of 11 prescriptions(see table 5.2). A large proportion of the cohort, 387 children and adults (73%), were dispensed at least one prescription for antihistamines, representing a total of 4855 antihistamine prescriptions dispensed over the 9-year period. The second most dispensed group of medicines was emollients, with 344 children and adults (65%) having 5364 emollient prescriptions dispensed. Pharmacies dispensed 1334 prescriptions for mild eczema to 265 patients (50%), 1395 prescriptions for moderate eczema to 254 patients (48%) and 1673 prescriptions for severe eczema to 217 patients (41%).

Prescriptions	**Total**	**2005-2013 No FLG mutation**	**FLG mutation**
	n=530	n=411	n=119
All eczema-related prescriptions			
Mean (SD)	27.6 (47.9)	24.3 (39.3)	39 (68.8)
Median (IQR)	11.5 (3-30)	11 (3-29)	15 (3-38.5)
Emollients			
Mean (SD)	10.1 (21.9)	8.9 (20)	14.4 (27.2)
Median (IQR)	2 (0-8.8)	2 (0-7)	3 (0-18.5)
Antihistamines			
Mean (SD)	9.2 (15)	8.5 (12.9)	11.4 (20.4)
Median (IQR)	3 (0-12)	3 (0-12)	2 (0-11.5)
Prescriptions for mild eczema			
Mean (SD)	2.5 (6)	2.2 (5.2)	3.4 (8.2)
Median (IQR)	1 (0-3)	0 (0-2)	1 (0-3.5)
Prescriptions for moderate eczema			
Mean (SD)	2.6 (9.2)	2.2 (6.8)	4 (14.8)
Median (IQR)	0 (0-2)	0 (0-2)	1 (0-3)
Prescriptions for severe eczema			
Mean (SD)	3.2 (10.4)	2.4 (8)	5.8 (15.9)
Median (IQR)	0 (0-2)	0 (0-2)	0 (0-2)

Table 5.2: Characteristics of the eczema-related prescriptions dispensed listed according to FLG mutations (n=530 children and adults with eczema and asthma), from 2005 to 2013. Standard deviation (SD), interquartile range (IQR).

Emollients and barrier preparations corresponded to 98% of the emollients dispensed, with the remaining 2% corresponding to emollients containing paraffin, urea, zinc oxide and benzyl benzoate. Cetirizine and chlorphenamine maleate were the two most dispensed antihistamines, corresponding respectively to 58% and 21% of the total antihistamines dispensed. Prescribing for mild eczema included hydrocortisone, which corresponded to 80% of the dispensed prescriptions for mild eczema. The remaining dispensed prescriptions corresponded to combined preparations of hydrocortisone with antibacterials and/or antifungals. Regarding prescriptions for moderate eczema, clobetasol butyrate accounted for 66%, and topical calcineurin inhibitors for 5% of the total prescriptions dispensed. Several medicines were dispensed to treat severe eczema, 34% of the total prescriptions for severe eczema dispensed were for betamethasone, 21% for topical corticosteroids combined with antibacterials or antifungals, 16% for bandages, 14% for mometasone furoate and 1.5% for immunosuppressants.

Looking at the number of eczema-related prescriptions for children and adults with eczema and asthma, the number of all eczema-related prescriptions, as well as the number of emollients and antihistamines, dispensed over the years, was stable. The number of prescriptions for mild eczema dispensed slightly decreased between 2005 and 2013, from a total of 213 prescriptions dispensed to 98 patients in 2005 to a total of 126 prescriptions dispensed to 73 patients in 2013. In 2005, 153 prescriptions for moderate eczema were dispensed to 60 patients and 153 prescriptions for severe eczema were dispensed to 60 patients. In 2013, there were 177 prescriptions for moderate eczema dispensed to 75 patients and 206 prescriptions for severe eczema dispensed to 70 patients. However, the average number of prescriptions dispensed per individual remained similar over the years.

Regardless of gender, children and adults with FLG mutations were dispensed more eczema-related prescriptions than children and adults without FLG mutations. Females with FLG mutations were dispensed more eczema-related prescriptions than males with FLG mutations. The gender difference was not as evident for children without FLG mutations (see figure 5.3, panel A). The effect of a FLG mutation on eczema-related prescribing was seen across all age groups. In those without FLG mutation, the number of eczema-related prescriptions in children with eczema and

asthma decreased as the children grew older (see figure 5.3, panel B).

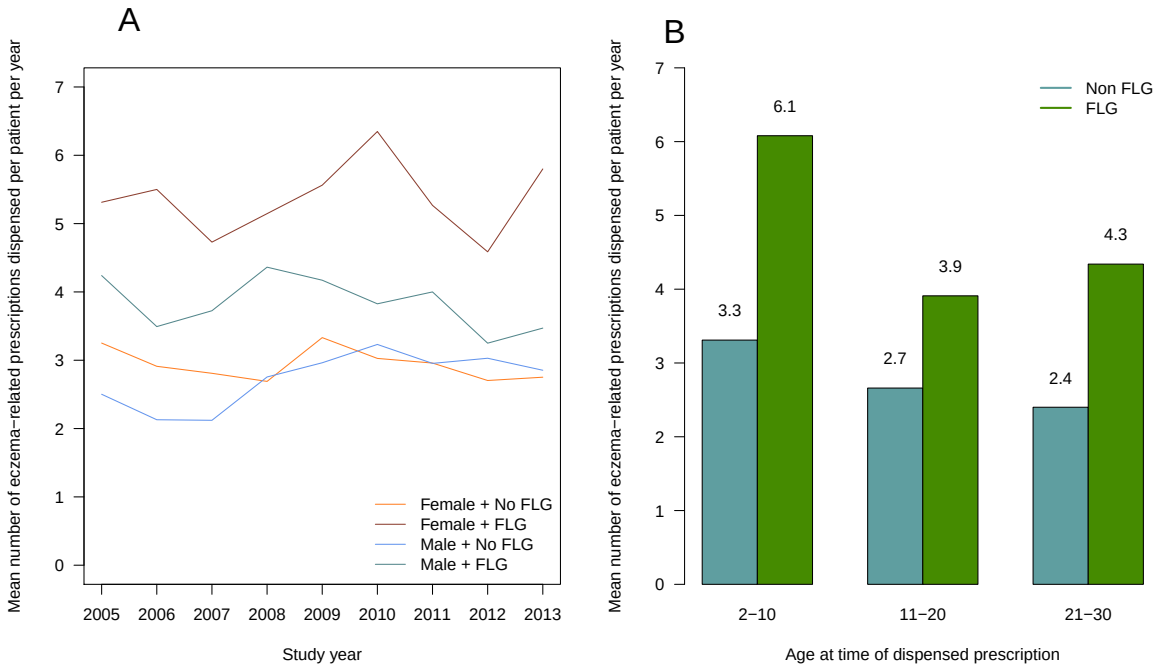

Figure 5.3: Mean number of eczema-related prescriptions dispensed per patient, over 9 years, according to FLG status, gender and age. **A**, mean number of eczema-related prescriptions dispensed according to gender. **B**, mean number of eczema-related prescriptions dispensed according to age.

Overall, the presence of a FLG mutation was significantly associated with the number of eczema-related prescriptions over the 9-year period of study (table 5.3). A weak-to-moderate association was found between age and gender and the number of eczema-related prescriptions over the studied period, with younger patients and females having more prescriptions dispensed than older patients and males (table 5.3). The incidence rate of dispensed prescriptions for emollients in children and adults with FLG mutations was 2.19 times that of children and adults who do not have FLG mutations (table 5.3). The incidence rate of dispensed prescriptions for severe eczema in children and adults with FLG mutations was 2.18 times that of children and adults who do not have FLG mutations (table 5.3). An association of weak-to-moderate significance was found between the presence of FLG mutations and the number of prescriptions for mild and moderate eczema, with patients with FLG mutations having more prescriptions dispensed than patients without FLG mutations (table 5.3). There was no evidence of an association between the

presence of FLG mutations and the number of prescriptions for antihistamines that were dispensed (table 5.3).

Variables	IRR	95% CI	P-value
All eczema-related prescriptions			
Study year	1.00	(0.96,1.05)	0.874
FLG (No vs. Yes)	1.55	(1.11,2.16)	0.011 [a]
Age (in years)	0.96	(0.93,0.99)	0.020 [a]
Gender (Male vs. Female)	1.52	(1.14,2.03)	0.004 [a]
Cat (No vs. Yes)	0.85	(0.61,1.20)	0.357
Antihistamines			
Study year	0.97	(0.92,1.03)	0.338
FLG (No vs. Yes)	1.23	(0.82,1.85)	0.318
Age (in years)	0.96	(0.92,1.00)	0.063
Gender (Male vs. Female)	1.46	(1.03,2.06)	0.035 [a]
Cat (No vs. Yes)	0.78	(0.52,1.17)	0.223
Emollients			
Study year	1.00	(0.93,1.07)	0.947
FLG (No vs. Yes)	2.19	(1.36,3.52)	0.001 [b]
Age (in years)	0.90	(0.86,0.95)	$<$0.001 [a]
Gender (Male vs. Female)	1.74	(1.15,2.63)	0.009 [a]
Cat (No vs. Yes)	0.76	(0.47,1.23)	0.273
Prescriptions for mild eczema			
Study year	0.93	(0.87,0.99)	0.028 [a]
FLG (No vs. Yes)	1.59	(1.03,2.45)	0.036 [a]
Age (in years)	0.93	(0.88,0.97)	0.001 [a]
Gender (Male vs. Female)	1.98	(1.36,2.88)	$<$0.001 [a]
Cat (No vs. Yes)	0.97	(0.63,1.51)	0.909
Prescriptions for moderate eczema			
Study year	0.93	(0.87,1.00)	0.060
FLG (No vs. Yes)	1.86	(1.17,2.96)	0.008 [a]
Age (in years)	0.99	(0.95,1.05)	0.852
Gender (Male vs. Female)	1.87	(1.24,2.81)	0.003 [a]
Cat (No vs. Yes)	1.06	(0.66,1.71)	0.795
Prescriptions for severe eczema			
Study year	0.98	(0.90,1.07)	0.634
FLG (No vs. Yes)	2.18	(1.22,3.91)	0.009 [b]

Continues overleaf

Table 5.3 – continued from the previous page

Variables	IRR	95% CI	P-value
Age (in years)	1.01	(0.95,1.07)	0.778
Gender (Male vs. Female)	1.39	(0.84,2.32)	0.203
Cat (No vs. Yes)	1.42	(0.80,2.55)	0.234

Table 5.3: Association between the number of eczema-related prescriptions dispensed and presence of FLG mutations in children and adults with eczema and asthma (n=530 children and adults), between 2005 and 2013. Incidence rate ratio (IRR), confidence interval CI. [a] - weak-to-moderate association, [b] - strong association.

Prescriptions for eczema associated with skin infections

Over the 9-year period, the mean number of antivirals dispensed per patient was 0.3 and the mean number of antibiotics for skin infections dispensed per patient was 2. Patients with FLG mutations were dispensed a mean of 3 antibiotics, and patients without FLG mutations were dispensed a mean of 2 antibiotics, while patients with and without FLG mutations were dispensed a similar number of antivirals (see table 5.4). In this cohort, 58 children and adults (11%) were dispensed 162 antivirals. In contrast, 329 children and adults (62%) were dispensed 1208 prescriptions of one or more antibiotics that cover bacterial pathogens likely to be found on the skin.

Prescriptions	Total	2005-2013 No FLG mutation	2005-2013 FLG mutation
	n=530	n=411	n=119
Antivirals			
Mean (SD)	0.3 (1.5)	0.3 (1.6)	0.2 (0.7)
Median (IQR)	0 (0-0)	0 (0-0)	0 (0-0)
Antibiotics skin pathogens			
Mean (SD)	2.3 (4.2)	2 (3.8)	3.1 (5.1)
Median (IQR)	1 (0-3)	1 (0-2)	2 (0-4)

Table 5.4: Characteristics of the antivirals and antibiotics dispensed listed according to FLG mutations (n=530 children and adults with eczema and asthma), from 2005 to 2013. Standard deviation (SD), interquartile range (IQR).

Regarding antivirals, 146 prescriptions dispensed were aciclovir, 15 were valaciclovir and 1 was penciclovir. The antibiotics most dispensed were oral flucloxacillin and topical fusidic acid (including betamethasone and hydrocortisone with fusidic acid), which were dispensed 304 and 588 times, respectively. The number of antivirals and antibiotics dispensed over the years is stable.

No apparent differences were observed between the number of antivirals and antibiotics dispensed in males versus females. Individuals with FLG mutations were dispensed more antibiotics for all age groups in comparison to individuals without FLG mutations. Individuals with FLG mutations were dispensed fewer antivirals for all age groups in comparison to individuals without FLG mutations (see figure 5.4).

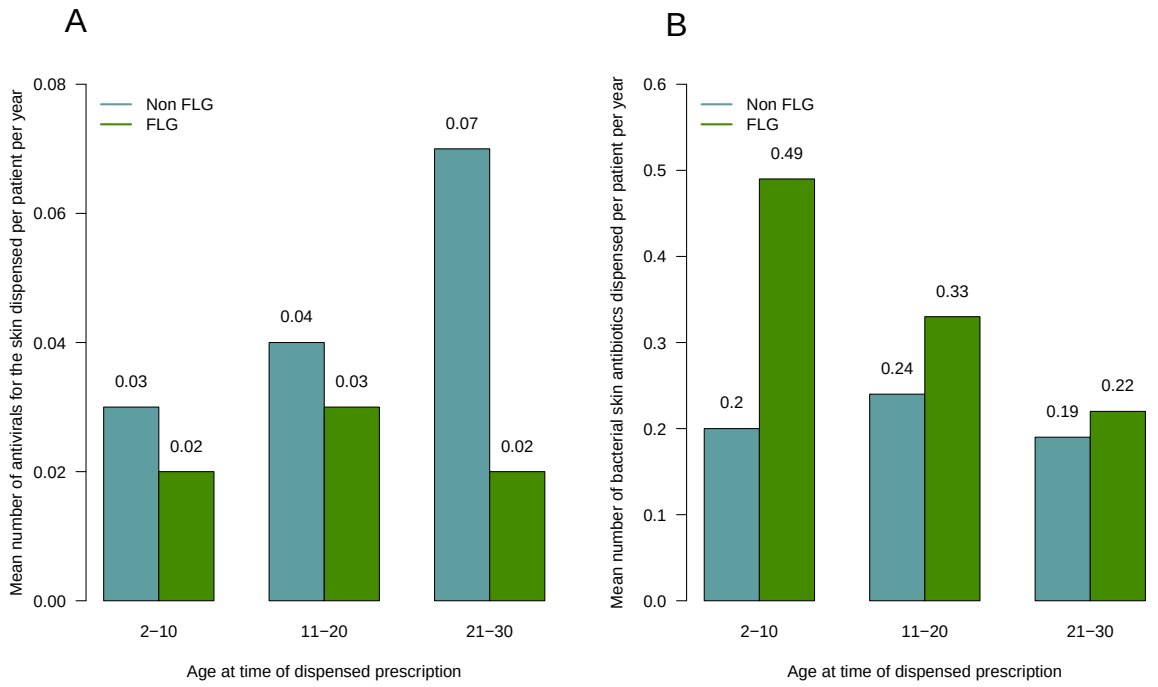

Figure 5.4: Mean number of antivirals and antibiotics dispensed per patient, over 9 years, according to FLG status and age. **A**, mean number of antivirals dispensed according to age. **B**, mean number of antibiotics dispensed according to age.

Overall, the presence of FLG mutations was associated with prescribing of antivirals and antibiotics (table 5.5). Gender was also associated with the number of antibiotics, with females having more antibiotics dispensed than males. However, these associations were weak-to-moderate.

Variables	IRR	95% CI	P-value
Antivirals			
FLG (No vs. Yes)	0.57	(0.33,0.98)	0.043 [a]
Age (in years)	1.03	(0.98,1.08)	0.201
Gender (Male vs. Female)	1.26	(0.82,1.94)	0.291
Cat (No vs. Yes)	0.77	(0.46,1.28)	0.315
Antibiotics			
Study year	0.97	(0.92,1.02)	0.202
FLG (No vs. Yes)	1.79	(1.31,2.44)	$<$0.001 [a]
Age (in years)	1.01	(0.97,1.04)	0.622
Gender (M vs. F)	1.33	(1.01,1.75)	0.041 [a]
Cat (No vs. Yes)	1.02	(0.74,1.40)	0.892

Table 5.5: Association between the number of antivirals and antibiotics dispensed and presence of FLG mutations in children and adults with eczema and asthma (n=530 children and adults), between 2005 and 2013 . Incidence rate ratio (IRR), confidence interval (CI). [a] - weak-to-moderate association.

5.1.2 Association between filaggrin (FLG) gene variation and asthma-related prescribing

Having found an association between FLG mutations and eczema-related prescribing, the association of FLG and asthma prescribing was also explored. Figure 5.5 shows the number of children and adults between 2005 and 2013. As expected, the number of children decreases while the number of adults increases. The proportion of individuals with FLG mutations remained constant over the years.

Over the 9-year period, the median number of asthma-related prescriptions dispensed per patient was 30. Patients with FLG mutations were dispensed a median of 40 prescriptions and patients without FLG mutations were dispensed a median of 29 prescriptions (see table 5.6). As expected in an asthma cohort, relievers were dispensed to the majority of patients (96%), corresponding to a total of 24596 reliever prescriptions dispensed. Inhaled Corticosteroid (ICS) were dispensed to 738 patients (75%), corresponding to 9213 dispensed prescriptions. The proportion of individuals to whom a combination of Long-acting β_2-agonists (LABA) and ICS and Leukotriene

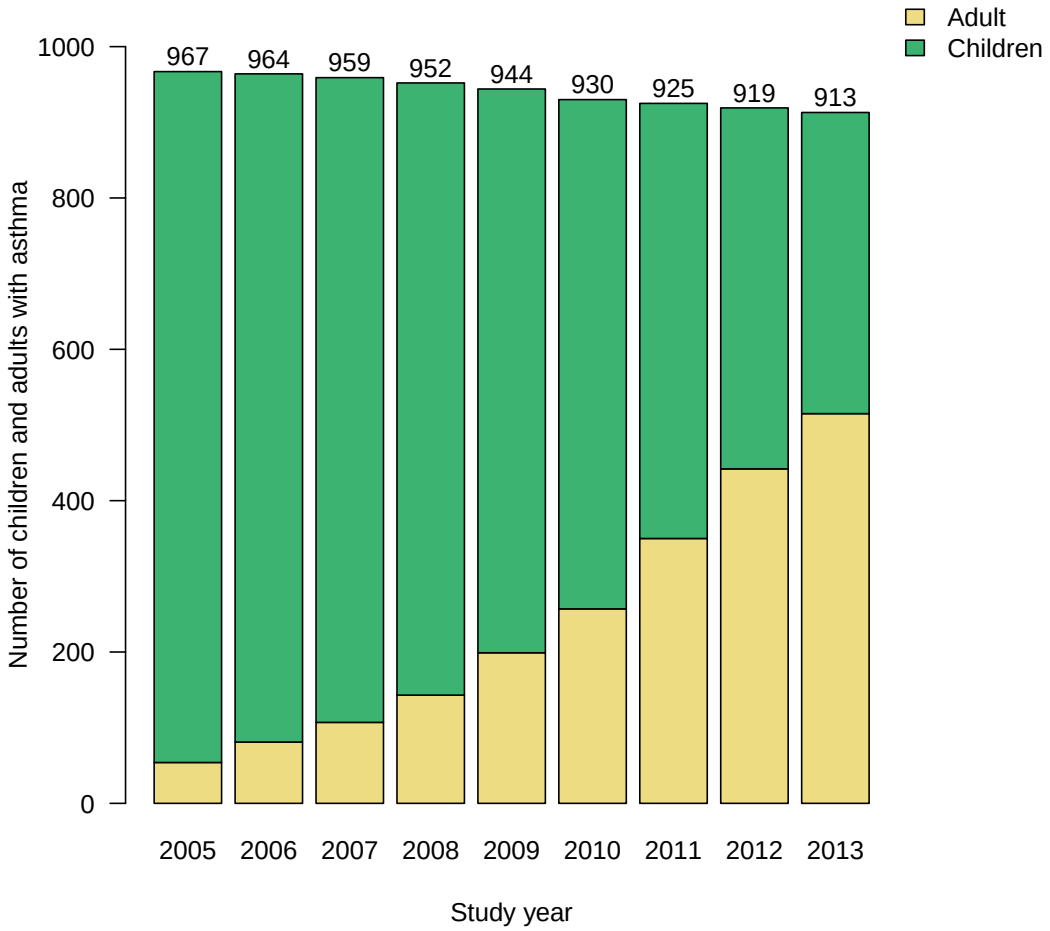

Figure 5.5: Number of children and adults with asthma per year, in the FLG analyses.

Receptor Antagonist (LTRA) prescriptions was dispensed was lower, 42% and 29% respectively. In this cohort, 414 individuals were dispensed 9286 prescriptions for a combination of LABA and ICS, and 282 individuals were dispensed 5115 prescriptions for LTRA. Comparing with the medication mentioned, fewer patients (6%) were dispensed separate LABA.

The majority of relievers (87%) dispensed was salbutamol. Regarding ICS, 75% of the prescriptions dispensed were for beclometasone dipropionate, 16% for fluticasone propionate and 9% for budesonide. The LABA prescriptions dispensed could be divided into those containing salmeterol, accounting for 84% of prescriptions dispensed, and formoterol fumarate, accounting for 16%. Regarding medications combining LABA and ICS, Seretide was dispensed the most (83%), followed by

Prescriptions	Total	2005-2013 No FLG mutation	FLG mutation
	n=978	n=815	n=163
All asthma-related prescriptions			
Mean (SD)	49.6 (55.5)	46.6 (51.1)	64.8 (71.8)
Median (IQR)	30 (12-68)	29 (11-64)	40 (20-97.5)
Relievers			
Mean (SD)	25.1 (31.6)	23.5 (29.0)	33.5 (41.0)
Median (IQR)	15 (7-31)	14 (6-30)	19 (9-45.5)
Inhaled Corticosteroid prescriptions			
Mean (SD)	8.5 (11.0)	8.5 (10.6)	8.7 (12.8)
Median (IQR)	5 (1-12)	5 (1-12)	5 (1-11.5)
Long-acting β_2-agonists prescriptions			
Mean (SD)	0.2 (1.3)	0.2 (1.5)	0.1 (0.5)
Median (IQR)	0 (0-0)	0 (0-0)	0 (0-0)
Combination of LABA and ICS prescriptions			
Mean (SD)	9.5 (16.9)	8.7 (16.0)	13.6 (20.1)
Median (IQR)	0 (0-13)	0 (0-11)	2 (0-23)
Leukotriene Receptor Antagonist prescriptions			
Mean (SD)	5.2 (12.8)	4.7 (11.8)	7.7 (16.8)
Median (IQR)	0 (0-2)	0 (0-1)	0 (0-4)

Table 5.6: Characteristics of the asthma-related prescriptions dispensed listed according to FLG mutations, from 2005 to 2013. Standard deviation (SD), interquartile range (IQR).

Symbicort (15%). The remaining 2% of prescriptions of combined LABA and ICS was made up of Fostair and Flutiform. Montelukast was the LTRA that was usually dispensed, with only 3 Zafirlukast prescriptions dispensed.

The number of all asthma-related prescriptions decreased over the years, as well as the number of ICS and LTRA that were dispensed. The number of reliever prescriptions also declined over the years, although the decrease was less marked, from 2888 dispensed prescriptions in 2005 to 2512 in 2013. Less than 30 LABA prescriptions were dispensed annually. In 2005, the number of inhalers combining LABA and ICS dispensed was 929, in 2008 the number increased to 1095 and

remained relatively stable until 2013.

Regardless of gender, children and adults with FLG mutations were dispensed more asthma-related prescriptions than children and adults without FLG mutations (see figure 5.6, panel A). The effect of a FLG mutation on asthma-related prescribing was seen across all age groups. In those without FLG mutation, the number of asthma-related prescriptions in children and asthma decreased as the children grew older (see figure 5.6, panel B).

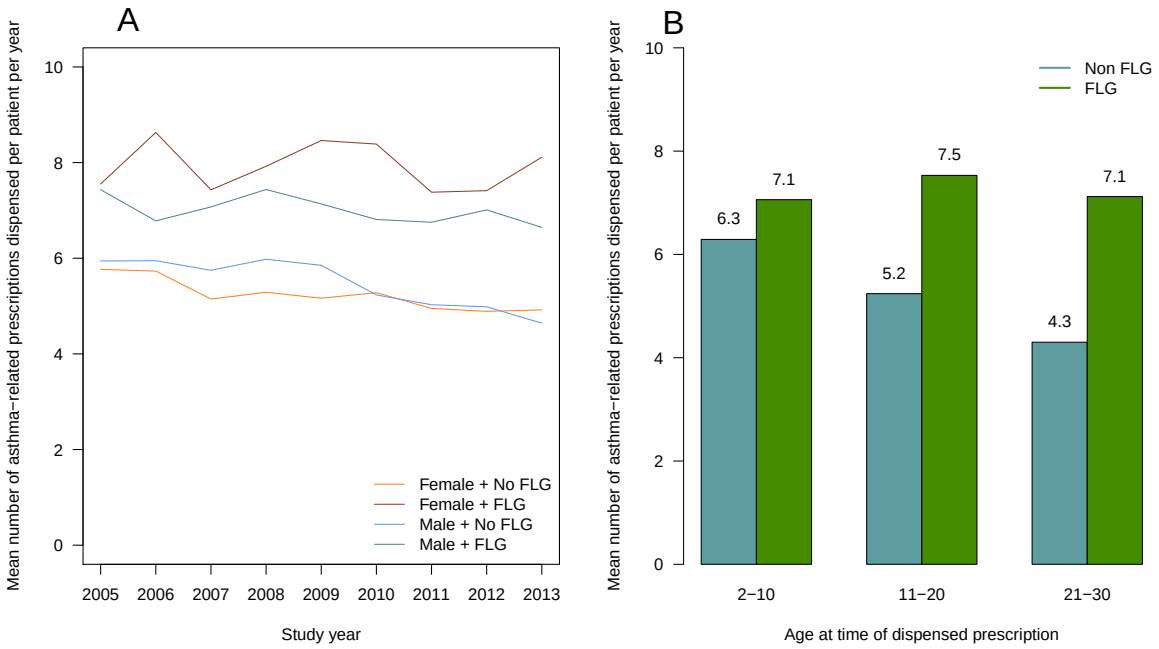

Figure 5.6: Mean number of asthma-related prescriptions dispensed per patient, over 9 years, according to FLG status, gender and age. **A**, mean number of asthma-related prescriptions dispensed according to gender. **B**, mean number of asthma-related prescriptions dispensed according to age.

Overall, the presence of FLG mutations was significantly associated with the number of asthma-related prescriptions over the 9-year period. The incidence rate of dispensed prescriptions for a combination of LABA and ICS in children and adults with FLG mutations was 2.67 times that of children and adults who do not have FLG mutations (table 5.7). Interestingly, the incidence rate of dispensed LTRA prescriptions in children and adults with a cat was 0.49 times that of children and adults who do not have a cat (table 5.7). A weak-to-moderate association was also found between age and the number of asthma-related prescriptions, with younger

patients having more prescriptions dispensed than older patients (table 5.7). A weak-to-moderate association was found between the presence of FLG mutations and the number of relievers dispensed, with individuals with FLG mutations having more prescriptions dispensed than individuals without FLG mutations (table 5.7). There was no evidence of an association between the presence of FLG mutations and the number of separate ICS, separate inhaled LABA, and oral LTRA dispensed (table 5.7).

Variables	IRR	95% CI	P-value
All asthma-related prescriptions			
Study year	0.93	(0.90,0.95)	$<$0.001 [a]
FLG (No vs. Yes)	1.30	(1.07,1.58)	0.008 [a]
Age (in years)	0.96	(0.94,0.98)	$<$0.001 [a]
Gender (Male vs. Female)	0.93	(0.80,1.08)	0.334
Cat (No vs. Yes)	0.98	(0.83,1.16)	0.861
Reliever prescriptions			
Study year	0.94	(0.92,0.97)	$<$0.001 [a]
FLG (No vs. Yes)	1.35	(1.13,1.61)	0.001 [a]
Age (in years)	0.97	(0.95,0.99)	$<$0.001 [a]
Gender (Male vs. Female)	0.94	(0.82,1.08)	0.390
Cat (No vs. Yes)	1.04	(0.89,1.21)	0.633
Inhaled Corticosteroid prescriptions			
Study year	0.85	(0.82,0.89)	$<$0.001 [a]
FLG (No vs. Yes)	0.90	(0.69,1.18)	0.455
Age (in years)	0.92	(0.90,0.94)	$<$0.001 [a]
Gender (Male vs. Female)	0.96	(0.78,1.18)	0.705
Cat (No vs. Yes)	1.17	(0.93,1.47)	0.176
Long-acting β_2-agonists prescriptions			
FLG (No vs. Yes)	0.47	(0.16,1.37)	0.166
Age (in years)	1.00	(0.96,1.04)	0.927
Gender (Male vs. Female)	1.11	(0.51,2.43)	0.927
Cat (No vs. Yes)	1.24	(0.48,3.17)	0.657
Combination of LABA and ICS prescriptions			
Study year	0.88	(0.82,0.94)	$<$0.001 [a]
FLG (No vs. Yes)	2.67	(1.44,4.96)	0.002 [b]
Age (in years)	1.01	(0.95,1.07)	0.756

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Table 5.7 – continued from the previous page

Variables	IRR	95% CI	P-value
Gender (Male vs. Female)	1.18	(0.73,1.90)	0.507
Cat (No vs. Yes)	0.66	(0.39,1.13)	0.129
Leukotriene Receptor Antagonist prescriptions			
Study year	0.89	(0.80,0.98)	0.021 [a]
FLG (No vs. Yes)	1.77	(0.85,3.69)	0.126
Age (in years)	0.84	(0.79,0.90)	$<$0.001 [a]
Gender (Male vs. Female)	0.97	(0.55,1.73)	0.930
Cat (No vs. Yes)	0.49	(0.25,0.93)	0.030 [b]

Table 5.7: Association between the number of asthma-related prescriptions dispensed and presence of FLG mutations in children and adults with asthma (n=978 children and adults), between 2005 and 2013. Incidence rate ratio (IRR), confidence interval (CI). [a] - weak-to-moderate association, [b] - strong association.

Asthma-related Accident & Emergency (A&E) visits/admissions

Out of 978 children and adults, 269 (27.5%) had a total of 1059 asthma-related A&E visits/admissions. Patients with FLG mutations had a mean of 2 asthma-related A&E visits/admissions, whereas patients without any FLG mutation had a mean of 1 asthma-related A&E visits/admissions (see table 5.8).

Asthma-related A&E visits/admissions	2005-2013 Total	No FLG mutation	FLG mutation
	n=978	n=815	n=163
Mean (SD)	1.1 (4.8)	0.9 (4.5)	1.7 (5.8)
Median (IQR)	0 (0-1)	0 (0-0)	0 (0-1)

Table 5.8: Characteristics of the asthma-related A&E visits/admission listed according to FLG mutations, from 2005 to 2013. Standard deviation (SD), interquartile range (IQR).

The number of asthma-related A&E visits/admissions decreased over the years. In 2005, 179 asthma-related A&E visits/admissions were recorded and in 2013 83 were recorded.

Until 2009, females with FLG mutations had slightly more asthma-related A&E visits/admissions per year than males and females without FLG mutations (see figure 5.7, panel A). From 2009 onwards, the mean number of A&E visits/admissions per year was similar between males and females. Individuals younger than 20 years old with FLG mutations had more A&E visits/admissions per year than individuals younger than 20 years without FLG mutations. In individuals older than 20 years, the opposite was found, individuals without FLG mutations had more A&E visits/admissions per year than individuals with FLG mutations (see figure 5.7, panel B).

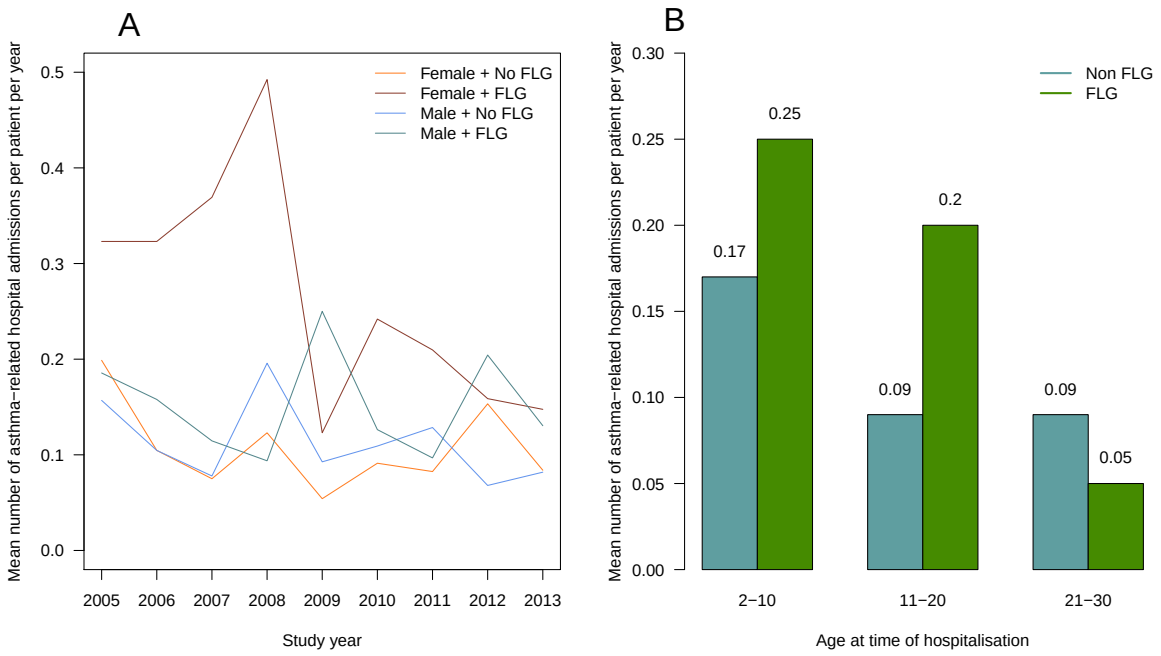

Figure 5.7: Mean number of asthma-related A&E visits/admissions per patient, over 9 years, according to FLG status, gender and age. **A**, mean number of asthma-related A&E visits/admissions according to gender. **B**, mean number of asthma-related A&E visits/admissions according to age.

Overall, FLG mutations were significantly associated with the number of asthma-related A&E visits/admissions. The incidence rate of asthma-related A&E visits/admissions in children and adults with FLG mutations was 2.37 times that of children and adults who do not have FLG mutations (table 5.9). Age and cat ownership were also associated with the number of asthma-related A&E visits/admissions over this period, with younger patients and patients without a cat

having more asthma-related A&E visits/admissions than older patients and patients with a cat; these associations were weak-to-moderate (table 5.9).

Variables	IRR	95% CI	P-value
Asthma-related A&E visits/admissions			
Study year	0.85	(0.78,0.94)	0.001 [a]
FLG (No vs. Yes)	2.37	(1.51,3.71)	$<$0.001 [b]
Age (in years)	0.89	(0.85,0.93)	$<$0.001 [a]
Gender (Male vs. Female)	1.35	(0.93,1.95)	0.112
Cat (No vs. Yes)	0.57	(0.37,0.88)	0.012 [a]

Table 5.9: Association between the number of asthma-related A&E visits/admissions and presence of FLG mutations in children and adults with asthma (n=978 children and adults), between 2005 and 2013. Incidence rate ratio (IRR), confidence interval (CI). [a] - weak-to-moderate association, [b] - strong association.

Prescribing of oral corticosteroids

Out of 978 children and adults, 483 (49.4%) were dispensed a total of 2628 oral prednisolone prescriptions. Over the 9-year period of study, the mean number of oral prednisolone prescriptions dispensed per individual was 3. Patients with FLG mutations were dispensed a mean of 4 oral prednisolone prescriptions, while patients without FLG mutations were dispensed a mean of 2 oral prednisolone prescriptions (see table 5.10).

Oral prednisolone prescriptions	2005-2013		
	Total	No FLG mutation	FLG mutation
	n=978	n=815	n=163
Mean (SD)	2.7 (7.2)	2.3 (5.9)	4.4 (11.8)
Median (IQR)	0 (0-3)	0 (0-2.7)	0 (0-4)

Table 5.10: Characteristics of the oral prednisolone prescriptions dispensed listed according to FLG mutations, from 2005 to 2013. Standard deviation (SD), interquartile range (IQR).

The number of oral prednisolone prescriptions dispensed over the years was stable. Females with FLG mutations were dispensed more oral prednisolone prescriptions than males and females without FLG mutations (see figure 5.8, panel A). The effect

of a FLG mutation on prescribing was seen across all age groups (see figure 5.8, panel B).

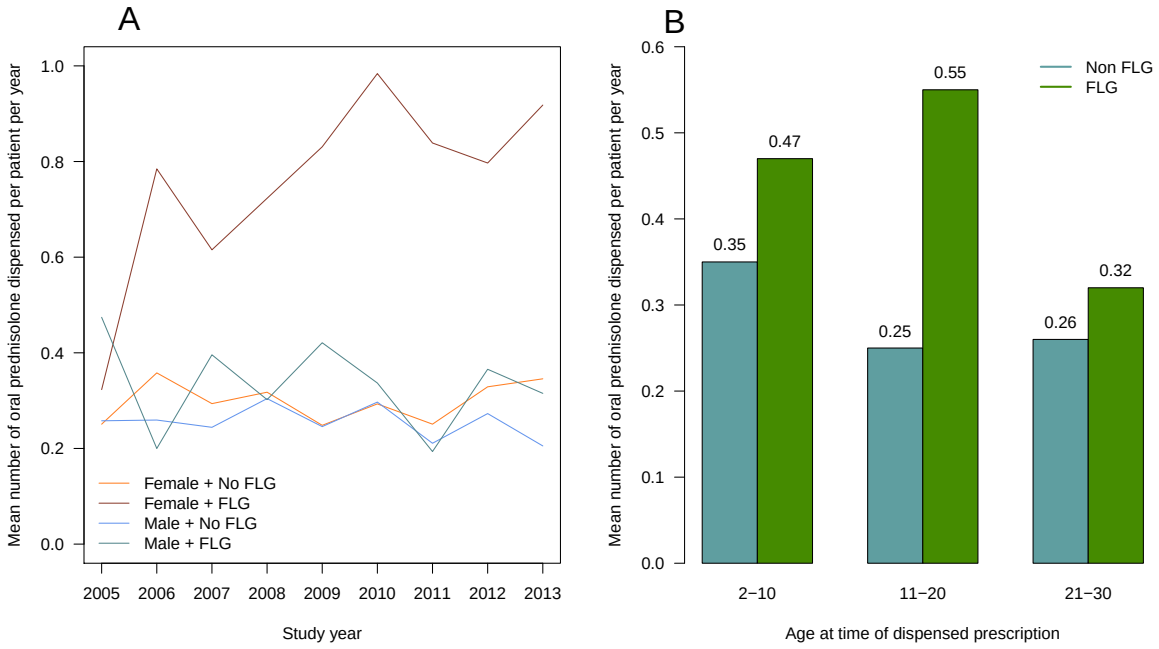

Figure 5.8: Mean number of oral prednisolone prescriptions dispensed per patient, over 9 years, according to FLG status, gender and age. **A**, mean number of oral prednisolone prescriptions dispensed according to gender. **B**, mean number of oral prednisolone prescriptions dispensed according to age.

Overall, the presence of FLG mutations were weakly-to-moderately associated with the number of oral prednisolone prescriptions dispensed (table 5.11). Age and cat ownership were also associated with the number of oral prednisolone prescriptions dispensed over this period, with younger patients and patients without a cat having more prescriptions than older patients and patients with a cat; these associations were weak-to-moderate.

Variables	IRR	95% CI	P-value
Oral prednisolone prescriptions			
Study year	0.96	(0.91,1.01)	0.128
FLG (No vs. Yes)	1.69	(1.19,2.39)	0.003 [a]
Age (in years)	0.90	(0.88,0.94)	$<$0.001 [a]
Gender (Male vs. Female)	1.19	(0.90,1.57)	0.215
Cat (No vs. Yes)	0.66	(0.48,0.90)	0.010 [a]

Table 5.11: Association between the number of oral prednisolone prescriptions dispensed and presence of FLG mutations in children and adults with asthma (n=978 children and adults), between 2005 and 2013. Incidence rate ratio (IRR), confidence interval (CI). [a] - weak-to-moderate association.

Asthma exacerbations

Out of 978 children and adults, 549 (56%) children and adults had a total of 3687 asthma exacerbations. Of the 978 children and adults included in the analysis, 281 (28.7%) were dispensed one or more prescriptions of oral prednisolone, 67 (6.8%) had one or more asthma-related A&E visits/admission, and 201 (20.5%) were dispensed one or more prescriptions of oral prednisolone and had one or more asthma-related A&E visits/admissions (see figure 5.9).

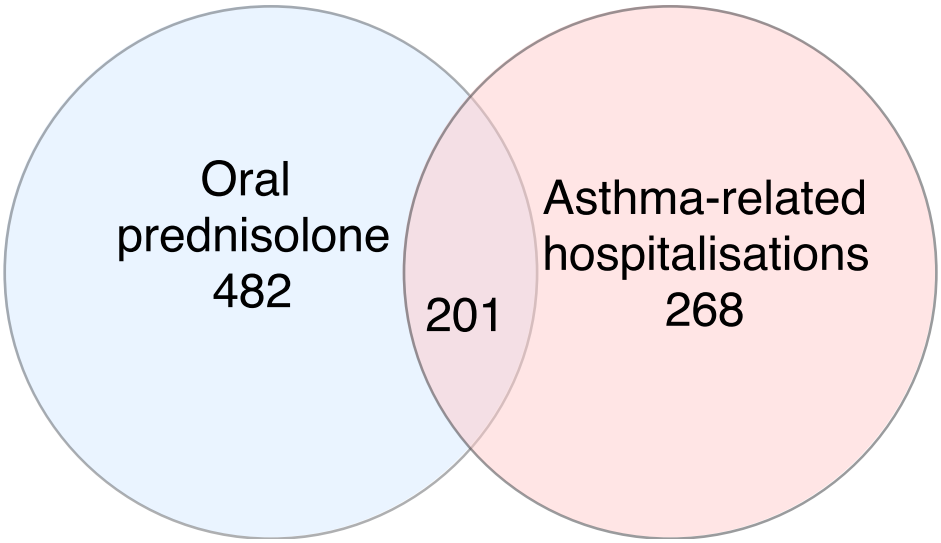

Figure 5.9: Number of children and adults with asthma (n=978 children and adults) in the FLG analyses that were dispensed oral prednisolone and/or had an asthma-related A&E visits/admissions, from 2005 to 2013

Females with FLG mutations had more asthma exacerbations than males, and females without FLG mutations. A similar effect was seen for asthma-related A&E visits/admissions and prednisolone prescriptions dispensed (see figure 5.7 and 5.8). Children with FLG mutations had a higher number of asthma exacerbations in comparison to children without FLG mutations. However, the number of asthma exacerbations for adults (21 to 30 years old) was similar, regardless of FLG status.

Overall, the presence of FLG mutations was weakly-to-moderately associated with the number of asthma exacerbations over the 9 year period of study (table 5.12). Age and cat ownership were also associated with the number of asthma exacerbations over this period; these associations were also weak-to-moderate.

Variables	IRR	95% CI	P-value
Asthma exacerbations			
Study year	0.95	(0.90,0.99)	0.023 [a]
FLG (No vs. Yes)	1.76	(1.27,2.45)	0.001 [a]
Age (in years)	0.90	(0.87,0.93)	$<$0.001 [a]
Gender (Male vs. Female)	1.19	(0.91,1.54)	0.194
Cat (No vs. Yes)	0.63	(0.47,0.85)	0.003 [a]

Table 5.12: Association between the number of asthma exacerbations and presence of FLG mutations in children and adults with asthma (n=978 children and adults), between 2005 and 2013. Incidence rate ratio (IRR), confidence interval (CI). [a] - weak-to-moderate association.

5.1.3 Association between filaggrin (FLG) gene variation and prescribing for acute allergic reactions and allergic rhinitis

Given the prevalence of allergic rhinitis and other allergic conditions in individuals with eczema and asthma, the role of FLG on acute allergic reactions and allergic rhinitis was investigated. Over the 9-year period of study, the mean number of allergic rhinitis prescriptions dispensed was 3 and the mean number of Adrenaline Auto-Injector (AAI) prescriptions dispensed was 1. The mean number of AAI and allergic rhinitis prescriptions dispensed was similar for individuals with and without FLG mutations (see table 5.13). Sixty-two (6.3%) patients were dispensed a total of

665 AAI prescriptions. Four hundred (40.9%) patients were dispensed a total of 2501 prescriptions for allergic rhinitis.

Prescriptions	Total	2005-2013 No FLG mutation	FLG mutation
Adrenaline Auto-Injector			
Mean (SD)	0.7 (3.1)	0.6 (2.9)	1.0 (3.7)
Median (IQR)	0 (0-0)	0 (0-0)	0 (0-0)
Allergic rhinitis prescriptions			
Mean (SD)	2.6 (7.4)	2.5 (7.3)	2.8 (7.7)
Median (IQR)	0 (0-2)	0 (0-2)	0 (0-2)

Table 5.13: Characteristics of the AAI and allergic rhinitis prescriptions dispensed listed according to FLG mutations (n=978 children and adults with asthma), from 2005 to 2013. Standard deviation (SD), interquartile range (IQR).

