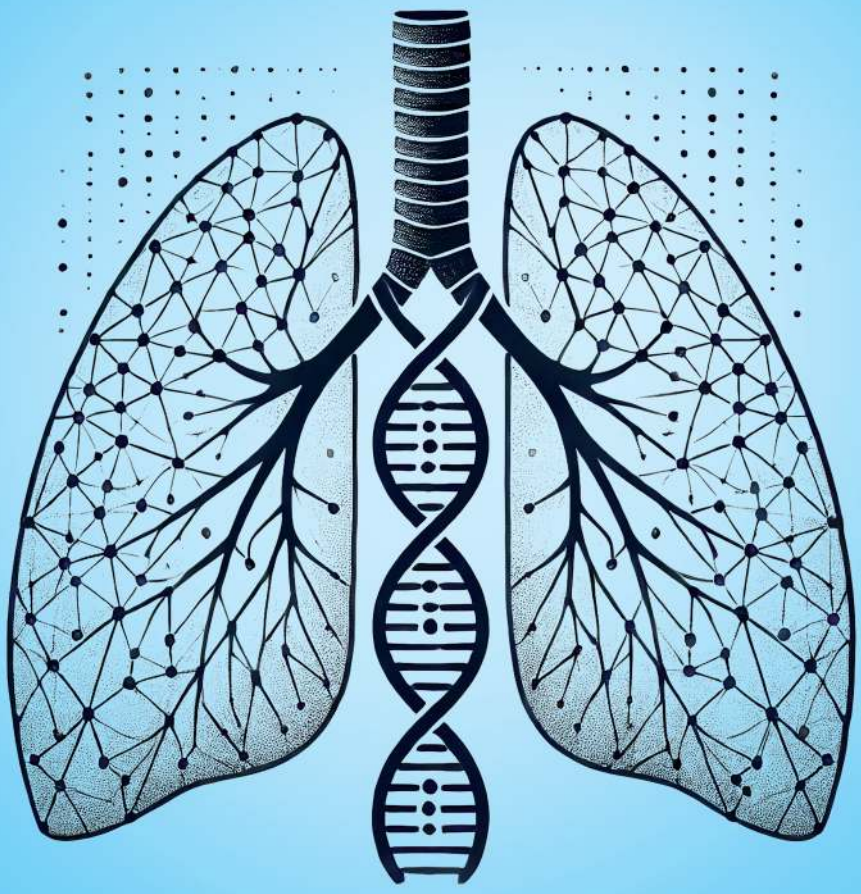
Management of Patients with Asthma or Chronic Obstructive Pulmonary Disease

The role of genetics



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Management of Patients with Asthma or Chronic Obstructive Pulmonary Disease
The role of genetics

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ISBN: 978-94-6510-202-3

Cover design: Leila Karimi Gazafroudi designed the cover page with the assistance of ChatGPT (personal communication, August 8, 2024).

Layout and printed by: Optima Grafische Communicatie (www.ogc.nl)

Management of Patients with Asthma or Chronic Obstructive Pulmonary Disease
The role of genetics

Behandeling van patiënten met astma of chronische obstructieve longziekte
De rol van genetica

Thesis
to obtain the degree of Doctor from the
Erasmus University Rotterdam, the Netherlands
by command of the
rector magnificus

Prof. dr. A.L. Bredenoord

and in accordance with the decision of the Doctorate Board.
The public defense shall be held on
Wednesday 2 October 2024 at 13:00 hours

by
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Erasmus University Rotterdam

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TABLE OF CONTENTS

1

General introduction

Asthma and chronic obstructive pulmonary disease (COPD) are two of the most prevalent chronic obstructive lung diseases worldwide. Both disorders are characterized by airway obstruction and chronic inflammation of the lungs; however, the type of inflammation varies considerably between the two diseases as well as within each disease, with a corresponding difference in clinical manifestations, pathology, clinical phenotypes, and response to treatment.[1,2]

Table 1. Some differences between COPD and Asthma[3,4]

Features	Asthma	COPD
Risk factor	Allergies and family history	Smoking
Main age group	Children and young adults	Older age
Symptoms	Wheezing and dyspnea outbreaks	Productive cough and chronic dyspnea
Predominant inflammatory cells	Eosinophils, mast cells, and CD4 (T helper 2 cell) lymphocytes	Neutrophils, macrophages, and CD8 (T cells) lymphocytes
Inflammatory patterns	Bronchoconstriction and airway hyperresponsiveness	Progressive airflow limitation, small airways fibrosis, and alveolar destruction
Spirometry (after taking BD*)	Lung functions improve (reversibility)	lung functions do not improve (absence of reversibility)

*BD, Bronchodilator

ASTHMA

Asthma is a global health problem and occurs in both children and adults. According to the Global Initiative for Asthma (GINA) guidelines, "Asthma is defined as a heterogeneous disease usually characterized by chronic airway inflammation. It is defined by the history of respiratory symptoms such as wheeze, shortness of breath, chest tightness, and cough that vary over time and in intensity, together with variable expiratory airflow limitation".[5]

Pathogenesis and pathophysiology of asthma

Asthma is a chronic inflammatory disorder which affects the lower respiratory tract, i.e., the trachea, the bronchi, and the bronchioles. Different inflammatory cells play an important role in airway inflammation in asthma i.e., mucosal mast cells, eosinophils, lymphocytes, dendritic cells, macrophages neutrophils, and CD8 (Tc1) lymphocytes.[1,2] The structural cells which contribute to inflammation in the airways are epithelial cells, smooth muscle cells, endothelial cells, fibroblast, myofibroblasts, and airway nerves. Key cellular mediators which mediate the complex inflammatory response are chemokines, cysteinyl leukotrienes, cytokines, histamine, nitric oxide (NO), and prostaglandin D2.[1,6] In addition to the inflammatory response, essential characteristics of structural changes and airway remodeling are linked to asthma severity.[5,7,8]

Airway narrowing which leads to symptoms and physiological changes in asthma might be the result of airway smooth muscle contraction, airway edema, airway thickening, and mucus overproduction.[9] Also, airway hyperresponsiveness can cause airway narrowing in patients with asthma.[10] Although the mechanism of airway hyperresponsiveness is not still clear, genetics, airway inflammation, extreme contraction of airway smooth muscles, thickening of the airway walls, and airway closure may contribute to this.[10,11]

Epidemiology of asthma

Asthma affects about 300 million individuals worldwide, and it is estimated that a further 100 million may be affected by 2025.[12,13] Although asthma prevalence and incidence are higher in children, asthma-related healthcare utilization and mortality are higher in adults.[14] Asthma is the most common chronic disease in children and the prominent cause of childhood morbidity.[5] The disease is the result of the complex interaction of host factors (i.e., genetic susceptibility, obesity, sex, and pre-term or/and small size for gestational age) and multiple environmental factors (i.e., allergens, indoor, outdoor, and occupational, microbiome, tobacco exposure, diet, and stress).[14-20] A significant social and economic burden has been attributed to asthma worldwide.[21-23] The economic burden of asthma includes direct costs (e.g. hospital admissions, treatments, and specialist visits) and indirect costs (e.g. school or working days lost, significant detriment of the quality of life, productivity loss of caregivers, and early death).[5,24-27]

Diagnosis of asthma

The diagnosis of asthma is mainly based on the presence of respiratory symptoms such as wheezing, dyspnea, cough or chest tightness, and fluctuating airflow limitation.[5] Airflow limitation can be assessed through different methods but is mainly assessed via a spirometry which measures the forced expiratory volume in 1 second (FEV_1), forced vital capacity (FVC), and their ratio (FEV_1/FVC). According to the GINA guidelines, a FEV_1/FVC value lower than 0.90 in children and 0.75-0.80 in adults shows the airway limitation.[5] However, asthmatic patients can have a normal lung function. A positive bronchodilator reversibility test, an increase in FEV_1 a few minutes after inhalation of a short-acting bronchodilator, such as salbutamol, can also confirm the diagnosis.[5]

Treatment of asthma

Asthma treatment aims primarily at controlling symptoms as well as preventing asthma-related mortality, exacerbations, and persistent airflow limitation.[5] Although there is no cure for asthma, asthma can be controlled through a stepwise treatment as recommended by the GINA guidelines.[5] Asthma medications can be classified as relievers that might be taken whenever needed to relief quickly symptoms and maintenance (controller) medications which are taken regularly to control the airway inflammation. The most common used maintenance therapy is Inhaled corticosteroids (ICS) which, since recent GINA guidelines can also be used as reliever therapy.[5]

The GINA guidelines 2023[5] recommend that all adults and adolescents with asthma should receive inhaled corticosteroids containing treatment, to minimize the risk of exacerbation, as described in Figure 1.

The cornerstone of asthma treatment in adults and children is ICS. If asthma is poorly controlled on low-dose ICS, according to GINA treatment guidelines, an increase in ICS dose or an addition of a long-acting $ß_2$-agonist (LABA) is recommended.[5] Both medications are effective in treating asthma symptoms, improving lung function, and/or reducing asthma exacerbations.[5] However, patient response to either ICS or ICS plus LABA varies significantly.[28,29]

Asthma exacerbations

People with asthma often have periods of worsening symptoms and airway obstruction, called exacerbations, attacks, or flare-ups.[5] According to the American Thoracic Society (ATS)/ European Respiratory Society (ERS) guidelines, a severe exacerbation is defined as worsening of asthma symptoms that requires hospitalization or emergency department visit or administration of systemic corticosteroids.[30] Regardless of the use of effective medications to control asthma, a severe asthma exacerbation is still associated with significant morbidity and mortality and results in direct and indirect costs linked with health care use and lost productivity.[31-34]

It has been proven that asthma exacerbations can vary greatly inter-and intra-individually in terms of severity and incidence and can range from mild symptoms to highly severe symptoms that may even prove fatal. The differences are due to both genetic and non-genetic factors such as clinical and demographic characteristics, as well as exposure to asthma risk factors, such as viral respiratory infections.[35] In light of this, an asthma exacerbation is considered one of the important target phenotypes in asthma pharmacogenetic studies.[36]

Asthma comorbidities

Patients with asthma frequently suffer from other comorbidities such as obesity, gastroesophageal reflux disease, rhinosinusitis, food allergies, depression, and anxiety in addition to their asthma symptoms.[5] There is a correlation between comorbidity and poor quality of life, more frequent use of healthcare services, and adverse effects associated with treatment.[37] Poor asthma control can also be attributed to certain comorbidities.[38]

Management of asthma and asthma exacerbations

Despite appropriate therapy adherence and correct inhalation technique, many patients with asthma keep experiencing exacerbations.[28] To improve asthma-guided treatment, there is a significant demand to use biomarkers. The non-invasive inflammatory markers, namely, blood eosinophils, blood IgE, septum eosinophils, and fractional exhaled nitric oxide (FENO), among others, are often measured as part of the management of asthma.[39] NO is produced in healthy

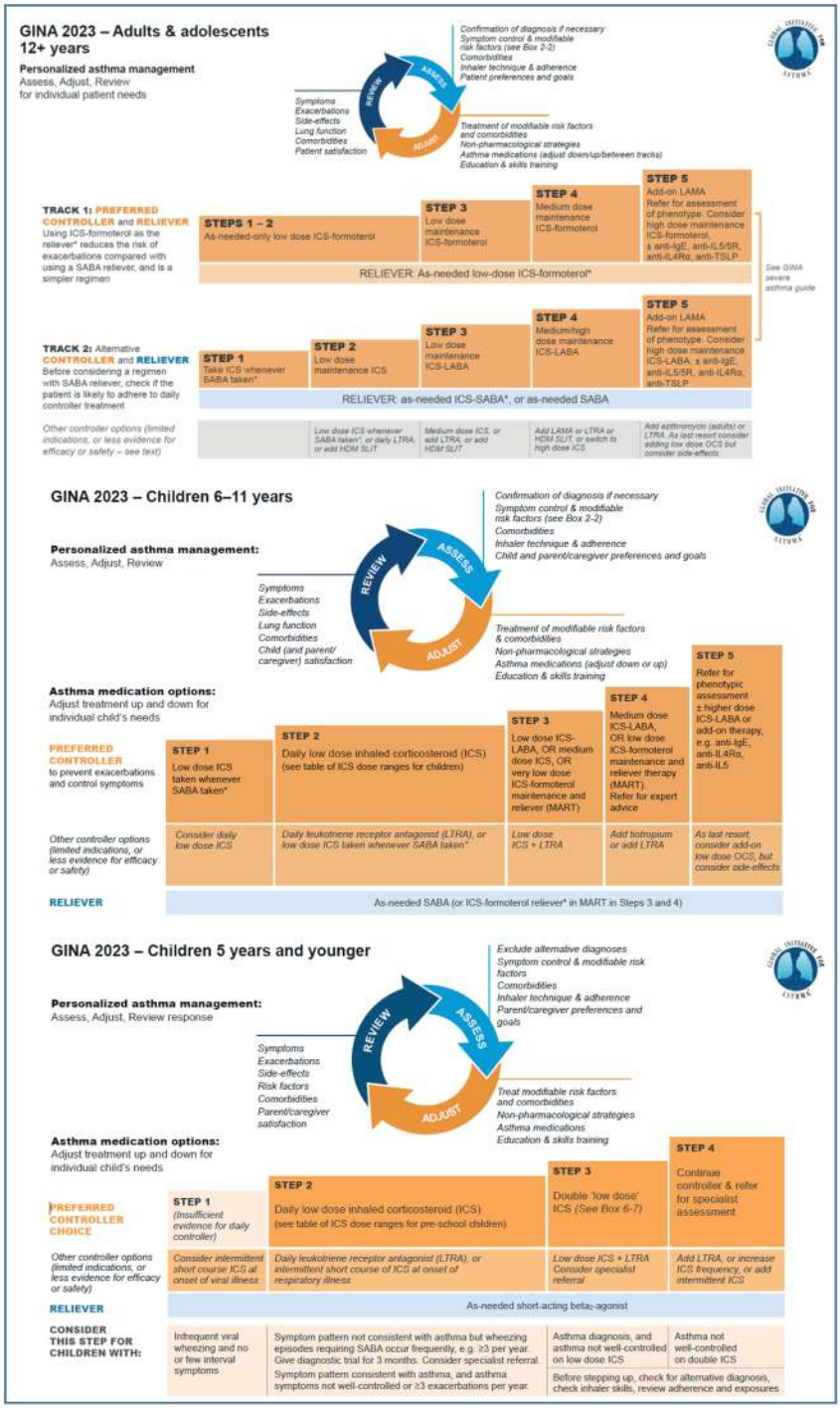

Figure 1. From Global Initiative for Asthma 2023, available from ginasthma.org, published in Fontana, WI, USA, all rights reserved.

lungs at a low level; however, in asthma, NO production is increased and is, in turn, resulting in a high level of FENO.[40] Although use of FENO to manage asthma control is currently not recommended by the GINA guidelines,[5] it might support management of children with asthma using ICS.[40] Furthermore, it has been demonstrated that FENO plays a role in the prediction of asthma exacerbations where higher levels of FENO are associated with an increased risk of asthma exacerbations.[41]

In addition, genetic variations might also be an important element in the variation of treatment response.[42-44]

CHRONIC OBSTRUCTIVE PULMONARY DISEASE (COPD)

As defined by the Global Initiative for Chronic Obstructive Lung Disease (GOLD) guidelines "COPD is a heterogeneous lung condition characterized by chronic respiratory symptoms (dyspnea, cough, sputum production) due to abnormalities of the airways (bronchitis, bronchiolitis) and/or alveoli (emphysema) that cause persistent, often progressive, airflow obstruction."[45]

Pathogenesis and pathophysiology of COPD

In patients with COPD, the augmented response to long term exposure to noxious particles and gases may lead to abnormal inflammatory immune responses of the lungs, resulting in mucous hypersecretion (chronic bronchitis), lung tissue parenchymal destruction (emphysema), and abnormal repair defense mechanisms resulting in small airway inflammation and fibrosis (bronchiolitis).[45-47] In COPD, neutrophils, macrophages, and T lymphocytes (CD8 and CD4) increase in the lung (Table 1).[46] In some patients with COPD, eosinophils may also increase.[47] The inflammatory cells release a variety of cytokines and mediators that are involved in the progression of the disease. Several inflammatory mediators are elevated in COPD, including leukotriene B4, chemotactic factors, pro-inflammatory cytokines, and growth factors.[1,46] In addition to inflammation, two other processes contribute to the pathogenesis of COPD, namely oxidative stress and proteases and antiproteases imbalance.[46,48] The above pathogenesis, in turn, results in mucous hypersecretion, airflow limitation, gas exchange abnormalities, pulmonary hypertension, and systemic effects of COPD.[45,49-53]

Epidemiology of COPD

According the GOLD guidelines, "COPD results from Gene (G)-environment (E) interaction occurring over the lifetime (T) of the individual (GET omics) that can damage the lung and/or alter their normal development/aging process".[45] To put it another way, COPD is due to the complex interaction of long-term exposure to noxious gases, host factors (i.e., genetic susceptibility, obesity, and poor lung growth during childhood), and airway hyper-responsiveness.[45,54-56]

COPD is a major public health burden that has been counted as the third leading cause of death worldwide, responsible for over 3 million deaths in 2019.[45,57] According to the Global Burden of Diseases (GBD) study in 2017, COPD was the seventh leading cause of years of life lost.[21] A systematic review and modelling analysis by Adeloye et al. reported a global prevalence of COPD among people aged 30–79 years of 10.3% (95% CI 8.2–12.8) in 2019.[58] In the coming decades, the burden and prevalence of COPD are expected to increase due to ongoing exposure to risk factors and the aging population worldwide.[59] Smoking is an important risk factor for COPD[45] reflected in the prevalence of COPD which is higher in current smokers and ex-smokers compared to non-smokers.[60] Data from the United States Risk Factor Surveillance System (BRFSS) for 2017 showed that the prevalence of COPD was 15.2% among current smokers, 7.6% among former smokers, and 2.8% among never smokers.[61] Also, the overall prevalence is higher among men than women and increases with age in both smokers and never smokers.[58,62]

Diagnosis of COPD

According to the GOLD guidelines, patients with dyspnea, chronic cough or sputum, and/or a history of exposure to COPD risk factors such as host factors, smoking, and household and/or occupational air pollution, should be considered as potential COPD patients and require further investigation.[45] Spirometry is required to establish the diagnosis where the post-bronchodilator ratio of $FEV_1/FVC < 0.7$ needed to confirm the airflow limitation.[45] As a consequence, the use of the fixed ratio (< 0.7) may lead to over-diagnosis of COPD among the elderly as well as a under-diagnosis among individuals younger than 45.[63] Accordingly, the ATS and ERS nowadays recommend using the lower limit of normal (LLN) as a cut-off value ($FEV_1/FVC<LLN$).[64] Similar to the reference values of age, height, sex, and race, the LLN values are based on the normal distribution where the lower 5% of the healthy population are considered as abnormal.[64] Clinicians require a simple and consistent diagnostic process. Therefore, the GOLD guidelines prefer fixed ratios to LLNs.[45]

Treatment of COPD

So far, COPD cannot be cured but pharmacological treatment can decrease symptoms, can reduce the risk and intensity of exacerbations and improve the health status and exercise capacity of COPD patients.[45]

Treatment consists primarily of maintenance therapy and reliever therapy. The purpose of reliever therapy is to relax the airway to make breathing easier, and is often used during an acute exacerbation. Reliever therapy consist of short-acting β_2-agonists (SABA) or short-acting muscarinic antagonists (SAMA). Maintenance therapy reduces airway inflammation and typically consists of LABA or long-acting muscarinic antagonists (LAMA), which may be combined with ICS, theophylline, or phosphodiesterase type 4 inhibitors. The GOLD guidelines recommend use of maintenance therapy over reliever therapy unless for patients with sporadic dyspnea and

for instant relief of symptoms in patients as already on maintenance therapy. Combined use of bronchodilators with diverse mechanisms and durations of activity may enhance bronchodilation with fewer adverse effects than extending the dose of a single bronchodilator.[45]

COPD exacerbations

COPD exacerbations are defined as episodes of acute worsening of respiratory symptoms requiring additional therapy.[45,65] Exacerbations of COPD are complicated events that are linked to an increase in inflammation of the airways, a rise in mucus production, and gas trapping.[45] Exacerbations are the leading cause of hospitalization and are responsible for 10% of all acute hospital admissions, increasing mortality and morbidity rates as well as the overall burden of disease.[66] According to the GOLD guidelines,[45] exacerbations are categorized as:

- Mild exacerbations requiring treatment only with short-acting bronchodilators.
- Moderate exacerbations requiring treatment with short-acting bronchodilators and antibiotics and/or oral systemic corticosteroids.
- Severe exacerbations requiring hospitalization or emergency room visits.

COPD comorbidity

Patients with COPD often experience other comorbidities, including cardiovascular disease, skeletal muscle dysfunction, osteoporosis, depression, anxiety, metabolic syndrome, and lung cancer.[67-69] It is unclear if this association is caused by common risk factors (e.g., smoking), genetic susceptibility, or impaired clearance of carcinogens.[45] Patients with multiple co-morbidities may experience a decline in health-related quality of life (HRQoL), and they are more likely to experience exacerbations.[70,71] Likewise, patients who suffer from frequent exacerbations tend to suffer from more comorbid conditions.[69] Also, co-morbidities affect mortality, hospitalization, and the need for particular treatment.[69] Thus, according to the GOLD guidelines, co-morbidities should routinely be evaluated in patients with COPD, and appropriate treatment should be provided.[45]

Management of COPD and COPD exacerbations

In the GOLD guidelines for COPD management, symptomatic assessment was combined with the patient's symptomatology and/or risk of exacerbation (i.e., the number of exacerbations and hospitalizations) into one tool known as the ABE (previously ABCD) assessment tool, as described in Figure 2.[45]

A patient with stable COPD has well-controlled symptoms and minimal pulmonary decline, whereas a patient with unstable COPD (who experiences frequent or severe exacerbations and a rapid decline in pulmonary function) may require more intensive management.[45] The management of stable COPD patients not only aims to reduce current symptoms, improve health status, and advance exercise tolerance but also aims to prevent disease progression, prevent

and treat exacerbation, and reduce mortality. The treatment of COPD exacerbations aims to reduce the negative effects of the current exacerbation and to prevent future exacerbations.[45] It should be noted that as per the recent GOLD guidelines, the LABA + LAMA combination has the highest treatment effect on reducing COPD exacerbations.[45,72] Furthermore, the use of LABA + ICS for COPD is not recommended. In cases where an ICS is indicated, LABA + LAMA + ICS has been demonstrated to be superior to LABA + ICS.[45,73]

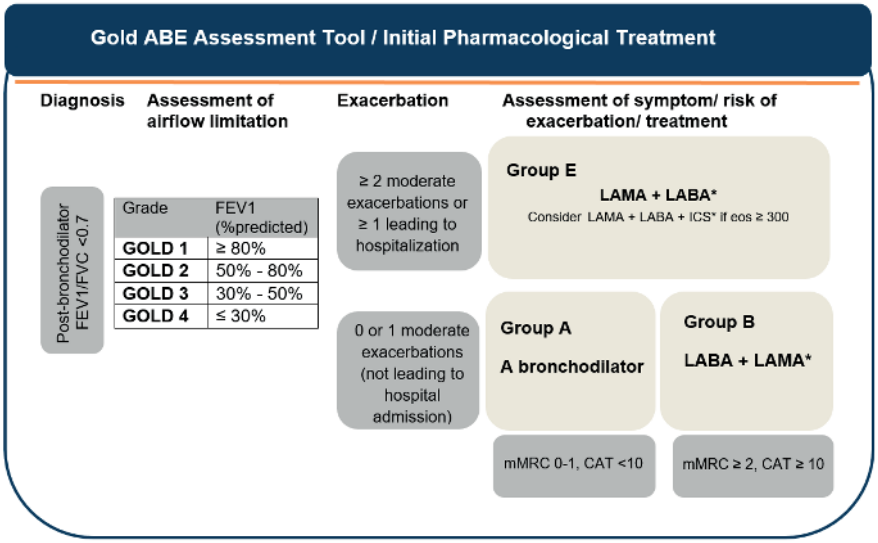

Figure 2. Reproduced from Global Initiative for Chronic Obstructive Lung Disease, 2024. Available from: https://goldcopd.org/. *Single inhaler therapy may be more convenient and effective than multiple inhalers. Abbreviations: eos, blood eosinophil count in cells per microliter; mMRC, modified Medical Research Count dyspnea questionnaire; CAT, COPD Assessment Test.

ROLE OF GENETICS

The term omics refers to various research areas in biology including genomics, epigenomics, transcriptomics, proteomics, and more. (Figure.3).

Genetics (genomics) is the study of changes to DNA (Deoxyribonucleic Acid) sequence, such as single nucleotide change in DNA sequence. Genetic factors are related to susceptibility to chronic obstructive lung diseases such as asthma and COPD, response to treatment, and clinical outcomes.[74-76]

Pharmacogenomics studies evaluate the association between variations in genes across the entire genome and treatment response.[78] The most prevalent type of genetic variation in the

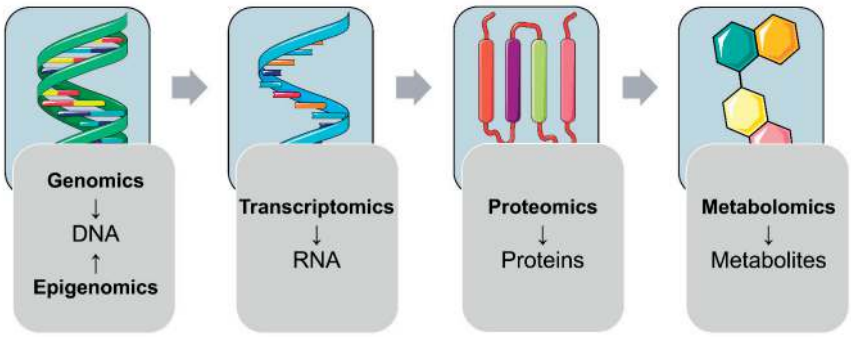

Figure 3. A brief overview of the various Omics fields that address different classes of molecules. A multi-Omics approach can be used to address interactions between different classes of molecules.[77] Reproduced from Shashikadze et al., 2021 Developmental Effects of (Pre-) Gestational Diabetes on Offspring: Systematic Screening Using Omics Approaches; Genes. 2021, 12, 1991, Issue 12, Fig. 1.

human genome are single nucleotide polymorphisms (SNPs), which are often examined through candidate gene and genome-wide association studies (GWAS).