Nasal corticosteroids corresponded to 76% of the rhinitis prescriptions dispensed, with fluticasone propionate, beclometasone dipropionate and mometasone furoate accounting for 79% of the nasal corticosteroids dispensed. Nasal sodium cromoglicate corresponded to 23% of the allergic rhinitis prescriptions dispensed. The number of AAI and prescriptions for allergic rhinitis dispensed, over the years, was stable.

Females with FLG mutations were dispensed more AAI and allergic rhinitis prescriptions than males with and without FLG mutations and females without FLG mutations. In 2007, there was an increase in the number of AAI prescriptions dispensed for females with FLG mutations, compared to males with and without FLG mutations and females without FLG mutations (see figure 5.10, panel A). Females with FLG mutations had been dispensed slightly more allergic rhinitis prescriptions than males with and without FLG mutations and females without an FLG mutation (Figure 5.10, panel B).

The effect of FLG mutations on AAI prescribing was seen across all age groups (Figure 5.11, panel A). Children with FLG mutations were dispensed a higher number of allergic rhinitis prescriptions than children without FLG mutation. However, as the

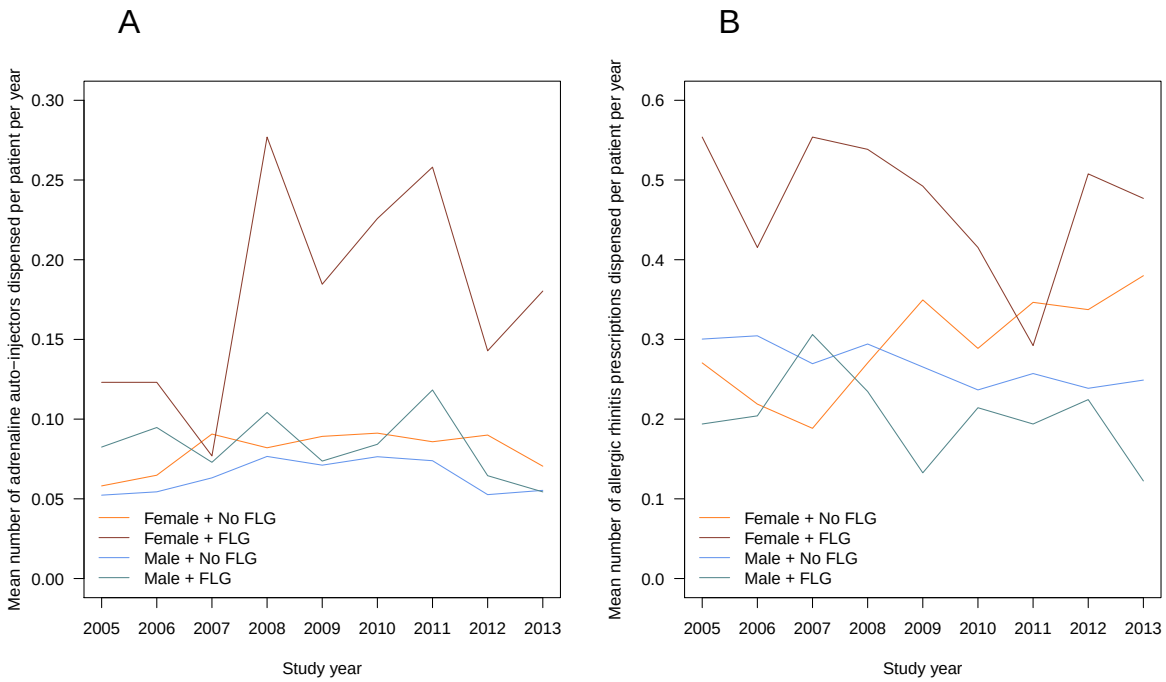

Figure 5.10: Mean number of AAI and allergic rhinitis prescriptions dispensed per patient, over 9 years, according to FLG status, gender and age. **A**, mean number of AAI dispensed according to gender. **B**, mean number of allergic rhinitis prescriptions dispensed according to gender.

children grew older, the situation was reversed: adults without FLG mutation were dispensed more allergic rhinitis prescriptions than adults with FLG mutations (Figure 5.11, panel B).

There was no evidence of an association between the presence of FLG mutations and the number of AAI and allergic rhinitis prescriptions dispensed (table 5.14).

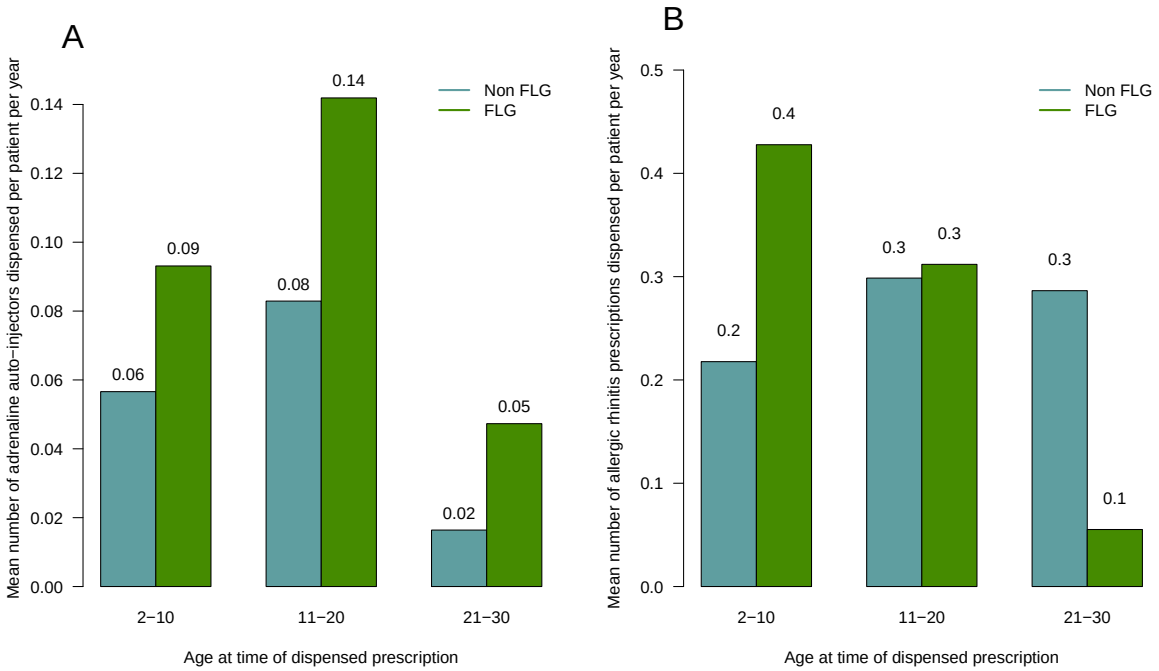

Figure 5.11: Mean number of AAI and allergic rhinitis prescriptions per patient, over 9 years, according to the FLG status and gender. **A**, mean number of AAI prescriptions according to the age. **B**, mean number of allergic rhinitis prescriptions according to age

Variables	**IRR**	**95% CI**	**P-value**
Adrenaline Auto-Injector			
FLG (No vs. Yes)	1.70	(0.48,6.08)	0.414
Age (in years)	0.89	(0.76,1.04)	0.150
Gender (Male vs. Female)	1.72	(0.63,4.68)	0.291
Cat (No vs. Yes)	0.45	(0.15,1.37)	0.160
Allergic rhinitis prescriptions			
Study year	0.88	(0.83,0.94)	$<$0.001 [a]
FLG (No vs. Yes)	1.43	(0.90,2.28)	0.131
Age (in years)	1.03	(0.98,1.08)	0.197
Gender (Male vs. Female)	1.01	(0.70,1.46)	0.955
Cat (No vs. Yes)	0.84	(0.55,1.26)	0.399

Table 5.14: Association between the number of AAI and allergic rhinitis prescriptions dispensed and presence of FLG mutations in children and adults with asthma (n=978 children and adults), between 2005 and 2013 . Incidence rate ratio (IRR), confidence interval (CI). [a] - weak-to-moderate association.

5.1.4 Pharmacoeconomics

Different prescribing patterns were found between children and adults with and without FLG mutations. Hence, the next step of the analysis was to understand whether these differences lead to different healthcare costs for the National Health Service (NHS).

The mean cost of eczema-related prescriptions for individuals with FLG mutations was £244, and the mean cost for individuals without FLG mutations was £150. Children and adults with FLG mutations have a significantly higher cost of prescribing for emollients and moderate and severe eczema compared to children and adults without FLG mutations (see table 5.15). There was no evidence of a difference between FLG status for the cost of antihistamines and prescribing for mild eczema (see table 5.15).

The mean cost of antivirals for individuals without FLG mutations was £4, and the mean cost of antivirals for individuals with FLG mutations was £1 (see table 5.15). This differences was not significant (see table 5.15). The mean cost of anti-bacterial skin antibiotics for individuals with FLG mutations was £20, and the mean cost of antibiotics for individuals without FLG mutations was £13; this difference was significant at the 5% level (see table 5.15).

The mean cost of asthma-related prescriptions for individuals with FLG mutations was £1443, and the mean cost for individuals without FLG mutations was £955. Children and adults with FLG mutations have a significantly higher cost of prescribing for relievers, combination of LABA with ICS, and LTRA than children and adults without FLG mutations (see table 5.15). There was no evidence of a difference between FLG status for the cost of ICS and LABA as single drug inhaler (see table 5.15).

The mean cost of asthma-related A&E visits/admissions for individuals with FLG mutations was £997, and the mean cost of asthma-related A&E visits/admissions for individuals without FLG mutations was £521. The mean cost of oral corticosteroids for individuals with FLG mutations was £16, and the mean cost of oral corticosteroids for individuals without FLG mutations was £7. The mean cost of asthma exacerbations for individuals with FLG mutations was £1013, and the mean cost of

asthma exacerbations for individuals without FLG mutations was £528. These differences were significant at a 5% level (see table 5.15). The mean cost of emergency General Practitioner (GP) consultations for individuals with FLG mutations was £150, and the mean cost of emergency GP consultations for individuals without FLG mutations was £80. These differences were significant at the 5% level (see table 5.15).

The mean cost of acute allergic reactions for individuals with FLG mutations was £63, and the mean cost of acute allergic reactions for individuals without FLG mutations was £36. The mean cost of allergic rhinitis prescriptions for individuals with and without FLG mutations was £22. There was no evidence of a difference at the 5% level (see table 5.15).

The mean cost for children and adults with eczema and asthma and with FLG mutations was £3306, and the mean cost for all conditions examined for children and adults with eczema and asthma and without FLG mutations was £2049. The mean cost for children and adults with asthma and with FLG mutations was £2938, and the mean cost for all conditions examined for children and adults with asthma and without FLG mutations was £1727. These differences were significant at the 5% level (see table 5.15).

Prescriptions	**Mean difference (in £)**	**95% BCa CI (in £)**
	Eczema	
All eczema-related	94.56	(17.05,208.76) [c]
Emollients	28.92	(0.47,69.21) [c]
Antihistamines	10.84	(-3.79,29.75)
Mild eczema	6.33	(-4.14,20.96)
Moderate eczema	12.94	(0.05,32.32) [c]
Severe eczema	35.53	(6.30,82.78) [c]
	Infected eczema	
Antivirals for the skin	-3.14	(-18.20,0.04)
Bacterial skin antibiotics	6.47	(0.02,17.76) [c]
	Asthma	
All asthma-related	487.67	(168.74,836.07) [c]

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Table 5.15 – continued from the previous page

Prescriptions	Mean difference (in £)	95% BCa CI (in £)
Reliever	79.06	(32.63,160.91) [c]
ICS	-11.81	(-36.82,21.89)
LABA	-17	(-40,40.85)
LABA and ICS	295.21	(107.76,491.15) [c]
LTRA	126.55	(13.69,246.44) [c]
Asthma exacerbations		
Asthma-related A&E visits/admissions	476	(49.46,1482.40) [c]
Oral corticosteroids	8.80	(3.26,20.57) [c]
Asthma exacerbations	485.11	(55.13,1502.65) [c]
Primary care emergency costs		
Emergency GP consultations	69.99	(22.11,145.96) [c]
Acute allergic reactions and allergic rhinitis		
AAI	27.72	(-0.62,74.85)
Allergic rhinitis	0.52	(-8.53,10.58)
Snapshot cost of the cohort		
Individuals with eczema and asthma	1258.58	(295.66,3109.55) [c]
Individuals with asthma	1210.66	(405.55,2646.33) [c]

Table 5.15: Difference in the mean cost of prescriptions dispensed according to FLG status of the patient, from 2005 to 2013. Figures prefixed with a minus sign indicate that the costs in children with FLG mutations are less than in children without these mutations. Bias corrected and accelerated (BCa), confidence interval (CI). [c] - significant association

5.2 Understanding the association between adrenoreceptor β_2 (ADRB2) gene variation and healthcare utilisation

Demographics

Of the 1100 children and young adults in BREATHE, 57 individuals were excluded due to genotyping failure for the Arg16 and Glu27 variant and 34 individuals were excluded due to missing information on one or more of the clinical variables considered. The final dataset corresponds to 1009 children and adults with asthma (see figure 5.12 for flowchart of the sample size).

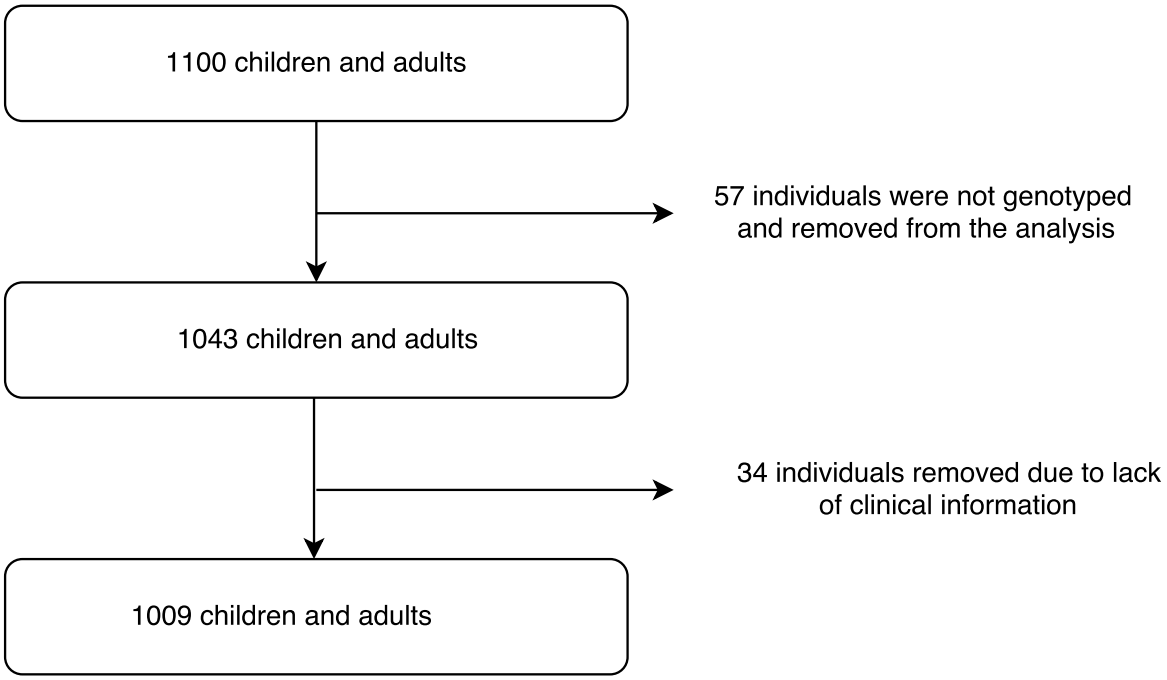

Figure 5.12: Flow diagram of the final sample included in the ADRB2 analysis.

Of the 1009 children and adults with asthma, 29 (2.9%) were not dispensed an asthma-related prescription from 2005 onwards, despite the physician diagnosis of asthma. These 29 patients had a mean age of 12 years in 2005, the youngest patient was 5 years old and the oldest patient was 20 years old.

The median age for participants in the cross-sectional dataset was 10 years, and the dataset had 606 males (60%). Regarding the Arg16 variant, the distribution of the

population is as follows: 45% Gly/Arg, 40% Gly/Gly and 15% Arg/Arg. For the Glu27 variant, the distribution of the population is: 50% Gln/Glu, 31% Gln/Gln and 19% Glu/Glu. A large number of individuals (80%) had one or more family members with asthma, and 35% smoked or were exposed to smoke at the time of data collection for BREATHE.

During data collection, the majority of the individuals were under the legal age to smoke. Although under-aged smoking can occur, it is unsurprising that only 21 individuals out of 1100 admitted to smoking. However, 380 patients were exposed to second hand exposure to smoke. Therefore, smoke and second hand exposure to smoke were combined for the analysis.

5.2.1 Association between adrenoreceptor β_2 (ADRB2) gene variation and asthma-related prescribing

Figure 5.13 shows the number of children and adults per year. Over the years, the proportion of individuals homozygous for the Arg16 and Glu27 variant remained constant.

Over the 9-year period, the median number of asthma-related prescriptions dispensed per patient was 31 (see table 5.16). Patients with the Arg/Arg genotype were dispensed a median of 41 prescriptions, patients with Gly/Gly genotype were dispensed a median of 30 prescriptions, and patients with Gly/Arg genotype were dispensed a median of 29 prescriptions. Regarding the Glu27 variant, patients with Gln/Gln genotype were dispensed a median of 36 prescriptions, patients with the Glu/Glu and Gln/Glu genotype were both dispensed a median of 29 prescriptions. The majority of the patients (96%) were dispensed a total of 25697 reliever prescriptions. ICS were dispensed to 757 patients (75%), corresponding to 8582 dispensed prescriptions. In this cohort, 427 individuals were dispensed 9731 prescriptions for a combination of LABA and ICS, and 304 individuals were dispensed 5632 prescriptions for LTRA. The proportion of individuals to whom a combination of LABA and ICS and LTRA prescriptions were dispensed was lower, 42% and 30% respectively. Fewer individuals 7% were dispensed separate LABA, with 243

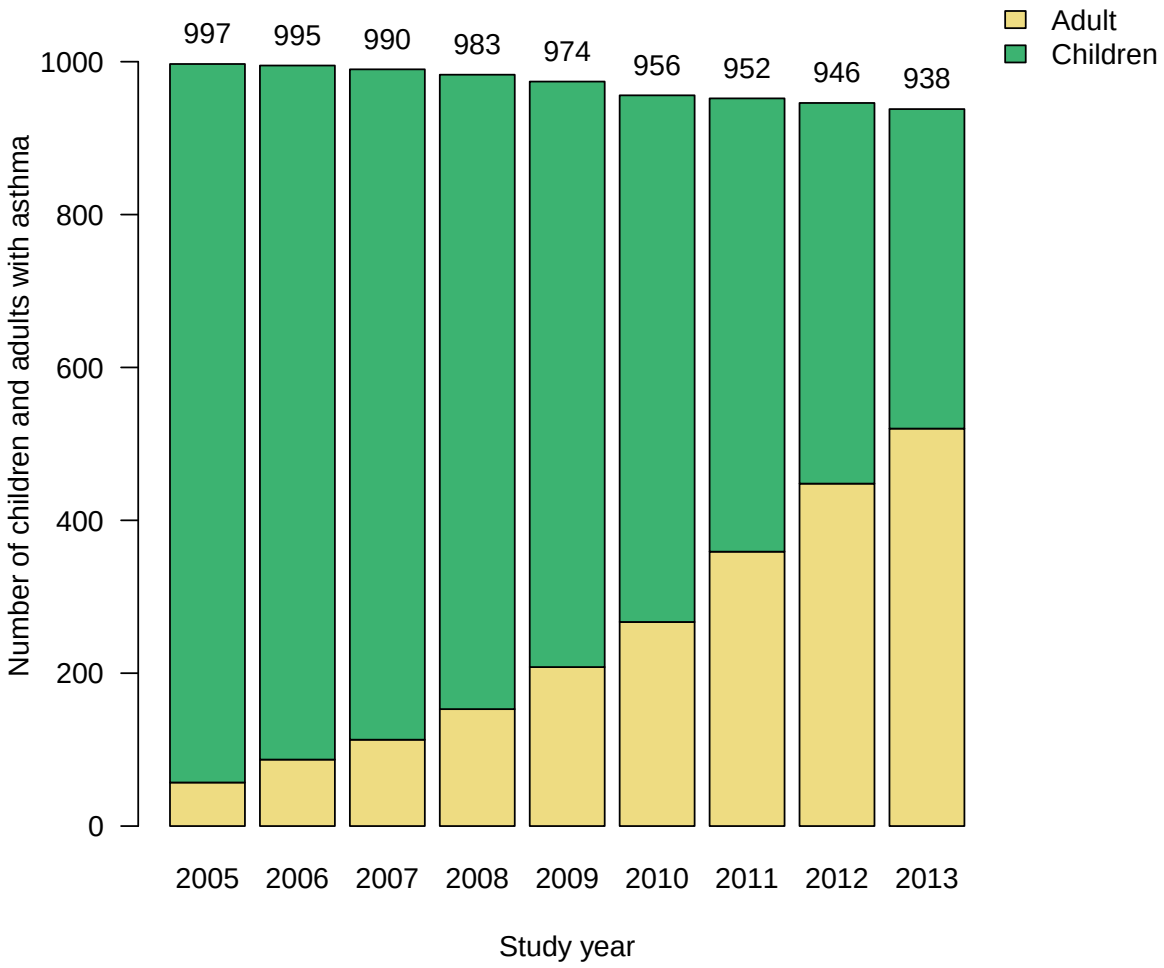

Figure 5.13: Number of children and adults with asthma per year, for the ADRB2 analyses.

prescriptions dispensed.

Regardless of genotype, children and adults who smoked and/or were exposed to second hand smoke were dispensed more asthma-related prescriptions than children and adults who did not smoke or were not exposed to second hand smoke. Regardless of the smoking status, patients with the Arg/Arg genotype were dispensed more asthma-related prescriptions than patients with Gly/Arg or Gly/Gly genotype (see figure 5.14, panel A). Regarding the Glu27 variant, patients who smoked and/or were exposed to second hand smoke with the Glu/Glu genotype were dispensed more prescriptions than patients with the Gln/Glu or Gln/Gln genotype. Patients who did not smoke and/or were not exposed to second hand smoke and had the Gln/Gln genotype were dispensed more asthma prescriptions than patients with the Gln/Glu or Glu/Glu genotype (see figure 5.14, panel B).

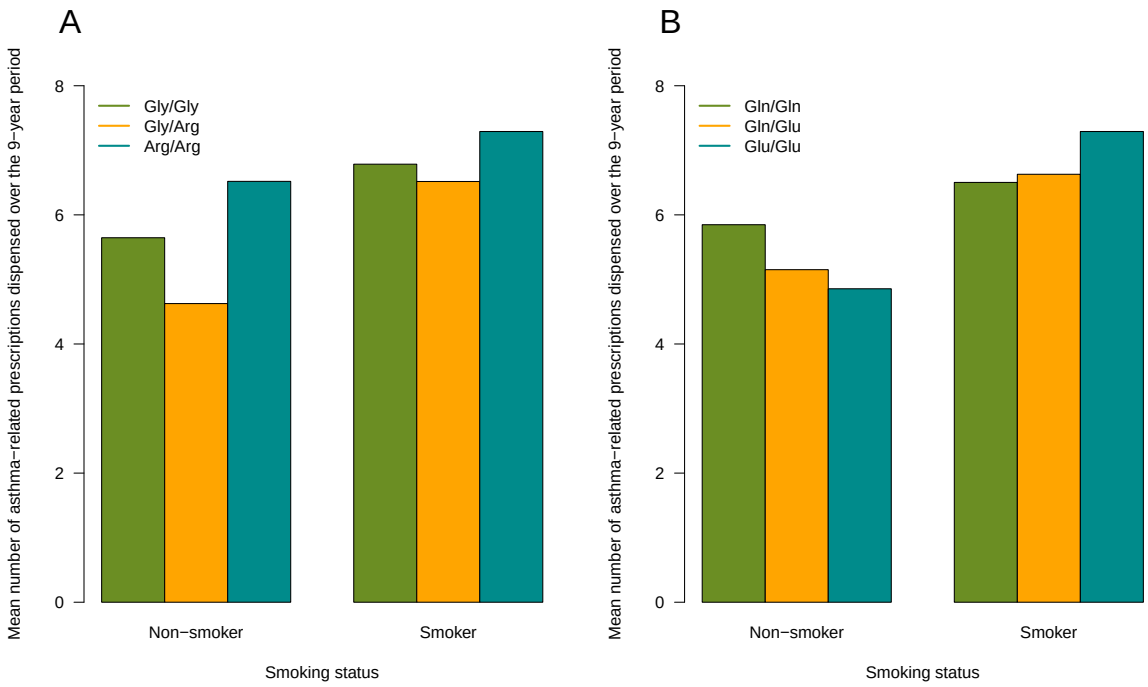

Figure 5.14: Mean number of asthma-related prescriptions dispensed per patient, over 9 years, according to the Arg16 and Glu27 variants and smoking status. **A**, mean number of asthma-related prescriptions dispensed according to the Arg16 variant. **B**, mean number of asthma-related prescriptions dispensed according to the Glu27 variant.

Overall, for both variants, the number of asthma-related prescriptions dispensed for children with asthma slightly decreased as the children grew older. Regarding the Arg16 variant, patients with the Arg/Arg genotype were dispensed a slightly higher

Prescriptions	Total	2005-2013					
		Arg16 variant			Glu27 variant		
		Gly/Gly	Gly/Arg	Arg/Arg	Gln/Gln	Gln/Glu	Glu/Glu
All asthma-related prescriptions							
Mean (SD)	50.5 (55.7)	52.7 (61.4)	45.6 (48.6)	59.0 (58.4)	52.3 (53.0)	49.4 (57.7)	50.4 (54.8)
Median (IQR)	31 (12-70)	30 (12-72)	29 (11-64.7)	41 (15-82.5)	36 (13-70)	29 (11-71)	29 (13-66)
Reliever prescriptions							
Mean (SD)	25.5 (31.6)	27.3 (35.4)	22.7 (26.6)	29.0 (34.1)	25.8 (30.7)	25.2 (33.0)	25.8 (29.5)
Median (IQR)	15 (7-32)	15 (7-33.5)	13 (7-29)	18 (8-35)	17 (7-33)	14 (6-32)	15 (8-30)
Inhaled Corticosteroid prescriptions							
Mean (SD)	8.5 (11.0)	8.7 (11.7)	8.0 (10.3)	9.6 (11.0)	8.8 (10.6)	8.3 (11.2)	8.6 (11.3)
Median (IQR)	5 (1-12)	5 (1-12)	5 (1-11)	6 (1-14)	5 (1-12)	5 (1-12)	4 (0-12)
Long-acting β_2-agonists prescriptions							
Mean (SD)	0.2 (1.4)	0.3 (1.4)	0.2 (1.5)	0.2 (0.8)	0.3 (1.7)	0.2 (1.1)	0.3 (1.5)
Median (IQR)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)
Combination of Long-acting β_2-agonists and Inhaled Corticosteroid prescriptions							
Mean (SD)	9.6 (17.1)	10.2 (18.7)	8.6 (15.1)	11.4 (17.8)	10.0 (16.8)	9.5 (17.1)	9.6 (17.6)
Median (IQR)	0 (0-13)	0 (0-13)	0 (0-12)	2 (0-17)	0 (0-14)	0 (0-14)	0 (0-12)
Leukotriene Receptor Antagonist prescriptions							
Mean (SD)	5.6 (13.1)	4.8 (12.5)	5.5 (12.9)	7.8 (14.9)	6.4 (13.3)	5.3 (13.2)	4.9 (12.6)
Median (IQR)	0 (0-2)	0 (0-1.5)	0 (0-1)	0 (0-7.5)	0 (0-4.5)	0 (0-1)	0 (0-1)

Table 5.16: Characteristics of the asthma-related prescriptions dispensed listed according to ADRB2 genotypes (n=1009 children and adults with asthma), from 2005 to 2013 . Standard deviation (SD), interquartile range (IQR).

number of prescriptions than patients with Gly/Arg and Gly/Gly genotype (see figure 5.15, panel A). For the Glu27 variant, patients with the Gln/Gln genotype were dispensed slightly more prescriptions than patients with the Gln/Glu and Glu/Glu genotype, except between 10 to 20 years old (see figure 5.15, panel B).

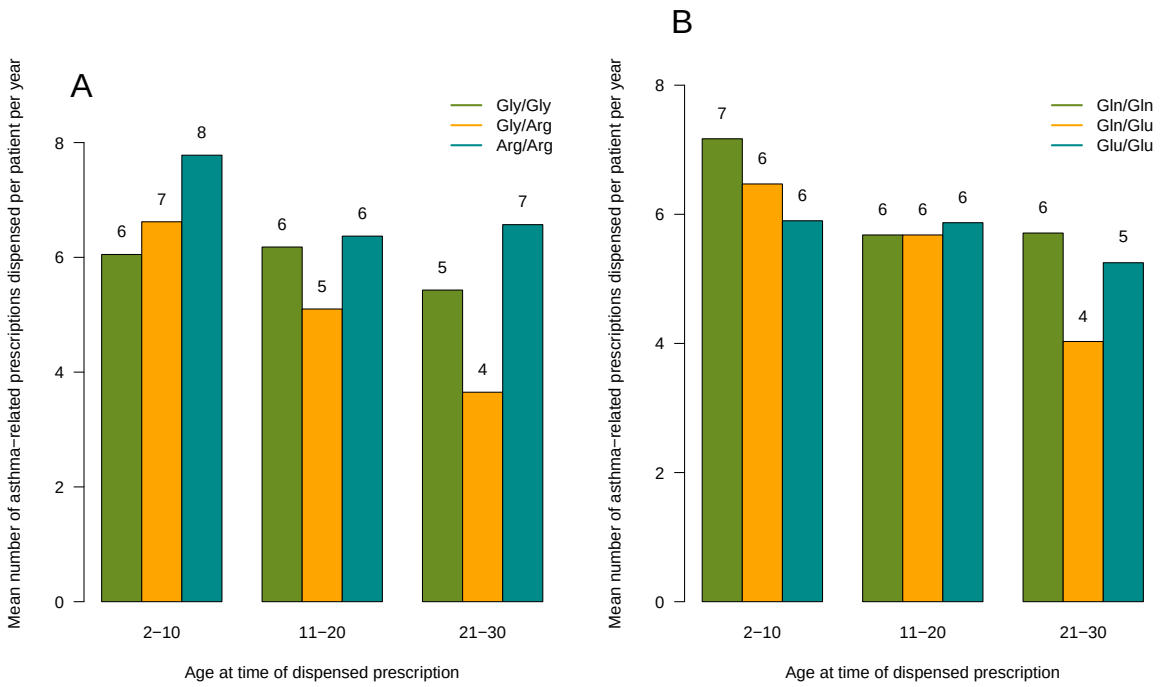

Figure 5.15: Mean number of asthma-related prescriptions dispensed per patient, over 9 years, according to the Arg16 and Glu27 variants and age. **A**, mean number of asthma-related prescriptions dispensed according to the Arg16 variant. **B**, mean number of asthma-related prescriptions dispensed according to the Glu27 variant.

A strong association was found between the Arg16 polymorphism and both the number of combined LABA and ICS prescriptions, and the number of LTRA prescriptions dispensed over the 9 year period of study (table 5.17). The incidence rate of dispensed prescriptions for a combination of LABA and ICS in children and adults with Arg/Arg genotype was 2.46 times that of children and adults with Gly/Gly genotype (table 5.17). The incidence rate of dispensed prescriptions for a combination of LABA and ICS in children and adults with Arg/Arg genotype was 2.50 times that of children and adults with Gly/Arg genotype (table 5.17). The incidence rate of dispensed prescriptions of LTRA in children and adults with Arg/Arg genotype was 2.33 times that of children and adults with Gly/Gly genotype (table 5.17). A weak-to-moderate association was found between the number of dispensed

prescriptions for a combination of LABA and ICS and smoking status (table 5.17). A weak-to-moderate association was also found between the number of asthma-related prescriptions and age and smoking status, with younger patients and patients who smoked and/or were exposed to smoke having more prescriptions dispensed than older patients and patients who did not smoke and/or were not exposed to smoke. A weak-to-moderate association was found for the number of reliever prescriptions dispensed between individuals Arg/Arg and Gly/Arg, with patients with Arg/Arg genotype having more prescriptions dispensed than individuals with Gly/Arg genotype (table 5.17). There was no evidence of an association between the Arg16 polymorphism and the number of ICS and inhaled separate LABA prescriptions dispensed (table 5.17).

Variables	IRR	95% CI	P-value
All asthma-related prescriptions			
Study year	0.93	(0.90,0.95)	$<$0.001 [a]
GlyGly vs. GlyArg	0.92	(0.79,1.08)	0.308
GlyGly vs. ArgArg	1.14	(0.92,1.42)	0.217
GlyArg vs. ArgArg	1.24	(1.00,1.53)	0.045 [a]
Age (in years)	0.96	(0.95,0.98)	$<$0.001 [a]
Smoking status (No vs. Yes)	1.18	(1.01,1.37)	0.032 [a]
Cat (no vs. Yes)	0.98	(0.83,1.15)	0.776
Reliever prescriptions			
Study year	0.94	(0.92,0.96)	$<$0.001 [a]
GlyGly vs. GlyArg	0.89	(0.77,1.02)	0.100
GlyGly vs. ArgArg	1.12	(0.92,1.36)	0.257
GlyArg vs. ArgArg	1.26	(1.04,1.53)	0.018 [a]
Age (in years)	0.97	(0.96,0.99)	$<$0.001 [a]
Smoking status (No vs. Yes)	1.21	(1.06,1.39)	0.005 [a]
Cat (No vs. Yes)	1.03	(0.88,1.19)	0.737
Inhaled Corticosteroid prescriptions			
Study year	0.85	(0.82,0.88)	$<$0.001 [a]
GlyGly vs. GlyArg	0.99	(0.80,1.22)	0.907
GlyGly vs. ArgArg	1.17	(0.87,1.56)	0.300
GlyArg vs. ArgArg	1.18	(0.89,1.57)	0.255
Age (in years)	0.92	(0.90,0.94)	$<$0.001 [a]

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Table 5.17 – continued from the previous page

Variables	IRR	95% CI	P-value
Smoking status (No vs. Yes)	0.89	(0.72,1.10)	0.271
Cat (No vs. Yes)	1.08	(0.86,1.36)	0.488
Long-acting β_2-agonists prescriptions			
GlyGly vs. GlyArg	0.75	(0.33,1.70)	0.491
GlyGly vs. ArgArg	0.62	(0.21,1.87)	0.398
GlyArg vs. ArgArg	1.56	(0.56,4.35)	0.394
Age (in years)	0.96	(0.89,1.04)	0.355
Smoking status (No vs. Yes)	0.63	(0.27,1.44)	0.272
Cat (No vs. Yes)	1.00	(0.42,2.39)	0.998
Combination of LABA and ICS prescriptions			
Study year	0.88	(0.82,0.94)	$<$0.001 [a]
GlyGly vs. GlyArg	0.98	(0.59,1.64)	0.953
GlyGly vs. ArgArg	2.46	(1.23,4.91)	0.010 [b]
GlyArg vs. ArgArg	2.50	(1.27,4.92)	0.008 [b]
Age (in years)	1.01	(0.96,1.07)	0.644
Smoking status (No vs. Yes)	1.91	(1.18,3.11)	0.008 [a]
Cat (No vs. Yes)	0.75	(0.44,1.28)	0.290
Leukotriene Receptor Antagonist prescriptions			
Study year	0.91	(0.83,1.00)	0.060
GlyGly vs. GlyArg	1.08	(0.60,1.95)	0.795
GlyGly vs. ArgArg	2.33	(1.06,5.13)	0.036 [b]
GlyArg vs. ArgArg	2.15	(0.99,4.70)	0.054
Age (in years)	0.84	(0.79,0.90)	$<$0.001 [a]
Smoking status (No vs. Yes)	1.76	(1.01,3.08)	0.047 [a]
Cat (No vs. Yes)	0.53	(0.28,1.00)	0.050

Table 5.17: Association between the number of asthma-related prescriptions dispensed and the Arg16 variant in children and adults with asthma (n=1009 children and adults), between 2005 and 2013 . Incidence rate ratio (IRR), confidence interval (CI). [a] - weak-to-moderate association, [b] - strong association.

A strong association was found between the Glu27 polymorphism and the number of LTRA prescriptions dispensed over the 9 year period of study (table 5.18). The incidence rate of dispensed prescriptions of LTRA in children and adults with Glu/Glu genotype was 0.43 times that of children and adults with Gln/Gln genotype (table 5.18). A weak-to-moderate association was found between the number of asthma-related prescriptions and age and smoking status, with younger patients and patients who smoked and/or were exposed to smoke having more prescriptions dispensed than older patients and patients who did not smoke and/or were not exposed to smoke (table 5.18). A weaker association was also found between the number of combined LABA and ICS prescriptions dispensed and the Glu27 polymorphism (table 5.18). There was no evidence of an association between the Glu27 genotypes and the number of relievers, ICS and inhaled LABA prescriptions dispensed (table 5.18).

Variables	IRR	95% CI	P-value
All asthma-related prescriptions			
Study year	0.93	(0.91,0.95)	$<$0.001 [a]
GlnGln vs. GlnGlu	0.91	(0.77,1.07)	0.269
GlnGln vs. GluGlu	0.92	(0.75,1.14)	0.446
GlnGlu vs. GluGlu	1.01	(0.83,1.23)	0.909
Age (in years)	0.96	(0.94,0.98)	$<$0.001 [a]
Smoking status (No vs. Yes)	1.18	(1.02,1.38)	0.028 [a]
Cat (No vs. Yes)	0.97	(0.82,1.14)	0.715
Relievers			
Study year	0.94	(0.92,0.96)	$<$0.001 [a]
GlnGln vs. GlnGlu	0.91	(0.78,1.05)	0.204
GlnGln vs. GluGlu	0.97	(0.80,1.17)	0.755
GlnGlu vs. GluGlu	1.07	(0.90,1.28)	0.458
Age (in years)	0.97	(0.96,0.99)	$<$0.001 [a]
Smoking status (No vs. Yes)	1.22	(1.06,1.40)	0.005 [a]
Cat (No vs. Yes)	1.02	(0.88,1.19)	0.798
Inhaled Corticosteroid prescriptions			
Study year	0.85	(0.82,0.88)	$<$0.001 [a]
GlnGln vs. GlnGlu	0.96	(0.77,1.20)	0.730
GlnGln vs. GluGlu	0.98	(0.73,1.30)	0.876

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Table 5.18 – continued from the previous page

Variables	IRR	95% CI	P-value
GlnGlu vs. GluGlu	1.02	(0.78,1.32)	0.901
Age (in years)	0.92	(0.90,0.94)	$<$0.001 [a]
Smoking status (No vs. Yes)	0.89	(0.72,1.09)	0.267
Cat (No vs. Yes)	1.08	(0.86,1.36)	0.498
Long-acting β_2-agonists prescriptions			
Study year	0.52	(0.44,0.62)	$<$0.001 [a]
GlnGln vs. GlnGlu	0.61	(0.27,1.36)	0.224
GlnGln vs. GluGlu	0.58	(0.20,1.64)	0.304
GlnGlu vs. GluGlu	0.96	(0.36,2.56)	0.929
Age (in years)	1.00	(0.91,1.08)	0.927
Smoking status (No vs. Yes)	0.72	(0.33,1.54)	0.394
Cat (No vs. Yes)	0.47	(0.19,1.15)	0.099
Combination of LABA and ICS prescriptions			
Study year	0.88	(0.83,0.94)	$<$0.001 [a]
GlnGln vs. GlnGlu	0.59	(0.35,1.00)	0.052
GlnGln vs. GluGlu	0.51	(0.26,1.01)	0.054 [b]
GlnGlu vs. GluGlu	0.87	(0.46,1.63)	0.657
Age (in years)	1.01	(0.95,1.07)	0.685
Smoking status (No vs. Yes)	1.95	(1.20,3.17)	0.007 [a]
Cat (No vs. Yes)	0.74	(0.43,1.27)	0.271
Leukotriene Receptor Antagonist prescriptions			
Study year	0.92	(0.84,1.01)	0.067
GlnGln vs. GlnGlu	0.53	(0.29,0.98)	0.044 [a]
GlnGln vs. GluGlu	0.43	(0.20,0.95)	0.037 [b]
GlnGlu vs. GluGlu	0.81	(0.39,1.69)	0.576
Age (in years)	0.84	(0.78,0.89)	$<$0.001 [a]
Smoking status (No vs. Yes)	1.81	(1.03,3.18)	0.038 [a]
Cat (No vs. Yes)	0.52	(0.28,0.99)	0.046 [a]

Table 5.18: Association between the number of asthma-related prescriptions dispensed and the Glu27 variant in children and adults with asthma (n=1009 children and adults), between 2005 and 2013. Incidence rate ratio (IRR), confidence interval (CI). [a] - weak-to-moderate association, [b] - strong association.

Asthma-related A&E visits/admissions

Out of 1009 children and adults, 289 (28.6%) had a total of 1143 asthma-related A&E visits/admissions. Over the 9-year period of study, the mean number of asthma-related A&E visits/admissions per individual was 1 (see table 5.19). The mean number of asthma-related A&E visits/admissions was similar across the different genotypes Adrenoreceptor β_2 (ADRB2) gene.

	2005-2013			
Prescriptions	**Total**	**Arg16 variant**		
		Gly/Gly	**Gly/Arg**	**Arg/Arg**
Mean (SD)	1.1 (4.8)	1.3 (5.0)	1.1 (5.2)	0.7 (1.9)
Median (IQR)	0 (0-1)	0 (0-1)	0 (0-1)	0 (0-1)
	Total	**Glu27 variant**		
		Gln/Gln	**Gln/Glu**	**Glu/Glu**
Mean (SD)	1.1 (4.8)	0.9 (4.0)	1.4 (5.8)	0.9 (2.3)
Median (IQR)	0 (0-1)	0 (0-1)	0 (0-1)	0 (0-1)

Table 5.19: Characteristics of the asthma-related A&E visits/admissions listed according to ADRB2 genotypes (n=1009 children and adults with asthma), from 2005 to 2013. Standard deviation (SD), interquartile range (IQR).

Children and adults who smoked and/or were exposed to second hand smoke had more asthma-related A&E visits/admissions than children and adults who did not smoke or were not exposed to second hand smoke (see figure 5.16). For both polymorphisms, individuals who smoked and/or were exposed to second hand smoke and were heterozygous had more A&E visits/admissions than individuals homozygous for the common and minor allele. On the other hand, individuals who did not smoke and/or were not exposed to second hand smoke and were wild-type (Gly/Gly or Gln/Gln) had more A&E visits/admissions than individuals with at least one minor allele.

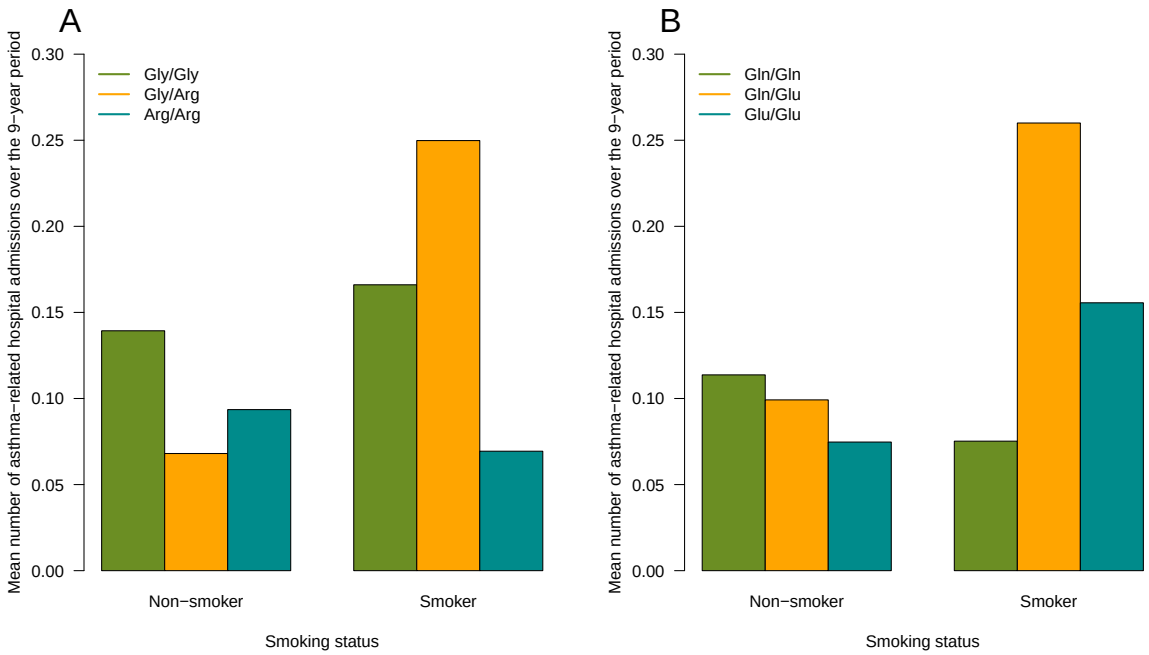

Figure 5.16: Mean number of asthma-related A&E visits/admissions per patient, over 9 years, according to the Arg16 and Glu27 variants and smoking status. **A**, mean number of asthma-related A&E visits/admissions according to the Arg16 variant. **B**, mean number of asthma-related A&E visits/admissions according to the Glu27 variant.