A SNP is an alteration to a single base of DNA.[79] There are approximately 10 million common SNPs (frequency of 1% or more) in the human genome.[80] SNPs that are more common in a particular population are referred to as major alleles (frequency >50%), while SNPs that are less common are referred to as minor alleles (frequency <50%).[81] SNPs in the protein-coding region may be synonymous which modify the DNA sequence however do not alter the protein coding sequence, or nonsynonymous implying an alternation of the DNA sequence in a coding region which result in changing the amino acid coding.[79] Candidate gene studies are hypothesis-driven where genes of interest are chosen on the basis of prior knowledge of the disease biology or the drug signaling pathway.[82] GWAS is a hypothesis-generating approach that helps to identify the association between SNPs and complex traits, as well as disease pathogenesis, to learn more about the disease process and optimize preventive and therapeutic interventions.[83]

In the last decades, candidate gene studies focused on genes that might be associated with COPD and asthma pathogenesis.[75,84] GWAS have also discovered several loci related to lung function, emphysema, COPD phenotypes, asthma susceptibility, asthma phenotypes, and mechanisms of obstructive pulmonary diseases.[85-94]

A large number of pharmacogenomics (GWAS and candidate genes) studies has investigated the influence of genetic variations on response to asthma medications (ICS, LTRA, SABA, and LABA).[95,96] In COPD however, only a few GWAS and candidate gene studies investigated the influence of genetic variations on treatment response.[74,75,97] It should also be noted that variation in *ADRB2*, which codes for the beta$_2$-adrenergic receptor, is usually assumed to be associated

with LABA or SABA treatment outcomes since it plays a central role in its mechanism of action.[75,96]

Epigenomics investigate genome-wide modifications of DNA or DNA-associated proteins, such as DNA methylation and histone acetylation that regulate gene expression but do not alter the DNA sequence.[98,99] DNA methylation has been the most extensively studied epigenetic mechanism to date. DNA methylation takes place when a methyl group (-CH3) is added to the carbon in the cytosine (C) that is next to guanine (G) in the DNA sequence, resulting in 5-methylcytosine. Approximately 70-80% of C-G dinucleotides (CpG, 5'-C-phosphate-G-3') in human DNA are methylated.[100] It has been primarily discovered that epigenetic variation, particularly DNA methylation, plays a role in the development of cancer.[98,101] Epigenomics studies like epigenome-wide association studies (EWAS), have also revealed differential DNA methylation in association with the risks of asthma and COPD across the life course, poor lung function, COPD severity, systemic corticosteroid use in patients with COPD, and allergic inflammation in asthma pathogenesis.[102-106]

THE OBJECTIVES OF OUR RESEARCH

- To investigate the association between the *FCER2* T2206C variant and FENO levels in a large cohort of asthmatic children treated with ICS
- To identify genetic variants associated with asthma exacerbations in admixed children treated with ICS and to validate the findings in European children
- To identify genetic variants associated with asthma exacerbations despite ICS use in European children and young adults and to validate the findings in non-Europeans
- To assess the association between the haplotypes of the *ADRB2* variants and the risk of asthma exacerbations in patients treated with ICS plus LABA
- To examine the association between the use of β-blockers and the risk of COPD exacerbations
- To investigate the association between the *ADRB2* variants and risk of exacerbations in COPD patients
- To evaluate whether DNA methylation is associated with COPD exacerbations
- To investigate different patterns of adherence to COPD maintenance therapy

DATA SOURCES

For most of our research questions on COPD we used data from the Rotterdam Study which is an ongoing prospective population-based cohort study comprising inhabitants of the Ommoord

district of Rotterdam, the Netherlands.[107] In addition to the Rotterdam Study, an investigation of treatment adherence in COPD patients was conducted using data from two primary care databases: the Integrated Primary Care Information database (IPCI)[108] and The Health Improvement Network (THIN).[109,110] The IPCI database (the Netherlands) contains 2.5 million patient electronic health records, with a median follow-up of 4.8 years and includes patient demographics, information about contacts with general practitioners, symptoms, diagnoses, laboratory and clinical measurements, prescriptions, and information about secondary care use.[111] It was estimated that there were 1385805 active patients as of 1 July 2021.[111] In THIN database, data were drawn from more than 700 general practices throughout the United Kingdom. A significant advantage of these databases (IPCI and THIN) is that they provide accurate data from real-life practice. For this thesis, we had also access to the data from the Pharmacogenomics in Childhood Asthma (PiCA) consortium.[112] The PiCA consortium was established in 2013 in order to enhance international collaborations and conduct large-scale pharmacogenomics studies in children with well-defined asthma across different ethnic groups. Data from the PiCA consortium includes amongst other data sources also data from the Effectiveness and Safety of Treatment with Asthma Therapy (ESTATe) study and from the Pharmacogenetics of Asthma Medication in Children: Medication with Anti-inflammatory effects (PACMAN). ESTATe used IPCI data from children and young adults with asthma in combination with genetic data to assess effectiveness and safety of treatment in children.[113] The PACMAN study in the Netherlands, is an observational cohort study that included children (age: 4-12 years) with self-reported regular use of asthma medication recruited through community pharmacies.[114]

Table 2. List of data sources that were used in this thesis

Chapter	Database	Population	Outcome
2.1	PACMAN Study	Children	FENO levels
2.2	PICA Consortium	Children and young adults	Asthma exacerbations
2.3	PICA Consortium	Children and young adults	Asthma exacerbations
2.4	PICA Consortium	Children and young adults	Asthma exacerbations
3.1	Rotterdam Study	Elderly population	COPD exacerbations
3.2	Rotterdam Study	Elderly population	COPD exacerbations
3.3	Rotterdam Study	Elderly population	COPD exacerbations
4	IPCI and THIN	Adults	Adherence to COPD maintenance therapy

OUTLINE OF THIS THESIS

We provided a general introduction in **Chapter 1.** In **Chapter 2.1,** we described the association between the *FCER2* T2206C variant and FENO levels in patients with asthma treated with ICS. In **Chapter 2.2**, a large-scale meta-analysis was performed to assess the association between the polymorphisms in *ADRB2* and risk of asthma exacerbations in patients treated

with ICS plus LABA. In **Chapter 2.3,** we performed a GWAS meta-analysis in admixed children and young adults to identify novel associations of genetic variants with ICS response in subjects with asthma exacerbations, and we validated the findings in European children. Then, in **Chapter 2.4,** we performed a GWAS meta-analysis of asthma exacerbations in children and young adults treated with ICS in European populations, and the results were validated in non-European children. In **Chapter 3.1,** we examined whether the association between the use of β-blockers and the risk of COPD exacerbations differed between patients with or without a cardiovascular indication for the use of β-blockers. In **Chapter 3.2,** we investigated the association between two functional SNPs of the *ADRB2* gene, rs1042713 and rs1042714, and risk of exacerbations in COPD patients treated with β_2-agonists. In **Chapter 3.3,** we examined the association between DNA methylation sites and exacerbation rate in COPD patients. In **Chapter 4**, we investigated adherence to COPD maintenance therapy. Finally, in **Chapter 5**, we discussed the key findings of the studies included in this thesis, addressed methodological considerations, and outlined recommendations for future research.

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2

The role of genetics in asthma

2.1

FCER2 T2206C variant associated with FENO levels in asthmatic children using inhaled corticosteroids: the PACMAN study

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Clin Exp Allergy . 2019 Nov; 49(11):1429-1436. doi: 10.1111/cea.13460.

ABSTRACT

Background: The *FCER2* gene, via encoding of the CD23 receptor, plays an important role in the regulation of IgE responses. A genetic variant of the *FCER2* gene (T2206C) was previously shown to be associated with IgE levels in asthmatic children. IgE sensitization has also been linked to increased levels of fractional exhaled nitric oxide (FENO).

Objective: To investigate whether the *FCER2* T2206C variant influences FENO levels in asthmatic children with a reported use of inhaled corticosteroids (ICS).

Methods: This cross-sectional study involved 593 asthmatic children with a reported use of ICS, availability of FENO measurements and genotyping data on the *FCER2* T2206C variant (rs28364072). An additive genetic model was assumed, and the association between the *FCER2* T2206C variant and the log-transformed (ln) FENO levels was evaluated using linear regression analysis, adjusted for age, sex, adapted British Thoracic Society (BTS) treatment steps, and atopy.

Results: The mean age of the population was 9.1 ± 2.2 years, and the median of FENO levels was 13.0 ppb with an interquartile range (IQR) of (8.0-27.5 ppb). The minor allele (G) frequency of rs28364072 was 29.6%, and each extra copy of the G allele was significantly associated with a lower level of the geometric mean of FENO (log scale, $\beta = -0.12$, 95% CI: −0.23, −0.02).

Conclusion and Clinical Relevance: Our results showed that the *FCER2* T2206C variant was significantly associated with lower FENO levels in carriers of the G allele. Nevertheless, this SNP contributed little to the variability in FENO levels in this patient population. Our findings contribute to the present knowledge on FENO in asthmatic children; however, future replication studies are required to establish the role of this gene in relation to FENO.

Keywords

Asthma, *FCER2* T2206C variant, fractional exhaled nitric oxide, inhaled corticosteroid

INTRODUCTION

Asthma in children is often atopic, IgE-mediated,[1-3] and characterized by chronic airway inflammation.[4] The key regulator of IgE in asthma is the low-affinity IgE receptor, also known as CD23. CD23, a C-type lectin, is expressed on B cells, macrophages, eosinophils, follicular dendritic cells, and platelets.[5] Activation of this receptor suppresses the production of IgE[6] that results in downregulation of the IgE-mediated immune response.[7] The CD23 receptor is encoded by the Fc fragment of the IgE receptor II (*FCER2*) gene which is an 11-exon gene located at chromosome 19p13.2. Variants of *FCER2* could modify the IgE synthesis.[7] Furthermore, in asthmatic patients, the rs28364072 polymorphism (T2206C) in *FCER2* has previously been shown to be associated with elevated IgE,[8,9] exacerbations,[8-10] increased daily need of inhaled corticosteroids (ICS),[8,9] and poor lung function.[9,10]

Nitric oxide (NO) is a biomarker used in respiratory disease and results from oxidation of L-arginine in the presence of nitric oxide synthase (NOS). Three different isoforms of NOS exist: two constitutive NOS (cNOS) isoforms and one inducible NOS (iNOS) isoform. The expression of iNOS is boosted by inflammatory stimulation.[11] NO, at higher concentrations, appears to function as an inflammatory agent.[12] Previous studies showed that the fractional exhaled nitric oxide (FENO) was raised in the airways of patients with asthma[11,13] and was positively associated with higher IgE levels in asthmatic patients.[14,15] Many factors such as age, height, race, use and adherence to asthma medication, diet, exercise, and environmental factors might affect FENO levels.[16,17] Dugas et al suggested that CD23 has a major regulatory effect on iNOS activation in human monocytes and leads to NO production.[18] Furthermore, Kolb et al[19] found that the NOS pathway is involved in IgE-mediated activation of monocytes. They also proposed that the increase of CD23-driven cAMP (cyclic adenosine monophosphate) in monocytes is partly linked to the NOS pathway.[19]

A study in Vietnamese children with uncontrolled and untreated asthma (n = 32)[20] showed that the levels of FENO were highest in the homozygous carriers (CC) of rs28364072 variation in the *FCER2* gene. Their results imply that polymorphisms in the *FCER2* gene are associated with FENO levels. However, no studies have evaluated this association in asthmatic children being treated with asthma medication.

In this study, we aimed to investigate the association between the *FCER2* T2206C variant and FENO levels in a large cohort of asthmatic children treated with inhaled corticosteroids (ICS).

METHODS

Study setting and population

The study was undertaken in the PACMAN (Pharmacogenetics of Asthma medication in Children: Medication with Anti-inflammatory effects) cohort study. Details of the study protocol have been described elsewhere.[21] In short, the PACMAN study includes children (aged 4-12 years) who were selected through pharmacies that belonged to the Utrecht Pharmacy Practice Network for Education and Research (UPPER).[22] Children who regularly used asthma medication (Anatomical Therapeutic Chemical code R03), namely ≥3 prescriptions within the last 2 years and ≥1 prescription(s) in the last 6 months, were recruited through community pharmacies in the Netherlands.[21] Selected children and their parents visited their own pharmacy, and during the visits, parents filled in a questionnaire that provided information about their children: respiratory symptoms, allergy, asthma diagnosis, medication use and adherence, socio-demographic factors, and environmental factors.[21] The Asthma Control Questionnaire (ACQ)[23] was also included in the questionnaire. In addition, FENO levels were measured (NIOX Mino; Aerocrine), and saliva samples were collected for DNA extraction (Oragene DNA Self-Collection kit; DNA Genotek, Inc., Kanata, ON, Canada). In the PACMAN study, daily ICS dosages (defined daily dosages of budesonide equivalent) were based on the last recorded refill prescription in the pharmacy prior to the study visit. The parents provided written informed consent, and the PACMAN cohort study was approved by the Medical Ethics Committee of the University Medical Centre Utrecht. The study population for our analysis consisted of all asthmatic children treated with inhaled corticosteroids (ICS) and who also had FENO measurements, genotyping, and questionnaire data.

Definition of fraction of nitric oxide in exhaled breath

FENO was measured during the baseline visit at the pharmacy by using a single-breath on-line (SBOL) technique which was performed with a hand-held electrochemical analyzer (NIOX Mino; Aerocrine, Solna, Sweden) during an exhalation time of 6 seconds.[24]

Genotyping

Genotyping was performed using Human Core-24 BeadChip Marker information, and the quality control (QC) procedures were applied to the genotype data. Data were imputed using the second release of the Haplotype Reference Consortium[25] (HRC) (realizes 1.1 2016) by mean Michigan Imputation Server.[26] We extracted genotype dosage for rs28364072 in the *FCER2* gene with a high imputation quality (Rsq score = 0.98). The Rsq score is defined as the ratio of the sample variance of the allele dosage during imputation to the expected variance under Hardy-Weinberg equilibrium (HWE).[27]

Potential confounders/co-variables

Potential confounders/covariates consisted of age, sex, (adapted) British Thoracic Society guidelines[28] treatment steps (BTS), and atopy. We defined treatment step as follows: step 0, no use of inhaled short-acting β_2-agonist; step 1, short-acting β_2-agonist as needed; step 2, step 1 plus regular ICS; step 3, step 2 plus long-acting β_2-agonist; step 4, step 3 plus oral leukotriene receptor antagonist. Since all patients were required to be on ICS, the study population was on BTS treatment step 2 or above.

Atopy was specified as a reported history of allergic rhinitis, eczema, or food allergy. Asthma control was measured with the help of the 6-item version of the Asthma Control Questionnaire (ACQ) (investigates respiratory symptoms and need of short-acting β_2-agonists).[29] A mean score of ACQ ≥ 0.75 indicated 'not well-controlled asthma' and an ACQ score of <0.75 indicated 'well-controlled asthma'.[23,30]

Functional annotation of SNPs in strong LD with rs28364072

We retrieved all proxy SNPs in high linkage disequilibrium (LD) ($R^2 > 0.8$, limit distance 100 kb, and population panel CEU using 1000 Genomes project) with rs28364072 in *FCER2* and checked their predicted functions, effects on protein structure, gene regulation, and splicing, using the HaploReg v4.1 (http://www.broadinstitute.org/mammals/haploreg/haplo reg.php; in the public domain).

Expression quantitative trait loci analysis

The correlation of the SNP rs28364072 and its proxies in high LD with the expression level of *FCER2* gene in whole blood was checked (expression quantitative trait loci [eQTL] analysis) using publicly available data from (GTEx) portal (GTEx portal: www.gtexp ortal.org/home/) and GeneNetwork.[31] Moreover, we checked the effect of the SNP on *FCER2* expression across different tissues using the GTEx portal.

Statistical analyses

Descriptive statistics were used to calculate means and standard deviations for continuous variables and percentages for categorical variables. For continuous variables that were not normally distributed, the median and interquartile range (IQR) were calculated. The chi-square test and Mann-Whitney U test were used to determine whether there was a significant difference in characteristics between children with well-controlled asthma and not well-controlled asthma. FENO values equal to zero parts per billion (ppb) were set at 2.5 ppb (the detection limit of the NIOX Mino = 5 ppb). Kruskal-Wallis test was used to assess whether there was a statistically significant difference in the concentration of FENO between three genotype categories of *FCER2* rs28364072 namely homozygous (GG), heterozygote (GA), and homozygous (AA). In pairwise comparison, Dunn's pairwise tests (also known as Dunn's post hoc tests) were used

on each pair of rs28364072 genotypes (GG vs AG, GG vs AA, and AG vs AA) to investigate the differences in FENO levels by genotype pairs. To control for multiple testing, we divided 0.05 by the number of tests (n = 3) being performed (Bonferroni correction); therefore, we considered a Dunn's P-value <0.016 (0.05/3) statistically significant. Mann-Whitney U test was used to evaluate whether there was a significant difference in FENO levels between the two categories: carrier of the mutant allele (GG and AG genotypes) and non-carrier of the G allele (AA genotype). To assess whether there was any effect of ICS dose on the levels of FENO in our study population, we tested the correlation between FENO levels and ICS dosage using the Spearman's rank test. We also tested this correlation in subgroups of carriers and non-carriers of the *FCER2* T2206C variant. Based on the GINA guideline[4] we categorized daily ICS dosage into three categories: low, medium, and high (Table S1). Kruskal-Wallis test was used to assess whether there was a statistically significant difference in the median FENO level between patients treated with low, medium, and high ICS dosages.

An additive genetic model was assumed, and the association between the *FCER2* T2206C variant and FENO concentrations was assessed using linear regression. As FENO was not normally distributed, FENO levels were used as log-transformed, natural logarithm (ln), for the regression analysis. The regression analysis was adjusted for age and sex in Model 1, and Model 2 was further adjusted for atopy as a dichotomous variable with two categories (0 = no and 1 = yes) and adapted BTS treatment steps. To investigate whether the association would differ between well-controlled asthma (ACQ < 0.75) and not well-controlled asthma (ACQ ≥ 0.75), we estimated the regression models in both. Next, we performed a sensitivity analysis and the FENO values equal to 0 ppb were set at 0 ppb and 5 ppb, respectively and the analyses were repeated. The HardyWeinberg package[32] (version 1.6.2) for R was applied to assess HWE using exact test with DOST (Double One-Sided Tail) P-value.[33] Statistical significance was considered at the P-value of <0.05 (two-sided). SPSS 24.0 software (IBM Corporation) was used for the analysis.

RESULTS

A data set of 593 children with reported use of ICS, FENO measurements, and *FCER2* genotype information was available within the PACMAN cohort. The general characteristics of the study population are shown in Table 1. The mean age of the population was 9.1 ± 2.2 years, and the majority of children were boys (62.1%). Of these children; 82% reported a history of atopy and 59% had well-controlled asthma. The median value of FENO was 13.0 ppb with an interquartile range (IOR) of 8.0-27.5 ppb. The median value of FENO was significantly lower in well-controlled asthma 13.0 ppb (IQR = 8.0-25.0 ppb) compared to not well-controlled asthma 16.0 ppb (IQR = 9.0-31.8 ppb) with P = 0.044. FENO values equal to zero ppb were found in 27 subjects

(19 boys) with a mean age of 7.9 ± 2.1.The minor allele (G) frequency of rs28364072 was 29.6%, and 10.5% of the participants were homozygous (GG) carriers of the *FCER2* T2206C variant. The SNP was in HWE in the total population (P = 0.051), in the category of well-controlled asthma (P = 0.121) and in the category of not well-controlled asthma (P = 0.219).

Table 1. Characteristics of study population

Baseline characteristics	**Total population**	**Well-controlled (ACQ < 0.75)**	**Not well-controlled (ACQ ≥ 0.75)**	**P[a]**
n	593	341/581[b]	240/581[b]	
Age, mean (± SD)	9.1 ± 2.2	9.0 ± 2.1	9.2 ± 2.2	
Male, % (n)	62.1 (368)	62.8 (127)	61.7 (148)	0.795
History of Atopy % (n)	82 (484/590)	82.4 (280/340)	82.5 (198/240)	0.963
Eczema, % (n)	66.9 (394/589)	65.9 (224/340)	69.9 (167/239)	0.313
Food allergy, % (n)	52.1 (306/587)	48.4 (164/339)	58.0 (138/238)	0.023
Hay fever, % (n)	46.4 (269/580)	45.4 (152/335)	48.5 (114/235)	0.460
Family history of Atopy				
Paternal asthma[C] % (n)	29.9 (163/546)	27.8 (88/317)	32.9 (72/219)	0.203
Paternal eczema[c] % (n)	27.6 (147/533)	28.0 (87/311)	27.7(59/213)	0.945
Paternal hay fever[c] % (n)	38.2 (206/539)	38.1(119/312)	39.2 (85/217)	0.811
Maternal asthma[c] % (n)	26.6 (147/553)	21.1 (66/313)	33.2 (76/229)	0.002
Maternal eczema[c] % (n)	39.9 (222/556)	36.5 (116/318)	44.1 (100/227)	0.075
Maternal hay fever[c] % (n)	44.3 (247/557)	43.2 (137/317)	46.3 (106/229)	0.476
Adapted BTS treatment step[d] % (n)				
2	69.1 (410)	74.7 (248)	66.8 (155)	
3	20.9 (124)	20.8 (69)	22.4 (52)	0.012
4	6.9 (41)	4.5 (15)	10.8 (25)	
Minor allele frequency *FCER2* variant %	29.6	29.5	29.9	
Genotype distribution T2206 variant				0.982
Homozygous wild type (AA) % (n)	51.4 (305)	51.6 (176)	50.8 (122)	
Heterozygous (AG) % (n)	38.1 (226)	37.8 (129)	38.3 (92)	
Homozygous variant (GG) % (n)	10.5 (62)	10.6 (36)	10.8 (26)	
FENO ppb, Median (IQR)	13.0 (8.0-27.5)	13.0 (8.0-25.0)	16.0 (9.0-31.8)	0.044
ACQ score, Median (IQR)	0.5 (0.17-1.17)	0.17 (0.0-0.5)	1.3 (1.0-1.8)	0.000

ACQ, Asthma Control Questionnaire; FENO, fractional exhaled nitric oxide; IQR, Interquartile range.

[a] P value; to evaluate the significant difference between the two categories, well-controlled asthma vs not well-controlled asthma, using chi square test or Mann Whitney U test.

[b] In the total population, data on the Asthma Control Questionnaire (ACQ) were missing in 12 subjects.

[c] Data were not available for the total population.

[d] 18 children were on ICSs plus leukotriene receptor antagonist treatment.

Levels of FENO in different *FCER2* variant genotypes

The levels of FENO were compared among the three genotypes of rs28364072 (Table 2 and Figure 1). The FENO levels were lowest in children with GG genotype (10.0 ppb, IQR: 7.0-22.2 ppb) and highest in AA genotype (16.0 ppb, IQR: 9.0-28.5 ppb). In pairwise comparison, there was significant evidence (unadjusted P-value) that the concentration of FENO was statistically significantly different between GG-AA genotypes and AG-AA genotypes (Table 2). After adjustment for multiple testing, the FENO levels remained significantly different between genotypes AA and AG (Table 2 and Figure 1). In the category of well-controlled asthma (n = 341), the FENO levels were significantly different between GG-AA genotypes and AG-AA genotypes; however, both were no longer significant after Bonferroni correction (Table 2). In the category of not well-controlled asthma (n = 240), there were no significant differences in FENO levels at all (P-value from Kruskal-Wallis test was equal 0.339; Table 2). We also found a significant difference in the FENO concentrations between the carriers of the mutant allele (GG and AG genotypes) and the non-carriers of the G allele (AA genotype) in the total population and well-controlled asthma category (Table 3).

Table 2. Concentration of FENO among genotypes of rs28364072

rs28364072 Genotypes	FENO ppb (IQR)	Pairwise comparison of FENO levels	P[b]
Total population (n = 593)[a]			
Homozygous variant (GG)	10.0 (7.0 - 22.2)	GG-AG	0.541
Heterozygous (AG)	12.0 (7.0 - 27.2)	GG-AA	0.029
Homozygous wild type (AA)	16.0 (9.0 - 28.5)	AG-AA	0.014
Well-controlled (n = 341)[a]			
Homozygous variant (GG)	10.0 (7.0 - 20.8)	GG-AG	0.526
Heterozygous (AG)	12.0 (7.0 – 23.0)	GG-AA	0.041
Homozygous wild type (AA)	14.5 (8.0 - 28.8)	AG-AA	0.029
Not well-controlled (n = 240)[a]			
Homozygous variant (GG)	14.0 (7.0 - 35.8)	GG-AG	
Heterozygous (AG)	13.0 (7.0 - 36.0)	GG-AA	0.339[c]
Homozygous wild type (AA)	18.0 (10.0 - 28.5)	AG-AA	

FENO: fractional exhaled nitric oxide.

[a]In the total population, data on the Asthma Control Questionnaire (ACQ) were missing in 12 subjects.

[b]Tested with Dun's P-value for pairwise comparison, a Bonferroni-corrected P-value lower than 0.016 (0,05/3) was considered statistically significant.

[c]P-value from Kruskal–Wallis test.

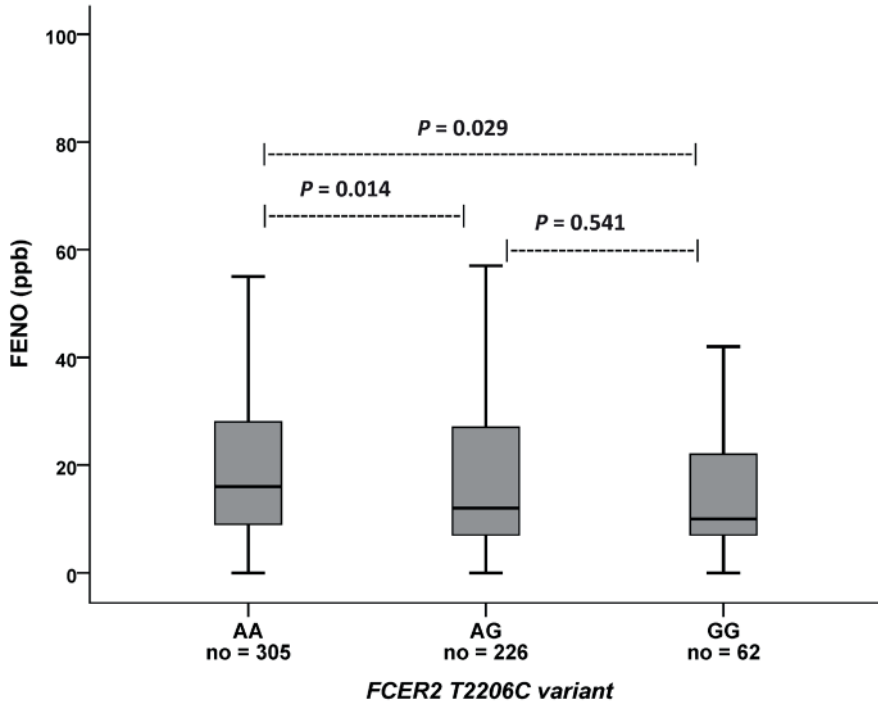

Figure 1. Concentration of FENO among genotypes of *FCER2* T2206C variant (rs28364072). FENO, fractional exhaled nitric oxide. The Kruskal–Wallis test was used and in pairwise comparison, a Dunn's *P*-value < 0.016 (0.05/3) was considered statistically significant.