For both variants, the number of asthma-related A&E visits/admissions in children with asthma decreased as the children grew older. Regarding the Arg16 variant, the number of A&E visits/admissions is similar between genotypes (see figure 5.17, panel A). For the Glu27 variant, patients with the Gln/Glu genotype had slightly more A&E visits/admissions than patients with the Glu/Glu or Gln/Gln genotype (see figure 5.17, panel B).

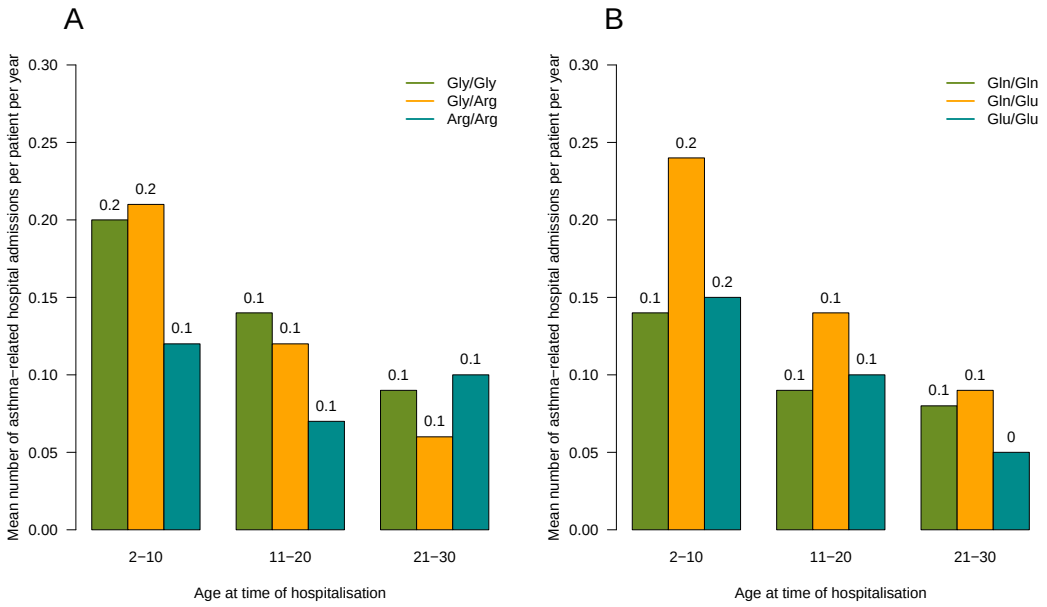

Figure 5.17: Mean number of asthma-related A&E visits/admissions per patient, over 9 years, according to the Arg16 and Glu27 variants and age. **A**, mean number of asthma-related A&E visits/admissions according to the Arg16 variant. **B**, mean number of asthma-related A&E visits/admissions according to the Glu27 variant.

Age and tobacco smoke exposure were associated with the number of asthma-related A&E visits/admissions over this period, with younger patients and patients who smoked and/or were exposed to smoke having more A&E visits/admissions than older patients and patients who did not smoke and/or were not exposed to smoke; these associations were weak-to-moderate (table 5.20). There was no evidence of an association between the number of asthma-related A&E visits/admissions and the Arg16Gly and Glu27Gln variants (table 5.20).

Variables	IRR	95% CI	P-value
Asthma-related A&E visits/admissions			
Arg16 variant			
Study year	0.86	(0.79,0.94)	$<$0.001 [a]
GlyGly vs. GlyArg	0.91	(0.62,1.33)	0.626
GlyGly vs. ArgArg	0.98	(0.58,1.65)	0.941
GlyArg vs. ArgArg	1.08	(0.64,1.80)	0.775
Age (in years)	0.89	(0.85,0.93)	$<$0.001 [a]
Smoking status (No vs. Yes)	1.74	(1.22,2.49)	0.002 [a]
Cat (No vs. Yes)	0.70	(0.46,1.06)	0.095
Glu27 variant			
Study year	0.87	(0.79,0.94)	0.001 [a]
GlnGln vs. GlnGlu	1.15	(0.77,1.72)	0.504
GlnGln vs. GluGlu	1.12	(0.68,1.87)	0.652
GlnGlu vs. GluGlu	0.98	(0.61,1.56)	0.929
Age (in years)	0.89	(0.85,0.93)	$<$0.001 [a]
Smoking status (No vs. Yes)	1.72	(1.20,2.46)	0.003 [a]
Cat (No vs. Yes)	0.70	(0.46,1.06)	0.088

Table 5.20: Association between the number of asthma-related A&E visits/admissions and the Arg16 and Glu27 variants in children and adults with asthma (n=1009 children and adults), between 2005 and 2013. Incidence rate ratio (IRR), confidence interval (CI).[a] - weak-to-moderate association.

Prescribing of oral corticosteroids

Out of 1009 children and adults, 506 (50.1%) were dispensed a total of 2719 dispensed prescriptions. Over the 9-year period of study, the mean number of oral prednisolone prescriptions dispensed per individual was 3 (see table 5.21). Patients with Arg/Arg genotype were dispensed a mean of 4 oral prednisolone prescriptions, patients with Gly/Gly genotype were dispensed a mean of 3 oral prednisolone prescriptions, and patients with Gly/Arg genotype were dispensed a mean of 2 oral prednisolone prescriptions. Regarding the Glu27 variant, patients with Gln/Gln or Gln/Glu genotype were both dispensed a mean of 3 oral prednisolone prescriptions and patients with Glu/Glu genotype were dispensed a mean of 2 oral prednisolone prescriptions.

	2005-2013			
Prescriptions	**Total**	**Arg16 variant**		
		Gly/Gly	**Gly/Arg**	**Arg/Arg**
Mean (SD)	2.7 (7.2)	2.8 (8.1)	2.3 (5.0)	3.7 (9.4)
Median (IQR)	0 (0-3)	0 (0-3)	0 (0-2)	0 (0-4)
	Total	**Glu27 variant**		
		Gln/Gln	**Gln/Glu**	**Glu/Glu**
Mean (SD)	2.7 (7.2)	3.0 (7.7)	2.7 (7.7)	2.2 (4.4)
Median (IQR)	0 (0-3)	0 (0-3)	0 (0-3)	0 (0-3)

Table 5.21: Characteristics of the oral prednisolone prescriptions dispensed listed according to ADRB2 genotypes (n=1009 children and adults with asthma), from 2005 to 2013. Standard deviation (SD), interquartile range (IQR).

Children and adults who smoked and/or were exposed to second hand smoke were dispensed more oral prednisolone prescriptions than children and adults who did not smoke or were not exposed to second hand smoke (see figure 5.18). For the Arg16 variant, individuals with the Arg/Arg genotype were dispensed more prescriptions than individuals with the Gly/Arg or Gly/Gly genotype, regardless of the smoking status. For the Glu27 variant, the number of dispensed prescriptions is similar for individuals who smoked and/or were exposed to second hand smoke, with Gln/Gln individuals receiving less prescriptions. For individuals who did not smoke and/or were not exposed to second hand smoke, Gln/Gln individuals were dispensed more prescriptions than Gln/Glu or Glu/Glu individuals.

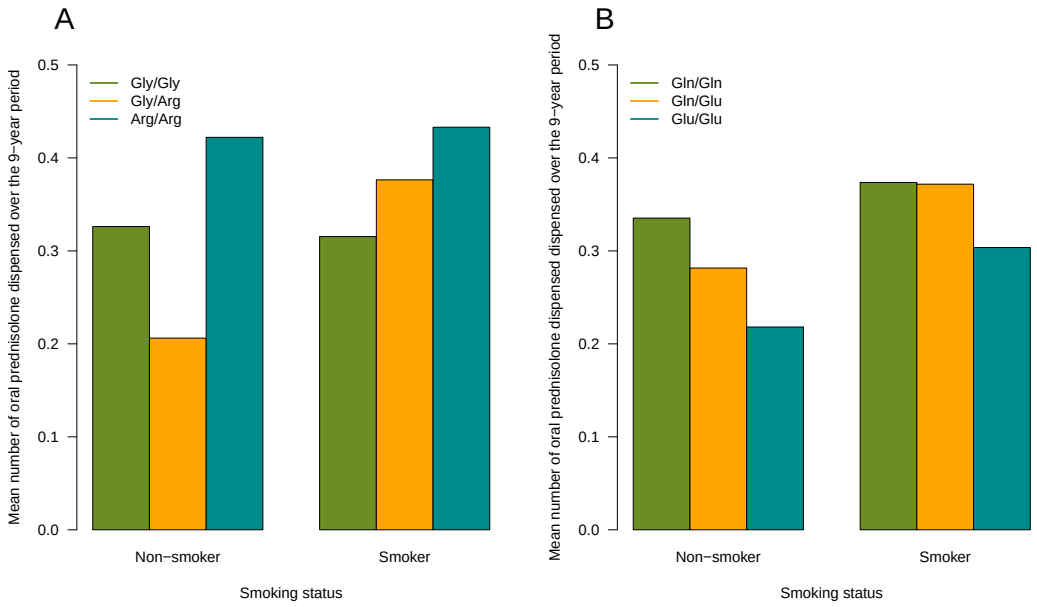

Figure 5.18: Mean number of oral prednisolone prescriptions dispensed per patient, over 9 years, according to the Arg16 and Glu27 variants and smoking status. **A**, mean number of oral prednisolone prescriptions dispensed according to the Arg16 variant. **B**, mean number of oral prednisolone prescriptions dispensed according to the Glu27 variant.

For both variants, individuals younger than 20 years old were dispensed a similar number of oral corticosteroids prescriptions between the different genotypes. Older individuals with the Arg/Arg genotype were dispensed more oral prednisolone than older individuals with the Gly/Gly and Gly/Arg genotype (see figure 5.19, panel A). Similarly, older individuals with the Gln/Gln genotype were dispensed more oral prednisolone than older individuals with the Gln/Glu and Glu/Glu genotype (see figure 5.19, panel B).

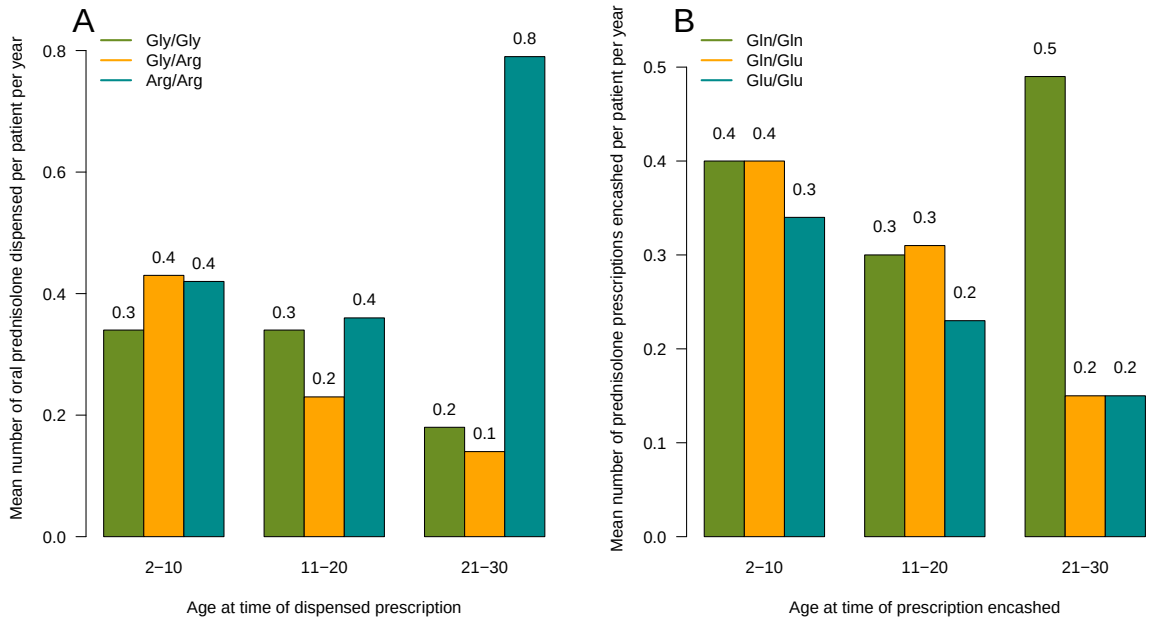

Figure 5.19: Mean number of oral prednisolone prescriptions dispensed per patient, over 9 years, according to the Arg16 and Glu27 variants and age. **A**, mean number of oral prednisolone prescriptions dispensed according to the Arg16 variant. **B**, mean number of oral prednisolone prescriptions dispensed according to the Glu27 variant.

Overall, Arg/Arg individuals were dispensed a higher number of oral prednisolone prescriptions than Gly/Gly or Gly/Arg individuals; the association was weak-to-moderate (table 5.22). Carriers of the Gln/Gln genotype also were dispensed a higher number of oral prednisolone prescriptions than carriers of the Glu/Glu genotype. Age, smoking status and cat ownership were associated with the number of oral prednisolone prescriptions dispensed over this period; these associations were also weak-to-moderate. Younger patients, patients without a cat, and patients who smoked and/or were exposed to smoke were dispensed more prescriptions than older patients, patients with a cat, and patients who did not smoke and/or were not exposed to smoke (table 5.22).

Variables	IRR	95% CI	P-value
Oral prednisolone prescriptions			
Arg16 variant			
Study year	0.96	(0.92,1.01)	0.122
GlyGly vs. GlyArg	1.06	(0.80,1.42)	0.673
GlyGly vs. ArgArg	1.64	(1.12,2.42)	0.011 [a]
GlyArg vs. ArgArg	1.54	(1.06,2.25)	0.024 [a]
Age (in years)	0.91	(0.88,0.94)	$<$0.001 [a]
Smoking status (No vs. Yes)	1.51	(1.15,1.98)	0.003 [a]
Cat (No vs. Yes)	0.67	(0.49,0.92)	0.014 [a]
Glu27 variant			
Study year	0.96	(0.92,1.01)	0.143
GlnGln vs. GlnGlu	0.75	(0.56,1.01)	0.057
GlnGln vs. GluGlu	0.63	(0.43,0.93)	0.020 [a]
GlnGlu vs. GluGlu	0.84	(0.59,1.21)	0.356
Age (in years)	0.91	(0.88,0.94)	$<$0.001 [a]
Smoking status (No vs. Yes)	1.48	(1.13,1.94)	0.004 [a]
Cat (No vs. Yes)	0.67	(0.49,0.92)	0.013 [a]

Table 5.22: Association between the number of oral prednisolone prescriptions dispensed and the Arg16 and Glu27 variants in children and adults with asthma (n=1009 children and adults), between 2005 and 2013. Incidence rate ratio (IRR), confidence interval (CI). [a] - weak-to-moderate association.

Asthma exacerbations

Out of 1009 children and adults, 582 (58%) children and adults had one or more asthma exacerbations, corresponding to 3834 asthma-related A&E visits/admissions and/or oral prednisolone prescriptions dispensed. Of the 1009 children and adults included in the analysis, 293 (29%) were dispensed one or more prescriptions of oral prednisolone, 77 (7.6%) had one or more asthma-related A&E visits/admissions, and 212 (21%) were dispensed one or more prescriptions of oral prednisolone and had one or more asthma-related A&E visits/admissions (see figure 5.20).

Over the 9-year period of study, the mean number of asthma exacerbations per individual was 4. Patients with Arg/Arg and Gly/Gly genotype had a mean of 4 asthma exacerbations, and patients with Gly/Arg genotype had a mean of 3 asthma

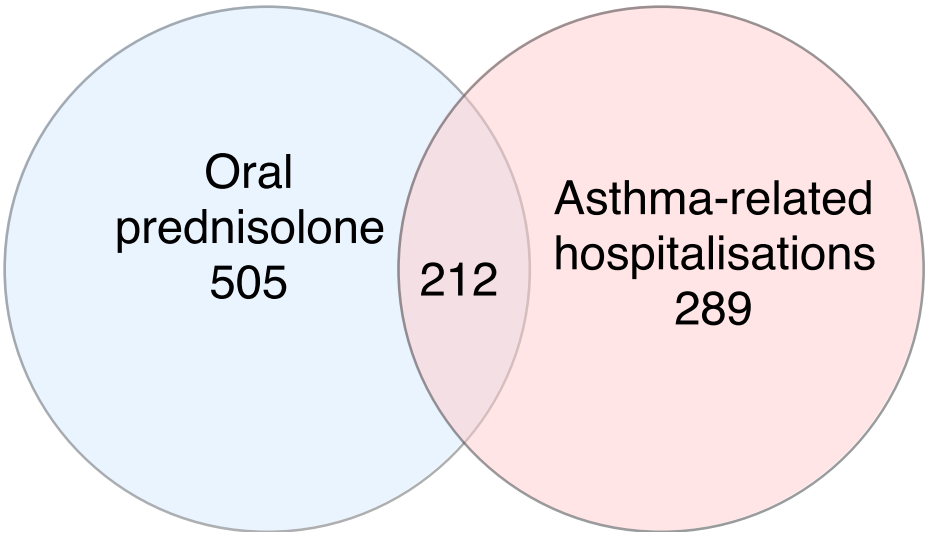

Figure 5.20: Number of children and adults with asthma (n=1009 children and adults) in the ADRB2 analyses that were dispensed oral prednisolone and/or had an asthma-related A&E visits/admission, from 2005 to 2013

exacerbations. Regarding the Glu27 variant, patients with Gln/Glu and Gln/Gln genotype had a mean of 4 asthma exacerbations, and patients with Glu/Glu genotype had a mean of 3 asthma exacerbations.

Children and adults who smoked and/or were exposed to second hand smoke had slightly more asthma exacerbations than children and adults who did not smoke or were not exposed to second hand smoke. Younger individuals had slightly more asthma exacerbations than older individuals.

Age, smoke exposure and cat ownership were weakly-to-moderately associated with the number of asthma exacerbations over this period, with younger patients, patients without a cat, and patients who smoked and/or were exposed to smoke having more prescriptions dispensed than older patients, patients with a cat, and patients who did not smoke and/or were not exposed to smoke. Overall, there was no evidence of an association between the number of asthma exacerbations and the Arg16Gly and Glu27Gln variants (table 5.23).

Variables	IRR	95% CI	P-value
Asthma exacerbations			
Arg16 variant			
Study year	0.95	(0.91,0.99)	0.018 [a]
GlyGly vs. GlyArg	1.03	(0.79,1.35)	0.811
GlyGly vs. ArgArg	1.41	(0.98,2.02)	0.065
GlyArg vs. ArgArg	1.36	(0.95,1.94)	0.091
Age (in years)	0.90	(0.88,0.93)	$<$0.001 [a]
Smoking status (No vs. Yes)	1.52	(1.18,1.96)	0.001 [a]
Cat (No vs. Yes)	0.69	(0.51,0.92)	0.012 [a]
Glu27 variant			
Study year	0.95	(0.91,0.99)	0.018 [a]
GlnGln vs. GlnGlu	0.84	(0.64,1.12)	0.238
GlnGln vs. GluGlu	0.73	(0.51,1.05)	0.087
GlnGlu vs. GluGlu	0.86	(0.62,1.21)	0.393
Age (in years)	0.89	(0.88,0.93)	$<$0.001 [a]
Smoking status (No vs. Yes)	1.53	(1.18,1.97)	0.001 [a]
Cat (No vs. Yes)	0.68	(0.51,0.91)	0.009 [a]

Table 5.23: Association between the number of asthma exacerbations and the Arg16 and Glu27 variants in children and adults with asthma (n=1009 children and adults), between 2005 and 2013. Incidence rate ratio (IRR), confidence interval (CI). [a] - weak-to-moderate association.

5.2.2 Pharmacoeconomics

Significant differences were found between patients with different genotypes. Patients with Arg/Arg or Gln/Gln genotype were dispensed more prescriptions for severe asthma than patients with Gly/Arg and Gly/Gly or Glu/Glu and Gln/Glu genotype. This section explores whether these differences translate into differences in cost for the NHS.

The mean cost of asthma-related prescriptions for individuals with the Arg/Arg genotype was £1302, while the mean cost for the Gly/Gly was £1092, and the mean cost for individuals with the Gly/Arg genotype was £955. Children and adults with Arg/Arg genotype have a significantly higher cost of prescribing for relievers and ICS compared to children and adults with Gly/Arg genotype (see table 5.24). Children and

adults with Gly/Arg genotype have a significantly lower cost of prescribing for relievers and separate LABA compared to children and adults with Gly/Gly genotype (see table 5.24). Children and adults with Arg/Arg genotype have a significantly higher cost of prescribing for LTRA compared to children and adults with Gly/Gly genotype (see table 5.24).

The mean cost of asthma-related A&E visits/admissions for individuals with the Gly/Gly genotype was £730, while the mean cost for individuals with the Gly/Arg genotype was £583, and the mean cost for individuals with the Arg/Arg genotype was £410. There was no evidence of a difference, at the 5% level (see table 5.24). The mean cost of oral corticosteroids prescribing for individuals with the Arg/Arg genotype was £12, while the mean cost for individuals with Gly/Gly genotype was £9, and the mean cost for individuals with the Gly/Arg genotype was £7. Children and adults with Arg/Arg genotype had a significantly higher cost of prescribing for oral corticosteroids compared to children and adults with Gly/Arg genotype (see table 5.24). The mean cost of asthma exacerbations for individuals with the Gly/Gly genotype was £740, while the mean cost of asthma exacerbations for individuals with the Gly/Arg genotype was £590, and the mean cost of asthma exacerbations for individuals with the Arg/Arg genotype was £422. There was no evidence of a difference, at the 5% level (see table 5.24). The mean cost of primary care emergency visits for individuals with the Arg/Arg genotype was £126, the mean cost of primary care emergency visits for individuals with the Gly/Gly genotype was £95, and the mean cost of primary care emergency visits for individuals with the Gly/Arg genotype was £77. Children and adults with Arg/Arg genotype had a significantly higher cost of primary care emergency visits compared to children and adults with the Gly/Arg genotype (see table 5.24).

The mean cost for all conditions examined for children and adults with asthma and with the Gly/Gly genotype was £1931, while the mean cost for children and adults with asthma and with the Arg/Arg genotype was £1850, and the mean cost for children and adults with asthma and with the Gly/Arg genotype was £1623. There was no evidence of a difference, at the 5% level (see table 5.24).

Prescriptions	Mean difference (in £)	95% BCa CI (in £)
All asthma-related prescriptions		
GlyGly vs. GlyArg	-136.64	(-365,64.93)
GlyGly vs. ArgArg	210.04	(-114.64,529.31)
GlyArg vs. ArgArg	346.68	(68.96,665.63) [c]
Relievers		
GlyGly vs. GlyArg	-31.72	(-70.89,-3.89) [c]
GlyGly vs. ArgArg	14.67	(-28.73,79.84)
GlyArg vs. ArgArg	46.40	(10.56,112.23) [c]
Inhaled Corticosteroid (ICS)		
GlyGly vs. GlyArg	-19.64	(-48.22,3.66)
GlyGly vs. ArgArg	24.94	(-14.76,67.04)
GlyArg vs. ArgArg	44.58	(8.41,87.35) [c]
Long-acting β_2-agonists (LABA)		
GlyGly vs. GlyArg	-33.84	(-70.19,-5.92) [c]
GlyGly vs. ArgArg	-16.25	(-54.43,22.85)
GlyArg vs. ArgArg	17.59	(-8.38,59.64)
Combination of LABA and ICS		
GlyGly vs. GlyArg	-76.77	(-229.15,52.53)
GlyGly vs. ArgArg	64.81	(-105.48,271.24)
GlyArg vs. ArgArg	141.58	(-18.05,326.38)
Leukotriene Receptor Antagonist (LTRA)		
GlyGly vs. GlyArg	29.45	(-43.28,92.20)
GlyGly vs. ArgArg	126.95	(15.53,244.27) [c]
GlyArg vs. ArgArg	97.49	(-5.31,220.37)
Asthma-related A&E visits/admissions		
GlyGly vs. GlyArg	-147.16	(-619.08,218.72)
GlyGly vs. ArgArg	-320.19	(-766.28,146.28)
GlyArg vs. ArgArg	-173.04	(-532.71,343.68)
Oral corticosteroids		
GlyGly vs. GlyArg	-2.53	(-7.11,0.20)
GlyGly vs. ArgArg	2.43	(-2.49,8.75)
GlyArg vs. ArgArg	4.96	(1.35,10.76) [c]
Asthma exacerbations		
GlyGly vs. GlyArg	-149.69	(-590.77,212.65)

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Table 5.24 – continued from the previous page

Prescriptions	Mean difference (in £)	95% BCa CI (in £)
GlyGly vs. ArgArg	-317.76	(-724.62,164.34)
GlyArg vs. ArgArg	-168.07	(-492.31,338.80)
Primary care emergency costs		
GlyGly vs. GlyArg	-17.37	(-55.52,10.72)
GlyGly vs. ArgArg	31.25	(-11.57,113.20)
GlyArg vs. ArgArg	48.62	(12.24,137.95) [c]
Snapshot cost of the cohort		
GlyGly vs. GlyArg	-308.24	(-1023.22,171.30)
GlyGly vs. ArgArg	-81.02	(-723.18,537.47)
GlyArg vs. ArgArg	227.22	(-239.89,859.19)

Table 5.24: Difference in the mean cost of prescriptions dispensed according to the Arg16 variant, from 2005 to 2013 (n=1009 children and adults with asthma). Figures prefixed with a minus sign indicate that the costs in children with FLG mutations are less than in children without these mutations. Bias corrected and accelerated (BCa), confidence interval (CI). [c] - significant association.

The mean cost of asthma-related prescribing for individuals with the Gln/Gln genotype was £1127, while the mean cost for individuals with the Gln/Glu genotype was £1038, and the mean cost for the Glu/Glu genotype was £1027. There was no evidence of a difference, at the 5% level, between participants with the Glu27 variant with respect to asthma-related prescribing (see table 5.25).

The mean cost of asthma-related A&E visits/admissions for individuals with the Gln/Glu genotype was £789, while the mean cost for individuals with the Glu/Glu genotype was £510, and the mean cost for individuals with the Gln/Gln genotype was £400. The mean cost of oral corticosteroids prescriptions for individuals with the Gln/Gln and the Gln/Glu genotype was £9, and the mean cost of oral corticosteroids prescriptions for the Glu/Glu genotype was £7. The mean cost of asthma exacerbations for individuals with the Gln/Glu genotype was £798, the mean cost of asthma exacerbations for individuals with the Glu/Glu genotype was £516, and the mean cost of asthma exacerbations for individuals with the Gln/Gln genotype was

£409. There was no evidence of a difference, at the 5% level. The mean cost of primary care emergency visits for individuals with the Gln/Gln genotype was £102, the mean cost of primary care emergency visits for individuals with the Gln/Glu genotype was £92, and the mean cost of primary care emergency visits for individuals with the Glu/Glu genotype was £74. There was no evidence of a difference, at the 5% level (see table 5.25).

The mean cost for all conditions examined for children and adults with asthma and with the Gln/Glu genotype was £1928, the mean cost for individuals with the Glu/Glu genotype was £1625, and the mean cost for children and adults with asthma and with the Gln/Gln genotype was £1638. There was no evidence of a difference, at the 5% level (see table 5.25).

Prescriptions	Mean difference (in £)	95% BCa CI (in £)
All asthma-related prescriptions		
GlnGln vs. GlnGlu	-89.30	(-331.39,130.07)
GlnGln vs. GluGlu	-99.83	(-358.14,213.82)
GlnGlu vs. GluGlu	-10.54	(-262.27,273.12)
Relievers		
GlnGln vs. GlnGlu	-5.75	(-43.75,26.11)
GlnGln vs. GluGlu	-11.47	(-48.20,23.96)
GlnGlu vs. GluGlu	-5.72	(-40.94,31.60)
Inhaled Corticosteroid (ICS)		
GlnGln vs. GlnGlu	-11.11	(-38,15.10)
GlnGln vs. GluGlu	-9.22	(-39.08,25.59)
GlnGlu vs. GluGlu	1.90	(-26.15,36.88)
Long-acting β_2-agonists (LABA)		
GlnGln vs. GlnGlu	-15.27	(-50.17,13.43)
GlnGln vs. GluGlu	5.75	(-32.94,48.35)
GlnGlu vs. GluGlu	21.01	(-12.34,58.95)
Combination of LABA and ICS		
GlnGln vs. GlnGlu	-16.44	(-158.98,108.18)
GlnGln vs. GluGlu	-18.92	(-190.86,180.44)
GlnGlu vs. GluGlu	-2.48	(-166.65,181.51)

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Table 5.25 – continued from the previous page

Prescriptions	Mean difference (in £)	95% BCa CI (in £)
Leukotriene Receptor Antagonist (LTRA)		
GlnGln vs. GlnGlu	-48.13	(-133.02,32.37)
GlnGln vs. GluGlu	-64.94	(-158.26,43.20)
GlnGlu vs. GluGlu	-16.81	(-91.76,82.87)
Asthma-related A&E visits/admissions		
GlnGln vs. GlnGlu	389.32	(-16.65,794.36)
GlnGln vs. GluGlu	109.76	(-304.55,385.19)
GlnGlu vs. GluGlu	-279.56	(-741.07,35.87)
Oral corticosteroids		
GlnGln vs. GlnGlu	-0.21	(-3.81,3.90)
GlnGln vs. GluGlu	-2.55	(-5.83,0.33)
GlnGlu vs. GluGlu	-2.34	(-6.30,0.46)
Asthma exacerbations		
GlnGln vs. GlnGlu	389.11	(-9.44,766.79)
GlnGln vs. GluGlu	107.21	(-239.17,402.33)
GlnGlu vs. GluGlu	-281.90	(-708.73,68.33)
Primary care emergency costs		
GlnGln vs. GlnGlu	-9.50	(-48.75,24.58)
GlnGln vs. GluGlu	-27.79	(-67.17,1.22)
GlnGlu vs. GluGlu	-18.30	(-57.06,8.78) [c]
Snapshot cost of the cohort		
GlnGln vs. GlnGlu	290.32	(-185.37,858.34)
GlnGln vs. GluGlu	-12.42	(-524.76,495.25)
GlnGlu vs. GluGlu	-302.74	(-922.01,197.43)

Table 5.25: Difference in the mean cost of prescriptions dispensed according to the Glu27 variant, from 2005 to 2013 (n=1009 children and adults with asthma). Figures prefixed with a minus sign indicate that the costs in children with FLG mutations are less than in children without these mutations. Bias corrected and accelerated (BCa), confidence interval (CI).

5.3 Understanding the association between Fc fragment of IgE receptor II (FCER2) genetic variation and healthcare utilisation

Demographics

Of the 1100 children and young adults in BREATHE, 139 individuals were excluded since they lacked genotyping information, and 34 individuals were excluded due to missing information on one or more of the clinical variables considered. The final dataset corresponds to 927 children and adults with asthma. Four hundred and ninety-four participants (53%) reported that they had eczema (see figure 5.21 for a flowchart of the sample size). Table 5.26 presents the characteristics of the study population. The distribution of the population is as follows: 54.7% wild-type - TT genotype, 37.1% heterozygous - TC genotype, and 8.2% homozygous - CC genotype.

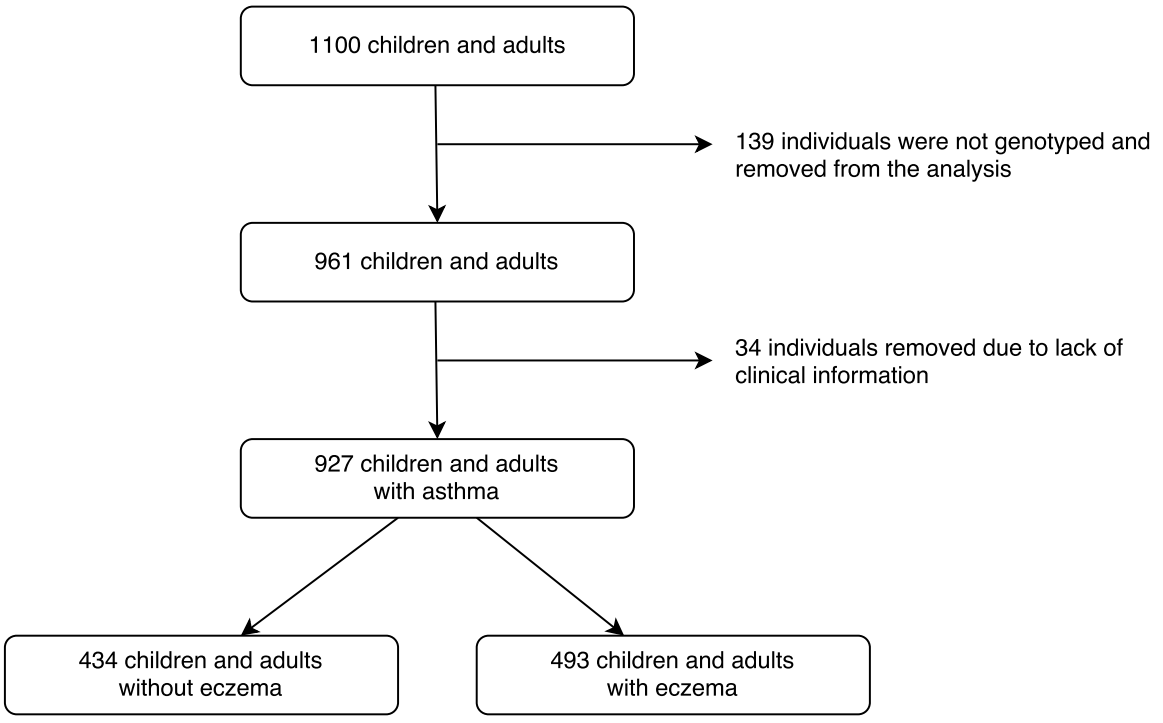

Figure 5.21: Flow diagram of the final sample included in the FCER2 analyses.

Variable	BREATHE N=927	Patients with eczema N=493	Patients without eczema N=434
Age, at data collection			
Median (IQR)	10 (7-13)	10 (7-13)	10 (7-13)
Number of males (%)	556 (60%)	302 (61.2%)	254 (58.5%)
Number of individuals TT (%)	507 (54.7%)	268 (54.4%)	239 (55.1%)
Number of individuals TC (%)	344 (37.1%)	186 (37.7%)	158 (36.4%)
Number of individuals CC (%)	76 (8.2%)	39 (7.9%)	37 (8.5%)
Number of cat owners (%)	243 (26.2%)	124 (24%)	119 (28.2%)
Number of children and young adults with family members with eczema (%)	499 (53.8%)	306 (62.1%)	193 (44.5%)
Number of children and young adults with family members with asthma (%)	570 (61.5%)	311 (63.1%)	259 (59.7%)

Table 5.26: Characteristics of the dataset used in the FCER2 analyses. Interquartile range (IQR).

5.3.1 Association between Fc fragment of IgE receptor II (FCER2) genetic variation and eczema-related prescribing

Figure 5.22 displays the number of children and adults per year. The proportion of individuals homozygous for the Fc fragment of IgE receptor II (FCER2) variant over the years was constant.

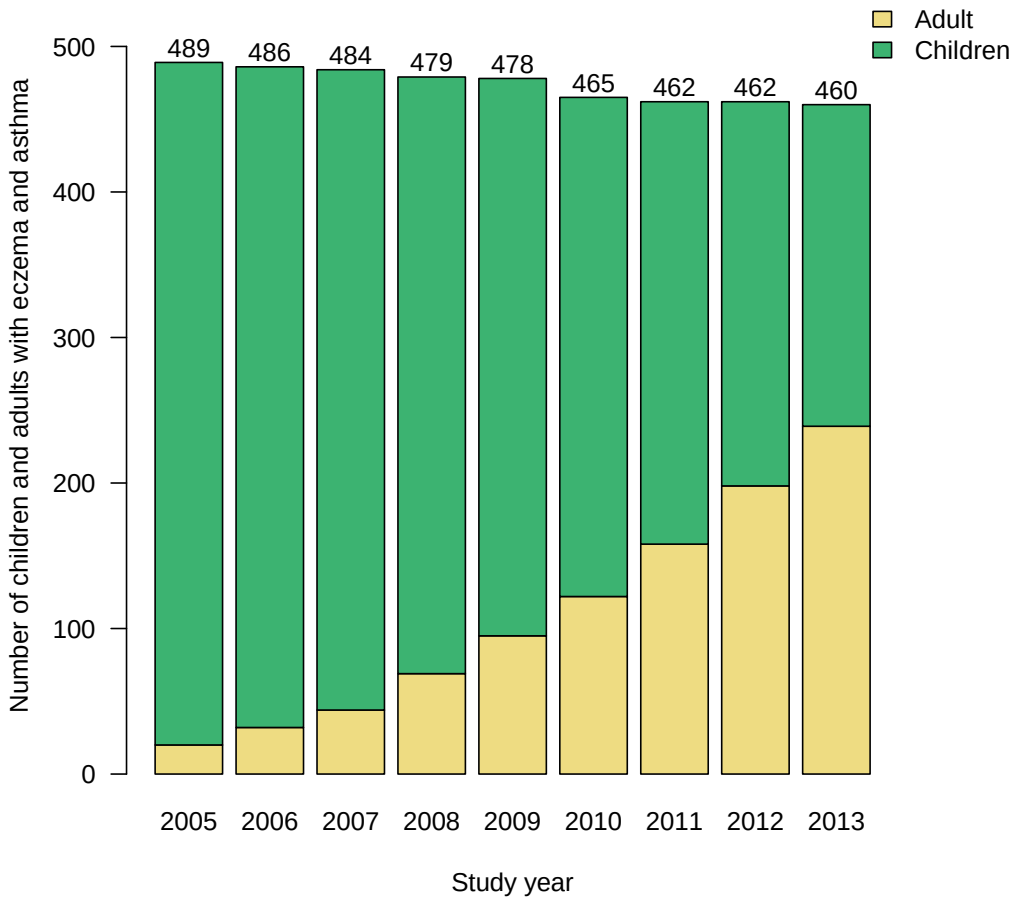

Figure 5.22: Number of children and adults with both eczema and asthma per year, for the FCER2-related analyses.

Over the 9-year period of study, the median number of eczema-related prescriptions dispensed per patient was 11. Patients with CC genotype were dispensed a median of 18 prescriptions, while patients with TC or TT genotype were both dispensed a median of 11 prescriptions (see table 5.27).

Prescriptions	2005-2013			
	Total	TT	TC	CC
All eczema-related prescriptions				
Mean (SD)	26.7 (47.6)	23.2 (42.5)	30.2 (55)	33.5 (41.6)
Median (IQR)	11 (3-30)	11 (3-27)	11 (3-35.7)	18 (4-37.5)
Emollients				
Mean (SD)	9.5 (21.5)	9.1 (21.7)	9.7 (21.7)	12 (19)
Median (IQR)	2 (0-8)	2 (0-7)	2 (0-8)	3 (0-16.5)
Antihistamines				
Mean (SD)	8.9 (14.4)	7.1 (11.8)	11.2 (17.4)	9.7 (14.1)
Median (IQR)	2 (0-12)	2 (0-9.2)	3 (1-13)	3 (1-13.5)
Prescriptions for mild eczema				
Mean (SD)	2.5 (6)	2.3 (5.3)	2.5 (6.6)	3.8 (7.4)
Median (IQR)	1 (0-2)	1 (0-3)	0 (0-2)	1 (0-3.5)
Prescriptions for moderate eczema				
Mean (SD)	2.7 (9.2)	2.1 (6.5)	3 (12.1)	4.7 (9.3)
Median (IQR)	0 (0-2)	0 (0-2)	1 (0-2)	1 (0-4.5)
Prescriptions for severe eczema				
Mean (SD)	3.1 (10.3)	2.6 (9.5)	3.8 (11.7)	3.4 (8.2)
Median (IQR)	0 (0-2)	0 (0-2)	0 (0-2)	0 (0-2.5)

Table 5.27: Characteristics of the eczema-related prescriptions dispensed listed according to the FCER2 genotypes (n=493 children and adults with eczema and asthma), from 2005 to 2013. Standard deviation (SD), interquartile range (IQR).

In this study, 354 patients (71.8%) were dispensed a total of 4373 prescriptions for antihistamines, and 315 patients (64%) were dispensed a total of 4709 prescriptions for emollients. Half of the cohort (50.3%) were dispensed 1217 prescriptions for mild eczema, 245 patients (49.7%) were dispensed 1326 prescriptions for moderate eczema, and 206 patients (41.8%) were dispensed 1529 prescriptions for severe eczema.

Until 2007, females with CC genotype were dispensed more eczema-related prescriptions than males and females with TT or TC genotype. From 2007 onwards, the mean number of prescriptions is similar for both genders (see figure 5.23, panel

A). Overall, individuals between 10 and 20 years old were dispensed less eczema-related prescriptions than children younger than 10 years old and adults older than 20 years old. For individuals older than 20 years old, patients with CC genotype were dispensed more eczema-related prescriptions than patients with TT or TC genotype (see figure 5.23, panel B).

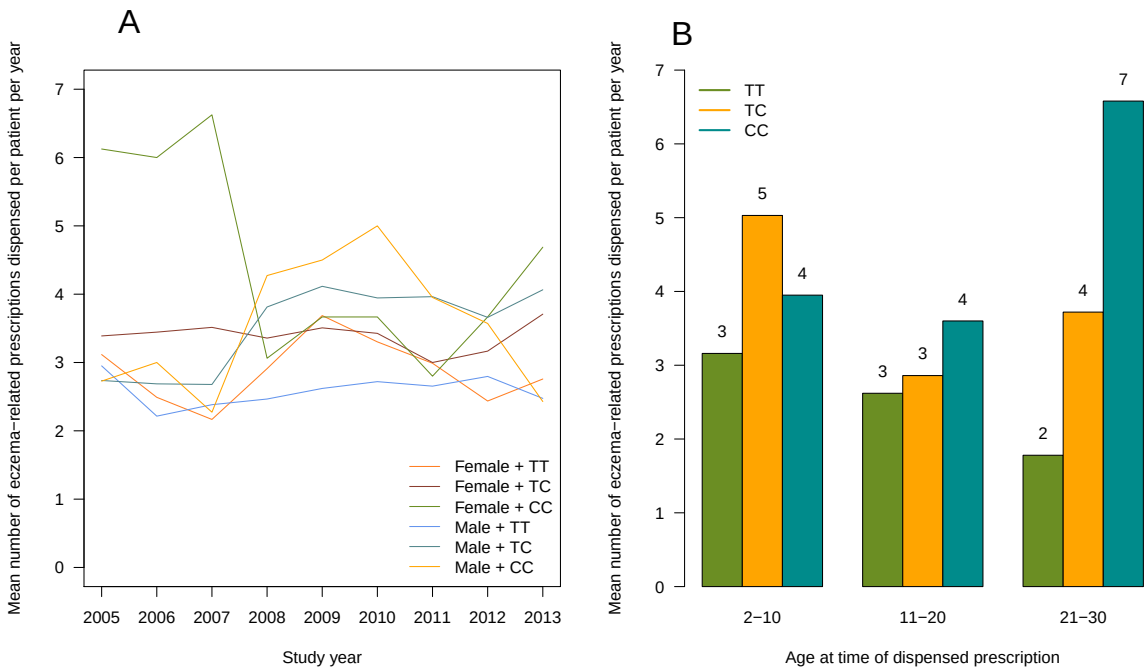

Figure 5.23: Mean number of eczema-related prescriptions dispensed per patient, over 9 years, according to the FCER2 variant, gender and age. **A**, mean number of eczema-related prescriptions dispensed according to gender. **B**, mean number of eczema-related prescriptions dispensed according to age.