Table 3. Concentration of FENO between carries and non-carriers of the G allele of rs28364072

rs28364072 variation	**n**	**FENO ppb** median (IQR)	**P**[a]
Total population (n = 593)			
Carriers of the variant (GG/AG)	288	12.0 (7.0 - 25.7)	0.004
Non-carriers of the variant (AA)	305	16.0 (9.0 - 28.5)	
Well-controlled category (n = 341)[b]			
Carriers of the variant (GG/AG)	165	11.0 (7.0 - 23.0)	0.010
Non-carriers of the variant (AA)	176	14.5 (8.0 - 28.8)	
Not well- controlled category (n = 240)[b]			
Carriers of the variant (GG/AG)	118	13.0 (7.0 - 36.0)	0.146
Non-carriers of the variant (AA)	122	18.0 (10.0 - 28.5)	

FENO: fractional exhaled nitric oxide; IQR: Interquartile range. [a]P-value. Tested with Mann–Whitney U test.
[b]In the total population, data on the Asthma Control Questionnaire (ACQ) were missing in 12 subjects.

Association of *FCER2* variant with FENO levels

The associations of the *FCER2* variant with FENO levels are presented in Table 4. In the crude model, each extra copy of the G allele of rs28364072 was significantly associated with a lower level of the geometric mean of FENO (log scale, β = −0.15, 95% CI: −0.26, −0.05). When we adjusted for age and sex (Model 1) and also after further adjustment for BTS treatment steps and a reported history of atopy (Model 2), the estimated effects remained statistically significant.

Table 4. Regression coefficients and 95% confidence intervals describing the association between rs28364072 variation of the *FCER2* gene (per copy of the G allele) and the concentration of FENO

FENO[a]	Effect allele	Crude		Model 1		Model 2	
		ß (95% CI)	p	ß (95% CI)	p	ß (95% CI)	p
Total population (n = 593)	G	-0.15 (-0.26 , -0.05)	0.005	-0.12 (-0.22 , -0.02)	0.021	-0.12 (-0.23 , -0.02)	0.018
Well-controlled (n = 341)	G	-0.18 (-0.32 , -0.05)	0.007	-0.16 (-0.29 , -0.03)	0.019	-0.17 (-0.30 , -0.04)	0.011
Not well-controlled (n = 240)	G	-0.12 (-0.29 , 0.06)	0.190	-0.09 (-0.25 , 0.08)	0.306	-0.08 (-0.25 , 0.09)	0.331

Note: In the total population, data on the Asthma Control Questionnaire (ACQ) were missing in 12 subjects. Crude, only SNP; Model 1, adjusted for age and sex; Model 2, Model 1 further adjusted for (adapted) British Thoracic Society (BTS) treatment steps and atopy. ACQ, Asthma Control Questionnaire; CI, Confidence Interval; FENO, Fractional exhaled Nitric Oxide; Not well-controlled, ACQ ≥ 0.75; Well-controlled, ACQ < 0.75.

[a]FENO levels were used as log-transformed (ln)

Association of *FCER2* variant with FENO levels stratified by ACQ scores

We tested the association between the variant and FENO levels when the total population was stratified in well-controlled asthma (ACQ < 0.75) and not well-controlled asthma (ACQ ≥ 0.75). In Model 2, adjusted for age, sex, BTS treatment steps, and atopy, each extra copy of the G allele of rs28364072 was significantly associated with a lower level of the geometric mean of FENO (log scale, β = −0.17, 95% CI: −0.30, −0.04) among children with well-controlled asthma. We did not observe any significant association (log scale, β = −0.08, 95% CI: −0.25, 0.09) between the *FCER2* variant and FENO levels in the not well-controlled asthma group (Table 4). However, the point estimate was in the same direction as well-controlled asthma category.

We performed sensitivity analyses whereby the FENO values equal to 0 ppb were set at 0 and 5 ppb, respectively. The results of these analyses were similar to the results of our original analyses. (Table S2).

Correlation of FENO levels and ICS daily dosage

Data on ICS dosage were available for 475 out of 593 subjects. There was no correlation between FENO levels and ICS dosage (Spearman's rank correlation coefficient [RS] = −0.016, P = 0.736). When subjects were grouped according to defined daily ICS dosage (low, medium, and high), higher FENO levels could be observed in the patients treated with low dosages

of ICS category (median FENO: 17.0ppb [IQR: 8.0-30.0]) compared to patients treated with medium ICS dosages (median FENO: 14.00 ppb [IQR: 8.0-23.0]) and high ICS dosages (median FENO: 11.5 ppb [IQR: 7.0-25.7]); however, the differences were not statistically significant. In addition, there was no significant correlation between FENO levels and ICS dosage in carriers or non-carries of the *FCER2* T2206C variant (RS = 0.025, P = 0.703 and RS = –0.038, P = 0.554, respectively).

Functional annotation and eQTL analysis of rs28364072

Functional annotation, using Haploreg v4.1 data, shows that rs28364072 has several proxy variants ($D' = 1$ and $R^2 > 0.8$), but they are all intronic and synonymous, without any predicted functions, (Table S3). Moreover, the cis-eQTL data from GTEx portal and GeneNetwork[31] showed that the minor allele G of rs28364072 is significantly associated with reduced expression levels of *FCER2* in whole blood (Table S4 and Figure S1 and Figure S2). Together, these data may support the notion that rs28364072 has an effect on *FCER2* gene expression and function.

DISCUSSION

In our population-based study, we assessed the association between the *FCER2* T2206C variant and FENO levels in children with asthma and reported use of ICS. Our results showed that the variation in the *FCER2* gene was significantly associated with lower levels of FENO in the total population and in well-controlled asthmatic patients. There was no statistically significant association between the *FCER2* polymorphism and levels of FENO in not well-controlled asthma group which might be explained by the small sample size of this group.

To the best of our knowledge, the current study is the first to evaluate the association between the *FCER2* T2206C variant and FENO levels among asthmatic children using ICS. So far only one study has investigated the same association but in mild-to-moderate uncontrolled and untreated asthmatic patients among Vietnamese children (n = 32), and they found that FENO levels were significantly higher in subjects with the *FCER2* gene mutation.[20] However, due to a small sample size they were not able to define a specific FENO level that was associated with the *FCER2* gene variation.[20] In the study by Nguyen-Thi-Bich et al,[20] all subjects with uncontrolled asthma were not on medication for at least one month before inclusion, while in the PACMAN study all subjects were on ICS treatment and ICS therapy is known to decrease FENO levels.[34] In addition, the frequency of the rs28364072 homozygous variant of the *FCER2* gene was slightly higher among Vietnamese children (15.6%) than (10.5%) in the mainly Caucasian children (91.4%) in the PACMAN study. Moreover, the sample size (n = 593) of the current study was roughly 19 times larger than the Vietnamese study (n = 32).

It has previously been shown that IgE is associated with FENO levels in children with asthma and allergy.[14,15] The low-affinity IgE receptor, CD23, by nature inhibits IgE production.[6] The *FCER2* gene affects the inflammatory mechanisms and results in the variability in the response to ICS in asthma.[35] The T2206C variant is located in splicing region of intron 9 of *FCER2* and might lead to alternative splicing and changes of the gene transcript length. Tantisira et al showed that the T2206C variant was associated with lower *FCER2* gene expression.[9] This was suggested to be a possible mechanism for the higher IgE levels among carriers of the mutant (C) allele in their findings.[9] In animal models, it was confirmed that absence of CD23 (CD23-/-) in the knockout mice resulted in elevated IgE-mediated response compared with wild-type CD23+/+.[36,37] In addition, use of corticosteroids might be associated with a decrease in CD23 expression,[38] as Tantisira et al[9] noticed that the highest IgE levels were detected in individuals who were homozygote (CC) for the *FCER2* T2206C variant and on ICS treatment. However, in our study population we did not observe a statistically significant effect of ICS dosage on FENO levels. There is some evidence from the literature that during an inflammatory response the CD23 receptor induces iNOS activation that in turn leads to NO production (Figure S3).[18] As shown previously, genetic expression of CD23 decreased in individuals homozygous for T2206C variant compared with the two other genotypes.[9] which is in line with eQTL data demonstrating decreased expression levels of *FCER2* in the carriers of T2206C variant (Table S3). This might lead to less NO production and consequently lower levels of FENO among carriers of the variant; hence, the observed lower FENO levels in carriers of the *FCER2* T2206C variant is plausible.

It should be noted that children in our study with not well-controlled asthma had a high percentage of food allergy and maternal asthma (Table 1). It has been shown that food allergy is associated with an increased risk of hospitalization and use of systematic corticosteroids due to asthma.[39,40] In addition, it has been reported that maternal asthma is a crucial risk factor for developing asthma in childhood.[41,42]

In our study, compared to non-carriers of *FCER2* T2206C variant, FENO levels in the carriers of the variant were significantly lower with a median difference of 4 ppb (Table 3). Further research is needed to monitor FENO over time in *FCER2* T2206C carriers to better understand the clinical relevance of this finding.

A major strength of this study was the availability of information on asthma symptoms and FENO measurements in a large real-life population of pediatric patients. Still, some limitations need to be addressed. First, atopy was defined based on parental reporting in the questionnaire and was not defined based on a skin prick test. The lack of an objective criterion such as the skin prick test thus might lead to both an underestimation (in case of asymptomatic patients) and an overestimation of atopy. Similarly, asthma symptoms were parent-reported which might be prone to over- or underestimation. Lastly, as gene expression is tissue specific, it would be

better to also check the association between T2206C variant and *FCER2* gene expression in lung tissue; however, so far, eQTL data from lung tissue is either not available or only in very small sample size.[31]

To conclude, we observed that the *FCER2* T2206C variant was significantly associated with lower levels of FENO in children with well-controlled asthma treated with ICS. However, this SNP contributed little to the variability in FENO levels in this patient population. We acknowledge that the exact mechanism by which the *FCER2* T2206C variant could modify FENO levels needs to be further explored and that future replication studies are required to establish the role of this gene in relation to FENO.

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SUPPLEMENTARY MATERIAL

Table S1. Low, medium and high daily doses of inhaled corticosteroids[S1]

Drug	Low	Medium	High
12 years and older			
Budesonide Daily dose (mcg)	200-400	>400-800	>800
Younger than 12 years			
Budesonide Daily dose (mcg)	100-200	>200-400	>400

Table S2. Regression coefficients and 95% confidence intervals describing the association between rs28364072 variation of the *FCER2* gene (per copy of the G allele) and the concentration of FENO

Outcome	n	Effect allele	Crude ß (95% CI)	P	Model 1 ß (95% CI)	P	Model 2 ß (95% CI)	P
FENO*	593	G	-0.16 (-0.27, -0.04)	0.008	-0.12 (-0.23, -0.01)	0.030	-0.12 (-0.23, -0.01)	0.033
FENO#	593	G	-0.15 (-0.25, -0.04)	0.005	-0.12 (-0.21, -0.02)	0.019	-0.12 (-0.22, -0.02)	0.014

FENO, Fractional exhaled Nitric Oxide; CI: Confidence Interval. FENO levels were used as log-transformed (ln).
*The FENO values equals to zero ppb were set at zero ppb. # The FENO values equals to zero ppb were set at 5 ppb. Crude, Only SNP; Model 1, Adjusted for age and sex; Model 2, Model 1 further adjusted for (adapted) British Thoracic Society (BTS) treatment steps and atopy

Table S3. Functional annotation of rs28364072 using the HaploRegv4.1

chr	pos (hg38)	LD (r^2)	LD (D')	variant	Ref	Alt	EUR freq	Enhancer histone marks	DNAse	Motifs changed	Selected eQTL hits	GENCODE genes / dbSNP func annot
19	7687177	0.82	0.99	rs1078625	A	G	0.32		BLD	11 altered motifs	4 hits	1.6kb 3' of *FCER2*
19	7689659	0.84	1	rs62110713	C	T	0.25	BLD		EBF		*FCER2*/ intronic
19	7689705	0.99	1	rs62110714	C	T	0.28	BLD		8 altered motifs		*FCER2*/ intronic
19	7689743	1	1	rs62110715	T	C	0.28	BLD	BLD	Ik-1,LUN-1		*FCER2*/ intronic
19	7689790	0.99	1	rs113483829	C	T	0.28	BLD	BLD,BLD	4 altered motifs		*FCER2*/ intronic
19	7689810	0.99	1	rs111674746	G	C	0.28	BLD	BLD,BLD			*FCER2*/ intronic
19	7689816	0.99	1	rs113453074	G	T	0.28	BLD	BLD	5 altered motifs		*FCER2*/ intronic
19	7689819	0.99	1	rs111740138	A	G	0.28	BLD	BLD	4 altered motifs		*FCER2*/ intronic
19	7689880	1	1	rs35260294	C	T	0.28	BLD	IPSC	NRSF		*FCER2*/ intronic
19	7689912	1	1	rs34288384	G	A	0.28	BLD	IPSC	ERalpha-a,Hic1,SP1		*FCER2*/ intronic
19	7690026	0.97	1	rs4996980	G	A	0.29	BLD, HRT		Rad21,Whn		*FCER2*/ intronic
19	7690031	0.97	1	rs4996979	T	C	0.29	BLD, HRT		4 altered motifs		*FCER2*/ intronic
19	7690056	0.97	1	rs4996978	A	C	0.29	BLD, HRT		4 altered motifs		*FCER2*/ intronic
19	7690078	0.97	1	rs4996977	T	C	0.29	BLD, HRT		PU.1		*FCER2*/ intronic
19	7690082	0.97	1	rs4996976	A	G	0.29	BLD, HRT		4 altered motifs		*FCER2*/ intronic
19	7690085	0.97	1	rs4996975	C	T	0.29	BLD, HRT	IPSC	AFP1,SIX5		*FCER2*/ intronic
19	7690170	1	1	rs2228138	G	A	0.28	BLD	ESC	5 altered motifs		*FCER2*/ synonymous
19	7690273	1	1	rs4996973	A	G	0.28	BLD	4 tissues	7 altered motifs		*FCER2*/ intronic
19	7690327	1	1	rs4996972	C	T	0.28	BLD, SPLN	4 tissues			*FCER2*/ intronic
19	7690399	1	1	**rs28364072**	A	G	0.28	BLD, SPLN		10 altered motifs		*FCER2*/ intronic
19	7690583	0.99	1	rs2277995	T	C	0.28	BLD, SPLN	BLD,BLD	25 altered motifs		*FCER2*/ intronic
19	7690586	0.99	1	rs74927160	G	T	0.28	BLD, SPLN	BLD,BLD	16 altered motifs		*FCER2*/intronic
19	7690599	0.87	1	rs76013233	G	A	0.26	BLD, SPLN	BLD,BLD	AP-2,SP1		*FCER2*/ intronic
19	7690632	1	1	rs2277994	A	C,G	0.28	BLD, SPLN	BLD,BLD			*FCER2*/ intronic
19	7690685	1	1	rs2277993	G	A	0.28	BLD, SPLN	BLD,BLD	AP-2		*FCER2*/ intronic
19	7690696	1	1	rs2277992	A	G	0.28	BLD, SPLN	BLD,BLD,BLD	Smad		*FCER2*/ intronic

Table S3. Continued

chr	pos (hg38)	LD (r^2)	LD (D')	variant	Ref	Alt	EUR freq	Enhancer histone marks	DNAse	Motifs changed	Selected eQTL hits	GENCODE genes / dbSNP func annot
19	7690830	1	1	rs2277991	A	G	0.28	BLD, SPLN	4 tissues	Maf,RREB-1	4 hits	*FCER2*/ intronic
19	7690859	1	1	rs2277990	A	G	0.28	BLD, SPLN	5 tissues	BCL,ELF1,Ets		*FCER2*/ intronic
19	7691182	1	1	rs73489945	G	A	0.28	BLD, SPLN	BLD,BLD,BLD	Myb,NRSF,Pax-4		*FCER2*/ intronic
19	7691202	1	1	rs66508756	T	C	0.28	BLD, SPLN	5 tissues	4 altered motifs		*FCER2*/ intronic
19	7691237	1	1	rs68063051	T	C	0.28	BLD, SPLN	5 tissues			*FCER2*/ intronic
19	7691305	1	1	rs67614954	C	T	0.28	BLD, SPLN	4 tissues			*FCER2*/ intronic
19	7691385	1	1	rs62110718	A	T	0.28	BLD, SPLN	4 tissues			*FCER2*/ intronic
19	7691425	1	1	rs62110719	C	T	0.28	BLD, SPLN	BLD,BLD,BLD	HNF1,Pbx-1		*FCER2*/ intronic
19	7691577	1	1	rs72998478	A	G	0.28	BLD	4 tissues	HDAC2,ZBRK1		*FCER2*/ intronic
19	7691591	1	1	rs66527560	C	G	0.28	BLD	4 tissues	5 altered motifs		*FCER2*/ intronic
19	7691709	0.93	0.97	rs62110724	G	A	0.28	BLD	4 tissues	LBP-1,Tgif1		*FCER2*/ intronic
19	7691748	0.96	0.99	rs62110725	A	C	0.29	BLD, OVRY	4 tissues	Ets,Mef2,RREB-1		*FCER2*/ intronic
19	7691771	0.99	1	rs62110726	T	C	0.28	BLD, OVRY	6 tissues	9 altered motifs		*FCER2*/ intronic
19	7691820	0.99	1	rs62110727	T	C	0.28	BLD, OVRY	4 tissues	COMP1,Myc		*FCER2*/ intronic
19	7692781	0.99	1	rs17159834	A	G	0.28	BLD, GI, SPLN	BLD,BLD,BLD	7 altered motifs	4 hits	*FCER2*/ intronic
19	7692842	0.99	1	rs62110730	C	T	0.28	BLD, GI, SPLN	5 tissues	HIF1		*FCER2*/ intronic
19	7692867	0.99	1	rs62110731	C	A	0.28	BLD, GI, SPLN	5 tissues	Pou2f2,Pou5f1		*FCER2*/ intronic
19	7692883	0.99	1	rs62110732	A	G	0.28	BLD, GI, SPLN	6 tissues	Foxp3		*FCER2*/ intronic
19	7693114	0.99	1	rs17159838	G	A	0.28	4 tissues	5 tissues	RREB-1		*FCER2*/ intronic
19	7693231	0.98	1	rs34613454	G	C	0.28	ESDR, BLD, GI	BLD,BLD	6 altered motifs		*FCER2*/ intronic
19	7698025	0.83	1	rs7249360	G	A	0.25	5 tissues	IPSC,BLD	4 altered motifs	4 hits	*FCER2*/ intronic
19	7698159	0.8	0.99	rs7249320	C	A	0.24	ESC, IPSC, BLD	ESC,IPSC,BLD	10 altered motifs	4 hits	*FCER2*/ intronic

Pos, position; LD, Linkage disequilibrium; ref, reference; Alt, alternative; EUR freq, European frequency; eQTL, expression quantitative trait loci; func annot, Functional annotation.

Table S4. The effect of rs28364072 on *FCER2* gene expression (using GETX portal,[52] http://www.gtexportal.org/home/)

Gene Symbol	Variant Id	SNP Id	P-Value	Normalized effect size	Tissue
FCER2	19_7755285_A_G_b37	rs28364072	1.30E-05	-0.16	Whole Blood

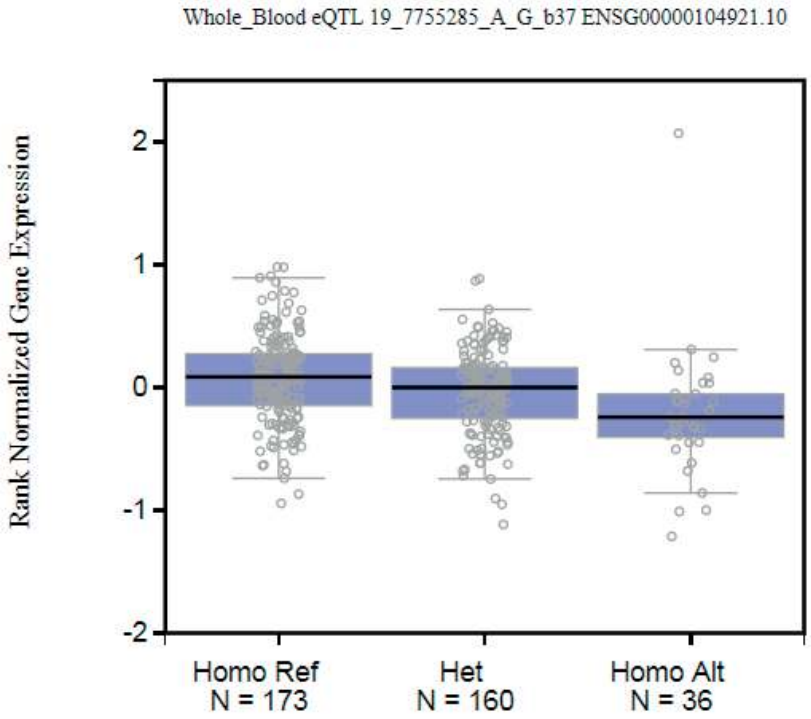

Figure S1. The genotypes of rs28364072 in GTEx Whole Blood.GTEx portal, http://www.gtexportal.org/home/
This figure is extracted from the GTEx portal. The legend on the top of the figure includes information on; tissue, analysis, chromosome_position_reference allele_effect allele_build and gene ID (GPR126).

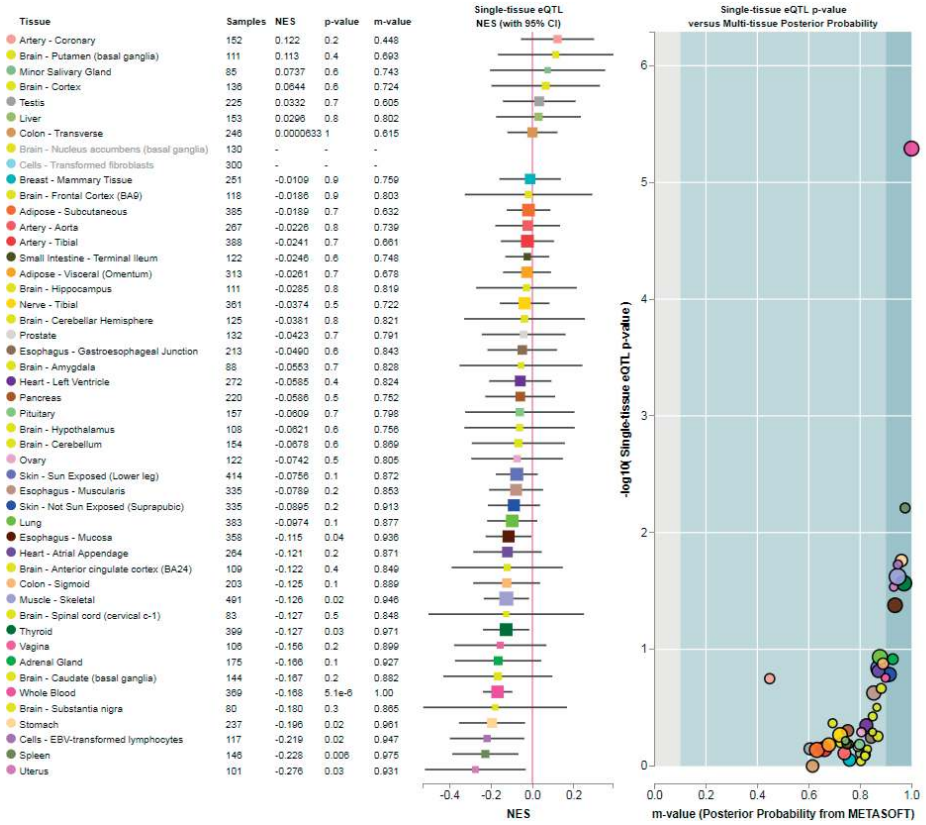

Figure S2. *FCER2* expression by rs28364072 in different tissues (using GTEx portal). www.gtexportal.org/home/[S2]
ENSG00000104921.10 *FCER2* and 19_7755159_A_G_b37 eQTL (Meta - Analysis RE2 P-Value: 1.91618e-11)
NOTE: using GTEx portal, http://www.gtexportal.org/home/
"NES, The slope of the linear regression of normalized expression data versus the three genotype categories using single-tissue eQTL analysis, representing eQTL effect size. The normalized expression values are based on quantile normalization within each tissue, followed by inverse quantile normalization for each gene across samples.
p-value, From a t-test that compares observed beta from single-tissue eQTL analysis to a null beta of 0.
m-value, The posterior probability that an eQTL effect exists in each tissue tested in the cross-tissue meta-analysis. The m-value ranges between 0 and 1 (Han and Eskin, PLoS Genetics 8(3): e1002555, 2012),
m-value interpretation:
Small m-value (e.g. <0.1); The tissue is predicted to NOT have an eQTL effect. Large m-value (e.g. >0.9); The tissue is predicted to HAVE an eQTL effect. Otherwise; The prediction of the existence of an eQTL effect is ambiguous." [2]

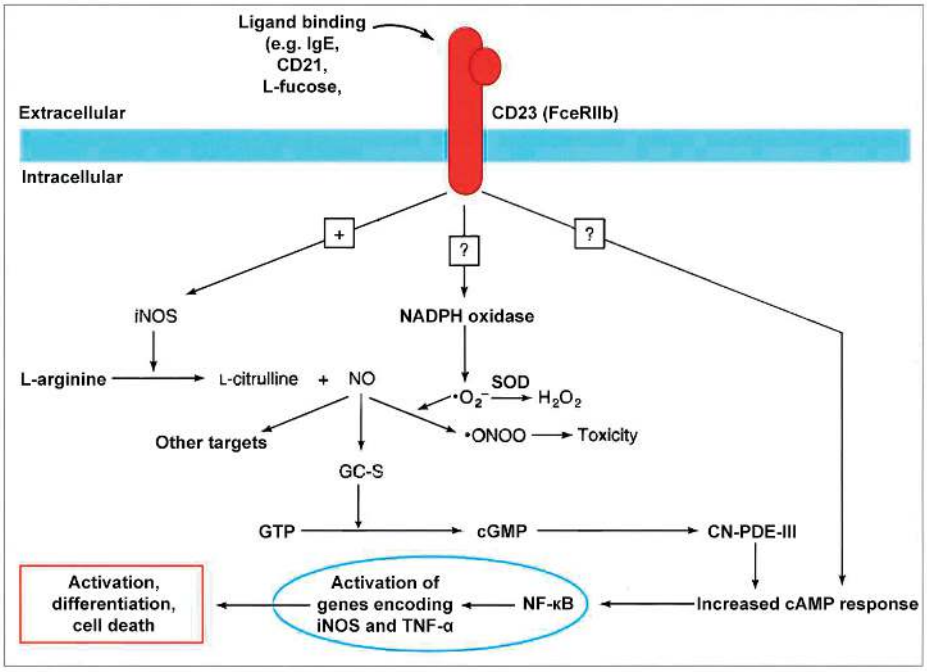

Figure S3. CD23 and NO production.[53]
Note: "Proposed sequence of events following the ligation of CD23 at the surface of monocytes. CD23 engagement, elicited through interaction with one of its multiple ligands, induces stimulation of the NOS and NADPH oxidase pathways".[53]

SUPPLEMENTARY REFERENCES

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S2. GTEx portal. www.gtexportal.org/home/.