Age was weakly-to-moderately associated with the number of eczema-related prescriptions over this time, with younger patients having more prescriptions dispensed than older patients (table 5.28). A weak-to-moderate association was found between the FCER2 polymorphism and the number of antihistamines dispensed (table 5.28). Overall, there was no evidence of an association between the FCER2 polymorphism and the number of prescriptions for emollients and the number of prescribing for mild, moderate and severe eczema (table 5.28).

Variables	IRR	95% CI	P-value
All eczema-related prescriptions			
Study year	1.02	(0.97,1.06)	0.403
TT vs. TC	1.19	(0.87,1.62)	0.277
TT vs. CC	1.54	(0.89,2.67)	0.122
TC vs. CC	1.30	(0.74,2.29)	0.366
Age (in years)	0.95	(0.92,0.98)	0.005 [a]
Gender (Male vs. Female)	1.33	(0.99,1.80)	0.058
Cat (No vs. Yes)	1.02	(0.72,1.43)	0.926
Antihistamines			
Study year	1.00	(0.95,1.06)	0.882
TT vs. TC	1.70	(1.17,2.47)	0.006 [a]
TT vs. CC	1.75	(0.91,3.39)	0.095
TC vs. CC	1.03	(0.52,2.03)	0.926
Age (in years)	0.94	(0.90,0.98)	0.005 [a]
Gender (Male vs. Female)	1.27	(0.89,1.83)	0.192
Cat (No vs. Yes)	0.98	(0.64,1.49)	0.926
Emollients			
Study year	0.99	(0.92,1.05)	0.694
TT vs. TC	0.95	(0.61,1.49)	0.826
TT vs. CC	1.78	(0.82,3.87)	0.143
TC vs. CC	1.88	(0.84,4.17)	0.123
Age (in years)	0.91	(0.87,0.96)	0.001 [a]
Gender (Male vs. Female)	1.51	(0.98,2.32)	0.062
Cat (No vs. Yes)	0.91	(0.56,1.50)	0.725
Prescriptions for mild eczema			
Study year	0.93	(0.86,0.99)	0.030 [a]
TT vs. TC	0.83	(0.55,1.24)	0.360
TT vs. CC	1.66	(0.84,3.27)	0.146
TC vs. CC	2.00	(0.99,4.07)	0.054
Age (in years)	0.93	(0.89,0.97)	0.002 [a]
Gender (Male vs. Female)	1.96	(1.33,2.88)	$<$0.001 [a]
Cat (No vs. Yes)	1.08	(0.69,1.69)	0.723
Prescriptions for moderate eczema			
Study year	0.93	(0.86,1.00)	0.049
TT vs. TC	1.17	(0.76,1.81)	0.475

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Table 5.28 – continued from previous page

Variables	IRR	95% CI	P-value
TT vs. CC	2.00	(0.97,4.15)	0.062
TC vs. CC	1.71	(0.81,3.63)	0.161
Age (in years)	0.99	(0.94,1.04)	0.779
Gender (Male vs. Female)	1.45	(0.96,2.20)	0.076
Cat (No vs. Yes)	1.18	(0.74,1.89)	0.490
Prescriptions for severe eczema			
Study year	0.97	(0.89,1.06)	0.493
TT vs. TC	1.57	(0.91,2.69)	0.105
TT vs. CC	1.64	(0.63,4.26)	0.312
TC vs. CC	1.04	(0.39,2.78)	0.929
Age (in years)	1.03	(0.97,1.10)	0.347
Gender (Male vs. Female)	1.17	(0.69,1.98)	0.548
Cat (No vs. Yes)	1.78	(0.99,3.21)	0.054

Table 5.28: Association between the number of asthma-related prescriptions dispensed and the FCER2 variant in children and adults with asthma (n=493 children and adults), between 2005 and 2013. Incidence rate ratio (IRR), confidence interval (CI). [a] - weak-to-moderate association, [b] - strong association.

Prescriptions for eczema associated with infections

Overall, the number of antivirals and antibiotics dispensed is similar for individuals, regardless of their genotype (see table 5.29). Fifty-four (11%) children and adults were dispensed a total of 152 prescriptions for antivirals, while 311 (63%) children and adults were dispensed a total of 1106 prescriptions for antibiotics.

There were no apparent differences between males and females for the pattern of prescribing either antivirals or antibiotics. Patients with TT genotype were dispensed more antivirals for all age groups in comparison to patients with TC or CC genotype (see figure 5.24, panel A). The opposite was found regarding antibiotics: patients with the CC genotype were dispensed more antibiotics than patients with TT or TC genotype (see figure 5.24, panel B). Older patients were dispensed more antivirals and antibiotics than younger patients.

Prescriptions	2005-2013			
	Total	TT	TC	CC
Antivirals				
Mean (SD)	0.3 (1.5)	0.4 (1.9)	0.2 (0.9)	0.2 (0.4)
Median (IQR)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)
Antibiotics				
Mean (SD)	2.2 (4.2)	2.2 (4.4)	2.2 (4)	2.6 (3.7)
Median (IQR)	1 (0-3)	1 (0-3)	1 (0-3)	1 (0-3.5)

Table 5.29: Characteristics of the antivirals and antibiotics dispensed listed according to the FCER2 genotypes (n=493 children and adults with eczema and asthma), from 2005 to 2013. Standard deviation (SD), interquartile range (IQR).

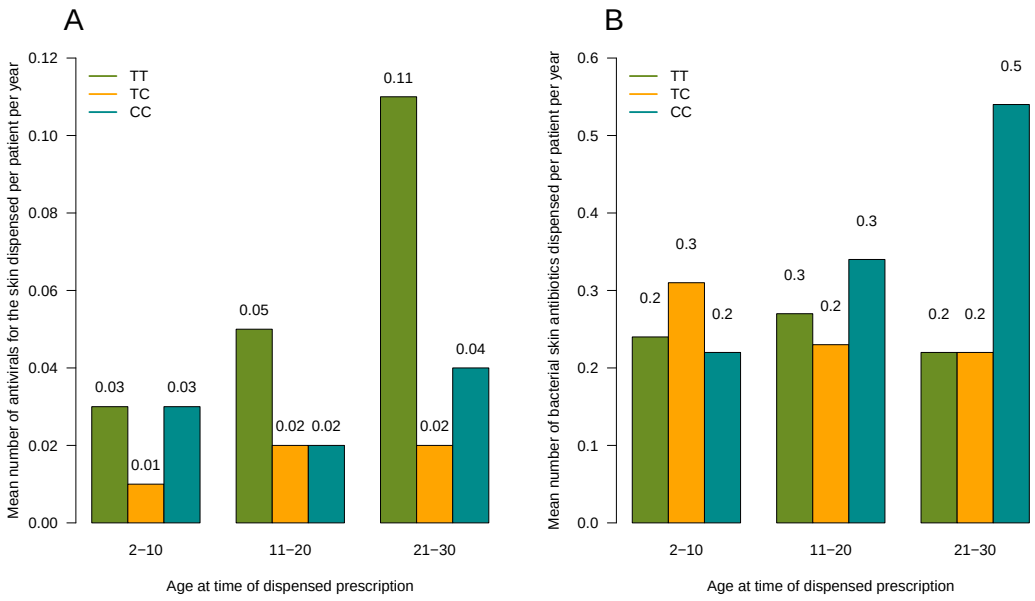

Figure 5.24: Mean number of antivirals and antibiotics dispensed per patient, over 9 years, according to the FCER2 variant and age. **A**, mean number of antivirals dispensed according to age. **B**, mean number of antibiotics according to age.

Overall, the FCER2 variant was inversely associated with the number of antivirals dispensed. Children and adults with TT genotype were dispensed more antivirals than children and adults with TC genotype (table 5.30). There was no evidence of an association between the FCER2 polymorphism and the number of prescriptions for antibiotics dispensed (table 5.30).

Variables	**IRR**	**95% CI**	**P-value**
Antivirals			
TT vs. TC	0.36	(0.22,0.60)	$<$0.001 [b]
TT vs. CC	0.42	(0.17,1.08)	0.073
TC vs. CC	1.18	(0.44,3.18)	0.747
Age (in years)	1.03	(0.99,1.08)	0.167
Gender (M vs. F)	1.20	(0.76,1.89)	0.438
Cat (No vs. Yes)	0.69	(0.41,1.18)	0.179
Antibiotics			
Study year	0.95	(0.90,1.01)	0.082
TT vs. TC	1.06	(0.79,1.43)	0.695
TT vs. CC	1.35	(0.80,2.28)	0.255
TC vs. CC	1.27	(0.74,2.19)	0.377
Age (in years)	1.02	(0.99,1.06)	0.218
Gender (M vs. F)	1.23	(0.92,1.64)	0.163
Cat (No vs. Yes)	1.20	(0.86,1.67)	0.273

Table 5.30: Association between the number of antivirals and antibiotics dispensed and the FCER2 variant in children and adults with asthma (n=493 children and adults), between 2005 and 2013. Incidence rate ratio (IRR), confidence interval (CI). [b] - strong association.

5.3.2 Association between Fc fragment of IgE receptor II (FCER2) genetic variation and asthma-related prescribing

Next, the association between the FCER2 polymorphism and asthma-related prescribing was explored. Figure 5.25 displays the number of children and adults per year. The proportion of individuals homozygous for the FCER2 polymorphism over the years was constant.

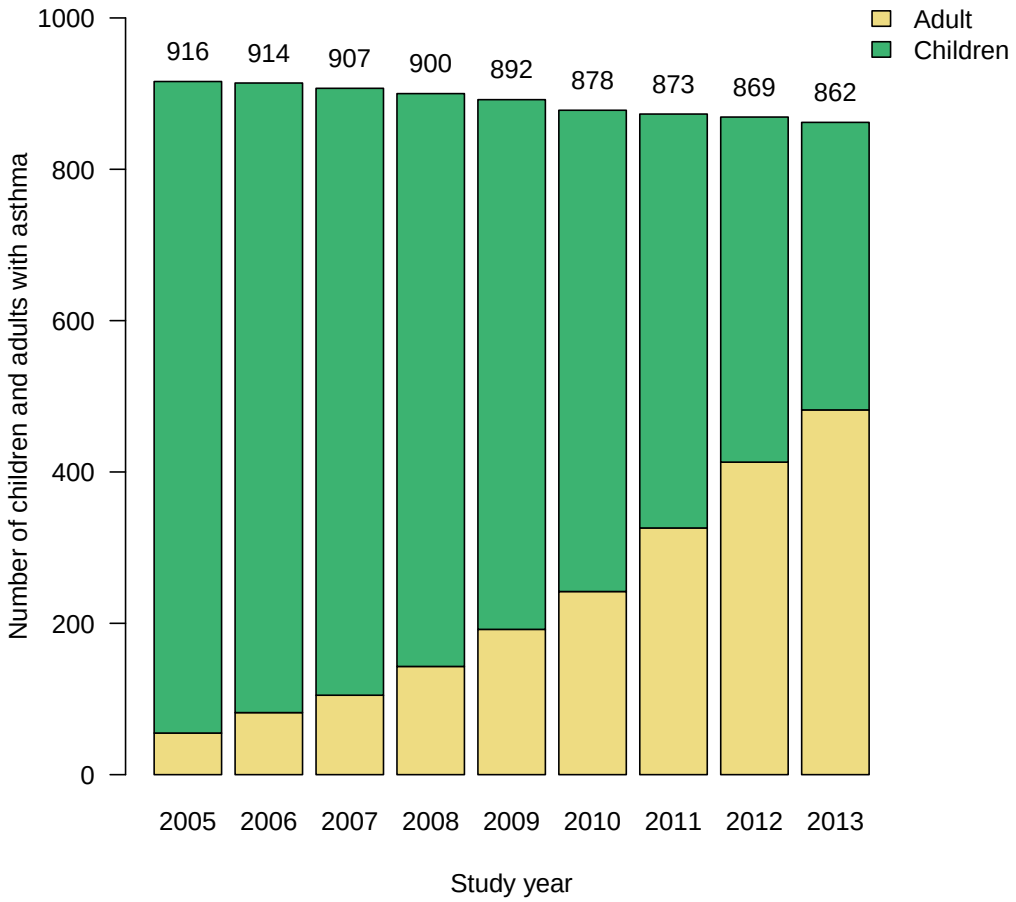

Figure 5.25: Number of children and adults with asthma per year, for the FCER2 analyses.

Over the 9-year period, the median number of asthma-related prescriptions dispensed per patient was 29. Patients with CC genotype were dispensed a median of 39 prescriptions, patients with TT genotype were dispensed a median of 29

prescriptions, and patients with TC genotype were dispensed a median of 28 prescriptions (see table 5.31).

Prescriptions	2005-2013			
	Total	TT	TC	CC
All asthma-related prescriptions				
Mean (SD)	47.4 (52.5)	46.5 (50)	46 (51.7)	59.5 (69.4)
Median (IQR)	29 (11-66)	29 (10-66.5)	28 (11-61.5)	39 (15-74)
Reliever prescriptions				
Mean (SD)	24.1 (30.2)	24 (28.3)	22.9 (28.4)	29.9 (45.6)
Median (IQR)	14 (6-31)	15 (6-31)	13 (7-29)	18 (9-33.2)
Inhaled Corticosteroid prescriptions				
Mean (SD)	8.7 (11.2)	8.6 (12.0)	8.6 (10.3)	9.3 (10.3)
Median (IQR)	5 (1-12)	5 (0-12)	5 (1-13)	6 (2-11.2)
Long-acting β_2-agonists prescriptions				
Mean (SD)	0.2 (1.3)	0.2 (1.5)	0.2 (0.8)	0.4 (1.7)
Median (IQR)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)
Combination of LABA and ICS prescriptions				
Mean (SD)	8.8 (16.2)	8.4 (15.2)	9.0 (17.3)	11.1 (17.4)
Median (IQR)	0 (0-12)	0 (0-11)	0 (0-11)	0 (0-19)
Leukotriene Receptor Antagonist prescriptions				
Mean (SD)	4.7 (11.6)	4.7 (12)	4.3 (10.9)	6.8 (12.3)
Median (IQR)	0 (0-1)	0 (0-1)	0 (0-1)	0 (0-7.2)

Table 5.31: Characteristics of the asthma-related prescriptions dispensed listed according to the FCER2 genotypes (n=927 children and adults with asthma), from 2005 to 2013 . Standard deviation (SD), interquartile range (IQR).

As expected, the majority of patients were dispensed one or more reliever prescriptions. A total of 22293 reliever prescriptions were dispensed among 890 (96%) patients, and 8024 ICS prescriptions were dispensed among 706 (76%) patients. In this study, 374 (40%) individuals were dispensed 8187 prescriptions of a combination of LABA and ICS, and 259 (28%) individuals were dispensed 4359 prescriptions of LTRA. Fifty patients (5.4%) were dispensed one or more separate prescriptions.

Smokers and/or individuals exposed to second hand smoke were dispensed more asthma-related prescriptions than non-smokers and/or individuals not exposed to second hand smoke. Patients with CC genotype were dispensed more asthma-related prescriptions than patients with TT or TC genotype, regardless of smoking status (see figure 5.26, panel A). Patients with CC genotype, between 11 and 20 years of age were dispensed more prescriptions than patients with TC or TT genotype, between 11 and 20 years of age. As the children grew older, the number of dispensed prescriptions slowly decreased (see figure 5.26, panel B).

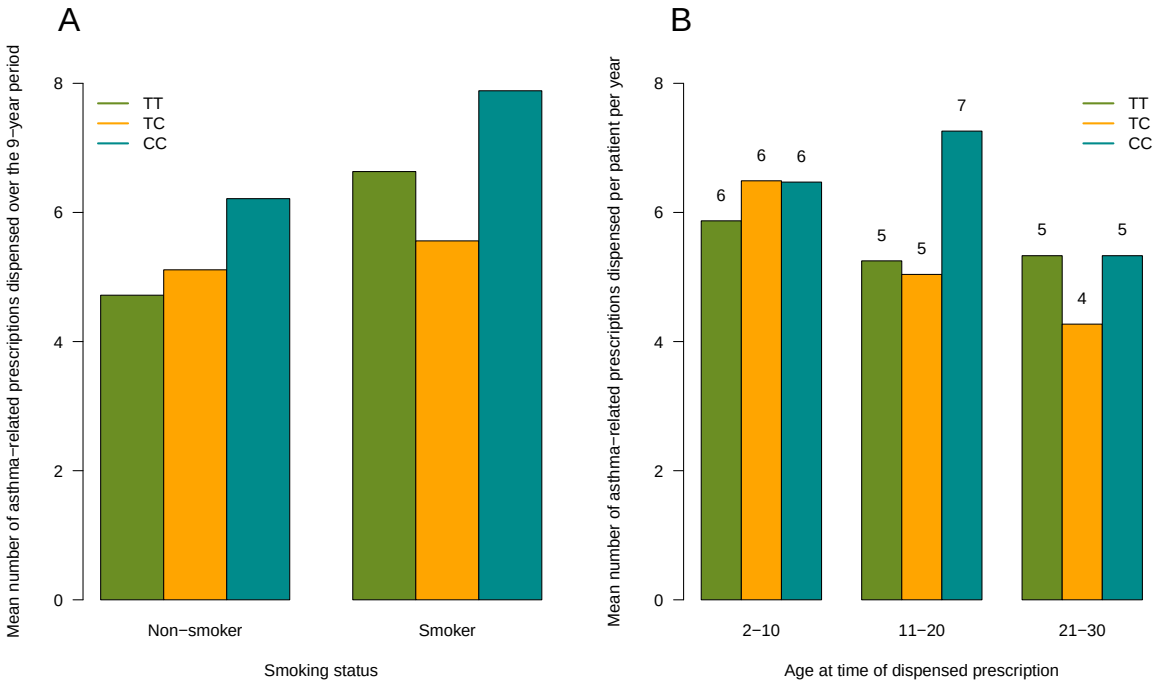

Figure 5.26: Mean number of asthma-related prescriptions dispensed per patient, over 9 years, according to the FCER2 variant, smoking status and age. **A**, mean number of asthma-related prescriptions dispensed according to smoking status. **B**, mean number of asthma-related prescriptions dispensed according to age.

The incidence rate of dispensed prescriptions of LTRA in children and adults with CC genotype was 4.12 times that of children and adults with TT genotype (table 5.32). The incidence rate of dispensed prescriptions of LTRA in children and adults with CC genotype was 5.81 times that of children and adults with TC genotype (table 5.32). The incidence rate of dispensed prescriptions of LTRA in children and adults with a cat was 0.48 times that of children and adults who do not have a cat (table 5.32). A weak-to-moderate association was found between age and the number of

asthma-related prescriptions dispensed, with younger patients having more prescriptions dispensed than older patients. There was no evidence of an association between the FCER2 polymorphism and the number of prescriptions dispensed for relievers, ICS, inhaled LABA, and a combination of LABA and ICS (table 5.32).

Variables	IRR	95% CI	P-value
All asthma-related prescriptions			
Study year	0.92	(0.90,0.95)	$<$0.001 [a]
TT vs. TC	1.02	(0.88,1.20)	0.778
TT vs. CC	1.30	(0.99,1.72)	0.061
TC vs. CC	1.27	(0.96,1.70)	0.097
Age (in years)	0.96	(0.94,0.98)	$<$0.001 [a]
Smoking status (No vs. Yes)	1.13	(0.97,1.32)	0.119
Cat (no vs. Yes)	1.00	(0.84,1.18)	0.978
Reliever prescriptions			
Study year	0.94	(0.92,0.96)	$<$0.001 [a]
TT vs. TC	0.99	(0.86,1.15)	0.925
TT vs. CC	1.20	(0.93,1.55)	0.165
TC vs. CC	1.21	(0.93,1.57)	0.162
Age (in years)	0.97	(0.96,0.99)	0.002 [a]
Smoking status (No vs. Yes)	1.20	(1.04,1.38)	0.013 [a]
Cat (No vs. Yes)	1.05	(0.90,1.23)	0.555
Inhaled Corticosteroid (ICS) prescriptions			
Study year	0.85	(0.82,0.88)	$<$0.001 [a]
TT vs. TC	1.17	(0.94,1.45)	0.157
TT vs. CC	1.20	(0.83,1.75)	0.329
TC vs. CC	1.03	(0.70,1.51)	0.881
Age (in years)	0.92	(0.90,0.95)	$<$0.001 [a]
Smoking status (No vs. Yes)	0.92	(0.74,1.13)	0.433
Cat (No vs. Yes)	1.14	(0.90,1.43)	0.277
Long-acting β_2-agonists (LABA) prescriptions			
TT vs. TC	0.92	(0.37,2.29)	0.853
TT vs. CC	2.22	(0.44,11.10)	0.333
TC vs. CC	2.42	(0.47,12.32)	0.288
Age (in years)	0.97	(0.89,1.07)	0.583
Smoking status (No vs. Yes)	0.55	(0.20,1.47)	0.233

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Variables	IRR	95% CI	P-value
Cat (No vs. Yes)	0.90	(0.32,2.51)	0.845
Combination of LABA and ICS prescriptions			
Study year	0.87	(0.81,0.94)	$<$0.001 [a]
TT vs. TC	1.00	(0.59,1.70)	0.996
TT vs. CC	1.78	(0.71,4.48)	0.222
TC vs. CC	1.78	(0.68,4.61)	0.237
Age (in years)	1.02	(0.96,1.09)	0.431
Smoking status (No vs. Yes)	1.70	(1.02,2.85)	0.043 [a]
Cat (No vs. Yes)	0.77	(0.44,1.37)	0.374
Leukotriene Receptor Antagonist (LTRA) prescriptions			
Study year	0.88	(0.79,0.98)	0.015 [a]
TT vs. TC	0.71	(0.38,1.31)	0.274
TT vs. CC	4.12	(1.48,11.45)	0.007 [b]
TC vs. CC	5.81	(2.00,16.85)	0.001 [b]
Age (in years)	0.85	(0.79,0.91)	$<$0.001 [a]
Smoking status (No vs. Yes)	1.51	(0.83,2.73)	0.173
Cat (No vs. Yes)	0.48	(0.25,0.95)	0.035 [b]

Table 5.32: Association between the number of asthma-related prescriptions dispensed and the FCER2 variant in children and adults with asthma (n=927 children and adults), between 2005 and 2013 . Incidence rate ratio (IRR), confidence interval (CI). [a] - weak-to-moderate association, [b] - strong association.

Asthma-related A&E visits/admissions

Out of 927 children and adults, 248 (26.7%) had a total of 816 A&E visits/admissions. Over the 9-year period of study, the mean number of asthma-related A&E visits/admissions per individual was 1 (see table 5.33). The mean number of asthma-related A&E visits/admissions was similar, regardless of the genotype.

Prescriptions	2005-2013			
	Total	TT	TC	CC
All asthma-related A&E visits/admissions				
Mean (SD)	0.9 (3.7)	0.9 (4.2)	0.9 (3.1)	1.0 (2.3)
Median (IQR)	0 (0-1)	0 (0-1)	0 (0-1)	0 (0-1)

Table 5.33: Characteristics of the asthma-related A&E visits/admissions listed according to FCER2 variant (n=927 children and adults with asthma), from 2005 to 2013. Standard deviation (SD), interquartile range (IQR).

Children and adults who smoked and/or were exposed to second hand smoke had more asthma-related A&E visits/admissions than children and adults who did not smoke or were not exposed to second hand smoke (see figure 5.27, panel A). Patients with CC genotype non-smokers and/or not exposed to second hand smoke had more A&E visits/admissions than patients with TT or TC genotype non-smokers and/or not exposed to second hand smoke, whereas patients with TC genotype smokers and/or exposed to second hand smoke had more A&E visits/admissions than patients with TT or CC genotype smokers and/or exposed to second hand smoke. Younger children had more A&E visits/admissions than adults, the number of A&E visits/admissions decreased as the children grew older (see figure 5.27, panel B). The number of A&E visits/admissions is similar across different genotypes for patients younger than 20 years old. Individuals older than 20 years with the TT genotype had more A&E visits/admissions than individuals with the TC and CC genotype.

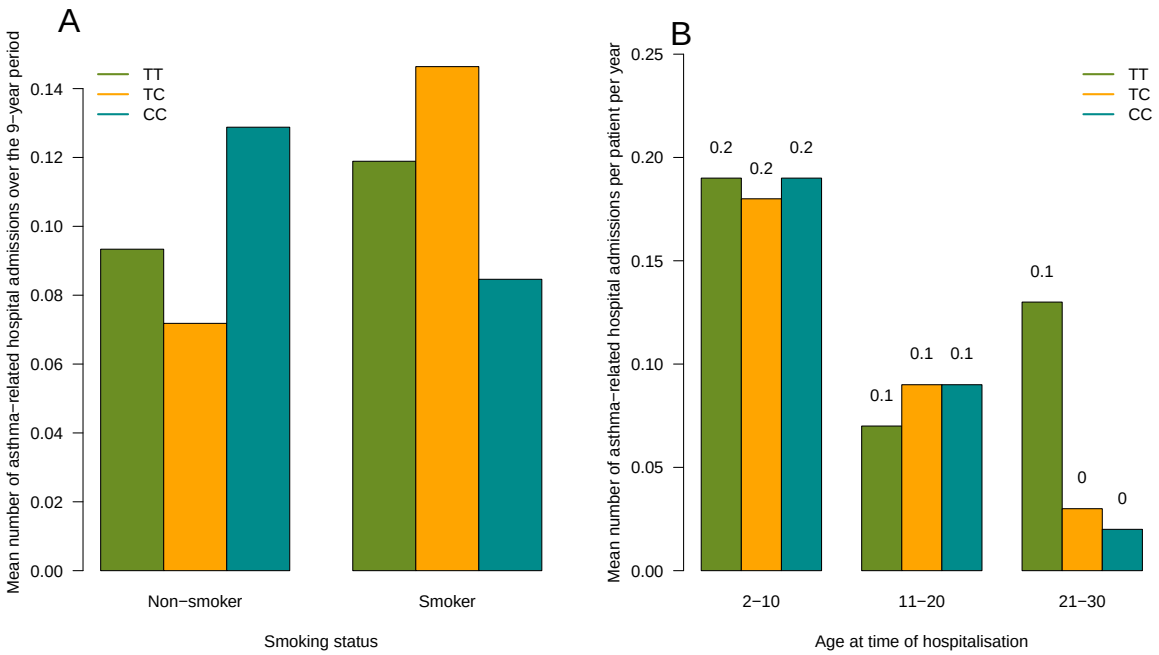

Figure 5.27: Mean number of asthma-related A&E visits/admissions per patient, over 9 years, according to the FCER2 variant, smoking status and age. **A**, mean number of asthma-related A&E visits/admissions according to smoking status. **B**, mean number of asthma-related A&E visits/admissions according to age.

Age and smoking status were weakly-to-moderately associated with the number of asthma-related A&E visits/admissions, with younger patients, and patients who smoked and/or were exposed to smoke having more A&E visits/admissions than older patients, and patients who did not smoke and/or were not exposed to smoke (table 5.34). Overall, there was no evidence of a difference in the number of asthma-related A&E visits/admissions between patients carrying different genotypes for the FCER2 polymorphism (table 5.34).

Variables	IRR	95% CI	P-value
Asthma-related A&E visits/admissions			
Study year	0.89	(0.81,0.97)	0.010 [a]
TT vs. TC	1.10	(0.75,1.63)	0.624
TT vs. CC	1.45	(0.76,2.79)	0.260
TT vs. CC	1.32	(0.67,2.58)	0.418
Age (in years)	0.90	(0.86,0.94)	$<$0.001 [a]
Smoking status (No vs. Yes)	1.54	(1.06,2.23)	0.023 [a]
Cat (No vs. Yes)	0.69	(0.45,1.05)	0.086

Table 5.34: Association between the number of asthma-related A&E visits/admissions and the FCER2 variant in children and adults with asthma (n=927 children and adults), between 2005 and 2013. Incidence rate ratio (IRR), confidence interval (CI). [a] - weak-to-moderate association.

Prescribing of oral corticosteroids

Out of 927 children and adults, 440 (47.5%) were dispensed a total of 2094 oral prednisolone prescriptions. Over the 9-year period of study, the mean number of oral prednisolone prescriptions dispensed per individual was 2 (see table 5.35). Patients with CC genotype were dispensed a mean of 3 prescriptions, and patients with TT or TC genotype were dispensed a mean of 2 prescriptions.

Prescriptions	2005-2013			
	Total	TT	TC	CC
Oral prednisolone				
Mean (SD)	2.3 (5.6)	2.1 (5.9)	2.2 (4.8)	3.5 (6.6)
Median (IQR)	0 (0-2)	0 (0-2)	0 (0-2)	0 (0-3)

Table 5.35: Characteristics of the oral prednisolone prescriptions dispensed listed according to FCER2 genotypes (n=927 children and adults with asthma), from 2005 to 2013. Standard deviation (SD), interquartile range (IQR).

Children and adults who smoked and/or were exposed to second hand smoke were dispensed more oral prednisolone prescriptions than children and adults who did not smoke or were not exposed to second hand smoke (see figure 5.28, panel A). Regardless of the smoking status, patients with CC genotype were dispensed more prescriptions than patients with TT or TC genotype. Younger children were dispensed

more prescriptions than adults, and the number of dispensed prescriptions decreased as the children grew older (see figure 5.28, panel B). Patients with CC genotype younger than 20 years were dispensed more prescriptions than patients with TT or TC genotype younger than 20 years. For patients older than 20 years, carriers of the TT genotype were dispensed more prescriptions than carriers of the TC or CC genotype.

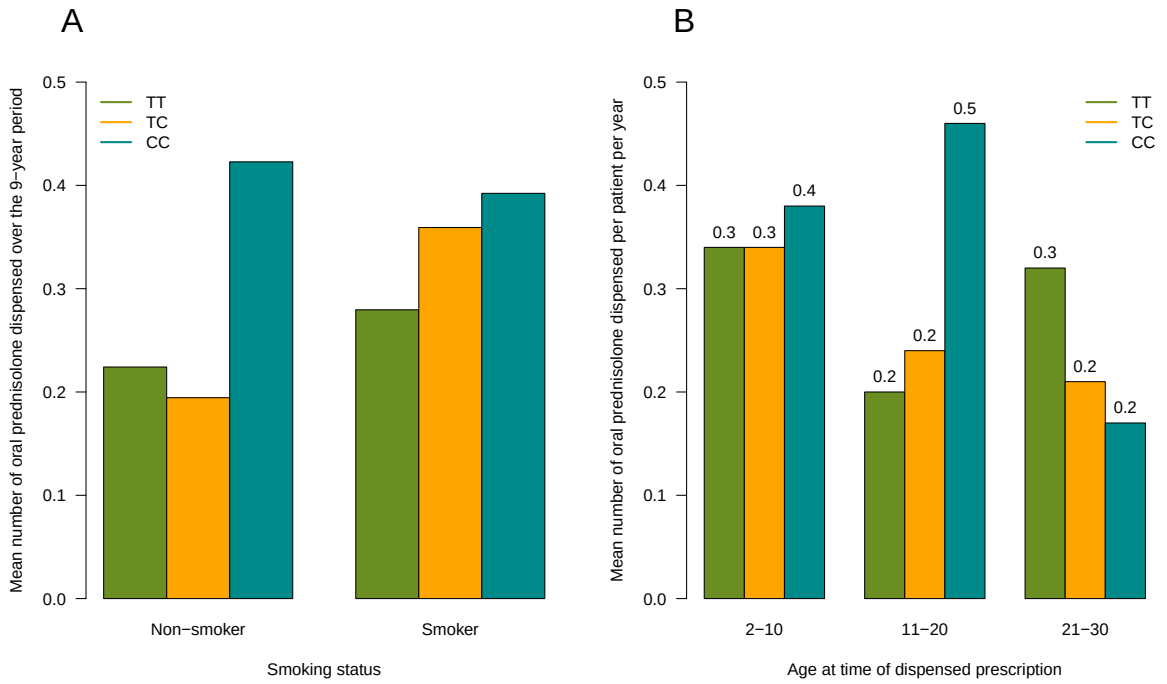

Figure 5.28: Mean number of oral prednisolone prescriptions dispensed per patient, over 9 years, according to the FCER2 variant, smoking status and age. **A**, mean number of oral prednisolone prescriptions dispensed according to smoking status. **B**, mean number of oral prednisolone prescriptions dispensed according to age.

Age, smoking status and cat ownership were weakly-to-moderately associated with the number of dispensed prescriptions over this period, with younger patients, patients who smoked and/or were exposed to smoke, and patients without a cat having more prescriptions dispensed than older patients, patients who do no smoke and/or were not exposed to smoke, and patients with a cat (table 5.36). Overall, there was no significant difference between the different genotypes and the number of prescriptions dispensed (table 5.36).

Variables	IRR	95% CI	P-value
Oral corticosteroids prescriptions			
Study year	0.96	(0.91,1.01)	0.147
TT vs. TC	0.99	(0.74,1.34)	0.958
TT vs. CC	1.49	(0.90,2.46)	0.118
TC vs. CC	1.50	(0.89,2.52)	0.124
Age (in years)	0.91	(0.88,0.95)	$<$0.001 [a]
Smoking status (No vs. Yes)	1.43	(1.08,1.91)	0.014 [a]
Cat (No vs. Yes)	0.71	(0.51,0.98)	0.039 [a]

Table 5.36: Association between the number of oral prednisolone prescriptions dispensed and the FCER2 variant in children and adults with asthma (n=927 children and adults), between 2005 and 2013. Incidence rate ratio (IRR), confidence interval (CI). [a] - weak-to-moderate association.

Asthma exacerbations

Out of 927 children and adults, 509 (55%) had a total of 2898 asthma exacerbations. Of the 927 children and adults included in the analysis, 262 (28.3%) were dispensed one or more prescriptions of oral prednisolone, 69 (7.4%) had one or more asthma-related A&E visits/admissions, and 178 (19.2%) were dispensed one or more prescriptions of oral prednisolone and had one or more asthma-related A&E visits/admissions (see figure 5.29).

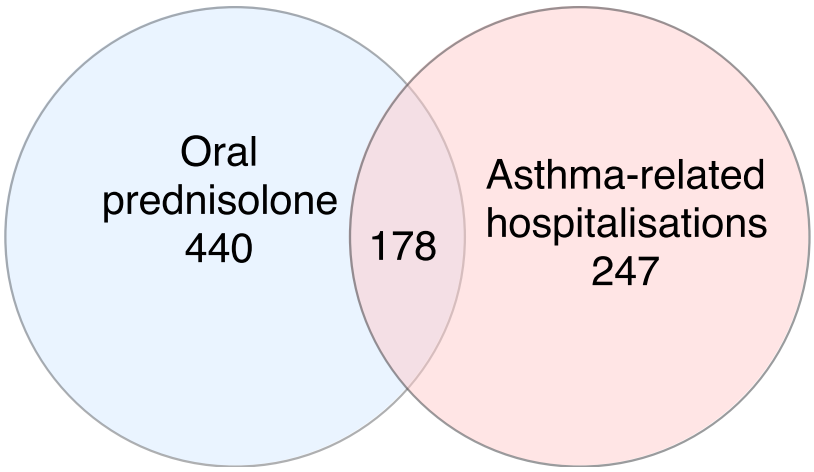

Figure 5.29: Number of children and adults with asthma (n=927 children and adults) in the FCER2-related analyses that were dispensed oral prednisolone and/or had an asthma-related A&E visits/admissions, from 2005 to 2013

Over the 9-year period of study, the mean number of asthma exacerbations per individual was 3. Patients with CC genotype had a mean of 4 asthma exacerbations, patients with TT or TC genotype had a mean of 3 asthma exacerbations.

Children and adults who smoked and/or were exposed to second hand smoke had more asthma exacerbations than children and adults who did not smoke or were not exposed to second hand smoke. Younger individuals had more asthma exacerbations than older individuals.

Age, smoking status and cat ownership were associated with the number of asthma exacerbations over this period; these associations were weak-to-moderate (table 5.37). Overall, there was no evidence of a significant difference between the FCER2 polymorphism and the number of asthma exacerbations (table 5.37).

Variables	**IRR**	**95% CI**	**P-value**
Asthma exacerbations			
Study year	0.95	(0.90,0.99)	0.032 [a]
TT vs. TC	0.98	(0.74,1.30)	0.907
TT vs. CC	1.40	(0.87,2.24)	0.167
TC vs. CC	1.42	(0.87,2.32)	0.161
Age (in years)	0.90	(0.88,0.93)	$<$0.001 [a]
Smoking staus (No vs. Yes)	1.41	(1.08,1.84)	0.012 [a]
Cat (No vs. Yes)	0.72	(0.53,0.98)	0.037 [a]

Table 5.37: Association between the number of asthma exacerbations and the FCER2 variant in children and adults with asthma (n=927 children and adults), between 2005 and 2013. Incidence rate ratio (IRR), confidence interval (CI). [a] - weak-to-moderate association.

5.3.3 Association between Fc fragment of IgE receptor II (FCER2) genetic variation and prescribing for acute allergic reactions and allergic rhinitis

In this section, the association between the FCER2 polymorphism and AAI and allergic rhinitis prescribing was explored. Over the 9-year period of study, the mean number of AAI prescriptions dispensed was 1, and the mean number of allergic rhinitis prescriptions dispensed per patient was 2 (see table 5.38). The mean number of prescriptions dispensed among patients with TT, TC or CC genotypes was similar. Fifty-four children and adults (5.8%) were dispensed a total of 567 AAI prescriptions, while 371 children and adults (40%) were dispensed a total of 2161 allergic rhinitis prescriptions.

		2005-2013		
Prescriptions	**Total**	**TT**	**TC**	**CC**
Adrenaline Auto-Injector				
Mean (SD)	0.6 (2.8)	0.7 (3.1)	0.4 (2.5)	0.6 (2.2)
Median (IQR)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)
Allergic rhinitis prescriptions				
Mean (SD)	2.3 (6.5)	2.3 (6.7)	2.4 (6.8)	2.4 (4)
Median (IQR)	0 (0-2)	0 (0-1.5)	0 (0-2)	0.5 (0-3)

Table 5.38: Characteristics of the AAI and allergic rhinitis prescriptions dispensed listed according to FCER2 variant (n=927 children and adults with asthma), from 2005 to 2013. Standard deviation (SD), interquartile range (IQR).

Regardless of the genotype, children and adults who did not smoke and/or were not exposed to second hand smoke were dispensed slightly more AAI and allergic rhinitis prescriptions than children and adults who smoked and/or were exposed to second hand smoke. Regarding AAI, patients with TC genotype non smoker and/or not exposed to second hand smoke were dispensed less prescriptions than patients with TT or CC genotype non smoker and/or not exposed to second hand smoke. Smokers and/or those exposed to second hand smoke with TT genotype were dispensed more AAI prescriptions than smokers and/or exposed to second hand smoke with TC or CC genotype (see figure 5.30, panel A). For allergic rhinitis prescriptions, the mean

number of prescriptions dispensed was similar between genotypes (see figure 5.30, panel B).

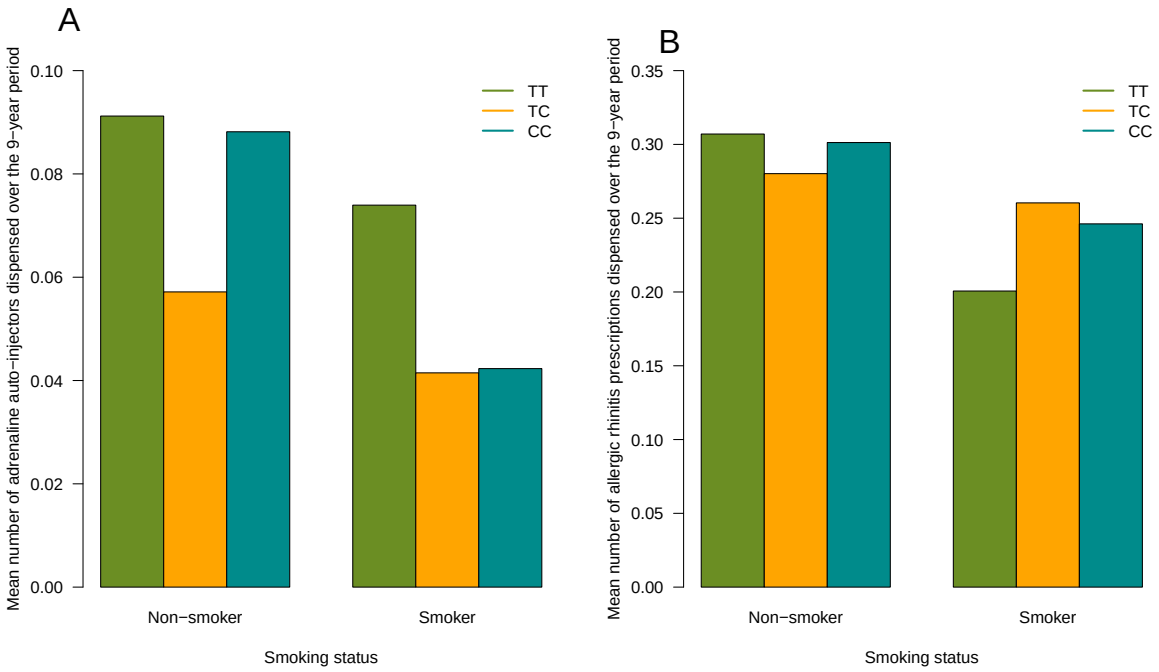

Figure 5.30: Mean number of AAI and allergic rhinitis prescriptions dispensed per patient, over 9 years, according to the FCER2 variant and smoking status. **A**, mean number of AAI dispensed according to smoking status. **B**, mean number of allergic rhinitis prescriptions dispensed according to smoking status.

Children aged between 10 and 20 years old were dispensed slightly more AAI prescriptions than younger children and older adults. Children younger than 10 years old and with TT genotype were dispensed more AAI prescriptions than children with TC or CC genotype. On the other hand, adults older than 20 years old and with CC genotype were dispensed more AAI prescriptions than adults with TC or TT genotype (see figure 5.31, panel A). The number of allergic rhinitis prescriptions slightly decreased as the children got older. Children younger than 10 years old with TT genotype were dispensed fewer allergic rhinitis prescriptions than children with TC or CC genotype. But as adults, patients with TT genotype were dispensed more allergic rhinitis prescriptions than patients with TC or CC genotype. Conversely, children with CC genotype were dispensed a higher number of allergic rhinitis prescriptions than children with TC or TT genotype, while adults with CC genotype were dispensed fewer allergic rhinitis prescriptions than adults with TC or TT genotype (see figure

5.31, panel B).

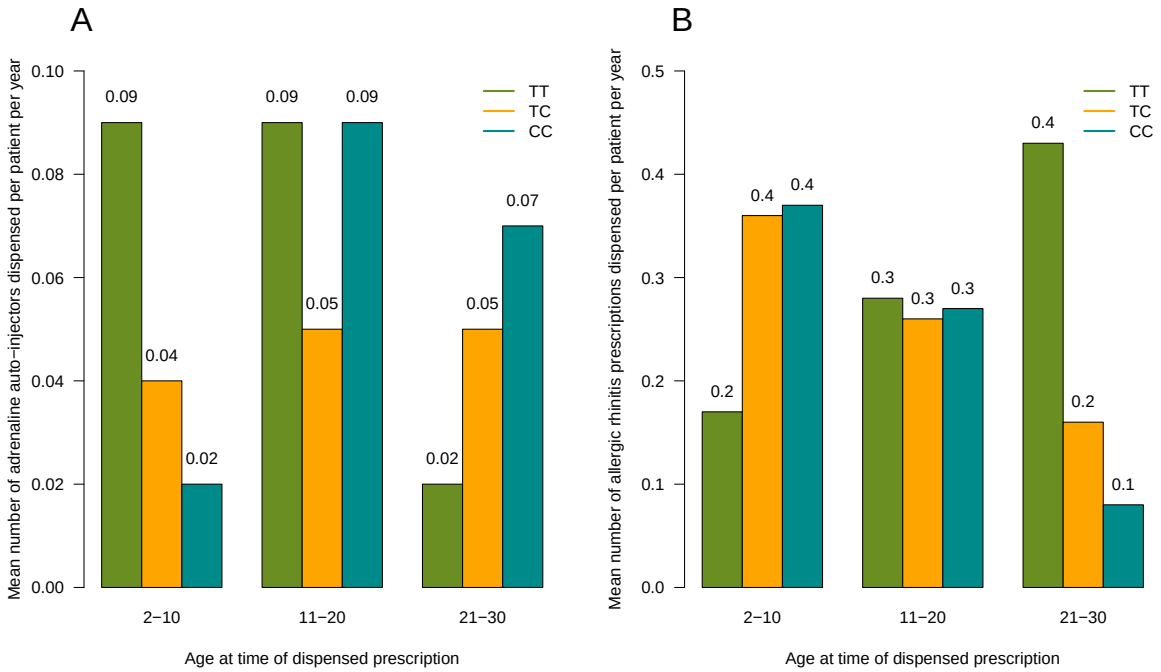

Figure 5.31: Mean number of AAI and allergic rhinitis prescriptions dispensed per patient, over 9 years, according to the FCER2 variant and age. **A**, mean number of AAI dispensed according to age. **B**, mean number of allergic rhinitis prescriptions dispensed according to age.