S3. Dugas B, Mossalayi MD, Damais C, Kolb JP. Nitric oxide production by human monocytes: evidence for a role of CD23. Immunology today. 1995; 16: 574-580.

2.2

ADRB2 haplotypes and asthma exacerbations in children and young adults: an individual participant data meta-analysis

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Clin Exp Allergy. 2021 Sep;51(9):1157-1171. doi: 10.1111/cea.13965.

ABSTRACT

Background: The polymorphism Arg16 in β_2-adrenergic receptor (*ADRB2*) gene has been associated with an increased risk of exacerbations in asthmatic children treated with long-acting β_2-agonists (LABA). However, it remains unclear whether this increased risk is mainly attributed to this single variant or the combined effect of the haplotypes of polymorphisms at codons 16 and 27.

Objective: We assessed whether the haplotype analysis could explain the association between the polymorphisms at codons 16 (Arg16Gly) and 27 (Gln27Glu) in *ADRB2* and risk of asthma exacerbations in patients treated with inhaled corticosteroids (ICS) plus LABA.

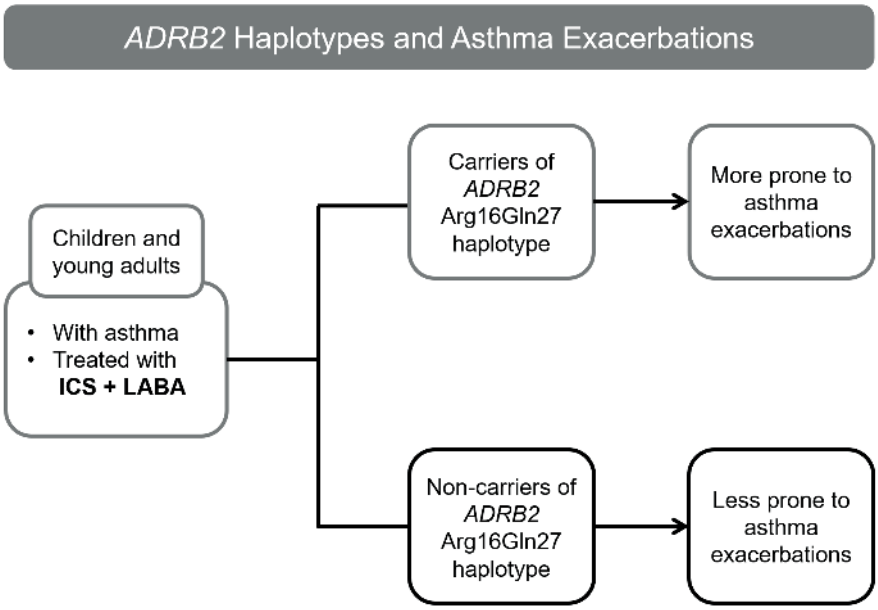
Methods: The study was undertaken using data from 10 independent studies (n = 5903) participating in the multi-ethnic Pharmacogenomics in Childhood Asthma (PiCA) consortium. Asthma exacerbations were defined as asthma-related use of oral corticosteroids or hospitalizations/emergency department visits in the past 6 or 12 months prior to the study visit/enrolment. The association between the haplotypes and the risk of asthma exacerbations was performed per study using haplo.stats package adjusted for age and sex. Results were meta-analyzed using the inverse variance weighting method assuming random-effects.

Results: In subjects treated with ICS and LABA (n = 832, age: 3–21 years), Arg16/Gln27 versus Gly16/Glu27 (OR: 1.40, 95% CI: 1.05–1.87, I^2 = 0.0%) and Arg16/Gln27 versus Gly16/Gln27 (OR: 1.43, 95% CI: 1.05–1.94, I^2 = 0.0%), but not Gly16/Gln27 versus Gly16/Glu27 (OR: 0.99, 95% CI: 0.71–1.39, I^2 = 0.0%), were significantly associated with an increased risk of asthma exacerbations. The sensitivity analyses indicated no significant association between the *ADRB2* haplotypes and asthma exacerbations in the other treatment categories, namely as-required short-acting β_2-agonists (n = 973), ICS monotherapy (n = 2623), ICS plus leukotriene receptor antagonists (LTRA; n = 338), or ICS plus LABA plus LTRA (n = 686).

Conclusion and clinical relevance: The *ADRB2* Arg16 haplotype, presumably mainly driven by the Arg16, increased the risk of asthma exacerbations in patients treated with ICS plus LABA. This finding could be beneficial in *ADRB2* genotype-guided treatment which might improve clinical outcomes in asthmatic patients.

Keywords

ADRB2, asthma exacerbations, haplotypes, inhaled corticosteroids, long-acting β_2-agonists



Graphical Abstract Text. Asthmatic children and young adults treated with inhaled corticosteroid (ICS) plus long-acting β_2-agonists (LABA) were more prone to asthma exacerbations if they were carriers of *ADRB2* haplotype (Arg16Gln27) compared to non-carriers. The *ADRB2* Arg16 haplotype, presumably mainly driven by the Arg16, increased the risk of asthma exacerbations in patients treated with ICS plus LABA. This finding could be beneficial in *ADRB2* genotype-guided asthma treatment and might improve patient outcomes.

KEY MESSAGE

- Response to treatment with inhaled corticosteroids (ICS) and long-acting β_2-agonists (LABA) varies inter-individually in asthmatic patients.
- The *ADRB2* Arg16 haplotype increased the risk of asthma exacerbations in patients treated with ICS plus LABA.
- This finding could be beneficial in *ADRB2* genotype-guided treatment in asthmatic patients.

INTRODUCTION

Asthma is a common, heterogeneous and chronic respiratory disease. Despite treatment, patients might experience exacerbations that can be life-threating. The combination therapy of inhaled corticosteroids (ICS) and long-acting β_2-agonists (LABA) is one of the recommended treatments for the control of asthma in children.[1] However, response to treatment with LABA varies inter-individually and this might be partly mediated by genetic variation.[2]

The β_2-adrenergic receptor is a member of the G protein-coupled transmembrane receptors broadly located on airway smooth muscle cells.[3] The β_2-adrenergic receptor (*ADRB2*) gene, a small intron-less gene on chromosome 5q31.32, encodes the receptor and contains different single nucleotide polymorphisms (SNPs). Of these SNPs, the coding non-synonymous variants rs1042713 (Arg16Gly), a Glycine-to-Arginine amino acid substitution at codon 16, and rs1042714 (Gln27Glu), a Glutamine-to-Glutamic acid amino acid substitution at codon 27, that are in linkage disequilibrium, have been found to be associated with asthma and asthma phenotypes.[4-6]

Although various studies have investigated the association between the *ADRB2* polymorphisms and response to LABA, the results are conflicting and inconclusive.[7-11] A recent meta-analysis in the Pharmacogenomics in Childhood Asthma[12] (PiCA) consortium showed that asthmatic children carrying 1 or 2 Arg allele(s) at rs1042713 and treated with ICS plus LABA have an increased risk of exacerbations.[10] Previous studies showed that the Gln allele at rs1042714 was a risk factor for asthma and associated with a less effective response to treatment with inhaled β_2-agonists during an acute asthma exacerbation.[6,13] Furthermore, most studies, as well as the recent meta-analysis in the PiCA consortium,[10] evaluated the effect of each variant independently but not the combined effect of their haplotypes that might yield additional insight into the association between the *ADRB2* variants and asthma exacerbations. Therefore, it is still unclear whether the combined effect of the *ADRB2* polymorphisms at codons 16 and 27 is associated with an increased risk of asthma exacerbations or whether the association is driven by just the single polymorphism at codon 16.

Therefore, we aimed to assess whether the haplotype analysis could explain the association between the polymorphisms at codons 16 and 27 of *ADRB2* and the risk of asthma exacerbations in patients treated with ICS plus LABA.

METHODS

Study population

Data from 10 independent studies participating in the PiCA consortium[12] were analyzed. BREATHE is an observational study that includes children and young adults (age: 3–22 years)[14] with physician-diagnosed asthma recruited from primary and secondary care units in Tayside, Scotland, and Brighton, United Kingdom. The Effectiveness and Safety of Treatment with Asthma Therapy in children (ESTATe) is a case-control study that includes children and young adults (4–19 years) with physician-diagnosed asthma recruited from primary care units in the Netherlands. The followMAGICS study is the follow-up study of the observational Multicenter Asthma Genetics in Childhood Study (MAGICS), which includes physician-diagnosed asthmatic children and young adults (age: 7–25 years)[15] recruited from secondary and tertiary centers in Germany and Austria. The Genes-Environment and Admixture in Latino Americans (GALA II) and the Study of African Americans, Asthma, Genes, and Environments (SAGE) studies are two independent case-control asthma cohorts (age: 8–21 years) that focus on two different racial/ethnic groups based on the self-identified ethnicity of the four grandparents of each subject: Hispanics/Latinos (GALA II) and African Americans (SAGE) in the United States and Puerto Rico.[16,17] The Pharmacogenetics of Asthma Medication in Children: Medication with Anti-inflammatory effects (PACMAN) study in the Netherlands,[18] is an observational cohort study that included children (age: 4–12 years) with self-reported regular use of asthma medication recruited through community pharmacies. Children were selected from community pharmacies in the Netherlands that belonged to the Utrecht Pharmacy Practice Network for Education and Research (UPPER).[19] The Pediatric Asthma Gene Environment Study (PAGES) is a cross-sectional observational study designed to relate asthma outcomes to environmental and genetic factors. Children (age: 5–16 years) with physician-diagnosed asthma were recruited from primary and secondary care centers across Scotland.[20] The Pharmacogenetics of Adrenal Suppression Study (PASS) in the United Kingdom (age: 5–18 years) is a multicenter cohort of asthmatic children. The study initially aimed to explore the association between use of corticosteroids and adrenal suppression and how genetic factors influence this association.[21,22] The Singapore Cross Sectional Genetic Epidemiology Study (SCSGES)[23] (age: 6 –31 years) is an ongoing cross-sectional genetic epidemiology study on allergic diseases among Singapore Chinese individuals. The ethnicity of subjects was self-reported Chinese and confirmed by principal component analysis. Asthma was defined by having a physician-diagnosis of symptoms prior to recruitment.[23,24] The SLOVENIA study is a case-control cohort (age: 5–18 years) and includes asthmatic children and young adults recruited from tertiary health centers from Murska Sobota, Slovenia.[25] Further details on the study population are described in the Supporting Information.

All studies have been approved by their local medical ethics committees/institutional review boards and parents or participants provided written consent. The Tayside Committee on Medi-

cal Research Ethics (Dundee, United Kingdom) approved BREATHE (reference number: NFB/FB/106/03). ESTATe was approved by the Medische Ethische Toetsings Commissie, Erasmus Medical Center (Rotterdam, The Netherlands) (reference number: MEC-2011- 474).GALA II and SAGE were approved by the Human Research Protection Program Institutional Review Board of the University of California, San Francisco (San Francisco, USA; reference numbers: 10-00889 and 10-02877 respectively). PACMAN was approved by the Medical Ethics Committee of the University Medical Centre Utrecht (Utrecht, The Netherlands, reference number: NL2124.021.08). PAGES has been approved by the Cornwall and Plymouth Research Ethics Committee (reference number: 07/H0203/204). PASS was approved by the Liverpool Pediatric Research Ethics Committee (Liverpool, United Kingdom, reference number: 08/H1002/56). SLOVENIA was approved by the Slovenian National Medical Ethics Committee (Ljubljana, Slovenia, reference number: 0120-569/ 2017/4). The Ethik-Kommission der Bayerischen Landesärztekammer (Munich, Germany; reference number: 01218) and ethics committee of the medical University of Hannover (reference number: 1021-2011) approved followMAGICS. The ethical approval for the SCSGES cohort was obtained from the Institutional Review Board of the National University of Singapore (NUS-IRB), reference numbers: 07-023, 09-256, 10-343,10-445 and 13-075 for the large scale epidemiology and genetics study and the Institutional Review Board of the National Healthcare Group Domain, Specific Review Board –B/04/055.

Medication data

Data on asthma treatment were collected either from pharmacy records, parent/patient-reported medication use or completed study questionnaires (PACMAN, followMAGICS, BREATHE, GALA II, PAGES, SAGE, and SCSGES) or physician prescriptions and pharmacy records (ESTATe, PASS, and SLOVENIA). Asthma treatment was categorized as follows: (1) as-required short-acting β_2-agonists (SABA) (2) ICS monotherapy, (3) ICS in combination with LABA, (4) ICS in combination with leukotriene receptor antagonists (LTRA), and (5) ICS in combination with LABA and LTRA. All children in categories 2–5 used as-required SABA.

Main outcome

Asthma exacerbations, the main outcome, were defined based on the American Thoracic Society (ATS)/European Respiratory Society (ERS) guidelines as episodes of worsening of asthma symptoms which require a short course (3–5 days) of oral systemic corticosteroids (OCS) use, hospitalizations or emergency department (ED) visits.[26] Cases were determined if subjects had at least one asthma exacerbation (described above) in the past 6 or 12 months prior to the study visit or enrolment.