A strong association was found between the FCER2 variant under study and the number of allergic rhinitis prescriptions dispensed. The incidence rate of dispensed prescriptions of allergic rhinitis in children and adults with CC genotype was 2.05 times that of children and adults with TT genotype (table 5.39). There was no evidence of associations between the FCER2 variant and the number of AAI prescriptions dispensed (table 5.39).

Variables	IRR	95% CI	P-value
Adrenaline Auto-Injector (AAI) prescriptions			
TT vs. TC	0.59	(0.19,1.77)	0.343
TT vs. CC	1.03	(0.15,6.94)	0.976
TC vs. CC	1.75	(0.24,12.70)	0.578
Age (in years)	0.94	(0.81,1.09)	0.401
Smoking status (No vs. Yes)	0.80	(0.28,2.27)	0.675
Cat (No vs. Yes)	0.60	(0.18,1.97)	0.398
Allergic rhinitis prescriptions			
Study year	0.86	(0.81,0.92)	$<$0.001 [a]
TT vs. TC	1.30	(0.88,1.92)	0.194
TT vs. CC	2.05	(1.06,3.97)	0.032 [b]
TC vs. CC	1.58	(0.80,3.11)	0.185
Age (in years)	1.03	(0.98,1.08)	0.204
Smoking status (No vs. Yes)	0.76	(0.52,1.13)	0.175
Cat (No vs. Yes)	0.86	(0.57,1.32)	0.503

Table 5.39: Association between the number of AAI and allergic rhinitis prescriptions dispensed and the FCER2 variant in children and adults with asthma (n=927 children and adults), between 2005 and 2013 . Incidence rate ratio (IRR), confidence interval (CI). [a] - weak-to-moderate association, [b] - strong association.

5.3.4 Pharmacoeconomics

The mean cost of eczema-related prescriptions for individuals with the CC genotype was £207, the mean cost of eczema-related prescriptions for individuals with the TC genotype was £189, and the mean cost of eczema-related prescriptions for individuals with the TT genotype was £147. There was no evidence of a difference between the FCER2 variant for the cost of eczema prescribing, at the 5% level (see table 5.40).

The mean cost of antivirals for individuals with the TT genotype was £6, the mean cost of antivirals for individuals with the CC and TC genotype was £1. Children and adults with TT genotype have a significantly higher cost of prescribing for antivirals compared to children and adults with TC or CC genotype (see table 5.40). The mean cost of antibiotics for individuals with the TT genotype was £15, the mean cost of antibiotics for individuals with the TC and CC genotype was £14. There was no

evidence of a difference at the 5% level (see table 5.40).

Prescriptions	Mean difference (in £)	95% BCa CI (in £)
Eczema		
All eczema-related prescriptions		
TT vs. TC	42.15	(-15.16,125.06)
TT vs. CC	60.69	(-28.75,187.43)
TC vs. CC	18.54	(-83.86,157.83)
Emollients		
TT vs. TC	3.77	(-19.95,33.71)
TT vs. CC	15.70	(-20.30,73.69)
TC vs. CC	11.93	(-27.66,73.63)
Antihistamines		
TT vs. TC	13.94	(-0.25,28.57)
TT vs. CC	14.38	(-6.12,44.01)
TC vs. CC	0.44	(-22.23,32.53)
Prescribing for mild eczema		
TT vs. TC	1.93	(-6.65,16.87)
TT vs. CC	7.63	(-6.16,32.23)
TC vs. CC	5.69	(-10.97,32.26)
Prescribing for moderate eczema		
TT vs. TC	5.92	(-3.21,20.90)
TT vs. CC	26.82	(0.98,83.53) [c]
TC vs. CC	20.90	(-6.81,80.41)
Prescribing for severe eczema		
TT vs. TC	16.57	(-5.15,53.74)
TT vs. CC	-3.85	(-20.89,20.35)
TC vs. CC	-20.42	(-53,7.26)
Infected eczema		
Antivirals for the skin		
TT vs. TC	-5.85	(-22.86,-1.10) [c]
TT vs. CC	-5.12	(-22.24,-0.34) [c]
TC vs. CC	0.73	(-0.31,2.88)

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Table 5.40 – continued from the previous page

Prescriptions	Mean difference (in £)	95% BCa CI (in £)
Bacterial skin antibiotics		
TT vs. TC	-1.28	(-7.80,6.42)
TT vs. CC	-1.67	(-9.25,6.33)
TC vs. CC	-0.39	(-8.02,8.07)

Table 5.40: Difference in the mean cost of eczema-related prescriptions dispensed according to the FCER2 polymorphism, from 2005 to 2013. Figures prefixed with a minus sign indicate that the costs in children with FLG mutations are less than in children without these mutations. Bias corrected and accelerated (BCa), confidence interval (CI). [c] - significant association.

The mean cost of asthma-related prescribing for patients with CC genotype was £1281, mean cost of asthma-related prescribing for patients with TC genotype was £958, and the mean cost of asthma-related prescribing for patients with TT genotype was £942. Children and adults with CC genotype have a significantly higher cost of prescribing for LABA compared to children and adults with TC and TT genotype (see table 5.41). There was no evidence of a difference between the FCER2 polymorphism for the cost of relievers, ICS, a combination of LABA and ICS, and LTRA (see table 5.41).

The mean cost of asthma-related A&E visits/admissions for patients with CC genotype was £450, the mean cost of asthma-related A&E visits/admissions for patients with TT genotype was £440, and the mean cost of asthma-related A&E visits/admissions for patients with TC genotype was £365. There was no evidence of a difference at the 5% level (see table 5.41).

The mean cost of oral corticosteroids prescribing for patients with CC genotype was £11 and the mean cost of oral corticosteroids prescribing for patients with TT or TC genotype was £7 (see table 5.41). The mean cost of asthma exacerbations for patients with CC genotype was £461, the mean cost of asthma exacerbations for patients with TT genotype was £447, and the mean cost of asthma exacerbations for patients with TC genotype was £372. There was no evidence of a difference at the 5% level (see table 5.41). The mean cost of primary care emergency visits for

individuals with the CC genotype was £120, the mean cost of primary care emergency visits for individuals with the TC genotype was £76, and the mean cost of primary care emergency visits for individuals with the TT genotype was £71. Children and adults with CC genotype have a significantly higher cost of primary care emergency visits compared to children and adults with TT genotype (see table 5.41).

The mean cost of acute allergic reactions for patients with TT genotype was £46 , the mean cost of acute allergic reactions for patients with CC genotype was £34, and the mean cost of acute allergic reactions for patients with TC genotype was £29. There was no evidence of a difference at the 5% level (see table 5.41). The mean cost of allergic rhinitis prescriptions for patients with CC genotype was £24, the mean cost of allergic rhinitis prescriptions for patients with TC and TT genotype was £20. There was no evidence of a difference at the 5% level (see table 5.41).

The mean cost for all conditions examined for children and adults with eczema and asthma and carriers of the CC genotype was £2665, the mean cost for children and adults with eczema and asthma and carriers of the TC genotype was £2033, and the mean cost for children and adults with eczema and asthma and carriers of the TT genotype was £1836. The mean cost for all conditions examined for children and adults with asthma and carriers of the CC genotype was £1921, the mean cost for children and adults with asthma and carriers of the TT genotype was £1555, and the mean cost for children and adults with asthma and carriers of the TC genotype was £1521. There was no evidence of a difference at the 5% level (see table 5.41).

Prescriptions	Mean difference (in £)	95% BCa CI (in £)
Asthma		
All asthma-related prescriptions		
TT vs. TC	15.71	(-178.98,213.83)
TT vs. CC	339.03	(-4.31,849.61)
TC vs. CC	323.32	(-42.94,846.04)
Relievers		
TT vs. TC	-14.83	(-39.54,10.62)
TT vs. CC	26.97	(-22.97,139.88)
TC vs. CC	41.80	(-6.25,152.13)
Inhaled Corticosteroid (ICS)		
TT vs. TC	4.76	(-19.43,30.46)
TT vs. CC	59.51	(-3.40,173.46)
TC vs. CC	54.75	(-4.30,180.06)
Long-acting β_2-agonists (LABA)		
TT vs. TC	9.72	(-13.94,40.29)
TT vs. CC	63.24	(5.85,183.05) [c]
TC vs. CC	53.52	(-8.30,172.60)
Combination of Long-acting β_2-agonists (LABA) and ICS		
TT vs. TC	27.39	(-96.96,166.36)
TT vs. CC	104.96	(-101.55,390.12)
TC vs. CC	77.57	(-152.19,361.79)
Leukotriene Receptor Antagonist (LTRA)		
TT vs. TC	-8.78	(-68.31,58.55)
TT vs. CC	86.89	(-25.09,235.83)
TC vs. CC	95.67	(-7.28,242.83)
Asthma exacerbations		
Asthma-related A&E visits/admissions		
TT vs. TC	-74.85	(-286.39,128.52)
TT vs. CC	10.20	(-262.97,576.60)
TC vs. CC	85.05	(-169.39,680.10)
Oral corticosteroids		
TT vs. TC	0.32	(-1.77,2.23)
TT vs. CC	4.26	(0.01,10.86) [c]
TC vs. CC	3.93	(-0.46,9.60)

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Table 5.41 – continued from the previous page

Prescriptions	Mean difference (in £)	95% BCa CI (in £)
Asthma exacerbations		
TT vs. TC	-74.53	(-291.04,110.65)
TT vs. CC	14.45	(-226.11,635.16)
TC vs. CC	88.98	(-171.01,749.74)
Primary care emergency costs		
TT vs. TC	4.75	(-22.62,26.72)
TT vs. CC	49.26	(4.15,114.45) [c]
TC vs. CC	44.51	(-2.07,108.35)
Acute allergic reactions and allergic rhinitis		
Adrenaline Auto-Injector (AAI)		
TT vs. TC	-16.97	(-39.13,4.51)
TT vs. CC	-11.33	(-37.38,26.40)
TC vs. CC	5.64	(-20.07,45.58)
Allergic rhinitis prescriptions		
TT vs. TC	0.61	(-8.29,9.63)
TT vs. CC	3.73	(-6.93,19.29)
TC vs. CC	3.12	(-7.42,18.10)
Snapshot cost of the cohort		
Individuals with eczema and asthma		
TT vs. TC	197.47	(-404.44,850.44)
TT vs. CC	829.07	(-140.12,2416.73)
TC vs. CC	631.60	(-383.12,2276.16)
Individuals with asthma		
TT vs. TC	-34	-343.02,347.57
TT vs. CC	365.87	(-180.75,1351.48)
TC vs. CC	399.88	(-159.58,1332.85)

Table 5.41: Difference in the mean cost of prescriptions dispensed according to the FCER2 variant, from 2005 to 2013. Figures prefixed with a minus sign indicate that the costs in children with FLG mutations are less than in children without these mutations. Bias corrected and accelerated (BCa), confidence interval (CI). [c] - significant association.

5.4 Summary

This chapter presents the results of the effect of genetic variation on health outcomes in children and adults in the BREATHE study. Specifically, the chapter discusses the effects of four FLG polymorphisms, two known variants, Arg16 and Glu27, of the ADRB2 gene and one variant of the FCER2 gene on dispensed prescriptions and asthma-related A&E visits/admissions.

Section 5.1 describes prescribing differences between FLG gene mutation carriers and non-carriers and shows that these differences lead to different healthcare costs.

The analysis showed that individuals with one or more FLG mutations were dispensed more emollients, prescriptions for severe eczema, and a combination of LABA with ICS prescriptions than individuals without FLG mutations. Individuals with one or more FLG mutations also had more asthma-related A&E visits/admissions than individuals without FLG mutations.

In addition, individuals with one or more FLG mutations were also dispensed more prescriptions for mild and moderate eczema, anti-bacterial skin antibiotics, relievers, and oral prednisolone than individuals without FLG mutations, however these associations were weak-to-moderate.

Section 5.2 describes prescribing differences between homozygous, heterozygous and wild-type individuals for the Arg16 and Glu 27 variants and shows that the presence of the Arg allele is associated with different healthcare costs.

Children and adults with the Arg/Arg genotype were dispensed more combined LABA and ICS than individuals with the Gly/Gly and Gly/Arg genotypes. A similar effect was found for LTRA, children and adults with the Arg/Arg genotype were dispensed more LTRA prescriptions than children and adults with the Gly/Gly genotype.

A weak-to-moderate association was also found between the Arg16 status and prescribing of relievers and oral prednisolone. Individuals with the Arg/Arg genotype were dispensed more relievers than individuals with the Gly/Arg genotype, and more oral prednisolone than individuals with the Gly/Arg and Gly/Gly genotype.

Regarding the Glu27 variant, children and adults with the Gln/Gln genotype were dispensed more LTRA prescriptions than children and adults with the Glu/Glu genotype. A weaker-to-moderate association was found between individuals with the Gln/Gln genotype and individuals with the Gln/Glu genotype. Children and adults with the Gln/Gln genotype were dispensed more prescriptions of combination of LABA and ICS than children and adults with the Glu/Glu genotype. A weak-to-moderate association was found between the Glu27 variant and oral corticosteroids prescriptions; individuals with the Glu/Glu genotype were dispensed more oral prednisolone prescriptions than individuals with the Gln/Gln genotype.

Section 5.3 describes prescribing differences between homozygous, heterozygous and wild-type individuals for one variant in the FCER2 gene and shows that these differences were also associated with different healthcare costs.

Children and adults with CC genotype for the FCER2 polymorphism were dispensed more prescriptions of LTRA than children and adults with TT or TC genotype. Children and adults with CC genotype also were dispensed more allergic rhinitis prescriptions than children and adults with TT genotype.

Younger individuals were dispensed more prescriptions for emollients, prescriptions for mild eczema, relievers, ICS, LTRA and oral prednisolone than older individuals. Younger individuals also had more asthma-related A&E visits/admissions than older individuals. However, these associations were weak-to-moderate.

A significant but weak-to-moderate association was found between prescriptions dispensed and gender. Females received more antihistamines, emollients, prescriptions for mild and moderate eczema and bacterial skin antibiotics than males.

Individuals with cat exposure received less LTRA and oral prednisolone prescriptions than individuals without a cat. Similarly, individuals with a cat had less asthma-related A&E visits/admissions than individuals without a cat, however, that association was only found in the FLG analysis, section 5.1.

A strong association was found between smoking status and dispensing of combined LABA and ICS. Children and adults who smoked and/or were exposed to second hand smoke received more LABA and ICS combined than children and adults who

did not smoke and/or were not exposed to second hand smoke. Smokers or individuals exposed to smoke also received more relievers, LTRA and oral prednisolone than non-smokers and had more asthma-related A&E visits/admissions.

Table 5.42 displays a summary of the hypothesis of the thesis along with the findings.

Hypothesis	Findings	Summary
FLG		
Children and adults with mutations were dispensed more prescriptions for **eczema** than children and adults without mutations, translating into a higher cost for the NHS	Individuals with mutations were dispensed more emollients and prescriptions for severe eczema than individuals without mutations. A weaker association was seen between FLG and prescriptions for mild and moderate eczema and anti-bacterial skin antibiotics. Individuals with FLG mutations had higher costs for the NHS for prescriptions for emollients and bacterial skin antibiotics, and prescribing for moderate and severe eczema, compared with individuals without FLG mutations.	Risk factor
Children and adults with mutations were dispensed more prescriptions for **asthma** and had more asthma exacerbations than children and adults without mutations, translating into a higher cost for the NHS	Individuals with mutations were dispensed more prescriptions combining LABA and ICS and had more asthma exacerbations than individuals without mutations. A weaker association was seen between FLG and relievers and oral prednisolone. Individuals with FLG mutations had higher costs for the NHS for prescriptions for relievers, LTRA, combination of LABA and ICS, asthma exacerbations and primary care emergency costs, compared with individuals without FLG mutations.	Risk factor
Children and adults with mutations were dispensed more prescriptions for **allergic reactions** than children and adults without mutations, translating into a higher cost for the NHS	No difference was found between the presence of FLG mutations and the prescribing for allergic reactions.	No association

Table 5.42 – continued from previous page

Hypothesis	Findings	Summary
Children and adults with mutations were dispensed more prescriptions for **allergic rhinitis** than children and adults without mutations, translating into a higher cost for the NHS	No difference was found between the presence of FLG mutations and the prescribing for allergic rhinitis.	No association
ADRB2		
Children and adults carriers of the Arg allele were dispensed more prescriptions for **asthma** and had more asthma exacerbations than carriers of the Gly allele, translating into a higher cost for the NHS	Carriers of the Arg allele were dispensed more prescriptions combining LABA and ICS, and more LTRA prescriptions, than carriers of the Gly allele. A weaker association was seen between the Arg allele and precriptions of relievers and oral prednisolone. Individuals with the Arg/Arg genotype had higher costs for the NHS for prescriptions for LTRA compared with individuals the Gly/Gly genotype. Individuals with the Arg/Arg genotype had higher costs for the NHS for prescriptions for relievers, ICS, oral corticosteroids, and primary care emergency costs compared with individuals the Gly/Arg genotype.	Risk factor

Table 5.42 – continued from previous page

Hypothesis	Findings	Summary
Children and adults carriers of the Gln allele were dispensed more prescriptions for **asthma** and had more asthma exacerbations than carriers of the Glu allele, translating into a higher cost for the NHS	Carriers of the Gln allele were dispensed more prescriptions combining LABA and ICS, and more LTRA prescriptions, than carriers of the Glu allele. A weaker association was seen between the Gln allele and oral prednisolone prescriptions. Individuals with the Gln/Glu genotype had higher costs for the NHS for primary care emergency costs compared with individuals the Glu/Glu genotype.	Risk factor
FCER2		
Children and adults carriers of the C allele were dispensed more prescriptions for **eczema** than carriers of the T allele, translating into a higher cost for the NHS	No difference was found between carriers of the C and T allele and the prescribing for allergic reactions. Individuals with the CC genotype had higher costs for the NHS for prescriptions for moderate eczema compared with individuals the TT genotype.	No association
Children and adults carriers of the C allele were dispensed more prescriptions for **asthma** and had more asthma exacerbations than carriers of the T allele, translating into a higher cost for the NHS	Carriers of the C allele were dispensed more LTRA prescriptions, than carriers of the T allele. Individuals with the CC genotype had higher costs for the NHS for prescriptions for LABA, oral corticosteroids, and primary care emergency visits compared with individuals the TT genotype.	Risk factor
Children and adults carriers of the C allele were dispensed more prescriptions for **allergic reactions** than carriers of the T allele, translating into a higher cost for the NHS	No difference was found between carriers of the C and T allele and the prescribing for allergic reactions.	No association

Table 5.42 – continued from previous page

Hypothesis	Findings	Summary
Children and adults carriers of the C allele were dispensed more prescriptions for **allergic rhinitis** than carriers of the T allele, translating into a higher cost for the NHS	Carriers of the C allele were dispensed more allergic rhinitis prescriptions, than carriers of the T allele.	Risk factor

Table 5.42: Summary of the hypothesis formulated and the results

5.5 Communicating personalised medicine to children and parents

The session at the Brighton Fringe festival was open to everyone, and 250 people participated in the activities throughout the day; 15 children, 5 parents and 5 medical students were interviewed and 15 parents and 7 children filled in a feedback questionnaire. In Portugal, the session took place in the primary schools in Bajouca; one school had 25 children, and 4 parents also participated in the activities, while the second school had 27 children and 10 parents participated in the activities. Each school had two teachers, who also completed paper questionnaires. Children's ages ranged between 5 and 10 years.

The activities were well received among the children. The favourites were the 'asthma inhalers', 'healing babies' and the 'genetic tree'. The 'skin barrier' activity was less popular at Bajouca. Figures 5.32, 5.33 and 5.34 display the activities performed in Bajouca.

Figure 5.32: Pictures of the 'healing babies' and 'skin barrier' activities taken in Bajouca

In the two sessions, 29 parents filled in a questionnaire, 14 from Bajouca and 15 from Brighton, 7 children filled in a questionnaire in Brighton and 4 teachers in Bajouca. Out of 40 questionnaires, 26 (65%) reported increased knowledge after the activities. Among 29 parents, 18 (62%) parents reported increased knowledge, the difference

Figure 5.33: Pictures of the 'asthma inhalers' activity taken in Bajouca

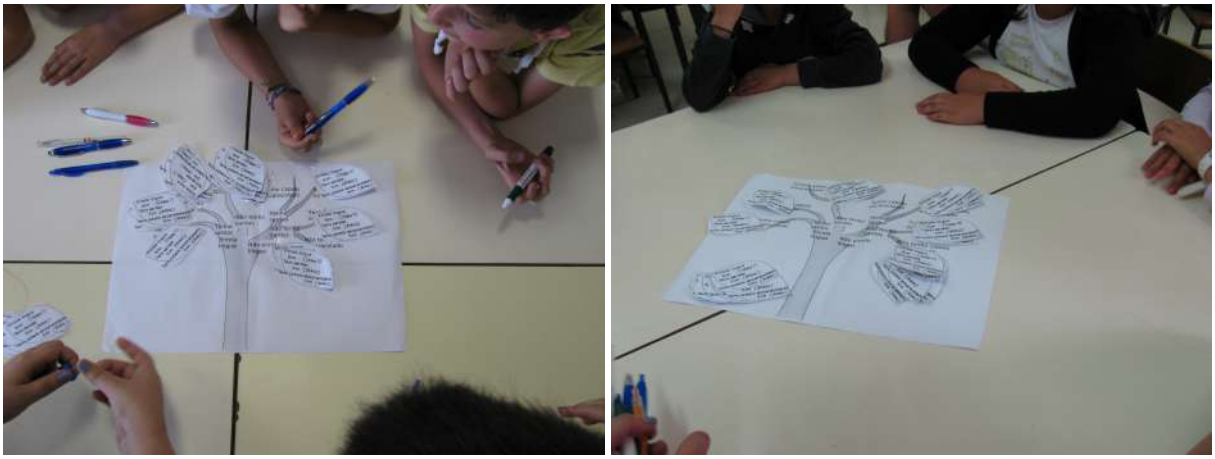

Figure 5.34: Pictures of the 'genetic tree' activity taken in Bajouca

was higher in Bajouca, where half of the parents reported increasing their knowledge after the activities. Only one teacher at Bajouca reported not to have increased her knowledge.

The output of combining knowledge and fun

Overall, both sessions yielded positive outcomes, where children and parents learned about the *concept of personalised medicine*, the *relationship between eczema and asthma* and the *effect of having FLG gene defects*. Several children and parents also learned about asthma, the different inhalers, DNA and heritability.

> "We cannot use the same medicine." (Child Bajouca)

> "You need different medicines for different people because they have different DNA. We are all different." (Child Brighton)

"I agree with personalised medicine because it can improve everyone's life." (Parent Bajouca)

"I think it's important to find out the right treatment for each disease." (Parents Bajouca)

"It would be possible to adapt the medication to each patient." (Teacher Bajouca)

"Any innovation in the treatment of diseases, accessible to all, would be a great progress." (Teacher Bajouca)

"(. . .) one size doesn't fit all. The person should dictate the medicine and not the other way around." (Parent Brighton)

"I find it quite cool that you have to have a very strong skin for all the stuff to not go through your body." (Child Brighton)

After one of the sessions in Bajouca, two parents wanted to learn more personalised medicine and whether this was already in practice. One girl in Bajouca showed interest in the FLG gene and the defective skin and how one could restore the epidermal barrier.

Overall, in Bajouca, the majority of the parents had a *positive attitude regarding genetic testing* and would test their child.

"Genetic testing can make us understand more about the diseases we have." (Parents Bajouca)

"More and more I agree that testing should be done to ascertain which medication is the best to be given." (Parents Bajouca)

"I agree that genetic testing is carried out. To know which is the best medication to be given to each person." (Parents Bajouca)

"Genetic testing is important so that each disease has the best possible treatment and its cure." (Parents Bajouca)

"There are very important to detect and cure genetic diseases." (Teacher Bajouca)

> "(. . .) genetic tests are not harmful to our health. On the opposite, they help control and fight diseases." (Teachers Bajouca)

However, some of the same parents expressed *concern regarding the cost of genetic testing* and the viability of implementing genetic testing in Portugal.

> "Yes, I think it should always be done. Without looking to financial questions to medical institutions, hospitals and health centres." (Parents Bajouca)

> "I'm just not sure if it's possible for all people since it will be expensive (. . .)."(Parents Bajouca)

> "Each individual is unique hence personalising the response to medications and treatments themselves would have in my opinion more positive outcomes. My doubts are with the costs of personalised medicine. I'm not sure if it would be viable in practical terms on an economic level and in practice." (Parents Bajouca)

> "My opinion is that this type of medicine is only accessible in rich countries." (Teacher Bajouca)

Although, the majority of the feedback was positive and several parents and children learned something new there was also some *confusion regarding what constitutes personalised medicine* and some *misunderstanding relating to the objective of genetic testing*. One parent perceived personalised medicine as a reduction in the medication side effects, another thought that genetic testing could provide a window into the genetic profile of their ancestries, and another parent mentioned stem cells, which could indeed be used in personalised medicine but was not referred to in the session.

Changes in the perception of science

A key message from these sessions is the discovery that *science can be fun*. Children noted that they learned more when presented with fun activities. Another interesting aspect was the *children's interest in Science, Technology, Engineering, Mathematics and Medicine (STEMM)* disciplines. Three children mentioned their interest in pursuing a medical career, while two children wanted to study chemistry and another one wanted to be a scientist.

"Serious fun." (Parent Brighton)

"Medicine is both an art and science." (Medical student Brighton)

"(. . .) I've noticed science can also be entertaining." (Teacher Bajouca)

"These activities have made the topic interesting and fun." (Teacher Bajouca)

"Communicating science through activities, especially for these ages, is the most motivating and desired way." (Teacher Bajouca)

"Science has a lighthearted side." (Parents Brighton)

The importance of public engagement

Parents and medical students pointed out the importance to interact with each other and to provide a *creative environment* to teach children about complex themes such as genetics. The medical students were also positively surprised by the children's knowledge.

"Medicine is for everyone, and I think doctors have too much power almost because people just put them on this pedestal and it's understanding that it's just science. Anyone should understand science." (Medical student Brighton)

"Rolling back to the essence of what is a gene, what is DNA, why it is important and putting in a language that means something to people, not just for the children but the parents and also explain why it is useful." (Medical student Brighton)

One parent was surprised about how little her child knew about asthma, despite being asthmatic.

"I was surprised about how little he knew. Because I've always as a mother been you know right, you take two puffs now and three puffs, but I never had actually really explained the problem, that it helps your lungs, so he didn't know. He didn't even know he had these inhalers and things at school." (Parent Brighton)

Chapter 6

Discussion

6.1 Understanding the association between filaggrin (FLG) gene variation and healthcare utilisation

Summary

This work has shown that Filaggrin (FLG) mutations is associated with different eczema and asthma phenotype, translating into increased prescribing patterns and healthcare costs for both eczema and asthma. Children and adults with FLG mutations were dispensed more prescriptions for emollients, more prescriptions for severe eczema, and more prescriptions for combined Long-acting β_2-agonists (LABA) and Inhaled Corticosteroid (ICS) than children and adults without FLG mutations. Children and adults with FLG mutations also had more asthma exacerbations than children and adults without FLG mutations.

Biological plausibility for findings

Regarding the greater number of emollients dispensed to patients with FLG mutations, a likely explanation relates to the key role of FLG in the epidermal barrier. FLG loss-of-function mutations are associated with disorganised keratin filaments and an impaired lamellar architecture, leading to an impaired epidermal barrier, lower levels of amino-acids, and TransEpidermal Water Loss (TEWL).[203,204] The association between FLG mutations and the dispensing of prescriptions for severe

eczema is concordant with previous studies.[87–89,92] In that sense, the association between FLG mutations and prescriptions for emollients could be because patients with severe eczema were dispensed more emollients. Similarly, a weak-to-moderate association was found between FLG mutations and prescriptions for antibiotics for the skin. Infections are more common in patients with greater severity of eczema, suggesting that patients with severe eczema could have been dispensed more antibiotics that act against skin pathogens compared to patients with mild or moderate eczema. It is also possible that an impaired skin barrier resulting from defective FLG directly contributes to an increased entry of bacterial pathogens, leading to an increased occurrence of infections, and hence increased prescribing of antibiotics, in comparison to FLG-sufficient individuals. While there is evidence supporting increased allergen entry in association with FLG mutations, studies are lacking regarding the possibility of increased pathogen entry in association with FLG defects. The thesis suggests that this area requires further study.

With regard to asthma medications, an association was found between the presence of FLG mutations and the prescribing of combined LABA and ICS. A strong association was also found between the presence of FLG mutations and asthma-related Accident & Emergency (A&E) visits/admissions. Although FLG is not expressed in the bronchial epithelium, the current hypothesis proposes that the sensitization triggered by the impaired skin barrier may lead to increased allergen entry and systemic inflammation in the lung and nasal tissues, thus resulting in increased asthma severity.[175]

Pharmacoeconomics

The findings that patients with FLG mutations cost more to the National Health Service (NHS) from the perspective of both eczema and asthma prescribing, and asthma exacerbations, than patients without FLG mutations, are of interest. This highlights the need for intervention studies in higher risk individuals, both to improve disease symptoms for patients and to reduce costs for the NHS. It is important to note that physicians and the patients involved were unaware whether or not the patients had FLG mutations, and prescribed medicine based solely on clinical need. Over a 9-year period, the healthcare system spent approximately $\pounds$1200 more on eczema

and asthma prescribing and asthma exacerbations, to treat an asthmatic individual with one or more FLG mutations, compared to an asthmatic individual without any FLG mutations.

In 2016, Scotland's population was estimated at 5.3 million, with 10.6 births per 1000 people.[205] In 2015, a study in Aberdeen estimated eczema prevalence at 29% and asthma prevalence at 19%.[206] Assuming that the prevalence of asthma and eczema in Aberdeen can be generalised to Scotland, one can extrapolate that of the 56,180 children born in 2016, around 16,292 may develop eczema, and 10,674 may develop asthma. Table 5.1 shows the proportion of children and adults with eczema and asthma. Assuming that 54% of children with asthma will also have eczema, around 5764 children born in 2016 could eventually suffer from both diseases.

Table 5.1 shows the proportion of children and adults with eczema and asthma with FLG mutations (22.4%), which corresponds to 1291 children with eczema and asthma and FLG mutations. Using that data one can extrapolate that over a 9-year period, the 1291 children with eczema and asthma and FLG mutations may cost the NHS a total of £315,000, and children with eczema and asthma and no FLG mutations may cost the NHS a total of £670,000. From this thesis, it can be seen that each child or adult with FLG mutations will cost approximately £95 more to the NHS than a child or adult without FLG mutations. The total costs are higher for those without mutations as most people do not carry a mutation. However, when one looks at a more personalised approach to the estimated 1291 individuals with eczema and asthma and FLG mutations, the NHS may spend an additional £122,000 on eczema-related prescriptions (estimates vary between £22,000 and £270,000) treating children with FLG mutations, with eczema and asthma, than children without FLG mutations, with eczema and asthma. The estimates and confidence intervals were taken from table 5.15.

Table 5.1 shows the proportion of children and adults with asthma and with FLG mutations (16.7%), which corresponds to 1783 children with asthma and FLG mutations and 8891 children with asthma and without FLG mutations. Again, by the same reasoning, over a 9-year period, children with asthma and FLG mutations may cost to the NHS a total of £2,573,000, and children with asthma and without FLG

mutations may cost to the NHS a total of £8,490,000. The NHS may spend an additional £869,000 (estimates vary between £301,000 and £1,491,000) on asthma-related prescriptions treating asthmatic children with FLG mutations than asthmatic children without FLG mutations.

Considering the prescribing cost for eczema, allergic rhinitis, asthma and the cost of A&E visits/admissions, over a 9-year period, children with eczema and asthma and FLG mutations may cost to the NHS a total of £4,268,000, and children with eczema and asthma and without FLG mutations may cost to the NHS a total of £9,166,000. The NHS may spend approximately an additional £1,625,000 (estimates vary between £382,000 and £4,014,000) treating children with eczema and asthma and FLG mutations than children with eczema and asthma and without FLG mutations.

To note that this cost analysis is a conservative estimate as it does not include additional General Practitioner (GP) and/or hospital appointment consultations. These consultation costs and additional loss of school and/or work days for children/adults and parents were considered out of the scope of this analysis. The cost analysis generated wide Confidence interval (CI), bringing a degree of uncertainty to the results, and indicating the considerable variability of costs among patients. A systematic review estimated that direct costs of eczema in the United States of America (USA) could be higher than 4 billion dollars per year.[207]

Limitations

A potential weakness of this study is that the definition of eczema relies on parent or patient report and may, on occasion, be subjective. Thus, although parents have identified their children as having eczema (n=530), 43 of these children did not have any eczema-related prescriptions over the 9 year period. These children could have had very mild eczema, could have outgrown their eczema, been misclassified, or moved outside Scotland. Another possibility is that the parents have identified their child or themselves as having eczema without a formal diagnosis. Civelek et al.[4] found that around 1% of children with current symptoms of eczema did not had a physician diagnosis of eczema and only 29% of children with eczema symptoms used medication. Of these 43 children and young adults, 41 had a family history of atopy (eczema, asthma, or rhinitis). The family history of atopy could have led these

patients to believe themselves to have eczema. This behaviour is not uncommon among patients suffering from allergies. Two Randomized Controlled Trials (RCT)[208,209] found that patients tend to over-represent their allergic triggers. In these studies, patients were asked about their allergic triggers and submitted to a skin prick test afterwards. For children, 13 to 36%, who believed to be allergic to grass pollen, tree pollen, cat or dog dander, or dust mite were incorrect in their assumptions demonstrated by skin prick test and thorough history. In adults, 8 to 39% believed to be allergic to grass pollen, tree pollen, cat or dog dander, or dust mite were also shown to be not allergic to these triggers. Another study[210] found that 46% of individuals who reported having allergic rhinitis never had a physician diagnosis.

Another important consideration is that all the emollients included in the analysis are available over-the-counter, which some patients may have found easier to obtain for mild eczema than attending a GP appointment and waiting for a prescription. In this thesis, 187 patients (35%) were identified as having eczema and were dispensed prescriptions for eczema but were not dispensed emollients, over a 9-year period. These 187 patients are likely to have had a mild form of eczema since they were dispensed fewer prescriptions (less than one) for moderate and severe eczema per year. This behaviour may be an indication of poor adherence, a misunderstanding regarding treatment by the patient and GP, or an indication that the patient bought the emollients over-the-counter. Hence, the effect size of the association between FLG mutations and prescriptions for emollients may be under or over-estimated, due to over-the-counter emollients.

Another limitation relates to the definition of allergic rhinitis. Instead of using an objective definition of rhinitis, the number of allergic rhinitis prescriptions were counted for all patients with asthma. It is worth noting that the number of dispensed prescriptions for allergic rhinitis was relatively low in this cohort raising the possibility that patients could have been dispensed prescriptions that were not considered in this analysis, or symptoms were mild therefore not requiring prescriptions, or patients bought over-the-counter oral antihistamines. For example, itchy and red eyes and runny nose are common symptoms in patients with allergic rhinitis, but many patients take over-the-counter prescriptions for this.[210] Interestingly, a study in 1992[210] found that 54% of individuals who reported having allergic rhinitis symptoms bought

over-the-counter medication, tablets, nasal sprays and eye drops. Only 31% of individuals with allergic rhinitis symptoms had a prescription in the past two years. The majority of these individuals found over-the-counter medication effective, which may explain the low number of prescriptions dispensed for allergic rhinitis. Several parents identified their children as having perennial or seasonal rhinitis (n=411), but 198 of these children (48%) were not dispensed prescriptions for allergic rhinitis over a 9-year period. On the other hand, some parents identified their children as not having perennial or seasonal rhinitis (n=567) but 187 of these children (33%) were dispensed prescriptions for allergic rhinitis. A study[47] found that a large proportion of asthmatics (80%) also had rhinitis, however, in this cohort only 40% of patients were dispensed one or more prescriptions for allergic rhinitis. A possible explanation for this difference could be attributed to patients with allergic rhinitis not using medication. A review found that between 34 and 61% of patients with allergic rhinitis believed that intranasal corticosteroids lost their efficacy after use.[211] This perception may lead patients to not use their medication for allergic rhinitis. No association was found between FLG mutations and prescribing for allergic rhinitis, although it is hypothesised that sensitization triggered by the impaired skin barrier may lead to increased inflammation in the lung and nasal tissues. The link between FLG mutations and allergic rhinitis should be further explored to understand whether an association exists, that this thesis was unable to find, or whether the lack of association is replicated, which leads to the question as to why might FLG mutations lead to increased asthma severity but not rhinitis?

All patients in BREATHE have a physician diagnosis of asthma. However, patients with both eczema and asthma are known to present a more severe form of both diseases. Therefore, this cohort may have a higher proportion of patients with severe eczema than one would find in the population of patients with eczema but without asthma. Hence, the results may not be generalisable to the entire population of individuals with just eczema, as it may over-inflate the cost.

Implications worldwide and for clinical practice

The translatability of these results to other Caucasian populations is another question. Studies across Europe found a similar Minor Allele Frequency (MAF) for the R501X and the 2284del4 FLG mutations between individuals without eczema, but Irish and British individuals with eczema had higher MAF than individuals with eczema from Germany and Denmark (combined genotype of R501X and 22del84 ~38% vs. ~18%).[182] Interestingly, the two most common polymorphisms are rare in Italian patients with eczema (MAF $<$0.01).[203] There are no data on other Mediterranean countries, but the difference in MAF could suggest that different genetic factors predispose to eczema in each of these populations. Interestingly, if FLG is indeed one of the key drivers of the 'atopic march', the different MAF could partially explain the high prevalence of eczema and asthma in the United Kingdom (UK).

Atopic diseases are prevalent in families, are associated with increased levels of Immunoglobulin E (IgE), and, usually, develop within the first years of life.[212] The identification of genetic biomarkers that may predict the development of these diseases is crucial for the individualization of treatments, aiming to reduce the severity of these diseases. Although FLG alone cannot fully explain the development of atopy-related diseases, FLG has the potential to be a predictive biomarker for eczema and asthma severity.

Hence, the next step should aim to translate these results into clinical practice. The implementation of genetic testing early in childhood could help identify carriers of FLG mutations. An interesting follow-up from the study presented on FLG is to understand whether screening children at higher risk of developing eczema is cost-effective. This can be achieved using a decision tree, which compares the cost of screening high-risk children versus children who are not screened.[102] An RCT[213] and a pilot study for an RCT[214] have found a delay in the onset of eczema in neonates exposed to daily emollients during an 8 and 6 month period of observation, respectively. The results are promising since the intensive use of emollients was reported to restore the hydration of the epidermal barrier. A promising RCT is currently under analysis.[207] This RCT, Barrier Enhancement for Eczema Prevention (BEEP), recruited at birth 1282 children at high-risk of developing eczema and

followed them up for 7 years. Children were randomised to skin care advice or skin care advice plus advice to use daily emollients. The primary objective is to assess whether daily use of emollients with skin care advice delays the onset of eczema, while secondary endpoints relate to the difference in time to onset of eczema and other allergic diseases, eczema severity, safety issues and cost-effectiveness analysis. However, no RCTs have explored the role of barrier enhancement in reducing the severity of eczema and asthma in children and young adults with FLG mutations and established atopic-related disease. Another interesting area of research for the future relates to the efficacy of emollients in individuals with and without FLG mutations. Data regarding the superiority and safety of emollients is currently lacking. A recent RCT published in 2018 did not report any benefit of using bath emollients.[215] The study did not collect information about the FLG gene status, thus it is not possible to infer that children with mutations would benefit from bath emollients compared to children without mutations. Interestingly, a report from 2016[216] has made it a top priority to understand which emollient is most effective and safest. It would be interesting to look at the effect of emollients on individuals with and without FLG mutations in the long-term, to understand whether any patient benefits from the use of daily emollients or, whether individuals with FLG mutations or severe eczema benefit more from using daily emollients.

6.2 Understanding the association between adenoreceptor β_2 (ADRB2) gene variation and healthcare utilisation

Summary

This work has shown that patients with Arg/Arg genotype were dispensed significantly more prescriptions for a combination of LABA and ICS than patients with Gly/Gly or Gly/Arg genotype. Individuals with Arg/Arg genotype also were dispensed more prescriptions for Leukotriene Receptor Antagonist (LTRA) than individuals with Gly/Gly genotype. Although prescribing differences between individuals with Arg/Arg

versus those with Gly/Arg for LTRA prescriptions were not significant, the Incidence rate ratio (IRR) was 2.15 and the CI (0.99,4.70), suggesting that children and adults with Arg/Arg genotype overall were dispensed more prescriptions for severe asthma than children and adults with Gly/Arg or Gly/Gly. Arg/Arg children and adults were also dispensed more oral corticosteroids than children and adults with Gly/Arg or Gly/Gly genotype. No association was found between genotypes and the number of asthma exacerbations, over a 9-year period of study.

Regarding the Glu27Gln polymorphism, this study has shown that patients with Gln/Gln genotype were dispensed significantly more prescriptions for LTRA than patients with Gln/Glu or Glu/Glu genotype. Individuals with Gln/Gln genotype were also dispensed more prescriptions for a combination of LABA and ICS than individuals with Glu/Glu genotype. Although prescribing for a combination of LABA and ICS between individuals Gln/Gln and Gln/Glu was not significant, the IRR was 0.59 and the CI (0.35,1.00), suggesting that children and adults with Gln/Gln genotype overall were dispensed more prescriptions for severe asthma than children and adults with Gln/Glu or Glu/Glu genotype. Interestingly, Gln is the common allele (wild-type), suggesting that Glu may act as a protective allele. A weaker association was found for prescribing of oral prednisolone. Children and adults with Gln/Gln genotype were dispensed more oral corticosteroids than children and adults with Glu/Glu genotype. No association was found between genotypes and the number of asthma exacerbations.

Biological plausibility for findings

The results found in this thesis regarding the greater number of prescriptions for severe asthma dispensed to patients with Gln/Gln genotype are concordant with previous studies, which found that individuals carrying the Gln allele may be more prone to inflammation, bronchodilation and bronchoconstriction than those carrying the Glu allele. The Glu allele is hypothesised to protect against down-regulation of the β_2 receptor, which leads to diminished clinical response, due to the decrease in the number of receptors.[75,76] However, the exact role of Glu27Gln polymorphism in the bronchodilation response to β_2-agonists is unclear. It is possible that the protective effect of the Glu allele is due in part to Linkage Disequilibrium between Glu27Gln and

Arg16Gly polymorphisms.

A possible explanation for the greater number of prescriptions for severe asthma dispensed to patients with Arg/Arg genotype is that the presence of Arg alleles enhance down-regulation of the β_2 receptor[75,76]Hence, the Arg allele may be associated with the development of tolerance to the bronchoprotective effects of β_2-agonists after long-term, chronic use of LABA, despite the use of ICS.[217,218] Chronic exposure to β_2-agonists results in the loss of receptor expression, eventually leading to tachyphylaxis, which is clinically manifested as reduced bronchodilation in response to bronchodilator.[219,220] A weaker association between individuals with Arg/Arg and Gly/Arg genotype was found for the dispensing of relievers. The diminished clinical responsiveness to LABA and not Short-acting β_2-agonists (SABA) could be due to the action time of each medication. SABA effects last 3 to 6 hours while LABA effects last more than 12 hours. As a follow-up, one could divide the reliever prescriptions into β_2-agonist reliever prescriptions and non-β_2-agonist reliever prescriptions to understand whether Arg/Arg individuals were dispensed significantly more β_2-agonist relievers than non-β_2-agonists in comparison to Gly/Arg and Gly/Gly individuals. The biological mechanism for the poor response to LABA in patients with the Arg allele does not explain why there is still diminished response in the presence of ICS. Glucocorticoids increase the number of β_2-receptors, blocking the down-regulation by the prolonged use of β_2-agonists.[118] In theory, ICS combined with LABA should up-regulate receptors, even after chronic use of β_2-agonist. However, this thesis and previous studies have found that individuals with the Arg allele taking combined LABA and ICS have poorer clinical response compared to individuals with the Gly allele taking combined LABA and ICS. Possible explanations could be the resistance to glucocorticoids in some patients, or an interplay of genes causing a decreased response to medication. Alternatively, perhaps, glucocorticoids are not able to overcome the down-regulation induced by the Arg allele as previously hypothesised.

No association was found between Arg16Gly and Glu27Gln Single nucleotide polymorphisms (SNPs) and the number of asthma exacerbations. A recent meta-analysis[126] found an increased risk of exacerbations for individuals on a combination of LABA and ICS. However, one could argue that the meta-analysis itself

has some limitations. The studies included classified asthma exacerbations as a dichotomous variable (at least one exacerbation vs. zero), whereas in this study, asthma exacerbations were classified as a count variable. Although dichotomizing variables is often acceptable, information about increases or decreases in the risk is lost, and dichotomization increases the risk of false positives or overestimation in the risk.[221] Contrary to this thesis, asthma exacerbations in the meta-analysis were classified based on parental recall, which is subjective, and the studies were cross-sectional. Interestingly, one of the studies in the meta-analysis was BREATHE, and the association between Arg16Gly polymorphism and asthma exacerbations in BREATHE was also not significant. Of note is that the meta-analysis included three UK studies (Arg16Gly MAF = 0.37 and 0.38), one study with Hispanic individuals (Arg16Gly MAF = 0.45) and a Dutch study, which classified Moroccans and Turkish as Caucasians (Arg16Gly MAF = 0.41). Although the meta-analysis was significant, the three UK studies were not significant when considered individually, which could suggest a smaller effect on the UK population in comparison to other populations.

Regarding the number of oral corticosteroids dispensed and the number of asthma-related A&E visits/admissions, a weak-to-moderate association was found between Arg16Gly genotypes and the number of prescriptions for oral prednisolone dispensed. However, no association was found between Arg16Gly genotypes and the number of asthma-related A&E visits/admissions. A higher number of oral corticosteroids and LTRA prescriptions were dispensed to patients with Arg/Arg genotype, compared to patients with Gly/Gly or Gly/Arg genotype. This might be due to the poorer clinical response to LABA in patients with the Arg allele. Patients who do not respond to the medication will need to step-up as a consequence of poor asthma control. The use of LTRA and/or oral corticosteroids may be enough to obtain control, without the need, in most patients, to attend the hospital with acute illness. This may explain the presence of an association between the Arg allele and prescriptions for oral corticosteroids, and the absence of an association between the Arg allele and asthma-related A&E visits/admissions. Similarly, patients with Gln/Gln genotype were dispensed more LTRA and oral corticosteroids compared to patients with Gln/Glu or Glu/Glu perhaps as a consequence of poor asthma control. The hypothesis that patients with Arg/Arg genotype may improve with oral corticosteroids,

without visiting a hospital, is supported by other observations. As previously mentioned, glucorticoids can up-regulate β_2 receptors and potentially taking oral steroids for acute illness could help up-regulate the receptors, while this up-regulation might not have happened with regular ICS. It would be interesting to explore this mechanism in further detail in the future.

This thesis did not present information about African-American children. However, it would be relevant to explore the association of the Arg allele and asthma severity in these children, defined by higher dispensing of combination LABA and ICS, LTRA, and oral corticosteroids. Previous studies found an increased number of asthma-related deaths and serious adverse events for children using monotherapy LABA compared to placebo.[222,223] Interestingly, African-Americans had worse outcomes than Caucasians, higher number of hospitalisations and lower Peak expiratory flow (PEF) rates.[222] It would be interesting to explore whether the higher number of hospitalisations is correlated with the use of β_2-agonists. Similarly, studies should explore the association of the Arg allele and clinical responsiveness to β_2-agonists in African-Americans. Given the high frequency of the Arg allele (37% in Caucasians) it is vital to understand whether LTRA is more effective than a combination of LABA and ICS in children with the Arg allele. This is even more important for African-Americans, whose Arg allelic frequency is 49%,[145] corresponding to half of these children potentially receiving inappropriate medication.

Pharmacoeconomics

Significant prescribing cost differences were found between participants carrying different genotypes for the Arg16Gly polymorphism. Children and adults with Arg/Arg genotype cost more to the NHS than children and adults with Gly/Arg genotype for dispensing of relievers, ICS and oral corticosteroids. However, no difference in costs was found between patients with Arg/Arg genotype and patients with Gly/Gly genotype. Individuals with Arg/Arg genotype also cost more to the NHS than individuals with Gly/Gly genotype for dispensing of LTRA. No difference in costs for LTRA prescribing was found between patients with Arg/Arg genotype and patients with Gly/Arg genotype.

As discussed previously, it is estimated that 56,180 children were born in 2016, in Scotland, of which, around 10,674 may develop asthma. In the results, 15% had the Arg/Arg genotype, which corresponds to 1601 children, 45% had the Gly/Arg genotype, which corresponds to 4803 children, and 40% had the Gly/Gly genotype, corresponding to 4270 children. In that sense, one can extrapolate the cost of LTRA prescriptions over a 9-year period. Asthmatic children with Gly/Gly genotype may cost to the NHS a total of £876,00, asthmatic children with Gly/Arg genotype may cost the NHS a total of £1,126,000, and asthmatic children with Arg/Arg genotype may cost to the NHS a total cost of £532,000. Over a 9-year period, the NHS may spend approximately an additional £203,000 (estimates vary between £25,000 and £391,000) more on LTRA prescriptions treating children with Arg/Arg genotype than children with Gly/Gly genotype.

As mentioned, these costs represent only a fraction of the amount NHS spend treating asthmatic patients. A recent cost analysis of asthma in the UK estimated an annual cost of £1 billion, of which 60% were due to prescribing. The remaining cost was divided into 14% for consultations, 12% for hospital care and 13% for disability claims.[38]

Limitations

In this thesis, children and adults who smoked and/or were exposed to smoke were dispensed more prescriptions for relievers and more prescriptions for severe asthma, such as a combination of LABA and ICS, LTRA and oral corticosteroids, than children and adults who did not smoke and/or were not exposed to smoke. Patients who smoked and/or were exposed to smoke also had more asthma-related A&E visits/admissions than patients who did not smoke and/or were not exposed to smoke. No apparent interaction between the Arg16Gly genotypes and smoking status on the number of asthma-related prescriptions was seen in this study (see figure 5.14) despite previous studies that found that an interaction between smoking and Adrenoreceptor β_2 (ADRB2) polymorphisms increased the risk of wheeze and asthma.[224] However, this thesis looked at asthma severity and not asthma susceptibility, and the majority of children in this study were not smokers but were exposed to second hand smoke. There is a possibility that direct smoking could lead

to a desensitisation and a down-regulation of the β_2-receptor response on stimulation by β_2-agonists. Nevertheless, one must be aware of the limitations of the variable 'smoke and/or second-hand smoking'. Information regarding second-hand exposure to smoke and direct smoking were collected between 2003 and 2005, at data collection. Members of the household could have stopped smoking during the 9-year period or adolescents reaching adulthood could have started smoking. Ideally, in a longitudinal study, patients would be followed-up annually to assess their smoking status and the information would be integrated into the model. With that information, the effect of direct smoking and second-hand smoking on asthma severity could be assessed, as well as the effect of smoking cessation on asthma severity.

The Arg/Gly polymorphic variation was not associated with the number of asthma exacerbations. There is a possibility that asthma exacerbation analyses were underpowered. No power calculations were performed in this thesis, since this thesis uses a dataset with data that was previously collected. Another point of consideration in the asthma exacerbations analyses is the model used. A count model was used to model the data assuming independence between asthma exacerbations in the same individual, and the order of asthma exacerbations is not considered important. However, if chronic use of β_2-agonists leads to a desensitisation of the β_2-receptor response in the airways, one could hypothesise that after the first asthma exacerbation the patient may be more prone to asthma exacerbations, creating dependence between asthma exacerbations. On the contrary, for patients without airway β_2-receptor desensitisation, sequential asthma exacerbations may be independent. This hypothesis can be tested in the future using a model for survival analysis with recurrent events.

The number of prescriptions dispensed should be interpreted with caution, especially regarding reliever prescriptions. Some asthmatics could have been dispensed several relievers, which they may spread over multiple locations, such as their house, school/workplace, relative's house, etc. Therefore, an increased number of relievers is not necessarily correlated with severity.[225]

Implications worldwide and for clinical practice

A collaboration with the Pharmacogenomics in Childhood Asthma (PiCA) consortium[226] has enabled access to the Pharmacogenetics of Asthma medication in Children: Medication with ANti-inflammatory effects (PACMAN) dataset, a cohort of asthmatic Dutch children with prescribing data since birth. Interestingly, a preliminary analysis of the Dutch data showed differences in prescribing patterns between the UK and the Netherlands. Children in the Dutch cohort were dispensed a higher number of ICS than children and adults in BREATHE. On the contrary, children and adults in BREATHE were dispensed a greater number of prescriptions for LTRA and oral corticosteroids than children in PACMAN.

An important consideration is that Arg16Gly and Glu27Gln are in strong Linkage Disequilibrium, making it difficult to ascertain whether Arg or Glu is causing an effect. In haplotype analyses, Arg16Gly appears to dominate the down-regulation compared with Glu27Gln. Several studies have shown different haplotypes in the ADRB2 gene. The role of Arg16Gly and Glu27Gln as a haplotype was not explored in this thesis but a haplotype analysis is planned.

The results of the ADRB2 study suggest that long-term use of β_2-agonists in children and adults with Arg/Arg genotype may be associated with poorer clinical response. The RCT by Lipworth et al. published in 2013[161] is encouraging since it shows that the use of montelukast instead of salmeterol was associated with an improvement in symptoms and quality of life and a reduced use of salbutamol, in children with Arg/Arg genotype. However, since the RCT was on children with Arg/Arg genotype it is still unclear whether heterozygous individuals may benefit from the use of montelukast instead of LABA, or whether the beneficial effects will be seen only in individuals with two copies of the Arg allele. An RCT, called Personalised medicine for Asthma ConTrol (PACT), is currently recruiting.[227] Patients are being randomised to current practice, or a combination of LABA and ICS (Gly/Gly) or montelukast (Gly/Arg or Arg/Arg). The primary outcome of this RCT is quality of life, measured using the Asthma Quality of Life Questionnaires with standardised activities (AQLQ(S)), and secondary endpoints are differences in asthma control, healthcare utilisation, and medication use over a one-year period.

6.3 Understanding the association between Fc fragment of IgE receptor II (FCER2) genetic variation and healthcare utilisation

Summary

This work has shown that patients with CC genotype were dispensed more prescriptions for LTRA and allergic rhinitis than patients with TT or TC genotype. A weaker association was found between individuals with TC and TT genotype and prescribing of antihistamines and antivirals, individuals with TC genotype were dispensed more antihistamines prescriptions and less antivirals against skin pathogens compared to individuals with TT genotype.

Biological plausibility for findings

A possible explanation for the greater number of LTRA prescriptions dispensed to patients with CC genotype in the Fc fragment of IgE receptor II (FCER2) gene could be related to an increased production of, and response to, IgE and consequently an increased release of histamines, leukotrienes and cytokines, in comparison to patients with the CT and TT genotypes. In particular, there is likely to be greater release of Interleukin 4 (IL-4), leading to increased allergy-related symptoms, which corticosteroids are unable to contain. The exact role of FCER2 in IgE regulation is unknown. However, FCER2 expression has been shown to be induced specifically by IL-4.[228] Corticosteroids inhibit the transcription of IL-4, which could be one of the reasons for their efficacy in chronic allergic inflammation.[229] However, some studies have found that corticosteroids may be less effective in the presence of elevated levels of IgE and IL-4. Wu et al.[230] found that the use of glucocorticoids was associated with increased levels of IgE in the presence of in vivo IL-4. Another study[231] found that the increase in IgE levels on glucocorticoids was only present in atopic asthmatic patients and not in non-atopic patients. The CC genotype for the FCER2 variant could be responsible for increased production of IL-4, leading to increased IgE, or a greater response to IgE, which corticosteroids may be unable to contain. Hence, it is possible that individuals with CC genotype will have a greater IgE

activity than individuals with TT or TC genotype. It is interesting that this is leading to a greater number of LTRA prescriptions being dispensed to individuals with CC genotype in comparison to patients with TT or TC genotype. What are the practical steps that could be leading to this increase in LTRA prescribing? It is possible that the increased symptoms in patients with CC genotype is leading to increased prescribing of different medicines such as ICS, nasal corticosteroids and LTRAs. However, the patient may be showing a greater response to LTRAs and this may be reported to the GPs. Perhaps, as a consequence, the GP is prescribing more LTRAs.

The association found for prescribing of LTRA between individuals with CC genotype and individuals with TC or TT genotype suggests that individuals treated with ICS, even in the presence of LABA, might not respond to the medication and need an LTRA medication. Curiously, patients with CC genotype were also dispensed more LABA prescriptions than patients with TC or TT genotype. Although this association was not significant, it corroborates the hypothesis that individuals with CC genotype were experiencing a poorer response to ICS in comparison to individuals with TC or CC genotype and hence were being dispensed more LTRA and also LABA. However, the biological mechanism behind the diminished clinical response to ICS is not fully understood. More research is needed to understand why individuals with a mutation in the FCER2 gene do not respond to ICS.

Regarding asthma exacerbations, no association was found between the studied FCER2 polymorphism and the number of oral corticosteroids prescriptions dispensed and also the number of asthma-related A&E visits/admissions. The lack of association does not disprove the hypothesised mechanism since it could suggest that these individuals are being prescribed LTRA and may be responding to these medications, and consequently not developing further asthma exacerbations. Koster et al.[81] found an increased risk of asthma-related A&E visits/admissions for children with CC genotype compared to children with TC or TT genotype on ICS treatment, reinforcing the idea that children with mutations on the FCER2 gene do not respond to ICS.

With regards to allergic rhinitis prescriptions, patients with CC genotype were dispensed a greater number of allergic rhinitis prescriptions compared with patients

with TT genotype. It is interesting to note that the majority of prescriptions (76%) included in the analysis were for nasal corticosteroids, strengthening the idea that individuals with two copies of the minor allele in the FCER2 polymorphism do not respond to corticosteroids. The effect of the CC genotype on prescribing could extend to eczema as children and adults with CC genotype were dispensed more prescriptions for mild (IRR:2.00, 95% CI:0.99,4.07) and moderate eczema (IRR:2.00, 95% CI:0.97,4.15) than children and adults with TC or TT genotype. However, these associations were not significant. This could be the result of a type II error as sample size was less than 500 participants. It would be interesting to look at the effect of FCER2 mutations on eczema and topical corticosteroids prescribing in a cohort with a larger sample size.

Pharmacoeconomics

Significant cost differences were also found between genotypes of the FCER2 polymorphism. The costs for patients with CC genotype are greater to the NHS than the costs for patients with TT genotype for prescriptions for moderate eczema, The same pattern is observed in asthma, for the prescribing costs for LABA and oral corticosteroids.

Using the previous estimate of 16,292 people possibly developing eczema, and 10,674 developing asthma, table 5.26 shows the proportion of children and adults with eczema and asthma. Assuming that 53% of children with asthma will also have eczema, around 5657 children born in 2016 could eventually suffer from both diseases. In the results, 8% had the CC genotype, which corresponds to 447 children with eczema and asthma, 2133 children with TC genotype and 3077 children with TT genotype. In that sense, one can extrapolate that over a 9-year period, children with eczema and asthma and TT genotype may cost to the NHS a total of £39,000, children with eczema and asthma and TC genotype may cost to the NHS a total of £40,000, and children with eczema and asthma and CC genotype may cost the NHS a total of £18,000 on prescribing for moderate eczema. Over a 9-year period, the NHS may spend an additional £12,000 (estimates vary between £438 and £37,000) on prescriptions for moderate eczema treating children with CC genotype than children with TT genotype.

However, since no association was found between individuals with CC and TT genotype for the number of prescriptions for moderate eczema, these cost differences are likely to not be as relevant as the numbers presented in the FLG and Arg16Gly analysis.

Limitations

Although an association was found between genotype and prescribing for allergic rhinitis, one must be aware of the previous discussed limitations of the allergic rhinitis data namely the low number of allergic rhinitis prescriptions dispensed. Nevertheless, the majority of prescriptions corresponded to nasal corticosteroids, which suggest that individuals with CC genotype may not respond to corticosteroids.

Implications worldwide and for clinical practice

The mechanism behind the poor response to corticosteroids, either inhaled, nasal, and possibly topical, by patients with the CC genotype is not entirely understood. For instance, it is unclear why the poor response to corticosteroids is only seen in individuals with two copies of the minor allele and not in individuals with one copy of the minor allele. More pathophysiological research is required to understand whether the use of corticosteroids in patients with mutations in the FCER2 gene causes an increased IgE responses, which corticosteroids are unable to suppress. If the hypothesised mechanism is indeed correct, FCER2 might become an important genetic biomarker for clinical responsiveness to ICS.

Omalizumab is an anti-IgE therapy, prescribed to individuals unresponsive to corticosteroids. Two RCTs[232,233] found that children on omalizumab had significantly fewer asthma exacerbations than children on placebo. One of the RCTs[232] also found that children on omalizumab had fewer symptoms, and improved asthma control with smaller doses of ICS, compared with patients on placebo. The other RCT[233] found that the use of omalizumab, compared with placebo or ICS, was associated with a decrease in asthma exacerbations for individuals who had an asthma exacerbation during the run-in period, suggesting a possible link between IgE activity and risk of asthma exacerbations. Since omalizumab reduces the levels of IgE, omalizumab could be a feasible option in patients with increased levels of IgE. The NHS guidelines recommend that omalizumab should only be started in specialist centres.[58]

There were no prescriptions for omalizumab dispensed in this cohort over the 9-year period. This could be because any patients prescribed omalizumab were dispensed the medicine from hospitals. It is possible that very few patients, or no patients, in this cohort were prescribed omalizumab. Although omalizumab has shown good results in some studies, it is still an expensive medicine (in 2016, an injection of omalizumab 150MG cost £256.15), whereas montelukast is cheaper (in 2016, one tablet of montelukast 10MG cost £4.02). Hence, it could be interesting to see whether children with asthma and the CC genotype have significantly better outcomes on LTRA compared to ICS. It would be interesting to design an RCT to compare the roles of LTRA and ICS on outcomes such as asthma exacerbations, quality of life and use of healthcare resources for individuals with uncontrolled asthma and to analyse the data as per genotype (CC, TC or TT). If children with CC genotype on LTRA have significantly better outcomes than children with CC genotype on ICS, it may be appropriate to consider implementing genetic testing in clinical practice in order to direct children towards treatments to which they may respond better. A decision tree can be drawn to assess the cost-effectiveness of such therapy.

Children in the Dutch (PACMAN) dataset have also have been genotyped for the FCER2 C/T polymorphic variation. This will provide an opportunity for at least a partial replication of the methodology. Since the number of LTRA prescriptions in the Dutch cohort is lower than the number of LTRA prescriptions in the Scottish cohort, it would be interesting to see whether children in PACMAN were dispensed a higher number of ICS prescriptions, and whether this was associated with an increased number of asthma exacerbations.

6.4 Communicating personalised medicine to children and parents

Findings

In the public engagement activities performed, parents and children increased their understanding about genetics, Deoxyribonucleic acid (DNA), heritability, the role of FLG gene defects in the skin, and the concept of personalised medicine following the session. Children enjoyed the activities, and parents and medical students appreciated the importance of engaging and sharing information.

Questions about genetic testing were included in the sessions in Bajouca, Portugal. Parents showed a positive attitude regarding genetic testing and would support the testing of their children upon recommendation from doctors. There were no concerns regarding ethical issues. This could be because the parents were unaware of the ethical issues, or because they felt it was outside the scope of the discussion. However, a consistent theme was concern regarding the cost of genetic testing and many thought it could not be implemented in clinical practice. Most of the parents were unaware that some forms of genetic testing are already prescribed in Portugal for some conditions, and are reimbursed by the Portuguese National Health System.

The difference in attitudes between parents from Brighton in the UK and Bajouca in Portugal was also interesting. Half of the participating parents in Bajouca stated having changed their awareness or increased their knowledge regarding personalised medicine, whereas, in Brighton, 11 out of the 15 interviewed parents said that the sessions have changed their awareness or increased their knowledge regarding personalised medicine. The Brighton session was included within a festival (the Brighton Fringe Festival), which meant that anyone with an interest in science could attend, while the sessions in Bajouca were attended by grandparents, unemployed parents and parents on maternity leave. The differences in "participation" and the fact that Bajouca is a village in a rural area of Portugal may be an indication of different social backgrounds. Another aspect to consider is that, in contrast to the UK, Portugal was ruled by a totalitarian regime until relatively recently, ending 43 years ago, which

means that most of its population faced education in a strict environment that was opposed to critical thinking. Although the literacy rate in Portugal is now around 96%, in 1935/40 the primary school enrolment ratio in Portugal was one of the lowest in Europe, 29%, compared to 73% in England and Wales.[234] A review[235] on the interest, motivation and attitudes towards science showed that parental education is correlated with attitude regarding science but not motivation. All of these factors could have played a role in the differences found between parents from Bajouca and Brighton.

Practical considerations

Several obstacles emerged from the public engagement sessions. One of them relates to the importance of having volunteers to help with such activity. Several activities were performed at the same time, and children are usually curious and enjoy interacting. Implementing a session without the help of volunteers would have been extremely difficult. For example, although the 'skin barrier' activity was well received in Brighton, in one of the sessions in Bajouca this activity was the least popular. The reason for this dislike was mainly due to the delivery of the activity. In both schools, teachers were fundamental to the implementation and functioning of the sessions. Teachers from the first school were more curious, helpful and engaging than teachers from the second school. On the other hand, children from the first school were shyer and more well-behaved during the activities. Before each session, the activities were explained to the volunteers as well as their role and possible questions the children may have. However, in the second session, the teacher responsible for the 'skin barrier' activity did not explain the purpose of the activity to the children, did not engage with the children, and passed the majority of the time chatting with the parents.

Another important challenge is to adapt the communication style according to individual needs, and particularly according to whether we are communicating with a child or a parent. Dr Christina Jones, who is a psychologist by training, conducted interviews in Brighton, and I conducted those in Bajouca. The interviewing style and differences are clear. In Bajouca, the second session was easier since children were more talkative and more engaging, while children in the first session were shy and it was hard to get information from them. The child interviews in Brighton yielded richer

information than those in Bajouca. The friendly speech and questioning style of Dr Christina Jones appeared to have placed children more at ease and made them more interactive and responsive, which underlines the importance of experience and interviewing skills in securing good public engagement.

Relying on cameras to record interviews can also create some technical issues. In the first session, children were interviewed in front of the camera so they were aware of the presence of the camera. To prevent any shyness prompted by the camera, the camera was left on a table for the second session. Although consent had been sought and obtained before the recording, the aim of lowering the presence of the camera was to reduce the children's perception of being recorded with the hope of putting them more at ease and making them more open to sharing ideas. The children were indeed more open during the second session in comparison to the first; however, placing the camera on the table and at a distance reduced the sound drastically making it extremely difficult to understand what they were saying.

Finally, children had fun with these activities; however, it was evident in some interviews that the child did not gain knowledge. Children may get too excited with the fun activities and ignore the main purpose and the explanations and just focus on having fun, which was more of an issue with the younger children. In that sense, there is a thin line and considerable overlap between fun and learning. The activities should be fun to engage children, but one should be aware of noting whether or not the children are actually learning as intended for the activity.

Personal reflections

The analysis of the data provided in these sessions has highlighted some issues. The "why" of this public engagement project needs to be refined. Why do we want to teach children and parents about personalised medicine? It is evident from these sessions that the notion of personalised medicine was understood, but it is not clear what parents and children will do with that information. It is always positive to gain more knowledge but perhaps learning is not the most interesting outcome. From my point of view, a fascinating 'why?' could relate to the changes we can achieve with a public engagement project on personalised medicine. Could the notion of personalised medicine engage parents in discussion with their physician about the

best treatment for their child? Could the understanding of the consequences of a broken skin, change parent's and children's attitudes regarding the use of emollients and treatment? Can an explanation about the disease, the treatment available and the consequences of not using emollients or the inhaler improve the children's adherence to medication? These are some interesting reasons to engage with the audience. Whether public engagement is part of the answer, is in itself another question. The only way to respond to this question is to perform another public engagement project and evaluate it.

All of the discussed points will help shape and strengthen a future public engagement project. Can an understanding of particular aspects of personalised medicine improve patient management by improving adherence? Moreover, can it start a discussion between patients and physicians? Patients should know more about their conditions and should play a more active role in their management. Obviously, this can only be obtained if physicians are aware of the latest scientific discoveries. Another public engagement project could be focused on the interaction between the community of health professionals, such as GPs, nurses and pharmacists, and the scientific community. These three groups of individuals should communicate with each other to understand what is happening in science and how that can shape medicine and clinical care. Future projects should include older children. Children in primary schools have no, or limited, knowledge of genetics. Learning genetics, heritability, mutations and personalised medicine in less than one hour is difficult under any circumstances. Hence, a longer period of time might need to be allotted for such activities. However, schools are a good place to perform similar activities. Using schools may reduce the selection bias found in public engagement activities in festivals or pubs.

6.5 Secondary findings

Prescribing patterns

Studies have pointed out differences between guidelines and clinical prescribing.[236,237] Although it is impossible to know whether guidelines were being followed, it is possible to observe eczema and asthma prescribing patterns in this cohort of Scottish children and adults.

Regarding eczema-related prescriptions, 73% of individuals with eczema and asthma were dispensed antihistamine prescriptions, followed by emollients (65%). Over the 9-year period, of the 387 patients that were dispensed an oral antihistamine prescription, the average number of antihistamines dispensed was 12 per patient, while of the 344 patients that were dispensed a prescription for emollients, the average number of emollient dispensed was 16 per patient. Half of the individuals with eczema and asthma were dispensed prescriptions for mild eczema, 48% were dispensed prescriptions for moderate eczema, while 41% were dispensed prescriptions for severe eczema. As expected, more patients were dispensed prescriptions for emollients and mild eczema compared with prescriptions for severe eczema. Over the 9-year period, of the 265 and 254 patients that were dispensed prescriptions for mild eczema and moderate eczema, the average number of prescriptions for mild and moderate eczema per patient was 5. Of the 217 patients that were dispensed prescriptions for severe eczema, the average number of prescriptions was 8 per patient, over 9-years. Although fewer children and adults were dispensed prescriptions for severe eczema, they were dispensed a higher number of prescriptions. A study in 2006 interviewed parents of school-children and found lower use of eczema-related prescriptions compared to the observations in this thesis. In this study, the medicine used most commonly was also antihistamines, as observed in this thesis, followed by emollients.[4] In this 2006 study, 16% of parents reported use of antihistamines, 14% reported use of emollients, 11% reported use of topical corticosteroids, and 6% reported use of topical calcineurin inhibitors and systemic corticosteroids. The difference in the proportion of medication use could be attributed to differences in severity, and differences in times, since most studies are

cross-sectional and in this thesis is a longitudinal study. The cohort studied in this thesis have asthma and eczema, and patients with both conditions are likely to have a severe form of eczema, hence may be exhibiting greater use of eczema-related medication.

With regard to asthma medication, more patients were dispensed relievers (96%), followed by ICS (75%), LABA and ICS 42%, and LTRA (30%). Half of the individuals with asthma were dispensed one or more oral prednisolone prescription over the 9-year period. Of the 939 patients that were dispensed reliever prescriptions, the average number of relievers per patient was 26 per patient, over 9-years. Regarding ICS, over 9-years, of the 757 patients that were dispensed ICS prescriptions, the average number of ICS was 11 per patient. Of the 427 patients that were dispensed prescriptions for combined LABA and ICS, and of the 282 patients that were dispensed LTRA prescriptions, the average number of combined LABA and ICS was 23, and the average number of LTRA was 18, per patient over 9-years.

Other studies have reported prescribing patterns for asthma populations. A 2008 Australian study interviewed parents of school-entry children about asthma medication use.[238] In 2006, a study in Finland examined the registry of reimbursements for children aged between 7 and 15 years old.[239] In the Netherlands, in 2006, pharmacy data was collected for children between 0 and 14 years old who had at least one asthma-related prescription dispensed.[240] Australian parents reported a similar use of relievers to the BREATHE cohort (90% of Australian parents reported use of relievers, compared to 96% of Scottish children). However, the number of children that were prescribed ICS varies per study. The highest proportion of children (89%) that were dispensed ICS was in the Netherlands. In BREATHE, 75% were dispensed at least one ICS prescription. Both the Australian and Finnish study, reported lower proportion of children using ICS, 38% and 41% respectively. The combination of LABA and ICS was also variable among these countries. The Finnish study reported that 47% of children were dispensed a combination of LABA and ICS. In BREATHE, 42% of patients were dispensed a combination of LABA and ICS, while 20% of Australian parents reported the use of combined LABA and ICS. LABA use in the Australian study was lower than the one reported here (3% vs. 7%). In the Netherlands, LABA was prescribed to 11% and of those, 9% were not

prescribed an ICS concomitantly. There is difficulty in establishing whether LABA prescriptions were prescribed with ICS, or whether these prescriptions were prescribed separately. An attempt was made to categorise concurrent use of LABA and ICS with separate inhalers. Separate prescriptions of LABA and ICS dispensed during the same calendar year were categorised as a combination of LABA and ICS. However, it is possible that prescriptions dispensed at the end of one year and prescriptions dispensed at the beginning of another year were prescribed at the same time by the GP or were intended to be used concurrently. Hence, the proportion of patients taking LABA separately could be smaller than that reported here. Similarly, the proportion of LABA and ICS users in BREATHE could be higher and closer to the proportion reported in the Finnish study. A study in Scotland, by Elkout et al. in 2012, used pharmacy records to identify Scottish children with at least one asthma-related prescription.[236] This study found a similar median of SABA prescriptions dispensed as for the study reported in this thesis. In BREATHE, a median of 15 reliever prescriptions were dispensed over a 9-year period, while the other Scottish study by Elkout et al. reported a median of 2 prescriptions per year, which would approximate to 18 prescriptions over a 9-year period.[236] However, the number of oral corticosteroids prescriptions dispensed were different between the two studies. In this thesis, an average of 3 short courses of oral prednisolone were dispensed over the 9-year period, while the Elkout study found a median of 1 short course of oral corticosteroids per year.

The differences found between these studies may be a consequence of different guidelines followed by health professional, differences in the populations under study, or differences in the methodologies used to explore prescribing patterns. A discussion between experienced respiratory physicians and experts on pharmacy databases could shed some light on how the use of pharmacy records could be optimised to understand which British Thoracic Society (BTS) step the patient is on, without using GP databases. For example, a study in the Netherlands used pharmacy records to estimate the asthma treatment step for asthmatic patients.[241] Medications dispensed on the same day were categorised into asthma steps. However, although physicians prescribe one or more medicines on the same day, patients may not collect all prescriptions at the same time. For instance, if one of the medication is out of stock,

the patient may have two different dispensing dates, although the medication were prescribed at the same time and belong to the same step. All of these points need to be addressed before using pharmacy records to estimate the asthma treatment step on which the patient lies at any point in time. Although it would be interesting to have access to that information, several obstacles need to be overcome first. One needs to be aware that the patient may only have one medication dispensed although two were prescribed. Under such circumstances, the patient could have one of the medications at home, or the patient might not request the dispensation of medication on the belief that it does not work. Hence, without discussing how to overcome all these issues and whether it is possible, pharmacy records cannot be reliably used to estimate the asthma treatment step on which the patient lies at any point in time.

Gender

Gender differences in health behaviour have previously been described. Some studies found that women were more likely to adhere to treatment than men.[186,187] Nyberg et al.[188] found that women used more prescriptions for two common skin diseases, eczema and psoriasis, than men. Ballardini et al.[86] also found a significant difference with gender on the use of emollients, 12-year old girls used emollients significantly more frequently than 12-year old boys. In this thesis, females were dispensed considerably more eczema-related prescriptions than males, over a 9-year period. Women were dispensed more antihistamines, emollients, and prescriptions for mild and moderate eczema than men. However, for this study, it is unclear whether women were actually prescribed more medication, whether women collected more often their prescriptions, or whether once dispensed that women were more adherent than men to the actual medication.

The association between asthma treatment adherence and gender is less clear than the association between eczema treatment adherence and gender. Sundberg et al.[242] found that men were more likely to not adhere to asthma treatment than women. However, some studies[243,244] found that men were more adherent to asthma treatment than women, while other studies[245–247] found no association between gender and adherence to asthma treatment. In this longitudinal study, no significant difference was found between females and males for the dispensing of

asthma-related prescriptions.

Cat ownership

As discussed in the introduction, there is conflicting information regarding the role of cat exposure during childhood on the development, and subsequent severity of eczema, asthma, and other allergic disease.[44,49,50,52] In this thesis, cat ownership (recorded at the time of data collection) was inversely associated with prescriptions for LTRA, oral corticosteroids and asthma exacerbations in patients with asthma. Patients who owned a cat (recorded at the time of data collection) were dispensed significantly fewer prescriptions for LTRA and oral corticosteroids and had significantly fewer asthma exacerbations than patients who did not own a cat during data collection. The fact that the presence of a cat was associated with a lower number of dispensed prescriptions does not necessarily mean that the presence of a cat acted as a protective factor. The prescribing of LTRA, oral corticosteroids and the occurrence of asthma exacerbations are a suggestion of relatively severe asthma. It is possible that individuals with a cat might have relatively severe asthma and/or eczema leading to different health behaviours compared to individuals without a cat.

One should also be aware of the limitation of this variable. Cat ownership was ascertained at the time of data collection. It was assumed that the ownership continued over time. It is possible that individuals who were dispensed severe asthma-related prescriptions could have re-housed their cat or altered their surrounding environment with the intent of reducing the impact of cat allergy. These hypothesis can be tested by following individuals over a longer period of time to understand the long-term impact of owning a cat.

Adherence

Adherence to treatment is generally low in chronic diseases, such as asthma and eczema.[225,248,249] Studies estimated that less than 50% of children use their controller medication as prescribed.[248–250] Other studies found lower adherence rates for inhaled devices, such as ICS, compared to oral medication, such as LTRA.[225,248] Interestingly, adherence to ICS was higher after the addition of long-acting inhaled β_2-agonists. This could indicate that adherence improves with severity of disease or that adherence improves with greater treatment efficacy. In this thesis, over a 9-year

period, patients were dispensed an average number of prescriptions for severe eczema that was higher than the average number of prescriptions for mild or moderate eczema. Similarly, over a 9-year period, patients were dispensed an average number of combined LABA and ICS higher than the average number of ICS prescriptions dispensed. These results could be an indication that severity may influence adherence. However, this suggestion is speculative as one cannot be sure if the patients used the medicine after collection as recommended. Another possible issue may relate to the misinterpretation that the medication is not working. Patients may stop treatment after a few days due to their perception that the medication is not working. However, maximum therapeutic efficacy is often obtained after several weeks on the controller medicine. Conversely, after a few days, patients may improve and stop taking medication due to the incorrect perception that the medication is no longer needed. Inhalers are effective when used properly, but some patients report poor inhaler technique.[225] In BREATHE, 3% of children and young adults reported poor inhaler technique and 17% did not respond to the question.

After the warning against the use of salmeterol alone in 2008 due to its association with asthma mortality,[63] the BTS/SIGN recommended prescribing LABA and ICS in a single inhaler device, to increase adherence. In this cohort, from the beginning of 2009, 117 separate LABA prescriptions were dispensed, which correspond to 48% of the total of separate LABA prescriptions dispensed over the 9-year period. The GP may have prescribed separate LABA or LABA and ICS in two devices. However, physicians should be aware that prescribing a combination of LABA and ICS in separate inhalers is associated with low adherence,[248] and monotherapy LABA has been associated with an increased number of asthma-related deaths and serious adverse effects.[222,223] Hence, the warning used against the use of salmeterol alone.[63]

In this thesis, no association was found between the three studied genes and dispensing of Adrenaline Auto-Injector (AAI) devices. AAI devices are used to treat anaphylaxis, a severe allergic reaction that can be life-threatening.[251] In this cohort, 6% of patients were dispensed prescriptions for an AAI, with an average of 1 AAI prescription dispensed per year. The A&E database showed only one case of anaphylaxis during the 9-year period. However, this number could be under-represented as anaphylactic episodes are often under-recognized and/or

under-treated in emergency departments.[252] Several anaphylactic episodes are misclassified due to the origin of the symptoms, such as skin-related, respiratory, and gastrointestinal.[253] A study in the Netherlands found that only 40% of asthmatic individuals at high risk of food allergy were prescribed an AAI.[254] A study in the UK from 2017 found an increase in the number of AAI devices prescribed over the years and an average number of 4 AAI devices prescribed per child, a number higher than the one found in this study.[255] Asthmatic patients might use their inhaler or antihistamines in case of anaphylaxis, which demonstrates the slight uncertainty regarding the precise indication for a prescription for inhalers or antihistamines in this cohort. Alternatively, the low number of AAI devices dispensed could be due to an 18 to 24 month expiration date; patients who did not use their device may not seek another prescription until 18 to 24 months later and may have only one AAI device and not two as currently recommended by Anaphylaxis UK.[256] However, British Society for Allergy & Clinical Immunology (BSACI) guidelines refer that evidence regarding the need for two AAI per patient is sparse and the decision to prescribe one or more devices should be individualised, based on the risk assessment of the child.[251]

Pharmacoeconomics

The cost of prescriptions is often an issue for patients, in particular for patients with chronic diseases. In 2011, the Scottish government decided to extend free prescriptions to the entire population. Before 2011, the only groups receiving free prescriptions were children, patients over 65 years, patients with cancer, diabetes or hypothyroidism, and patients from a low socioeconomic background.

The cost analysis in the thesis focused on prescription and A&E visits/admissions costs. However, as seen in the prescription cost tables in the Appendix, prescription costs vary over the years, with remarkable increases and decreases. The variation in prices over the years is mainly due to patents.[101] In 2014, Symbicort and Singulair patents expired, which has facilitated the development of new generic medicines which will hopefully decrease the cost of medication. For instance, in 2002, the NHS Scotland estimated the cost per unit of Symbicort 200/6 turbohaler at £38.77 but in 2014, it was estimated to be £5.74 more expensive,£44.51.

Another limitation for this cost analysis is the lack of information regarding GP visits. One might expect that a higher number of prescriptions is associated with a higher use of healthcare resources. Uncontrolled and severe asthma is more expensive than controlled and mild asthma.[257] A study published in 2006 explored the costs of scheduled and unscheduled asthma visits in 7 European countries, including the UK.[257] The authors estimated that half of the annual cost (average total annual costs: 789 € for infants, 463 € for children and 566 € for adults) is due to unscheduled visits, such as hospital admissions and A&E visits, which were associated with severe asthma. However, estimates widely differ among studies. Other studies have slightly differing cost analyses including an annual estimate of $2697 for adults, with medication costs alone representing 50% of the total cost,[258] while a UK study found that prescription costs represented only 20% of the total cost for children.[259]

6.6 Strengths and limitations

Longitudinal data are a valuable resource for understanding disease progression and evaluating the long-term effects of treatment. Individuals affected by chronic diseases, like eczema and asthma, can experience periods when they are well-controlled on no medication, or on preventive medicine alone; however, at any time symptoms can reappear, and more intensive treatment may again be necessary. One of the advantages of longitudinal studies is the ability to analyse both within-person and between-person variation, unlike cross-sectional studies which can explore only between-person variation. Another advantage of longitudinal studies is the ability to inform on the variation that occurs over a larger period of time, which is especially important for atopic diseases. As previously mentioned, asthma exacerbations are more common in September/October, at the beginning of school, and during the spring. This is often due to increased exposure to viruses or allergens. Hence, cross-sectional studies recruiting during those periods may find a greater number of asthma exacerbations during these specific time periods and not due to a true genetic association. With longitudinal studies, it is possible to study whether the association is seen over longer periods of time. Longitudinal studies allow us to describe and predict individual differences in symptoms over time, e.g., which

patients benefit most from a particular treatment. The linkage of BREATHE with routine healthcare databases allowed a longitudinal analysis of the data, over a 9-year period, representing a major strength of this thesis.

However, as previously mentioned, one needs to be aware of the limitations of using pharmacy records. Dispensed prescriptions provide more information than prescribed prescriptions, as one knows that the prescription was dispensed, but it is impossible to determine whether the patient followed the doctor's recommendation, used the medicine at all, or whether the medication was shared among members of the household. It is also impossible to determine whether or not the patient bought additional over-the-counter medication or alternative medicine. Although prescriptions have always been free for children in Scotland, and since 2011 for everyone, patients may not go to the GP to get a prescription and instead buy over-the-counter prescriptions. It is also important to note that although this is an asthma cohort with a physician diagnosis, while the same cannot be said for eczema or rhinitis. These conditions were self-reported and could be based on patient belief in having one or both conditions and not on formal diagnosis.

Although routine healthcare data constitutes a valuable resource for research, one needs to be aware that most outcomes are not validated. In this thesis, three different diagnoses were used. In the case of eczema, self-report of the disease was used and it is unclear whether the parents of, or the patients themselves answered positively based on a physician diagnosis or based on an assumption that they had the disease. Asthma was the only condition that was physician-diagnosed. However, as previously mentioned, there are cases of misdiagnosis, although the number is low. Patients were classified as suffering from allergic rhinitis based on the medications prescribed. Several studies classify asthma severity based on self-report of medication use. In this thesis, asthma severity was classified based on the dispensing of medication. Both approaches have limitations as it is unclear whether the patient followed the doctor's recommendation. In order to improve reproducibility and assessment of asthma severity, outcomes should be validated, harmonised, and guidelines for research using routine healthcare databases developed.[260]

Modelling longitudinal count data with a large amount of zeros is challenging. Different models were used to model the number of prescriptions and asthma-related A&E visits/admissions, a Generalised Linear Mixed Models, Generalised Estimated Equations and Zero Inflated Models with a Poisson and a negative binomial family. The residuals and predicted values of these models were compared to find the most appropriate model for this data. One study found that the Zero Inflated Model with a random intercept performed better than the mixed model.[261] However, when adding random slopes, the Poisson mixed model performed better than the Zero Inflated Model. Other studies have found that the Zero Inflated Models performed better than mixed models.[262,263] For this thesis, the most appropriate model was the Generalised Linear Mixed Models with a negative binomial family. Comparing the observed and predicted values of all the fitted models, the Generalised Linear Mixed Model with a negative binomial family estimated more zeros than the other models and the maximum number of prescriptions predicted was closest to the observed maximum number of prescriptions. Although the predicted values from the Generalised Linear Mixed Model with a negative binomial family were the closest to the observed values, the model estimated a smaller amount of zeros and a smaller maximum value than the observed value, modelling a lower degree of variation than real life. Hence, the results presented should be interpreted with caution.

In this thesis, no adjustments were made to P-values. The debate between correcting or not correcting the P-value for multiple testing has been ongoing for decades, and both sides have compelling arguments although no consensus has been reached.[264] Several authors argue against the correction of P-values, and against excessive importance being given to P-values.[190,265] In this thesis, the effect size and the precision obtained guided the interpretation of the results, instead of the P-value. Importantly, there was no sample size calculation, which also counts against using P-values. Interpreting effect sizes based on P-values may lead to rejecting a strong effect size, simply because the P-value was higher than 0.05 and vice-versa, accept a small effect size simply because the P-value was small. CI can give more information beside the significance of a finding; the CI provides information about the magnitude of the effect size and the degree of precision.[190,265] Another issue in adjusting the P-value refers to the definition of multiple comparisons. Although one

can assume it refers to the number of outcomes to be tested, should exploratory and univariate models where one checks the significance of multiple covariates be included in this count? Should the researcher include the number of hypotheses tested since the first publication? However, these questions remain unanswered, especially due to the difficulty in accounting for all possible inferences.[265] As seen previously, several papers were already published with BREATHE data, therefore, adjusting the P-value only for the hypotheses tested in this thesis may not be the most appropriate choice. Results were interpreted considering the biological mechanism for each finding and considering the effect size and precision found.

6.7 Future work

The ability to follow the progression of asthmatic children over an extended period of time opens the door to several important areas of research. A one-year study[266] found that children who used 5 or more rescue medications, such as SABA, had more asthma exacerbations than children who used 0 to 5 rescue medications. An area for possible exploration is whether children using a large number of rescue medications during early or mid-childhood continue to use an increased number of asthma rescue medication and suffer from an increased number of asthma exacerbations in their adolescence or adulthood? Using this method it would also be possible to follow-up children without asthma-related prescriptions. A related question would be do some children out-grow their asthma in terms of medication use and are there any factors that may influence this process? Conversely, do children who out-grow their asthma dispensed more severe asthma-related prescriptions three or four years later and what are the factors correlated with this outcome? Asthma has a seasonal pattern, with an increased use of medication and a higher number of asthma exacerbations during school entry, autumn, and spring.[173,232,267,268] It is possible to further explore these patterns with longitudinal data to understand whether dispensing of prescriptions and asthma exacerbations are more common during seasonal peaks and whether children and adults with different genotypes, e.g. carrying specific FCER2 polymorphisms have different patterns of asthma-related prescription uptake or asthma exacerbations during different seasons. Respiratory

infections, usually viral, constitute one of the main triggers for asthma exacerbations and therefore it would be interesting to explore the association between the dispensing of antibiotics for respiratory infections in patients with asthma longitudinally.[232,233,267,269] It is known that antibiotics are not usually required, but often prescribed, for viral respiratory infections and further exploration of antibiotic use, considering genotypic variation, may shed light on this important area.

Another interesting research area would be the comparison of self-reported number of asthma-related A&E visits/admissions and medication use with the numbers found from pharmacy records and the records on the Scottish Morbidity Records (SMR)-01 database. How parents report asthma-related A&E visits/admissions? Do they only consider an asthma-related A&E visits/admissions when their child is admitted to A&E, or on the opposite, do they report asthma-related A&E visits/admissions, which were not directly caused by asthma? How do parents perceive asthma severity in their children? Does this perception correlate with a "higher step" on the doctor prescribed asthma treatment pathway? Does dispensing of prescription correlate with how parents/patients perceive asthma severity in their child/themselves?

The analyses in this thesis focussed on a group of children and adults with a physician diagnosis of asthma. However, as discussed in the Introduction, section 1.2, misdiagnosis of asthma is common. There have been no recent studies exploring the extent of misdiagnosed asthma in children and further studies should address this problem as both under- and over-diagnosis are problematic.

Asthma and eczema are both diseases with a stepped care approach. Unfortunately, with pharmacy data it is difficult to understand whether physicians are following guidelines or not. An interesting project would be to compare the number of prescribed medications, the number of dispensed medications, and the number actually used as a proportion of those dispensed, e.g. by urine testing as a marker of medicine levels in the body, to understand patient behaviour, as adherence is a serious problem in asthma, increasing healthcare usage and costs, and resulting in loss of productivity.[225,249] Patients may ignore physicians' recommendations for a number of reasons, often not using the medication or stopping medication because they feel the medication is not working, or conversely because they fell much better

and feel it is no longer required. Further understanding of the reasons behind adherence patterns are crucial since this could lead to initiatives to improve adherence, consequently improving the patient quality-of-life and the efficient functioning of the healthcare system. Public engagement activities may be crucial to helping adherence as once the public are engaged and understand the disease more fully, adherence may improve. Patients may share some reasons for non adherence and initiatives can be discussed with patients before they are considered for implementation.

6.8 Concluding remarks

In this thesis, three genes with different biological functions were studied in relation to asthma and eczema severity in humans. The first gene, FLG, has a role in hydrating the epidermal barrier and keeping allergens and infections out of the host. Results showed that children and adults with FLG mutations were dispensed more prescriptions for emollients, more prescriptions of medications for severe eczema, more prescriptions for a combination of LABA and ICS, and had more asthma-related A&E visits/admissions than children and adults without FLG mutations. The second gene studied, ADRB2, is involved in bronchial smooth muscle cell relaxation. Two SNPs were considered: Arg16Gly and Glu27Gln. Children and adults with Arg/Arg or Gln/Gln genotype were dispensed more prescriptions for severe asthma, such as a combination of LABA and ICS, LTRA and oral corticosteroids, than children and adults with Gly/Arg and Gly/Gly genotype or Gln/Glu and Glu/Glu genotype. The final gene considered in this body of work was the FCER2 gene, which is involved in the regulation of IgE responses. One polymorphism in the FCER2 gene was considered on the basis of a systematic review of current evidence. Results showed that children and adults with CC genotype were dispensed more prescriptions for oral LTRA, and more allergic rhinitis prescriptions than children and adults with TC and TT genotype. To take this work forward into the public arena, the results of the FLG and ADRB2 genes were presented in a science festival in Brighton, UK, and in a primary school in Bajouca, Portugal. Overall, children and parents understood the role of personalised medicine in clinical practice and engaged well with this work.

Asthma, eczema, and allergies have been defined by broad definitions, and treated as homogeneous disease, despite the range of different phenotypes. Stratifying asthma into different subgroup of patients that have different characteristics and that respond differently to medication may improve treatment and outcomes. The identification of genetic biomarkers has the potential to change clinical practice. Contrary to the belief that "one size fits all", personalised medicine uses genetic information to aid the prediction, prevention, diagnosis, and treatment of certain diseases.[270–272] This thesis advocates for a personalised approach and provides examples of how asthma can be improved by targeting treatment to specific sub-groups. For instance, an intensive use of emollients after birth in children with FLG mutations might delay the onset of eczema and possibly postpone or prevent the progression of allergic diseases such as asthma and allergic rhinitis. Such use of emollients could also reduce the severity of asthma, potentially reducing the entry of allergens and thus reducing the number of asthma exacerbations. This hypothesis is already being tested in the BEEP trial. In the case of the Arg16Gly polymorphism, children with one or two copies of the Arg allele may respond better to montelukast than to a combination of LABA and ICS. This hypothesis is being tested in the PACT trial. Lastly, there is a possibility that children with the CC genotype in the FCER2 polymorphism may have better outcomes with LTRA than with ICS. A study, published in 2018, compared the costs of screening tests available for detecting breast and ovarian cancer, and the subsequent treatment, with the costs of new genetic tests and treatment.[273] The authors tested mutations in the BRCA1/2, RAD51C/D and PALB2 and found that testing was more cost-effective for the NHS than the family history screening, currently offered. The test cost an estimated £175. With the constant advance of technology, the prices of genetic testing have reduced and possibly testing for FLG, ADRB2 or FCER2 gene mutations could be even cheaper. Further RCTs are needed to confirm the results obtained in this thesis, but combining these results with previous research highlights the importance of the variation in these three genes in the future development of personalised medicine-related approaches in the management of atopic conditions. Current guidelines focus on the approach "one size fits all". However, that approach is not the most adequate, as shown here and in previous studies. Individuals with mutations at

certain genes respond differently to medication than individuals without such mutations. The approach "one size fits all" ignores these patients and is currently prescribing medicine that may not work. Guidelines and RCTs need to incorporate genetic traits, and other relevant characteristics. However, to push for a change, genetic association studies need to collect and report data that may be translated into clinical practice. P-values do not have clinical relevance, and measures of risk should be preferred over P-values. Similarly, authors of genetic association studies should collaborate to increase sample size and increase the representation of different ethnic groups, and should adhere to guidelines to improve the quality and transparency of the studies. Funders of scientific research also play a role as replication studies are mandatory to confirm, or reject, an association. Licensing bodies should also address the question "for whom does this intervention work?". Is it expected to work similarly across all individuals? Should we expect differences between individuals from different ethnicities? Or have studies reported different responses to an intervention based on different genetic traits?

Another important aspect of personalised medicine relies on greater doctor-patient communication and greater communication between researchers and the public. Patients should be involved in decision making and should be informed regarding the latest scientific discoveries. Their feedback is invaluable in implementing new management modalities and guiding future research. In the public engagement activities performed in this thesis, parents were open to the concept of personalised medicine and welcomed more communication with their doctors. Parents were also keen on genetic testing, and would test their child to know whether the child had a genetic mutation that may potentially aid their management. Although parents did not mention any ethical concerns regarding genetic testing, ethical issues should be discussed alongside the evolution of science. One important question concerns the right that parents have to genotype their child without their consent. Obviously, children are unaware of the implications of genetic testing, but later in life, children might not be interested in knowing their genotype, and their predisposition to diseases. An example would be a mother having her daughter tested for a BRAC1/2 mutation, which increases the risk of developing breast and ovarian cancer. Does the parent even have the right to make this decision for her child? How best to

communicate such a decision to other family members, who may or may not be interested in knowing that they could be a carrier of the mutation? This decision may seem trivial in the case of asthma and eczema. However, having a mutation is not synonymous with having the disease. Another pertinent question is when to test the child? Children with FLG mutations may benefit from daily use of emollients from birth, but having an FLG mutation is not synonymous with developing the disease. Does the knowledge that your child has the CC genotype in the FCER2 gene, and may not respond to commonly prescribed ICS, increase parental stress, even though there is a chance that it could a false positive?[274] Another important aspect to consider is the implementation of genetic testing in clinical practice. In countries with national public health insurance, with the goal of reducing inequalities, one may expect that the national public health system would provide genetic testing, if it is cost-effective and within budget. However, in countries without national public health insurance, insurance companies may increase the premium based on the genetic background of the individual, or even exclude individuals with certain mutations.[275]

The clinical findings in this thesis improve our understanding of the impact of genetic mutations in an individual and on the healthcare system in general, with respect to atopic diseases in children and young adults. The increased understanding of the three genes and their variations studied in this thesis could help significant advances in clinical practice in the future, saving costs for the NHS, improving disease control, and improving the quality-of-life of the patients.

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Appendices

MEDLINE search

Search strategy – MEDLINE

1. exp ASTHMA/
2. exp POLYMORPHISM, SINGLE NUCLEOTIDE/ OR exp POLYMORPHISM, GENETIC/ OR exp GENOTYPE/
3. exp MUTATION/
4. (geno* OR genome-wide OR variant* OR polymorphism* OR SNP OR protein* OR gene*).ti,ab
5. 2 OR 3 OR 4
6. exp PHARMACOGENETICS/
7. exp INDIVIDUALIZED MEDICINE/
8. pharmacogenomic*.ti,ab
9. 6 OR 7 OR 8
10. 1 AND 5 AND 9
11. 10 [Limit to: Humans and (Age Groups All Child 0 to 18 years or Infant 1 to 23 months or Preschool Child 2 to 5 years or Child 6 to 12 years or Adolescent 13 to 18 years)]

Quality assessment checklist

The following checklist is divided into two sections. Each section is evaluated by a set of questions, which are answered by *yes*, *no*, *unclear* or *limited*, or *NA (Not applicable)*.

The first section concerns the design and validity of the study, while the second part evaluates the results obtained.

1. **Section 1: Are the methods of the study valid?**

    1.1. Did the study use population stratification? (*Population stratification refers to differences in allele frequencies between sub-populations due to genetic ancestry differences. Ignoring population stratification may lead to incorrect associations. Hence, it is important that studies mention the ethnicity of the individuals studied and if needed correct the stratification. Answer yes if the ethnicity is reported and corrected, when necessary. Answer unclear if the ethnicity is reported, different ethnicities are present but no correction was done. Answer no if the ethnicity is not reported.*)

    1.2. Did the study include a comparison of individuals with and without the mutation? *To assess the validity of the study, individuals with and without mutations should be properly represented and have similar demographic characteristics. Answer yes if demographical and clinical data was reported for each sub-group. Answer limited if limited information was provided. Answer no if demographic and clinical data was not reported for each sub-group.*

    1.3. Was the outcome of the study clearly defined? *Answer yes if the outcome was clearly defined. Answer no if the definition of the outcome was unclear.*

1.4. Was the sample size appropriate? *A small sample size will report inaccurate results. Hence the importance of calculating the sample size prior to study initiation. Answer yes if a sample size calculation was done or the sample size is equal or above 500. Answer no if no sample size calculation was performed and the sample size is below 500.*

1.5. Was the accession number or were the flanked sequences provided? *Correct identification of the polymorphisms studied is crucial for replication. Answer yes if these were reported. Answer no if an ambiguous identification was used or no identification was provided.*

1.6. Was the minor allele frequency (MAF) reported? *Different genetic backgrounds may have different allelic frequencies, hence it is important to report the MAF to understand whether the MAF is representative of the population under study. Answer yes if the MAF, or allelic frequencies, were reported. Answer no if the MAF was not reported nor allelic frequencies.*

1.7. Was the Hardy-Weinberg Equilibrium (HWE) reported? *HWE states that the genotype frequency will remain constant across generations in the absence of evolutionary forces. Deviations from the HWE may suggest genotyping errors. Answer yes if HWE was reported and if applicable deviations were discussed. Answer unclear if HWE was reported but deviations were ignored. Answer no if HWE was not mentioned. Answer NA if the cohort is a population of individuals with the disease, without controls.*

2. **Section 2: Are the results of the study reliable?**

2.1. Were the P-values adjusted for multiple testing, where appropriate? *Multiple testing is crucial when analysing a large number of polymorphisms as it can lead to false associations. Answer yes if the P-values were adjusted for multiple testing and the adjusted P-value or the method was provided. Answer unclear if the P-values were adjusted for multiple testing but the adjusted P-value or the method was not provided. Alternatively, only for candidate gene association studies, answer unclear if the P-values were not adjusted but other approaches were adopted, such as*

consideration of the magnitude and precision of the estimate. Answer no if the P-values were not adjusted, or it was not reported. Answer NA if a single polymorphism was studied.

2.2. Was the genetic model used stated? *Identification of the genetic model used is crucial for replication. Answer yes if the genetic model used was reported. Answer unclear if more than one genetic model was used and it is unclear to which one the results refer to. Answer no if the genetic model used was not reported.*

2.3. Was the rationale for choice of model given? *Assuming an incorrect genetic model may lead to bias and unreliable results. Answer yes is the reason for using the model was stated. Answer no if no reasons are provided.*

2.4. Have the authors reported any risk estimate, such as relative risk (RR), odds-ratio (OR), hazard ratio (HR), rate, proportion, difference? *It is important to understand the risk or protection of each allele or genotype to draft recommendations for clinical practice. Answer yes if a risk estimate has been reported. Answer no if no risk estimate was reported. Report of P-values is not adequate.*

2.5. Have the authors considered the magnitude and precision of the estimates obtained? *This item refers to the confidence surrounding the results. A wide confidence interval and/or a short risk estimate may be a sign of variation across patient, few events or a small sample size. Answer yes if the magnitude of the estimate is large and the precision of the estimate is narrow, or the discussion interpret the results with caution, mentioning the limitations due to a large confidence interval and/or a small magnitude. Answer unclear if confidence intervals are not provided. Answer no if the magnitude is small, the precision of the estimate is wide and results are exaggerated.*

A summary of each study is included at the end of the checklist. A study can be *adequately described* if more than 50% of the applicable items are answered positively, otherwise it will be considered *inadequately described*.

Prices of medication and hospital admissions

Generic name	Formulation	Strength	2005	2006	2007	2008	2009	2010	2011	2012	2013
ANTIHISTAMINES											
ALIMEMAZINE TARTRATE	SYRUP	7.5MG/5ML	5.97	5.54	7.03	7.68	7.65	8.01	11.34	11.68	24.21
ALIMEMAZINE TARTRATE	TABS	10MG	5.94	5.6	6.88	7.17	7.26	8.97	12.01	11.04	11.49
ALLERIEF	SOLN	2MG/5ML	2.5	2.34	2.35	2.31	2.28	2.29	2.26	2.38	2.55
ATARAX	TABS	10MG	1.43	1.48	1.43	1.31	1.18	1.78	1.65	1.67	1.67
ATARAX	TABS	25MG	2.22	2.23	2.19	2.09	2.03	2.08	1.98	1.91	1.87
BENADRYL ALLERGY	SOLN	5MG/5ML						3.08	2.98	3.16	3.20
BENADRYL FOR CHILDREN ALLERGY	SOLN	5MG/5ML	4.05		4.05	1.09	3.11	3.15	3.74	3.04	3.34
CETIRIZINE HYDROCHLORIDE	SOLN	1MG/ML	12.04	10.2	8.25	5.52	3.34	2.61	2.72	2.36	2.04
CETIRIZINE HYDROCHLORIDE	TABS	10MG	5.59	4.57	2.15	1.6	0.89	1.3	1.41	1.3	1.41
CHLORPHENAMINE MALEATE	ELIX	2MG/5ML	2.64	2.56	2.57	2.53	2.49	2.4	2.51	2.54	2.63
CHLORPHENAMINE MALEATE	INJ	10MG/ML	8.72	8.32	8.47	8.5	8.52	8.6	9.89	11.56	14.03
CHLORPHENAMINE MALEATE	SOLN	2MG/5ML				2.59	2.59	2.47	2.43	2.4	2.34
CHLORPHENAMINE MALEATE	SYRUP	2MG/5ML	2.63	2.64	2.61	2.57	2.66	2.8	2.85	2.92	2.87
CHLORPHENAMINE MALEATE	TABS	4MG	2	1.38	3.42	2.66	1.76	2.16	2.41	2.03	1.99
DESLORATADINE	SOLN	500MCG/ML					10.49	10.01	9.93	9.98	12.29
DESLORATADINE	SYRUP	500MCG/ML	10.08	9.35	9.4	9.67	9.88	9.67			
DESLORATADINE	TABS	5MG	9.35	8.97	9.24	9.55	9.74	9.71	9.79	9.71	8.98
HYDROXYZINE HYDROCHLORIDE	SYRUP	10MG/5ML	2.48	2.37	2.69	2.41	2.53	2.52	2.47	2.6	2.59
HYDROXYZINE HYDROCHLORIDE	TABS	10MG	1.42	1.43	1.39	1.14	1	1.62	1.68	1.64	1.55
LEVOCETIRIZINE DIHYDROCHLORIDE	TABS	5MG	9.32	9.65	9.88	7.46	7.68	6.67	6.92	7.07	7.23
LORATADINE	SOLN	5MG/5ML	11.77	6.78	5.59	8.4			4.54	3.72	3.33
LORATADINE	TABS	10MG	6.27	4.65	2.71	2.17	1.4	1.76	1.73	1.71	1.72
NEOCLARITYN	SOLN	500MCG/ML					10.06	10.06	9.05	9.46	10.1
NEOCLARITYN	SYRUP	500MCG/ML	9.71	9.06	9.37	9.16	8.9	9.24			
NEOCLARITYN	TABS	5MG	8.82	8.47	8.53	8.65	8.96	9.21	9.76	10.17	10.2
PHENERGAN	ELIX	5MG/5ML	2.43	2.58	2.91	3.14	3.12	3.67	4.12	5.17	5.58
PHENERGAN	TABS	10MG	1.49	1.52	1.84	2.08	2.05	2.78	2.76	2.77	2.74

Table 1 – continued from previous page

Generic name	Formulation	Strength	2005	2006	2007	2008	2009	2010	2011	2012	2013
PHENERGAN	TABS	25MG	1.75	1.84	2.08	2.51	2.66	3.85	3.54	3.17	3.33
PIRITON	SYRUP	2MG/5ML	2.62	2.64	2.56	2.48	2.51	2.57	2.6	2.72	2.72
PIRITON	TABS	4MG	0.51	0.52	0.51	0.51	0.52	0.46	0.45	0.47	0.45
PROMETHAZINE HYDROCHLORIDE	ELIX	5MG/5ML	2.68	2.93	3.46	3.83	4.24	5.31	5.88	6.07	6.92
PROMETHAZINE HYDROCHLORIDE	TABS	10MG	1.59	1.64	1.9	2.01	2	2.65	2.66	2.66	2.68
PROMETHAZINE HYDROCHLORIDE	TABS	25MG	1.59	1.64	2.13	2.16	2.29	3.09	3.1	3.05	3.26
VALLERGAN	SYRUP	7.5MG/5ML	5.73	5.43	6.55	7	7.14	7.19	6.9	6.37	
VALLERGAN	TABS	10MG	6.47	6.05	7.46	7.94	7.76	8.31	7.97	8.26	
XYZAL	TABS	5MG	8.66	8.9	9.36	6.77	7.54	6.64	6.94	7.39	7.65
ZIRTEK ALLERGY	SOLN	5MG/5ML	10.61	9.76	9.47	8.08	8.64	8.82	9.08	9.49	7.58
ZIRTEK ALLERGY	TABS	10MG	9.94	9.89	10.23	10.11	10.57	10.86	11.15	11.16	12.09
EMOLLIENTS											
AQUEOUS	CREAM		0.96	1.26	2.21	1.78	1.43	1.72	2.85	3.17	4.15
ARJUN CREAM 1%										12.77	11.89
AVEENO	CREAM		7.03	7.31	7.59	7.55	7.48	7.86	8.11	8.11	8.32
AVEENO	LOT		6.91	6.7	7.19	7.21	7.27	7.33	7.42	7.53	7.53
AVEENO	OIL		5.82	6.01	6.13	6.16	6.07	6.07	6.2	6.21	6.15
AVEENO	SACH		13.14	14.63	15.02	15.25	14.03	13.94	13.26	12.70	14.69
BALNEUM	OIL		13.14	14.63	15.02	15.25	14.03	13.94	13.26	12.7	14.69
BALNEUM CREAM EMOLLIENT								8.93	8.53	8.21	8.6
BALNEUM PLUS	CREAM		6.36	6.42	6.15	5.73	5.8	5.87	5.82	5.74	5.74
BALNEUM PLUS	OIL		7.85	9.26	8.84	7.05	5.55	5.83	6.03	6.52	6.28
CALMURID	CREAM		11.93	11.46	11	11.63	11.53	9.19	9.13	8.94	10.29
CETRABEN			8.24	8.29	8	7.47	7.52	7.5	7.48	7.48	7.35
CETRABEN	CREAM		2.99	3.15	3.05	3.06	4.15	12.49	13.12	14.37	15.39
CETRABEN (PUMP DISPENSER)	CREAM		5.41	5.39	5.42	5.49	5.49	5.81	6.18	6.27	6.19
DERMALO BATH	EMULS		3.89	3.67	3.71	3.70	3.88	3.75	3.56	3.61	3.68

Table 1 – continued from previous page

Generic name	Formulation	Strength	2005	2006	2007	2008	2009	2010	2011	2012	2013
DERMOL	CREAM		2.99	3.15	3.05	3.06	4.15	12.49	13.12	14.37	15.39
DERMOL 200	LOT		5.59	5.32	5.42	5.47	5.52	5.4	5.36	5.24	5.22
DERMOL 500	LOT		7.29	6.91	6.94	6.98	6.96	6.76	6.67	6.66	6.67
DERMOL 600	LIQ		9.1	8.62	8.56	8.68	8.67	8.45	8.35	8.24	8.19
DIPROBASE	CREAM		3.4	2.99	2.94	3.14	3.32	3.23	5.04	4.6	4.22
DIPROBASE	OINT		4.22	3.88	3.9	3.5	3.56	3.4	3.43	4.51	5.21
DIPROBATH	OIL		8.14	7.66	7.72	7.78	7.69	7.55	7.41	6.49	4.39
DOUBLEBASE	EMULS					6.03	5.86	5.75	5.69	5.83	5.91
DOUBLEBASE	GEL		4.47	4.15	4.17	5.33	5.05	5.81	6.34	5.16	4.91
DOUBLEBASE DAYLEVE	GEL									4.99	5.89
E45	CREAM		2.75	2.81	2.97	3.12	3.11	3.23	3.45	3.41	3.79
E45 (EMOLLIENT WASH)	CREAM		3.68	3.7	3.89	4.25	4.22	4.19	4.16	4.32	4.37
E45	LOT		4.32	4.39	4.4	4.39	4.41	4.53	5.22	4.89	4.62
E45	OIL		4.87	4.95	5.3	5.47	5.42	5.5	5.53	5.5	5.49
E45 ITCH RELIEF	CREAM		4.85	4.73	4.66	5.03	7.6	8.73	8.87	8.72	8.76
EMOLLIN						13.22	10.04	8.74	9.17	8.68	8.25
EMULSIDERM	LOT		8.34	8.1	8.08	8.16	8.18	7.63	7.69	7.79	8.05
EMULSIFYING OINTMENT			1.49	2.19	3.85	3.03	2.32	2.3	2.59	2.36	2.29
EPADERM CREAM EMOLLIENT							6.22	6.22	6.29	6.17	6.39
EPADERM OINTMENT EMOLLIENT			6.01	6.07	6.35	6.35	6.31	6.52	6.52	6.34	6.42
EUCERIN	CREAM	10.00%	9.89	11.23	11.64	11.56	12.77	12.62	12.2	11.87	11.85
EUCERIN	CREAM	5%	6.23	8.40	8.57	8.55	9.05	8.97	9.03	8.83	8.58
EUCERIN	OIL		6.19	6.24	6.16	6.6	7.1	7.52	7.96	7.83	7.57
HYDROMOL			6.07	6.22	6.33	6.4	6.34	5.48	5.4	5.21	5.05
HYDROMOL	CREAM	2.50%	7.48	7.06	7.09	6.47	5.91	6.79	8.75	8.28	7.4
HYDROMOL OINTMENT			5.18	4.49	4.61	4.86	4.93	5.06	5.06	5.06	
LIQ PARAFFIN 50%	OINT		6.23	4.3	3.78	4.25	4.92	5.1	5.56	3.54	3.08

Table 1 – continued from previous page

Generic name	Formulation	Strength	2005	2006	2007	2008	2009	2010	2011	2012	2013
NUTRAPLUS	CREAM	10%	6.76	6.62	5.04	5.03	5.81	5.80	7.44	6.18	6.02
OILATUM (SHOWER GEL)								7.48	6.85	6.84	6.42
OILATUM (SCALP)			6.2	6.36	6.6	6.76	7.13	7.26	7.36	7.4	7.56
OILATUM	CREAM		4.55	4.79	4.76	5.19	5.5	4.44	4.62	4.6	4.74
OILATUM	EMULS		4.58	4.62	4.62	4.58	4.58	4.56	4.58	4.57	4.67
OILATUM	GEL		7.72	7.12	7.28	7.41	7.4	7.52	7.75	7.51	6.8
OILATUM JUNIOR	CREAM			6.78	6.78	6.99	7.1	5.57	5.35	5.09	5.15
OILATUM JUNIOR	LIQ		5.63	5.76	5.88						
OILATUM JUNIOR BATH			5.88	6.2	6.33	5.94	5.82	5.86	5.9	5.72	5.71
OILATUM PLUS	EMULS		8.96	8.18	8.28	8.23	8.09	8.07	8.05	7.77	7.06
PARAFFIN SOFT WHITE			0.93	1.34	2.73	2.42	2.01	1.7	1.92	2.06	2.3
QV GENTLE WASH										5.89	4.94
SUDOCREM	CREAM		2.91	2.9	2.89	2.87	2.94	2.96	2.94	3.09	3.02
ULTRABASE	CREAM		2.62	2.4	2.76	3.26	3.15	5.75	7.81	6.47	5.32
UNGUENTUM M	CREAM		5.54	5.62	5.63	5.5	5.6	5.67	5.81	5.79	6.13
UREA	OIL		5.71	5.66	6.06	6.15	6.1	6.49	6.96	6.79	
VASELINE DERMACARE	LOT		3.5	3.66	3.71	3.84	3.69				
ZINC AND CASTOR OIL	OINT			2.11	2.09	1.99	1.90	2.03	2.14	1.89	2.72
PRESCRIPTIONS FOR MILD ECZEMA											
ALPHADERM	CREAM		5.22	4.39	4.72	4.62	4.81	5.62	5.46	5.48	5.43
DAKTACORT	CREAM		2.58	2.47	2.27	2.26	2.41	2.14	2.51	2.82	2.84
DAKTACORT	OINT		2.62	2.53	2.5	2.51	2.5	2.39	2.58	2.89	2.88
DAKTACORT HC	CREAM		4.47	4.52	4.4	4.42	4.4	4.43	4.33	4.01	4.01
DIODERM	CREAM	0.10%	3.06	2.89	2.83	2.78	2.75	2.66	2.58	2.66	2.67
EFCORTELAN	CREAM	0.50%	0.8	0.72	0.7	0.72	0.66				
EFCORTELAN	CREAM	1.00%	1.06	1	1.7	1.01	0.9				
EFCORTELAN	OINT	1.00%	1.48	1.25	1.14	1.09	0.91	0.93			

Table 1 – continued from previous page

Generic name	Formulation	Strength	2005	2006	2007	2008	2009	2010	2011	2012	2013
EFCORTELAN	OINT	2.50%	2.86	2.55	2.4	2.55	2.71				
HYDROCORTISONE	CREAM	0.50%	0.63	1.39	7.09	7.67	5.67	4.06	3.46	3.15	2.51
HYDROCORTISONE	CREAM	1.00%	0.82	1.59	7.59	7.6	4.57	2.97	2.58	2.44	2.13
HYDROCORTISONE	CREAM	2.50%	2.03	3.86	34.19	62.37	61.53	40.13	43.69	44.29	46.16
HYDROCORTISONE	OINT	0.50%	0.67	1.61	8.71	9.79	7.12	4.75	5.19	5.21	5.09
HYDROCORTISONE	OINT	1.00%	0.86	1.73	7.68	8.16	5.31	3.48	4.16	4.98	3.74
HYDROCORTISONE	OINT	2.50%	2.77	4.97	34.86	66.06	78.15	55.46	61.29	61.27	57.37
LOCOID C	CREAM		4.16	3.94	3.99	3.89	4.03	3.23			
MILDISON LIPOCREAM	CREAM	1.00%	3.65	3.51	3.58	3.73	3.89	3.85	2.82	2.43	2.38
NYSTAFORM HC	CREAM	0.50%	3.04	3.05	3.09	3.09	3.08	3.09	3.05	3.03	2.93
TIMODINE	CREAM		2.75	2.73	2.75	2.74	2.7	2.32	2.62	2.76	2.75
VIOFORM HYDROCORTISONE	CREAM		1.72	1.72	1.7	1.66	1.63	1.5			
PRESCRIPTIONS FOR MODERATE ECZEMA											
BETNOVATE RD	CREAM	0.03%	3.9	3.67	3.7	3.77	3.74	3.61	3.44	3.41	3.4
BETNOVATE RD	OINT	0.03%	4.17	3.92	3.93	3.97	4.02	3.88	3.7	3.65	3.64
CALMURID HC	CREAM		7.18	7.34	7.26	7.61	7.71	8.47	9.63	9.85	10.59
CLOBETASONE BUTYRATE	CREAM	0.05%	3.72	3.56	3.59	3.61	3.61	3.45	3.35	3.3	3.27
CLOBETASONE BUTYRATE	OINT	0.05%	4.31	4.13	4.1	4.19	4.15	3.95	3.79	3.74	3.81
ELIDEL	CREAM	10MG/G	33.2	30.31	32.73	32.44	31.46	29.72	31.95	31.32	30.7
EUMOVATE	CREAM	0.05%	3.72	3.54	3.53	3.49	3.45	3.26	3.31	3.16	3.14
EUMOVATE	OINT	0.05%	4.53	4.38	4.42	4.37	4.21	4	4.07	3.61	3.57
FLUDROXYCORTIDE	OINT	0.01%	2.9	3.34	3.5	3.76	3.71	3.51	3.35	3.26	3.75
FLUDROXYCORTIDE	TAPE		20.49	20.88	20.13	23.07	22.18	22.75	21.73	23.96	22.86
FUCIDIN	CREAM	2.00%	3.86	3.13	3.09	2.99	2.92	2.77	2.69	2.53	2.58
FUCIDIN	OINT	2.00%	3.34	3.39	3.45	3.35	3.29	3.12	3.02	2.87	2.97
FUCIDIN H	CREAM		6.25	6.24	6.25	6.27	6.19	5.88	5.74	5.59	5.58
FUCIDIN H	OINT		5.07	4.02	4.03	3.99	3.99	3.92	3.95		

Table 1 – continued from previous page

Generic name	Formulation	Strength	2005	2006	2007	2008	2009	2010	2011	2012	2013
FUSIDIC ACID	CREAM	2.00%	3.57	2.88	2.85	2.85	2.82	2.69	2.72	2.57	2.53
HAELAN	CREAM	0.01%	3.13	4.06	3.77	3.8	3.82	3.86	3.62	3.71	3.73
HAELAN	OINT	0.01%	2.3	3.26	3.55	4.23	3.59	3.67	3.87	3.72	3.73
HAELAN	TAPE		20.63	21.59	35.06	27.77	29.88	23.86	24.92	22.96	21.18
PIMECROLIMUS	CREAM	10MG/G	35.47	34.23	35.86	35.86	32.5	33.47	31.87	31.44	28.48
PROTOPIC	OINT	0.03%	34.45	31.99	35.3	32.14	31.43	29.4	27.8	28.22	28.3
PROTOPIC	OINT	0.10%	38.41	36.23	34.97	39.27	40.25	36.85	34.86	33.3	33.68
SODIUM FUSIDATE	OINT	2.00%	2.97	3	2.97	3.04	2.99	2.95	2.99	2.98	2.75
TACROLIMUS	OINT	0.03%	39.2	36.84	35.74	37.28	34.89	32.9	31.45	31.33	29.16
TACROLIMUS	OINT	0.10%	41.38	40.75	38.69	37.56	37.25	35.37	34.89	34.49	35.64
TRIMOVATE	CREAM		4.45	4.17	4.19	4.19	4.16	3.97	3.9	3.85	3.82
PRESCRIPTIONS FOR SEVERE ECZEMA											
ACTI-FAST 2WS STOCK				10.02	10.02	10.02	10.02	10.02	10.02		10.02
AZATHIOPRINE	TABS	25MG	19.7	18.75	14.56	12.74	11.56	13.78	12.78	13.1	9.87
AZATHIOPRINE	TABS	50MG	11.72	12.95	15.88	15.2	12.34	11.43	9.79	8.52	6.77
BECLOMETASONE DIPROPIONATE	CREAM	0.025%	2.86	2.76	2.99						80.36
BETACAP	APPL	0.10%	4.47	4.24	4.26	4.26	4.25	4.12	4.07	4.05	4.03
BETAMETHASONE DIPROPIONATE	OINT	0.05%	5.53	4.93	4.9	5.09	5.02	5.05	4.9	5.2	4.95
BETAMETHASONE VALERATE	CREAM	0.03%	3.78	3.57	3.58	3.59	3.59	3.45	3.43	3.43	3.42
BETAMETHASONE VALERATE	CREAM	0.10%	2.65	2.56	2.63	2.74	2.81	3.37	3.89	3.57	4.49
BETAMETHASONE VALERATE	LOT	0.10%	5.47	5.21	5.24	5.21	5.26	5.04	5.09	5.15	5.47
BETAMETHASONE VALERATE	OINT	0.03%	4.06	3.83	3.85	3.93	3.92	3.67	3.69	3.69	3.76
BETAMETHASONE VALERATE	OINT	0.10%	3.08	3.19	3.24	3.33	2.99	3.22	3.98	4.14	6.37
BETAMETHASONE WITH CLIOQUINOL	CREAM										13.76
BETAMETHASONE WITH CLIOQUINOL	OINT										13.08
BETNOVATE	APPL	0.10%	6.2	5.83	5.84	5.85	5.82	5.59	5.47	5.45	5.45
BETNOVATE	CREAM	0.10%	2.74	2.64	2.67	2.71	2.68	2.66	2.69	2.51	2.52

Table 1 – continued from previous page

Generic name	Formulation	Strength	2005	2006	2007	2008	2009	2010	2011	2012	2013
BETNOVATE	OINT	0.10%	2.89	2.74	2.77	2.79	2.84	2.85	2.87	2.71	2.74
BETNOVATE C	CREAM		2.55	2.39	2.44	2.48	2.44	2.41	2.4	2.37	2.31
BETNOVATE C	OINT		2.87	2.69	2.75	2.77	2.75	2.82	2.83	2.73	2.66
BETNOVATE N	CREAM		3.43	3.12	3.13	3.26	3.34	3.36	3.29	3.39	3.27
BETNOVATE N	OINT		3.29	3.12	3.13	3.18	3.27	3.34	3.51	3.41	3.34
BETTAMOUSSE		0.10%	9.54	10.61	10.72	10.7	10.55	10.05	10.26	10.53	10.49
CALABAND BAN			17.43	24.28	21.07	20.93	14.54	4.93			
CLINIFAST SG (GLOVES CHILD)									14.91	18.04	16.63
CLINIFAST SG (LEG)							23.87	32.98	27.79	36.04	28.82
CLINIFAST SG (VEST)							21.58	26.62	23.74	32.01	28.92
CLINIFAST SG (SOCKS)								9.62	9.2	13.38	10.41
CLINIFAST SG (GLOVES ADULT)									14.91	18.04	16.63
CLOBETASOL PROPIONATE	CREAM	0.05%	6.08	5.75	5.85	5.84	5.82	5.42	5.25	5.09	5.11
CLOBETASOL PROPIONATE	OINT	0.05%	6.22	6.03	6.06	6.02	6.24	5.91	6.12	6.16	5.65
COMFIFAST EW SG (MITTENS)				11.83	11	11.33	11.33	10.49	10.21	9.9	8.99
COMFIFAST EW SG (LEG)				38.13	38.55	36.23	37.6	35.07	30.59	31.63	33.65
COMFIFAST EW SG (VEST)				32.79	30.07	33.7	28.59	26.42	28.52	27.96	30.24
COMFIFAST MULTISTRETCH STOCK								18.77	18.84	16.4	14.62
CUTIVATE	CREAM	0.05%	8.25	7.92	8.05	7.82	8.46	7.66	7.15	6.3	6.1
CUTIVATE	OINT	0.005%	9.44	8.58	9.09	8.56	9.50	9.36	8.73	8.16	8.08
DERMOVATE	APPL	0.05%	11.62	10.74	11.32	10.76	10.54	9.59	9.11	8.68	9.14
DERMOVATE	CREAM	0.05%	5.97	5.71	5.71	5.52	5.51	5.15	5.15	4.89	4.87
DERMOVATE	OINT	0.05%	6.2	5.96	5.95	5.96	5.87	5.51	4.94	5.11	5.43
DERMOVATE NN	CREAM		5.64	5.30	5.32	4.43		5.71	5.25	4.59	
DERMOVATE NN	OINT		5.69	4.9	5.09			5.62	5.54	4.91	
DIPROSONE	CREAM	0.05%	6.01	5.92	5.98	5.82	5.54	5.59	5.3	4.9	4.88
ELAST VISC STOCK (COMFIFAST)			9.02	8.65	10.91	11.07	11.17	10.16	10.07	9.36	9.62

Table 1 – continued from previous page

Generic name	Formulation	Strength	2005	2006	2007	2008	2009	2010	2011	2012	2013
ELAST VISC STOCK (TUBIFAST 2W)			11.27	15.81	16.11	19.34	19.67	21.81	21.12	18.58	15.68
ELAST VISC STOCK (CLINIFAST)			8.91	9.41	8.79	9.6	9.6	9.79	9.18	9.17	9.06
ELAST VISC STOCK	9				9	9	8.98	9.22	9.38	8.68	8.72
ELAST VISC SG (TIGHTS)			34.62	33.43	38.51	40.28	37.49	47.49	47.85	67.74	
ELAST VISC SG (VEST 5-8YRS)				39.62	42.64	40.57	40.56	35.57	36.19	42.43	
ELAST VISC SG (LEG 8-11YRS)				48.35	48.85	41.88	44.04	42.04	40.78	32.85	
ELAST VISC SG (LEG 5-8YRS)						29.69	26.39	28.38	30.38	34.25	
ELAST VISC SG (GLOVES CHILD)								15.15	16.89	23.05	
ELAST VISC SG (LEG 2-5YRS)			38.73	52.94	57.38	64.63	68.32	73.26	71.04	57.82	
ELAST VISC SG (VEST 8-11YRS)			35.99	42.41	51.65	54.13	56.12	64.67	61.77	52.64	
ELAST VISC SG (SOCKS)			10.31	13.94	15.5	16.43	18.31	21.79	22.89	16.89	
ELOCON	CREAM	0.10%	8.97	8.57	8.77	8.8	8.8	8.41	7.42	8.39	8.37
ELOCON	LOT	0.10%	7.04	6.54	6.57	6.61	6.81	6.32	6.20	5.75	5.71
ELOCON	OINT	0.10%	10.17	9.6	9.93	10.05	10.34	10.13	9.97	9.73	9.93
ETRIVEX	APPL	500MCG/G				14.45	14.22	18.42	18.29	18.24	18.57
FLUOCINOLONE ACETONIDE	CREAM	0.01%	3.76	3.84	4.77	5.08	6.49	6.98	6.46	6.6	7.58
FLUOCINOLONE ACETONIDE	OINT	0.01%	4.38	4.34	5.7	5.92	7.54	7.91	7.68	7.84	8.22
FLUOCINOLONE ACETONIDE	OINT	0.03%	4.15	4.69	5.88	5.78	7.69	7.96	8.22	8.98	11.19
FUCIBET	CREAM		9.37	8.86	8.95	8.96	8.9	8.5	8.28	7.97	7.95
FLUTICASONE PROPIONATE	CREAM	0.05%	8.03	7.66	7.87	7.43	7.67	8.71	7.70	7.25	7.19
FLUTICASONE PROPIONATE	OINT	0.005%	8.24	7.67	8.25	7.67	9.01	8.12	9.93	7.18	7.02
HYDROCORTISONE BUTYRATE	CREAM	0.10%	4.2	3.97	4.2	4.2	4.22	4.04	3.1	3.04	3.03
HYDROCORTISONE BUTYRATE	LOT	0.10%	11.12	10.51	10.55	10.35	10.32	9.76	7.15	7.32	7.4
HYDROCORTISONE BUTYRATE	OINT	0.10%	4.35	4	4.23	3.98	3.94	3.86	2.75	2.58	2.55
ICHTHOPASTE BAN			26.22	31.09	27.33	29.25	29.85	28.29	29.07	27.42	26.53
ICTHABAND BAN			21.99	18.7	17.99	17.25	20.24	17.35	13.74	23.16	19.06
LOCOID	CREAM	0.10%	4.65	4.46	4.39	4.33	4.43	4.2	3.03	3	3.08

Table 1 – continued from previous page

Generic name	Formulation	Strength	2005	2006	2007	2008	2009	2010	2011	2012	2013
LOCOID	LOT	0.10%	11.04	10.54	11.07	10.83	10.77	10.38	7.82	7.42	7.54
LOCOID CRELO	EMULS	0.10%	9.92	9.39	9.46	9.36	9.48	8.96	6.89	6.93	6.78
LOTRIDERM	CREAM		8.03	8.16	8.11	8.11	8.13	8.21	8.2	8.05	7.93
METHOTREXATE	TABS	2.5MG	3.42	3.4	3.58	4.05	4.24	4.42	4.35	3.43	3.54
MOMETASONE FUROATE	CREAM	0.10%	9.09	8.89	8.88	9.04	9.1	8.88	9.25	8.81	8.74
MOMETASONE FUROATE	LOT	0.10%	6.31	5.88	5.91	6.01	5.97	5.77	5.77	6.15	5.75
MOMETASONE FUROATE	OINT	0.10%	10.1	9.7	9.74	10.13	10.22	10.22	11.39	9.47	11.14
NEORAL	CAPS	10MG	49.34	45.38	51.95	58.43	56.46	60.1	57.97	57.8	54.35
NEORAL	CAPS	100MG	171.18	121.17	130.92	139.69	169.26	188.67	171.2	167.38	168
SKINNIES SG (SOCKS)									10.87	12.98	12.23
SKINNIES SG (GLOVES)									13.16	14.38	10.5
SKINNIES SG (VEST)									41.73	47.73	39.1
SKINNIES SG (LEG)									34.96	40.15	36.87
STERIPASTE BAN			25.72	27.27	25.9	27.64	30.19	24.88	23.82	26.14	26.51
SYNALAR	CREAM	0.01%	4.02	4.24	5.19	5.42	6.8	6.73	6.89	6.69	7.15
SYNALAR	CREAM	0.03%	3.85	3.82	5.08	4.94	6.27	6.05	5.74	5.68	6.22
SYNALAR	GEL	0.03%	3.73	5.2	6.25	6.45	8.42	8.34	8.46	8.14	8.21
SYNALAR	OINT	0.01%	4.69	5.42	5.68	6.68	7.35	8.19	7.29	7.47	7.7
SYNALAR	OINT	0.03%	3.96	4.27	5.34	5.86	7.24	7.62	7.6	7.7	8.99
SYNALAR C	CREAM		3.38	3.41	4.37	4.51	5.42	5.37	5.52	5.62	5.83
SYNALAR C	OINT		3.6	3.96	4.81	5.22	6.27	6.37	6.51	6.19	6.54
SYNALAR N	OINT		3.05	3.39	4.12	4.23	5.38	5.02	5.09	5.2	5.57
TUBIFAST 2WS STOCK			13.18	18.45	16.06	21.22	21.55	21.63	20.73	18.44	18.64
TUBIFAST 2WS SG (VEST)			37.07	42.82	44.21	45.98	42.61	42.61	42.46	42.43	39.42
TUBIFAST 2WS SG (GLOVES)					14.63	12.99	16.89	16.89	17.41	16.63	17.01
TUBIFAST 2WS SG (LEG)			42.09	49.73	49.84	53.37	50.92	50.92	48.38	42.59	43.03
VISCOPASTE PB 7 BAN			26.29	33.28	29.15	31.45	30.43	31.99	29.77	29.31	28.16

Table 1 – continued from previous page

Generic name	Formulation	Strength	2005	2006	2007	2008	2009	2010	2011	2012	2013
ZP AND ICHTHAMMOL BAN BP			19.22	23.95	27.63	26.58	25.52	24.67	28.14	25.04	24.2
ZP BAN BP (VISCOPASTE PB7 10%)			26.12	32.38	31.13	32.29	31.24	29.27	29.39	28.18	25.68
ZP BAN BP (ZINCABAND 15%)			27.34	25.84	13.67	16.46	16.83	26.56	13.47	29.8	29.8

Table 1: List of prices for eczema-related prescriptions dispensed. No price was found for the medicines that have one or more years marked in violet. The price used for the analysis corresponds to the price found for the year before or after. Prices are presented in pounds sterling. EW - EASY WRAP. SG - STOCKINETTE GARMENT. 2W - 2-WAY. 2WS - 2-WAY STRETCH. VISC - VISCOSE. BAN - BANDAGE. ZP - ZINC PASTE. ELAST - ELASTICATED.

Generic name	Formulation	Strength	2005	2006	2007	2008	2009	2010	2011	2012	2013
BACTERIAL SKIN ANTIBIOTICS											
FLOXAPEN	SYRUP	125MG/5ML	4.92	5.06	5.08	5.90	8.61	8.14	7.82		
FLOXAPEN	SYRUP	250MG/5ML	11.67	11.37	11.66	12.79	24.06	24.62	27.50		
FLUCLOXACILLIN	CAPS	250MG	3.04	3.31	3.86	3.17	2.47	2.09	2.74	2.16	2.04
FLUCLOXACILLIN	CAPS	500MG	5.18	5.84	7.29	6.4	5.51	3.73	4.2	3.64	2.95
FLUCLOXACILLIN	SOLN	125MG/5M						29.79	31.98	29.66	29.88
FLUCLOXACILLIN	SOLN	250MG/5ML						50.09	52.98	49.35	48.9
BACTROBAN	CREAM	2.00%	6.33	6.07	5.87	5.88	5.75	5.78	5.24	5.03	5.09
BACTROBAN	OINT	2.00%	6.89	6.8	6.76	7.17	7.15	7.05	6.86	6.02	5.84
MUPIROCIN	CREAM	2.00%	5.36	5.06	5.05	5.06	5.02	5.02	5.03	4.99	4.87
MUPIROCIN	OINT	2.00%	5.46	5.16	5.09	5.15	5.16	5.12	5.11	5.28	5.16
ANTIVIRALS FOR THE SKIN											
ACICLOVIR	CREAM	5.00%	4.86	4.43	2.49	1.77	1.29	1.43	1.57	1.5	2.05
ACICLOVIR	OINT	3.00%	11.5	11.09	11.01	11.03	10.95	10.49	10.31	10.24	10.17
ACICLOVIR (DISPERSIBLE)	TABS	200MG	6.39	5.86	5.95	4.55	3.55	3.33	3.33	3.01	4.09
ACICLOVIR	TABS	200MG	6.03	6.19	6.21	6.27	6.36	6.39	7.18	6.85	4.7
ACICLOVIR	TABS	400MG	8.22	8.35	8.43	8.66	8.72	8.61	9.61	9.52	6.91
ACICLOVIR (DISPERSIBLE)	TABS	400MG	17.2	15.44	10.7	9.37	9.16	7.56	8.47	9.03	11.77
ACICLOVIR	TABS	800MG	48.9	48.92	9.44	9.35	9.49	9.43	10.32	10.16	7.74
ACICLOVIR (DISPERSIBLE)	TABS	800MG	16.87	15.34	11.76	9.69	8.55	6.46	7.78	9.02	10.15
CLEARSORE	CREAM	5.00%	2.8	2.8	2.77	2.83	2.92	2.78	2.76	2.79	2.44
GALPHARM COLD SORE	CREAM	5.00%							0.78	0.78	0.79
NUMARK COLD SORE	CREAM	5.00%							1.15	1.15	0.91
SOOTHELIP	CREAM	5.00%	2.78	2.79	2.74	2.67	2.62	2.59	2.61	2.87	
ZOVIRAX	CREAM	5.00%	8.34	8.09	8.21	8.6	9.39	8.76	8.26	9.27	8.91
ZOVIRAX (COLD SORE)	CREAM	5.00%	4.26	4.34	4.13	4.02	4.02	4.01	4	4.13	4.13
ZOVIRAX	OINT	3.00%	12.12	11.09	10.96	11.14	11.18	10.41	10.13	9.96	10
VECTAVIR	CREAM	1.00%	5.08	4.94	5.03	5.03	5.19	5.21	5.11	5.13	5.18

Table 2 – continued from previous page

Generic name	Formulation	Strength	2005	2006	2007	2008	2009	2010	2011	2012	2013
VALACICLOVIR	TABS	500MG	81.69	79.43	81.3	87.4	82.93	88.81	82.1	84.98	71.84
VALTREX	TABS	500MG	66.17	74.38	68.36	73.12	84.2	82.9	83.5	92.89	95.21

Table 2: List of prices for the antivirals for skin and bacterial skin antibiotics dispensed. No price was found for the medicines that have one or more years marked in violet. The price used for the analysis corresponds to the price found for the year before or after. Prices are presented in pounds sterling.

Generic name	Formulation	Strength	2005	2006	2007	2008	2009	2010	2011	2012	2013
RELIEVERS											
ABLE SPACER (WITH MASK)			7.29	7.44	6.76	7.23	6.94	6.91	7.1	7.27	6.74
ABLE SPACER			4.62	4.81	4.76	4.74	4.72	4.7	4.8	4.88	4.6
AEROCHAMBER PLUS			4.87	4.86	4.91	4.96	4.97	4.98	5.07	5.15	5.26
AEROCHAMBER PLUS (WITH MASK)			8.57	8.55	8.62	8.7	8.73	8.92	9.03	9.12	9.4
AIROMIR	INHAL	100MCG/DOSE	3.01	3.07	3.03	3.05	3.07	3.02	3.02	2.9	2.91
AIROMIR AUTOHALER	INHAL	100MCG/DOSE	8.06	8.19	8.3	8.39	8.39	8.21	8.27	8.42	8.39
ASMASAL CLICKHALER	INHAL	95MCG/DOSE	7.8	7.47	7.55	7.74	7.87	7.7	7.77	7.62	7.09
ATROVENT		40MCG/DOSE	15.99	15.68	15.82	15.75	15.37	14.82	15.73	13.71	
ATROVENT	INHAL	20MCG/DOSE	5.63	5.56	5.63	5.69	6.03	7.4	7.42	7.49	7.34
ATROVENT	SOL	250MCG/ML	30.8	24.92	25.33	25.85	24.4	19.89	18.28	18.27	17.87
BRICANYL NEBUHALER			4.4	4.28	4.18	3.6					
BRICANYL	INHAL	250MCG/DOSE	8.42	7.69	4.37						
BRICANYL TURBOHALER	INHAL	500MCG/DOSE	9.15	9.23	9.35	9.52	9.67	9.7	9.71	9.7	9.79
COMBIVENT	INHAL		9.64	9.7	9.88	10.33	9.73				
COMBIVENT	SOL		48.67	40.33	40.01	39.93	40.45	39.14	39.56	39.29	39.06
DEVICES WITH PA (AEROCHAMBER)			8.44	7.27	7.56	7.62	7.4	7.82	8.55	7.81	
DEVICES WITH PA (VOLUMATIC)			2.75	2.73	2.82	2.75	2.91	2.7	3.05	3.08	2.86
DEVICES WITH PA (NEBUHALER)			4.28	4.36	4.28	3.89					
EASYHALER SALBUTAMOL	INHAL	100MCG/DOSE		4.1	4.21	4.36	4.34	4.3	4.36	4.37	4.24
EASYHALER SALBUTAMOL	INHAL	200MCG/DOSE		8.65	8.92	8.75	8.78	8.8	8.86	8.51	8.33
IPRATROPIUM BROMIDE	INHAL	20MCG/DOSE	5.71	5.77	5.79	5.87	6.04	7.22	7.27	7.33	7.16
IPRATROPIUM BROMIDE	SOL	250MCG/ML	33.84	33.87	33.76	33.38	32.8	32.65	28.89	24.11	22.63
NEBUHALER			4.28	4.35	4.75	4.53	4.07	3.42			
OPTICHAMBER ADVANTAGE				4.05	4.47	3.96	4.57	4.2	4.14	4.16	
OPTICHAMBER				4.43							4.43
POCKET CHAMBER VALVED AEROSOL			4.64	5.43	4.47	6.92	6.95	10.47	7.53	8.81	6.59
PULVINAL SALBUTAMOL	INHAL	200MCG/DOSE	6.55	6.64	6.78	6.9	6.97	6.79	6.87	6.95	7.14

Table 3 – continued from previous page

Generic name	Formulation	Strength	2005	2006	2007	2008	2009	2010	2011	2012	2013
SALAMOL	INHAL	100MCG/DOSE	2.41	2.15	2.1	2.09	2.08	2.07	2.09	2.11	2.1
SALAMOL EASI-BREATHE	INHAL	100MCG/DOSE	8.33	8.43	8.56	8.63	8.73	8.76	8.82	8.89	8.83
SALAMOL STERI-NEB	SOL	1MG/ML	9.28	6.88	6.8	6.64	6.57	6.5	6.19	6.5	6.37
SALAMOL STERI-NEB	SOL	2MG/ML	19.27	14.22	14.84	14.77	13.99	14.18	13.63	13.85	13.81
SALBUTAMOL (WITH DEVICE)	DISKS	200MCG/DOSE	8.38	7.94	7.81						
SALBUTAMOL (REFILL)	DISKS	200MCG/DOSE	8.24	7.66	7.68						
SALBUTAMOL (200D)	INHAL	100MCG/DOSE	2.7	2.72	4.43	4.48	4.35	4.35	2.27	2.26	2.24
SALBUTAMOL (200D DP)	INHAL	100MCG/DOSE	4.3	4.11	4.25	4.34	4.48	4.37	4.36	4.41	4.49
SALBUTAMOL (60D DP)	INHAL	200MCG/DOSE	7.61	7.36	7.5	7.68	7.71	7.47	7.53	7.54	5.19
SALBUTAMOL (100D DP)	INHAL	200MCG/DOSE	6.33	6.41	6.51	6.58	6.57	6.42	6.45	6.6	6.82
SALBUTAMOL (200D DP)	INHAL	200MCG/DOSE	9.45	8.76	8.54	8.94	9.15	9.18	9.21	9.38	9.39
SALBUTAMOL	INHAL	95MCG/DOSE	7.99	7.51	7.6	7.65	7.66	7.45	7.58	7.55	6.5
SALBUTAMOL	SOL	1MG/ML	18.62	18.64	13.97	8.93	13.07	7.08	6.63	6.9	7.39
SALBUTAMOL	SOL	2MG/ML	41.47	42	31.71	16.68	19.78	14.3	14.03	14.43	14.36
SALBUTAMOL	SOLN	2MG/5ML	0.88	2.05	2.6	2.21	2.26	2.3	2.79	2.63	1.77
SPACE CHAMBER PLUS											6.59
TERBUTALINE SULFATE	INHAL	250MCG/DOSE	8.47	8.57							
TERBUTALINE SULFATE	INHAL	500MCG/DOSE	9.27	9.34	9.39	9.54	9.69	9.66	9.69	9.72	9.76
VENTMAX SR	CAPS	8MG	12.2	12.21	12.69	12.72	12.95	12.09	11.91	12.44	12.62
VENTODISKS (WITH DISKHALER)	DISKS	200MCG/DOSE	8.16	7.74	7.67						
VENTODISKS (REFILL)	DISKS	200MCG/DOSE	8.13	7.58	7.42						
VENTOLIN	SOL	1MG/ML	11.47	6.75	5.82	5.81	5.81	5.7	5.58	5.72	5.94
VENTOLIN	SOL	2MG/ML	25.37	13.25	10.84	10.92	10.7	10.15	9.87	9.98	10.16
VENTOLIN	SYRUP	2MG/5ML	0.8	0.79	0.81	0.91	0.93	0.92	0.98	0.93	1.02
VENTOLIN ACCUHALER	INHAL	200MCG/DOSE	7.89	7.52	7.64	7.81	7.95	7.66	7.71	7.66	5.12
VENTOLIN EVOHALER	INHAL	100MCG/DOSE	3.78	2.7	2.55	2.57	2.56	2.54	2.53	2.51	2.48
VENTOLIN VOLUMATIC SPACER			3.09	2.89	2.81	2.86	2.87	3.1	2.83	3.01	6.53

Table 3 – continued from previous page

Generic name	Formulation	Strength	2005	2006	2007	2008	2009	2010	2011	2012	2013
VOLUMATIC SPACER			2.88	2.86	2.93	2.92	2.94	3.01	3.04	3.09	3.2
Long-acting anti-muscarinic (LAMA)											
EKLIRA GENUAIR	INHAL	322MCG/DOSE									32.32
SPIRIVA	CAPS	18MCG	41.75	42.73	43.57	44.39	44.39	41.25	39.81	40.3	44.07
SPIRIVA RESPIMAT	INHAL	2.5MCG				43.6	44.95	44.13	44.68	45.86	45.69
TIOTROPIUM (INHALER)	CAPS	18MCG	43.77	45.36	47.06	48.61	48.76	45.91	43.53	44.58	42.95
TIOTROPIUM (REFILL)	CAPS	18MCG	48.56	49.65	47.82	48.63	49.31	47.7	46.71	47.65	52.09
TIOTROPIUM	INHAL	2.5MCG				62.33	44.13	43.91	45.36	46.97	48.69
Inhaled Corticosteroid											
AEROBEC 100 AUTOHALER	INHAL	100MCG/DOSE	10.17	9.7	9.65	9.7	9.69	7.66			
AEROBEC 50 AUTOHALER	INHAL	50MCG/DOSE	5.15	4.88	4.95	4.99	5.47				
ALVESCO	INHAL	160MCG/DOSE		18.84	21.32	21.18	21.9	22.49	25.74	26.82	23.87
ALVESCO	INHAL	80MCG/DOSE	28.56	31.14	39.67	33.2	33.55	32.98	37.44	39.28	38.72
ASMABEC CLICKHALER	INHAL	100MCG/DOSE	12.56	11.9	12.08	12.24	12.68	12.31	11.26	11.96	12.6
ASMABEC CLICKHALER	INHAL	250MCG/DOSE	16.98	15.61	16.01	16.4	16.96	16.58	16.3	16.12	16.75
ASMABEC CLICKHALER	INHAL	50MCG/DOSE	8.18	7.94	8.14	8.31	8.39	7.86	7.12	6.3	7.1
BECLAZONE 100	INHAL	100MCG/DOSE	10.22	4.34	4.65	9.34	10.4	9.9	7.88		
BECLAZONE 100 EASI-BREATHE	INHAL	100MCG/DOSE	10.05	10.11	11.71	12.79	13.01	12.83			
BECLAZONE 200	INHAL	200MCG/DOSE	22.5	12.12	10.71	20.43	22.76	22.38	18.27		
BECLAZONE 250 EASI-BREATHE	INHAL	250MCG/DOSE	22.34	22.51	24.36	25.64	26.11	25.68			
BECLAZONE 50	INHAL	50MCG/DOSE	5.34	2.56	3.22	4.85	5.5	5.1	4.21		
BECLAZONE 50 EASI-BREATHE	INHAL	50MCG/DOSE	5.29	5.33	5.32	5.43	5.5	5.43			
BECLOFORTE (200D)	INHAL	250MCG/DOSE	30.09	12.90	9.29	9.51					
BECLOMETASONE DIP (200D CLICK)	INHAL	100MCG/DOSE	12.83	12.22	12.38	12.3	12.34	12.04	11.44	11.97	12.37
BECLOMETASONE DIP (200D)	INHAL	100MCG/DOSE	10.72	10.74	9.09	10.92	15.63	7.94	10.82	10.58	
BECLOMETASONE DIP (100D DP)	INHAL	100MCG/DOSE	6.62	6.58	6.65	6.74	6.9	6.82	6.81	6.96	6.94

Table 3 – continued from previous page

Generic name	Formulation	Strength	2005	2006	2007	2008	2009	2010	2011	2012	2013
BECLOMETASONE DIP (200D DP)	INHAL	200MCG/DOSE		20.73	19.77	22.86	23.33	22.15	18.87	19.25	20.79
BECLOMETASONE DIP (100D DP)	INHAL	200MCG/DOSE	12.51	12.38	12.47	12.65	12.96	12.59	12.56	12.51	12.48
BECLOMETASONE DIP (100D CLICK)	INHAL	250MCG/DOSE	16.49	15.67	15.78	15.88	16.12	15.52	15.32	16.04	17.33
BECLOMETASONE DIP (200D)	INHAL	250MCG/DOSE	23.53	23.42	20.85	25.28	33.26	17.16	22.33		
BECLOMETASONE DIP	INHAL	50MCG/DOSE	5.33	5.72	6.16	6.7	8.75	4.89	5.76		
BECODISKS	DISKS	200MCG/DOSE	26.4	24.78	25.34	24.53	24.61	23.05	23.72	24.15	24.47
BECOTIDE 100	INHAL	100MCG/DOSE	13.23	5.11	3.66	3.44					
BECOTIDE 200	INHAL	200MCG/DOSE	25.85	13.63	10.98	10.9					
BECOTIDE 50	INHAL	50MCG/DOSE	6.48	2.85	2.17	2.1					
BUDELIN NOVOLIZER	INHAL	200MCG/DOSE	21.92	16.32	19	16.55	15.88	16.45	21.53	21.79	20.61
BUDESONIDE (200D DP BA)	INHAL	100MCG/DOSE	23	23.28							
BUDESONIDE (120D CFC FREE)	INHAL	100MCG/DOSE					12.61	11.85	11.92		
BUDESONIDE (200D)	INHAL	200MCG/DOSE	26.95	27.74	28.11	28.41	28.49	28.78	24.33	24.08	24.24
BUDESONIDE (100D DP BA)	INHAL	200MCG/DOSE	25.08	25.26	25.36	25.22	25.24	24.05	16.33	16.31	16.99
BUDESONIDE (100D REFILL)	INHAL	200MCG/DOSE	14.98	14.45	12.98	12.79	12.74	13.17	12.29	12.08	13.8
BUDESONIDE (120D CFC FREE)	INHAL	200MCG/DOSE					17.08	18.23	17.98		
BUDESONIDE (50D DP BA)	INHAL	400MCG/DOSE	30.01	30.16	29.94	30.31	30.2	29.1	22.7	22.86	23.41
BUDESONIDE (100D DP BA)	INHAL	400MCG/DOSE		30.94	25.77	26.31	26.01	25.6	25.62	27.04	25.88
BUDESONIDE	INHAL	50MCG/DOSE	8.66	9.07	9.16	9.1	9.07				
CICLESONIDE	INHAL	160MCG/DOSE	33.6	36.34	38.53	40.5	41.39	41.51	48.09	48.69	46.93
CLENIL MODULITE 100	INHAL	100MCG/DOSE			9.67	9.89	9.97	9.66	9.72	9.76	9.73
CLENIL MODULITE 200	INHAL	200MCG/DOSE			20.52	21.6	21.91	21.33	21.37	21.51	21.46
CLENIL MODULITE 250	INHAL	250MCG/DOSE			21.13	21.92	21.91	21.28	21.54	21.9	21.98
CLENIL MODULITE 50	INHAL	50MCG/DOSE			4.33	4.68	4.72	4.55	4.61	4.62	4.62
EASYHALER BECLOMETASONE	INHAL	200MCG/DOSE		18.33	18.66	18.79	18.8	18.2	18.61	18.5	18.02
EASYHALER BUDESONIDE	INHAL	100MCG/DOSE		10.57	11.2	11.33	11.37	11.15	11.3	11.17	11.04
EASYHALER BUDESONIDE	INHAL	200MCG/DOSE			20.3	21.4	22.86	22.78	22.82	22.41	22.73

Table 3 – continued from previous page

Generic name	Formulation	Strength	2005	2006	2007	2008	2009	2010	2011	2012	2013
FLIXOTIDE	DISKS	250MCG/DOSE	36.6	32.98	33.51	33.73	36.81	33.32	31.62	25.73	
FLIXOTIDE ACCUHALER	INHAL	100MCG/DOSE	12.77	12.37	12.48	12.66	12.61	12.79	12.98	13.14	13.02
FLIXOTIDE ACCUHALER	INHAL	250MCG/DOSE	31.28	29.37	30.09	29.86	30.31	31.07	30.83	30.79	30.78
FLIXOTIDE ACCUHALER	INHAL	50MCG/DOSE	9.1	8.72	8.91	9.02	8.86	9.06	9.32	8.77	8.99
FLIXOTIDE ACCUHALER	INHAL	500MCG/DOSE	56.53	52.23	52.18	51.7	51.35	52.44	53.25	52.5	54.25
FLIXOTIDE EVOHALER	INHAL	125MCG/DOSE	28.49	27.25	27.91	27.74	28.28	28.18	28.02	28.58	28.23
FLIXOTIDE EVOHALER	INHAL	250MCG/DOSE	50.71	48.08	48.62	48.72	49.81	50.68	49.7	50.32	50.6
FLIXOTIDE EVOHALER	INHAL	50MCG/DOSE	7	6.54	6.71	6.8	6.99	6.93	7.05	7.24	7.22
FLUTICASONE PROP (REFILL)	DISKS	100MCG/DOSE	20.08	19.04	19.11	19.7	18.92	17.26	18.99		
FLUTICASONE PROP (WITH DEVICE)	DISKS	100MCG/DOSE	17.02	15.52	15.36	15.25	18.01	17.54	17.48		
FLUTICASONE PROP (REFILL)	DISKS	250MCG/DOSE	42.3	38.51	38.35	39.4	37.11	37.82	32.48		
FLUTICASONE PROP (WITH DEVICE)	DISKS	250MCG/DOSE	37.83	33.08	33.79	32.41	33.84	37.6	36.43		
FLUTICASONE PROP (WITH DEVICE)	DISKS	50MCG/DOSE	9.48	8.94	8.74	9.63	9.99				
FLUTICASONE PROP (REFILL)	DISKS	50MCG/DOSE	10.84	10.81	10.4	11.67	11.87				
FLUTICASONE PROP	INHAL	100MCG/DOSE	12.79	12.16	12.38	12.51	12.72	12.91	13.01	13.12	13.18
FLUTICASONE PROP	INHAL	125MCG/DOSE	29.05	27.63	28.19	28.39	28.69	28.71	28.88	28.88	29.08
FLUTICASONE PROP (120D CFC FREE)	INHAL	250MCG/DOSE	52.01	48.78	49.18	49.05	49.46	49.71	49.78	50.22	49.95
FLUTICASONE PROP (60D DP)	INHAL	250MCG/DOSE	32.02	30.39	30.56	30.18	30.29	30.31	30.84	30.55	30.57
FLUTICASONE PROP (120D CFC FREE)	INHAL	50MCG/DOSE	7.55	7.15	7.2	7.2	7.29	7.25	7.12	7.32	7.25
FLUTICASONE PROP (60D DP)	INHAL	50MCG/DOSE	8.98	8.64	8.79	8.92	8.97	9.01	8.87	8.59	8.55
FLUTICASONE PROP	INHAL	500MCG/DOSE	56.41	53.52	54.3	53.83	54.51	54.09	54.68	54.27	54.76
PULMICORT	INHAL	100MCG/DOSE					12.42	12.04	12.01	10.97	
PULMICORT (200D)	INHAL	200MCG/DOSE	26.26	27.22	27.5	27.74	28.1	28.37			
PULMICORT (120D CFC FREE)	INHAL	200MCG/DOSE					15.68	17.92	18.02	16.41	
PULMICORT LS	INHAL	50MCG/DOSE	8.45	8.83	8.99	9.06	8.72	7.59			
PULMICORT TURBOHALER	INHAL	100MCG/DOSE	22.63	22.74	23.18	23.47	23.52	22.21	15.4	15.26	14.91
PULMICORT TURBOHALER	INHAL	200MCG/DOSE	24.59	24.75	24.92	24.99	25.09	23.82	16.44	16.46	16.89

Table 3 – continued from previous page

Generic name	Formulation	Strength	2005	2006	2007	2008	2009	2010	2011	2012	2013
PULMICORT TURBOHALER	INHAL	400MCG/DOSE	29.17	29.34	29.47	29.82	29.58	28.48	22.21	22.31	22.63
PULVINAL BECLOMETASONE DIP	INHAL	100MCG/DOSE	6.68	6.91	6.93	7.03	7.14	6.96	6.85	7.13	7.33
PULVINAL BECLOMETASONE DIP	INHAL	200MCG/DOSE	13.18	13.48	13.45	13.64	13.67	13.29	13.38	13.34	13.21
QVAR 100	INHAL	100MCG/DOSE	21.49	21.28	21.14	21	21.04	21.17	21.41	21.35	21.19
QVAR 100 AUTOHALER	INHAL	100MCG/DOSE	21.97	22.09	22.11	22.27	22.64	22.61	22.52	22.43	22.33
QVAR 100 EASI-BREATHE	INHAL	100MCG/DOSE	22.61	21.3	20.5	21.2	21.5	21.48	21.36	21.61	21.45
QVAR 50	INHAL	50MCG/DOSE	9.27	9.23	9.15	9.17	9.35	9.43	9.53	9.56	9.54
QVAR 50 AUTOHALER	INHAL	50MCG/DOSE	9.45	9.37	9.6	9.89	9.96	9.96	9.93	9.91	9.88
QVAR 50 EASI-BREATHE	INHAL	50MCG/DOSE	10.3	9.02	9.07	9.24	9.41	9.42	9.45	9.57	9.62
Long-acting β_2-agonists											
ATIMOS MODULITE	INHAL	12MCG		35.03	37.42	39.83	41.17	38.85	38.7	38.29	39.93
EASYHALER FORMOTEROL	INHAL	12MCG/DOSE				28.23	27.53	27.4	28.75	27.75	26.94
FORMOTEROL FUMARATE	INHAL	12MCG/DOSE	33.92	34.1	34.5	34.66	34.73	35.06	35.25	35.74	35.97
FORMOTEROL FUMARATE	INHAL	6MCG/DOSE	33.33	34.58	34.62	35.02	34.98	34.63	36.08	37.5	37.86
OXIS TURBOHALER 12	INHAL	12MCG/DOSE	33.13	33.18	33.58	33.82	34.3	34.34	35.54	36.76	37.72
OXIS TURBOHALER 6	INHAL	6MCG/DOSE	32.36	33.14	32.96	32.94	34.43	35.07	34.76	35.26	34.57
SALMETEROL (REFILL)	DISKS	50MCG/DOSE	50.97	47.89	49.24	48.62	48.97	49.48	49.23	48.93	51.57
SALMETEROL (WITH DEVICE)	DISKS	50MCG/DOSE	45.81	42.26	41.98	42.22	42.1	42.58	42.7	42.99	42.28
SALMETEROL	INHAL	25MCG/DOSE	39.91	37.81	38.1	38.29	38.48	38.68	39	39.61	37.09
SALMETEROL	INHAL	50MCG/DOSE	41.47	39.16	39.38	39.77	39.93	39.84	40.35	41	41.83
SEREVENT	INHAL	25MCG/DOSE	38.58	37.11	37.92						
SEREVENT ACCUHALER	INHAL	50MCG/DOSE	40.91	39.39	40.31	40.17	40.25	39.8	40.02	40.6	40.75
SEREVENT EVOHALER	INHAL	25MCG/DOSE		38.07	38.07	37.86	38.27	38.62	38.54	39.6	40.09
Long-acting β_2-agonists with corticosteroids											
FLUTIFORM	INHAL	125MCG/5MCG								35.79	35.79
FLUTIFORM	INHAL	250MCG/10MCG									54.52

Table 3 – continued from previous page

Generic name	Formulation	Strength	2005	2006	2007	2008	2009	2010	2011	2012	2013
FOSTAIR 100/6	INHAL					32.33	36.83	35.9	37.07	37.16	38.01
SERETIDE 100 ACCUHALER	INHAL		44.38	42.43	43	43.51	43.98	44.32	44.66	40.29	25.86
SERETIDE 125 EVOHALER	INHAL		51.14	48.98	49.96	50.45	50.68	48.83	49.07	49.47	49.37
SERETIDE 250 ACCUHALER	INHAL		51.89	49.38	50.16	50.99	51.32	49.37	49.69	49.87	50.06
SERETIDE 250 EVOHALER	INHAL		84.96	81.03	82.04	83.25	83.79	80.78	81.21	81.46	81.35
SERETIDE 50 EVOHALER	INHAL		25.29	24.29	25.03	25.42	25.86	25.79	25.86	26	25.93
SERETIDE 500 ACCUHALER	INHAL		57.38	54.07	54.83	55.31	56.03	56.12	56.53	56.61	56.77
SYMBICORT 100/6 TURBOHALER	INHAL		40.42	40.82	41.74	42.91	43.85	43.85	44.21	44.53	44.54
SYMBICORT 200/6 TURBOHALER	INHAL		48.31	48.82	49.38	50.15	51.13	51.43	52.04	52.5	52.86
SYMBICORT 400/12 TURBOHALER	INHAL		49.28	51.43	52.79	54.02	54.94	55.12	55.83	56.29	56.88
Leukotriene Receptor Antagonist											
ACCOLATE	TABS	20MG	35.22	35.88	36.15	35.84	34.35	23.07	23.13	24.54	25.39
MONTELUKAST	SACH	4MG	29.01	29.49	31.28	31.54	32.48	32.54	32.81	33.1	33.88
MONTELUKAST	TABS	10MG	37.6	39.25	39.93	40.48	40.93	41.2	41.6	40.78	38.98
MONTELUKAST	TABS	4MG	33.24	34.36	35.2	35.79	36.26	36.5	36.81	36.48	35.82
MONTELUKAST	TABS	5MG	35.68	36.28	36.95	37.27	37.96	38.32	38.63	39.72	39.05
SINGULAIR	SACH	4MG	29.14	29.08	28.94	32.56	31.04	31.46	32.6	33.34	32.95
SINGULAIR	TABS	10MG	34.97	36.36	37.2	37.66	38.3	38.44	40.5	42.83	43.17
SINGULAIR	TABS	4MG	31.56	31.52	32.56	33.79	32.55	33.57	32.68	33.34	36.6
SINGULAIR	TABS	5MG	32.58	32.87	33.75	33.88	35.27	35.31	35.01	42.83	38.74
ZAFIRLUKAST	TABS	20MG	36.69	37.45	37.71	37.64	36.21	23.95	23.66	23.68	22.45

Table 3: List of prices for asthma-related prescriptions dispensed. No price was found for the medicines that have one or more years marked in violet. The price used for the analysis corresponds to the price found for the year before or after. Prices are presented in pounds sterling. PA - PRESSURISED AEROSOLS, DP - DRY POWDER, BA - BREATH ACTUATED, DIP - DIPROPIONATE, PROP - PROPIONATE

Generic name	Formulation	Strength	2005	2006	2007	2008	2009	2010	2011	2012	2013
ANAPEN	INJ	300MCG/0.3ML		49.44	55.29	54.12			55.76	57.04	54.39
ANAPEN JUNIOR	INJ	150MCG/0.3ML		65.25	67.47	68.54	67.61	64.46	71.13	57.19	60.53
EPINEPHRINE	INJ	150MCG/0.3ML	57.61	58.15						63.29	
EPINEPHRINE	INJ	300MCG/0.3ML	45.16	46.2							
EPIPEN	INJ	300MCG/0.3ML	46.75	48.22	49.96	50.72	51.54	52.65	54.1	48.72	48.53
EPIPEN JR	INJ	150MCG/0.3ML	55.47	56.06	59.27	59.66	61.03	62.67	62.28	56.1	56.01
JEXT	INJ	300MCG/0.3ML								68.61	51.99
MINIJET ADRENALINE	INJ	100MCG/ML	13.05	14.05	16.08	14.53	16.12	19.67	20.53	21.54	20.99
MINIJET ADRENALINE	INJ	100MCG/ML	13.05	14.05	16.08	14.53	16.12	19.67	20.53	21.54	2099

Table 4: List of prices for asthma-related prescriptions dispensed. Prices are presented in pounds sterling.

Generic name	Formulation	Strength	2005	2006	2007	2008	2009	2010	2011	2012	2013
AZELASTINE HYDROCHLORIDE	DROPS	0.05%				8.05	7.27	7.27	7.19	7.6	7.75
AZELASTINE HYDROCHLORIDE	SPRAY	0.10%	4.42	4.39	4.45	4.64	5.08		3.01	2.88	2.9
AZELASTINE HYDROCHLORIDE	SPRAY	140MCG/DOSE	13.85	12.83	12.69	12.66	12.49	9.98	11.02	12.29	12.11
OPTILAST	DROPS	0.05%	8.03	7.4	7.46	7.37	7.63	7.72	7.59	7.25	7.3
RHINOLAST (136D)	SPRAY	140MCG/DOSE	14.15				14.15		14.15	14.15	
BECLOMETASONE DIP (200D)	SPRAY	50MCG/DOSE	3.97	4.19	5.87	5.26	4.09	3.48	3.2	2.68	2.66
BECLOMETASONE DIP (180D)	SPRAY	50MCG/DOSE				6.1	5.8	5.85	6.3	6.49	6.49
BECONASE	SPRAY	50MCG/DOSE	4.52	2.83	2.53	2.53	2.52	2.49	2.47	2.45	2.43
BECONASE HAYFEVER	SPRAY	50MCG/DOSE	4.07	4.11	4.16	4.05	3.95				
BECONASE HAYFEVER ALLERGY	SPRAY	50MCG/DOSE	4.82	4.87	4.94	4.79	4.81	4.86	5	5.26	5.23
NASOBEC	SPRAY	50MCG/DOSE				6.21	6.03	4.04	3.81	4.5	6.27
NASOBEC AQUEOUS	SPRAY	50MCG/DOSE	3.08	3.18	3.81	3.16	3.25	2.89	3.42	3.56	3.56
BETNESOL	DROPS	0.10%		2.64	2.81	2.7	2.68	4.15	2.67	2.59	2.7
VISTAMETHASONE	DROPS	0.10%	1.43	1.37	1.35	1.3	1.29	1.36	1.38	1.33	1.35
BETNESOL N	DROPS		2.52	2.54	2.61	2.53	2.51	2.46	2.39	1.81	1.58
BUDESONIDE (100D)	SPRAY	100MCG/DOSE	7.14	6.82	7.2	7.23	6.37	5.9			
BUDESONIDE (200D)	SPRAY	100MCG/DOSE	7.53	7.04	10.82	6.08	6.28	5.94			
BUDESONIDE	SPRAY	64MCG/DOSE	5.69	5.61	5.59	5.68	5.84	5.82	5.83	5.56	4.44
RHINOCORT AQUA	SPRAY	64MCG/DOSE	5.63	5.63	5.74	5.79	5.83	5.19	3.46	4.45	4.44
AVAMYS	SPRAY	27.5MCG/DOSE					6.61	7.42	7.42	7.12	7.21
FLUTICASONE FUROATE	SPRAY	27.5MCG/DOSE					8.67	7.23	7.6	7.41	7.44
FLIXONASE	SPRAY	50MCG/DOSE	14.32	13.73	13.87	13.91	13.93	13.47	13.3	13.04	12.88
FLIXONASE NASULE	DROPS	400MCG	25.61	24	23.37	22.91	23.35	22.9	22.15	21.65	21.54
FLUTICASONE PROPIONATE	DROPS	400MCG	25.44	23.24	23.52	23.44	23.22	22.47	22.23	21.19	21.44
FLUTICASONE PROPIONATE (150D)	SPRAY	50MCG/DOSE	14.63	13.83	13.92	13.98	14.09	13.58	13.36	13.11	13.03
FLUTICASONE PROPIONATE (60D)	SPRAY	50MCG/DOSE	4.6	4.6	4.87		5.25	5.25	5.21	7.42	8.59
NASOFAN	SPRAY	50MCG/DOSE	12.31	12.31	12.52	12.67	10.8	10.17	10.37	9.77	9.65
NASOFAN ALLERGY	SPRAY	50MCG/DOSE			4.84	5.72	5.93	6.18	5.77	5.53	6.18

Table 5 – continued from previous page

Generic name	Formulation	Strength	2005	2006	2007	2008	2009	2010	2011	2012	2013
PIRINASE HAYFEVER	SPRAY	0.05%	4.62	5.33	4.94	5	5	4.87	4.87	4.72	4.57
IPRATROPIUM BROMIDE	SPRAY	0.03%	5.46	5.51	5.57	5.73	5.75	5	5.11	4.99	5.05
RINATEC	SPRAY	0.03%	5.35	5.45	5.51	5.54	5.38	4.77	4.87	4.9	4.88
MOMETASONE FUROATE	SPRAY	50MCG/DOSE	11.69	8.83	8.9	8.96	9.04	8.92	9.01	9.11	9.21
NASONEX	SPRAY	50MCG/DOSE	11.82	8.84	8.91	8.96	8.97	8.86	8.98	9.11	9.01
OPTICROM (AQ)	DROPS	2.00%	9.07	9.63	9.6	9.76	9.65	9.24	9.4	9.53	9.97
OPTICROM (Allergy)	DROPS	2.00%	3.16	3.19	3.1	3.12	3.12	3.15	3.26	3.25	3.26
OPTREX ALLERGY	DROPS	2.00%	3.16	3.11	3.24	3.45	3.52	3.64	3.76	3.93	3.98
RYNACROM	SPRAY	4.00%	21.6	19.86	20.52	20.8	20.95	20.46	20.95	19.93	19.47
SODIUM CROMOGLICATE	DROPS	2.00%	2.23	2.48	3.55	3.14	2.11	2.3	2.7	2.65	2.69
SODIUM CROMOGLICATE	SPRAY	2.00%	10.04	10	10.01	10.88	11.46	10	10.91	11.5	9.91
SODIUM CROMOGLICATE	SPRAY	4.00%	23.44	21.54	21.22	21.24	21.27	20.84	20.96	20.72	20.32
NASACORT	SPRAY	0.06%	10.67	8.52	8.61	8.58	8.65	8.66	8.72	8.82	8.85
TRIAMCINOLONE ACETONIDE	SPRAY	0.06%	10.72	8.64	8.71	8.77	8.87	9.03	9.14	9.12	9.14

Table 5: List of prices for allergic rhinitis prescriptions dispensed. No price was found for the medicines that have one or more years marked in violet. The price used for the analysis corresponds to the price found for the year before or after. Prices are presented in pounds sterling.

Generic name	Formulation	Strength	2005	2006	2007	2008	2009	2010	2011	2012	2013
DELTACORTRIL	TABS	5MG	0.8	0.77	0.76	1.31	1.62	1.83	1.91	2.09	1.89
PREDNISOLONE	TABS	1MG	2.02	3.22	5.88	3.64	2.46	3.68	3.92	3.56	3.75
PREDNISOLONE	TABS	2.5MG	0.61	0.61	1.88	5.53	8.26	10.61	16.84	18.81	11.06
PREDNISOLONE	TABS	25MG	2.6	2.58	3.3	3.89	4.76	6.06	7.15	7.72	7.71
PREDNISOLONE	TABS	5MG	1.42	2.12	3.67	2.38	1.61	2.13	2.23	3.79	3.45

Table 6: List of prices for oral prednisolone prescriptions dispensed. Prices are presented in pounds sterling.

Speciality	Patient Type	Costs per admission				Costs per day								Overhead
		Med	Rad	Lab	Total	Med	Nursing	Pharmacy	Other	Rad	AHP	Lab	Total	
General Medicine	Inpatient	37	9	35	81	37	208	64	7	1	9	4	330	66%
General Medicine	Daycase					286	314	214	6	1	1	39	861	0%
Paediatrics (Medical Paediatrics)	Inpatient	33	21	43	97	33	302	94	14	3	25	6	477	25%
Paediatrics (Surgical Paediatrics)	Daycase					14	47	5	2	1	1	7	77	1%
Respiratory Medicine	Inpatient	26	44	38	108	26	86	32	8	2	13	1	168	121%
Respiratory Medicine	Daycase					127	55	158	3	0	0	15	0	-1%
General Surgery (excl. Vascular)	Inpatient	21	23	30	74	21	172	33	8	2	19	3	258	48%
General Surgery (excl. Vascular)	Daycase					70	26	12	3	1	1	28	141	-4%
Accident & Emergency	Inpatient	214	2	49	265	214	682	136	10	1	7	20	1,070	5%
Accident & Emergency	Daycase													
Anaesthetics (Medical Other)	Inpatient	87	93	59	239	87	245	34	7	1	10	1	385	54%
Ear Nose & Throat	Inpatient	26	14	7	47	26	427	108	13	3	26	2	605	24%
Ear Nose & Throat	Daycase					29	31	5	2	0	1	5	73	13%
Plastic Surgery (Scotland)	Inpatient	40	19	88	147	40	219	48	18	2	15	8	350	44%

Table 7: List of prices for asthma-related hospital admissions. The prices collected are from Fife. An assumption was made that prices between Fife and Tayside were equal. The cells in blue corresponds to speciality names now obsolete. The name in brackets correspond to the name of the speciality considered instead. Prices are presented in pounds sterling. Med - Medical, Rad - Radiology, Lab - Laboratory

Consent forms

CONSENT FORM FOR FILMING / PHOTOGRAPHY OF 'EVERY CHILD IS DIFFERENT' BRIGHTON FESTIVAL FRINGE EVENT ATTENDEES

The BSMS Communications Department can be contacted for advice on: 01273 877308

- I have agreed to be filmed/photographed or for my child to be filmed/photographed
- I understand that my identity/my child's identity will be revealed on camera
- I understand that the footage/photography will be used to produce a short film and promotional photographs of this event and may be used within promotional materials for future events and public engagement by BSMS, The Royal Alexandra Children's Hospital (BSUH NHS Trust) and University of Brighton.
- I understand that the film and photographs will be posted online and information from my interview may be used within future research projects into personalised medicine and proposals by the researcher.

NAME OF PERSON FILMED / PHOTOGRAPHED:

..... IF A

CHILD, AGE: .....

LOCATION: ..... SIGNED:

..... DATE:

.....

PRINT NAME:
.....

Thank you.

A copy of this form will be retained by:
Communications Department, Brighton and Sussex Medical School, University of Sussex, Falmer, Brighton, BN1 9PX

For official use

DATE:.....

NAME OF FILMMAKER / PHOTOGRAPHER: Silverbox Productions, Morna Dick

NAME OF PRODUCTION / PUBLICATION: Every Child Is Different

brighton and sussex medical school

Vimos por este meio pedir a sua autorização para a utilização de imagens das actividades que vamos promover com os vossos filhos e usar as respectivas fotografias imprimidas para possível publicação e divulgação.

Eu, abaixo-assinado, autorizo a utilização das imagens obtidas no decorrer do evento.

Nome do filho: _____

Nome do encarregado de educação: _____

Assinatura do encarregado de educação: _____

Data: _____

Translation

We hereby ask your permission to use images of the activities which we will promote with your children and to use the printed photos to promotion and possible publication.

I, the undersigned, allow the use of images obtained during the event.

Name of the child: _____

Name of the parent: _____

Signature of the parent: _____

Date: _____

Questionnaires

brighton and sussex medical school

Every Child is Different – Brighton Fringe Event Feedback

Did you enjoy the event "Every Child is Different"?

- ○ Yes
- ○ No

What did you enjoy most about the event?

What could we have done to improve the event?

What was the most important thing you have learnt from today?

Did the event change your awareness knowledge, your opinion or your mind in any way?

- ○ Yes
- ○ No

If yes, please tell us what changed and how?

How did you hear about the event? _____

If you are happy to be contacted by the Brighton & Sussex Medical School in the future about personalised medicine or providing parent and child feedback on the research we are planning, please leave your contact details below

Name:
Telephone number:
Email address:

Many thanks for attending our event and providing feedback!

brighton and sussex medical school

Formulário pais / Parents Questionnaire

Gostou das actividades? / Did you enjoy the activities?

☐ Sim / Yes ☐ Não / No

Tem alguma sugestão a dar-nos para melhorar? / Do you have any suggestion for improvement?

As actividades realizadas hoje aumentaram o seu conhecimento, mudaram a sua opinião ou pensamento? / The activities performed today improved your knowledge, changed your opinion or thought?

☐ Sim / Yes ☐ Não / No

Se sim, pode nos explicar de forma? / If yes, can you tell us how?

Qual a sua opinião sobre medicina personalizada? / What is your opinion about personalised medicine?

Tendo em conta o que ouviu hoje sobre medicina personalizada, qual a sua opinião sobre testes genéticos? / Bearing in mind what you heard today about personalised medicine, what is your opinion about genetic testing?

Se o seu médico lhe perguntar se pode efectuar testes genéticos à sua criança qual seria a sua resposta? Pode explicar o porquê dessa resposta? / If your doctor asked you to perform genetic testing to your child, what would your answer be? Can you explain why?

Muito obrigada por participar e partilhar as suas opiniões! / Thank you very much for your participation and sharing your opinions!

Formulário professoras / Teacher questionnaire

Gostou das actividades? / Did you enjoy the activities?

☐ Sim / Yes ☐ Não / No

Tem alguma sugestão a dar-nos para melhorar? / Do you have any suggestion for improvement?

As actividades realizadas hoje aumentaram o seu conhecimento, mudaram a sua opinião ou pensamento? / The activities performed today improved your knowledge, changed your opinion or thought?

☐ Sim / Yes ☐ Não / No

Se sim, pode nos explicar de forma? / If yes, can you tell us how?

Muitas vezes existe uma ponte entre cientistas e não cientistas. Comunicar ciência através de actividades mudou a sua percepção sobre ciência de alguma forma? Tornou o tópico mais acessível ou mais interessante? / A bridge is often present among scientists and non scientists. Communicating science through activities has changed your perception of science in any way? Has it made the topic more interesting or accessible?

Consideraria fazer actividades deste genéro durante o ensino? / Would you consider performing similar activites during school period?

Qual a sua opinião sobre medicina personalizada? / What is your opinion about personalised medicine?

Tendo em conta o que ouviu hoje sobre medicina personalizada, qual a sua opinião sobre testes genéticos? / Bearing in mind what you heard today about personalised medicine, what is your opinion about genetic testing?

Se o seu médico lhe perguntar se pode efectuar testes genéticos à sua criança qual seria a sua resposta? Pode explicar o porque dessa resposta? / If your doctor asked you to perform genetic testing to your child, what would your answer be? Can you explain why?

Muito obrigada por participar e partilhar as suas opiniões! / Thank you very much for your participation and sharing your opinions!