Data on asthma exacerbations, asthma-related OCS use or hospitalizations/ED visits, were reported by the parent/child at the study visit or based on study questionnaires or physician records: (1) BREATHE and PASS: hospitalizations or OCS use in the past 6 months preceding

the study visit; (2) PACMAN: ED visits or OCS use in the past 12 months preceding the study visit; (3) GALA II, SLOVENIA, ESTATe, SAGE, PAGES, and SCSGES: hospitalizations/ED visits or OCS use in the past 12 months preceding the study visit. In followMAGICS, only data on asthma-related hospitalizations or ED visits were available in the past 12 months preceding the study visit.[12]

Genotyping

In BREATHE and PAGES, genotypes were determined by using Taqman-based allelic discrimination assays on an ABI 7700 sequence detection system (Applied Biosystems).[4,27] In followMAGICS, samples were genotyped using Illumina Sentrix HumanHap300 BeadChip array (Illumina, Inc.)[15] In both GALA II and SAGE, samples were genotyped using the Axiom® LAT1 array (Affymetrix Inc.) and quality control (QC) procedures were performed as described previously.[28,29] In PACMAN and ESTATe, samples were genotyped using the Illumina Infinium CoreExome-24 BeadChip (Illumina, Inc.).[30] In PASS, genotyping was performed using the Illumina Omni Express 8v1 array (Illumina, Inc.). QC procedures and imputation are described elsewhere.[22] In SCSGES, genotyping was conducted using Kompetitive Allele Specific PCR (KASP) genotyping platform (LGC, Inc). QC was performed based on the quality of clustering.[23] In the SLOVENIA study, genotyping of 336 samples was performed with the Illumina Global Screening Array-24 v1.0 BeadChip (Illumina). QC procedures and imputation described elsewhere.[30]

Functional annotation of variants and expression quantitative trait loci (eQTL) analysis

We used HaploRegv4.1 (http://www.broadinstitute.org/mammals/haploreg/haplo reg.php)[31] to retrieve all proxy SNPs in strong linkage disequilibrium (LD) (r^2 threshold > 0.8, limit distance 100 kb and population panel CEU using 1000 Genomes project) with rs1042713 and rs1042714 in *ADRB2* and to assess the predicted functions of the variants including protein structure, effects on gene regulation, and splicing. We also checked the correlation of the SNPs and their proxies with the expression level of *ADRB2* in whole blood using expression quantitative trait loci (eQTL) data from Genenetwork.[32]

Statistical analyses

Descriptive statistics were used to calculate means and standard deviations (SD) for continuous variables and percentages for categorical variables. Hardy–Weinberg equilibrium (HWE) was assessed for each SNP using a web program (http://www.oege.org/software/hwe-mr- calc.shtml) which uses the Pearson chi-squared test for HWE testing.[33] In our main analysis, we analyzed the association between haplotype combinations of polymorphisms at codons 16 and 27 of the *ADRB2* gene and asthma exacerbations in the category of children treated with ICS plus LABA. We used the haplo.stats package (version 1.7.7)[34] in R adjusting for age and sex in each study separately, and the resulting odds ratios (ORs) were meta-analyzed. The statistical methods of the

haplo.stats package assume that all subjects are unrelated and linkage phase of the genetic markers remains unknown.[34] To address potential heterogeneity between studies, we used the inverse variance weighting method assuming random-effects. We also reported I^2 and Cochran's Q-test of the meta-analysis.[35] Forest plots were made using the "metafor" package in R (version 3.3.3).[36]

Data on asthma-related OCS use were not available in followMAGICS. Therefore, in a sensitivity analysis, we repeated the haplotype analysis (as described above) separately for asthma-related hospitalizations/ED visits outcome as well as for asthma-related OCS use outcome. Furthermore, to test the robustness of our result in the treatment category of ICS plus LABA, we repeated the haplotype analysis (as described above) in the other treatment categories as follows; as-required SABA, ICS monotherapy, ICS plus LTRA, and ICS plus LTRA plus LABA. Since we investigated the association of haplotype combinations of two polymorphisms and asthma exacerbations, we considered a p-value< 0.025 (0.05/2) for our main meta-analysis to be statistically significant.

RESULTS

Study characteristics

The characteristics of the study populations (for each study) are presented in Table 1. Data on age, sex, and treatment were available for 5903 children and young adults. Out of these 5903 subjects, data on asthma exacerbations were available in 5726 subjects.

Asthma exacerbations occurred in 2494 patients (43.5%) and the proportion of asthma exacerbations ranged from 9.7% (PACMAN) to 86.2% (PASS) across the studies. The mean age (SD) of the patients ranged between 8.7 (2.3) years for PACMAN and 17.3 (3.0) years for followMAGICS, and in all studies, the majority of patients were male. The percentage of subjects treated with ICS plus LABA differed across the studies and ranged from 10.2% in GALA II to 50.3% in followMAGICS. In addition, all patients in SLOVENIA and SCSGES were treated with ICS monotherapy.

Table 2 shows the *ADRB2* genotype and haplotype data. The risk allele (Arg) frequency for rs1042713 was highest in SCSGES (0.55) followed by SAGE (0.51). The risk allele (Arg) frequency for rs1072713 ranged between (0.34) for ESTATe and (0.41) for PACMAN across the European studies. The risk allele (Gln) frequency for rs1042714 was highest in SCSGES (0.93) followed by SAGE (0.82). The risk allele (Gln) frequency for rs1042714 was similar across the European studies and ranged between (0.54) for PASS and (0.60) for ESTATe and SLOVENIA. Both SNPs were in HWE in all studies and they showed a complete LD (D' ~1) with r^2 that ranged from 0.10 in SCSGES to 0.50 in PASS.

Table 1. Characteristics of the study populations

Characteristics	BREATHE	ESTATe	Follow MAGICS	GALA II	PACMAN	PAGES	PASS	SAGE	SLOVENIA	SCSGES
n	998	101	167	1618	791	722	384	740	212	170
Male sex, %	60.0	58.0	62.3	55.7	62.3	57.6	56.0	52.3	56.1	68.2
Mean age, y (SD)	10.2 (4.0)	10.6 (4.2)	17.3 (3.0)	12.4 (3.2)	8.7 (2.3)	9.8 (3.7)	11 (3.3)	13.8 (3.5)	10.8 (3.4)	14.0 (6.4)
Ethnicity, n. (%)										
Caucasian	998 (100)	96 (95)	167 (100)	N/A	711 (89.9)	360 (50)	384 (100)	N/A	212 (100)	N/A
Hispanic	N/A	N/A	N/A	1618.(100)	3 (0.4)	N/A	N/A	744 (100)	N/A	N/A
Asian	N/A	1 (1)	N/A	N/A	6 (0.8)	11 (1.5)	N/A	N/A	N/A	170 (100)
African	N/A	0 (0)	N/A	N/A	9 (1.1)	N/A	N/A	N/A	N/A	N/A
Mixed	N/A	2 (2)	N/A	N/A	53 (6.7)	15 (2)	N/A	N/A	N/A	N/A
Unknown (missing)	N/A	2 (2)	N/A	N/A	9 (1.1)	336 (46.5)	N/A	N/A	N/A	N/A
Treatment group, n. (%)										
SABA alone	173 (17.3)	0 (0.0)	25 (15.0)	576 (35.6)	80 (10.1)	79 (10.9)	0 (0.0)	207 (27.9)	N/A	N/A
ICS alone	562 (56.3)	65 (64.0)	39 (23.3)	538 (33.2)	497 (62.8)	271 (37.6)	29 (7.5)	367 (49.6)	212 (100)	170 (100)
ICS + LABA	142 (14.3)	34 (34.0)	84 (50.3)	165 (10.2)	148 (18.7)	135 (18.7)	126 (33.0)	98 (13.2)	N/A	N/A
ICS + LTRA	37 (3.7)	0 (0.0)	4 (2.4)	208 (12.9)	21 (2.7)	65 (9.0)	0 (0.0)	35 (4.7)	N/A	N/A
ICS + LABA + LTRA	84 (8.4)	2 (2.0)	15 (9.0)	131 (8.1)	45 (5.7)	172 (23.8)	229 (59.5)	33 (4.6)	N/A	N/A
Asthma exacerbations in the past year or in the last six months prior to the study visit/enrolment										
Hospitalizations/ED*, n. (%)[a]	147 (14.7)	13 (12.9)	11 (6.6)	865 (54.8)	42 (5.5)	151 (21.7)	290 (75.5)	272 (39.0)	49 (27.7)	34 (20.0)
OCS use, n. (%)[a]	234 (23.4)	36 (35.6)	N/A	587 (37.4)	46 (5.8)	316 (45.7)	198 (51.6)	162 (22.4)	23 (12.9)	36 (21.2)
Asthma exacerbations*, n. (%)[a]	250 (25.0)	49 (48.5)	N/A	1,013 (64.3)	75 (9.7)	346 (50.0)	331 (86.2)	317 (45.8)	54 (30.3)	59 (34.7)

Abbreviations: Asthma exacerbations, asthma-related hospitalizations/ED visits or oral corticosteroids use; ED, emergency department visits; N/A, Not Applicable; OCS use, oral corticosteroids use.
[a]Data on asthma-related hospitalizations/ED visits outcomes were missing in 40 subjects in GALA II, 24 subjects in PACMAN, 27 subjects in PAGES, 43 subjects in SAGE and 35 subjects in SLOVENIA; data on asthma-related oral OCS use were missing in 49 subjects in GALA II, 30 subjects in PAGES, 16 subjects in SAGE, and 34 subjects in SLOVENIA, data on asthma exacerbations were missing in 44 subjects in GALA II, 21 subjects in PACMAN, 30 subjects in PAGES, 48 subjects in SAGE, and 34 subjects in SLOVENIA. In followMAGICS, only data on asthma-related hospitalizations/ED visits were available.

Table 2. *ADRB2* genotype and haplotype data

Characteristics	BREATHE	ESTATe	Follow MAGICS	GALAII	PACMAN	PAGES	PASS	SAGE	SLOVENIA	SCSGES
Subjects with data on rs1042713. n.	998	101	167	1618	791	720	384	740	212	170
Risk allele (Arg) frequency (rs1042713)	0.37	0.34	0.38	0.44	0.41	0.37	0.37	0.51	0.37	0.55
rs1042713 genotype, no. (%)										
Arg/Arg	154 (15.4)	14 (13.9)	25 (15.0)	306 (18.9)	124 (15.7)	101 (14.1)	59 (15.4)	198 (26.7)	35 (16.5)	46 (27.0)
Arg/Gly	436 (43.7)	40 (39.6)	78 (46.7)	819 (50.6)	402 (50.8)	330 (45.8)	167 (43.5)	355 (48.0)	87 (41.0)	96 (56.5)
Gly/Gly	408 (40.9)	47 (46.5)	64 (38.3)	493 (30.5)	265 (33.5)	289 (40.1)	158 (41.1)	187 (25.3)	90 (42.5)	28 (16.5)
Subjects with data on rs1042714. n.	998	101	167	1,622	791	722	384	744	212	169
Risk allele (Gln) frequency (rs1042714)	0.56	0.60	0.58	0.78	0.63	0.56	0.54	0.82	0.60	0.93
rs1042714 genotype, no. (%)										
Gln/Gln	307 (30.8)	36 (35.6)	57 (34.1)	971 (59.9)	313 (39.6)	232 (32.1)	115 (30.0)	497 (66.8)	81 (38.2)	144 (85.2)
Gln/Glu	495 (49.6)	50 (49.5)	79 (47.3)	576 (35.5)	376 (47.5)	349 (48.4)	184 (47.9)	223 (30.0)	91 (42.9)	25 (14.8)
Glu/Glu	196 (19.6)	15 (14.9)	31 (18.6)	75 (4.6)	102 (12.9)	141 (19.5)	85 (22.1)	24 (3.2)	40 (18.9)	0 (0.0)
Subjects with data on both SNPs. n.	998	101	167	1618	791	714	384	740	212	169
Haplotype frequency										
Arg16/Gln27	0.37	0.34	0.38	0.44	0.41	0.37	0.37	0.51	0.37	0.55
Gly16/Gln27	0.18	0.27	0.20	0.34	0.22	0.19	0.17	0.31	0.23	0.37
Gly16/Glu27	0.45	0.39	0.42	0.22	0.37	0.44	0.46	0.18	0.40	0.08
Linkage disequilibrium between rs1042713 and rs1042714										
r^2 (D`)	0.47 (~1)	0.33 (1)	0.43 (0.98)	0.23 (1)	0.40 (~1)	0.46 (~1)	0.50 (~1)	0.23 (1)	0.40 (1)	0.10 (1)

Three haplotypes were determined at positions 16 and 27 and haplotype frequencies were as follows: Arg16/Gln27 (ranged from 0.34 to 0.55), Gly16/Gln27 (ranged from 0.17 to 0.37) and Gly16/Glu27 (ranged from 0.08 to 0.46; Table 2).

Risk of asthma exacerbations in children treated with ICS plus LABA

Data on the outcome, asthma exacerbations (asthma-related OCS use or hospitalizations/ED visits), haplotypes and ICS plus LABA treatment were available in seven studies (n = 832, age = 3–21 years). The meta-analysis indicated that Arg16/Gln27 versus Gly16/Glu27 (OR: 1.40, 95% CI: 1.05–1.87, I^2 = 0.00%, p = 0.022) and Arg16/ Gln27 versus Gly16/Gln27 (OR: 1.43, 95% CI: 1.05–1.94, I^2 = 0.00%, p = 0.023) were significantly associated with an increased risk of asthma exacerbations (Figure 1). However, Gly16/Gln27 versus Gly16/Glu27 (OR: 0.99, 95% CI: 0.71–1.39, I^2 = 0.00%, p = 0.946) was not associated with the risk of asthma exacerbations.

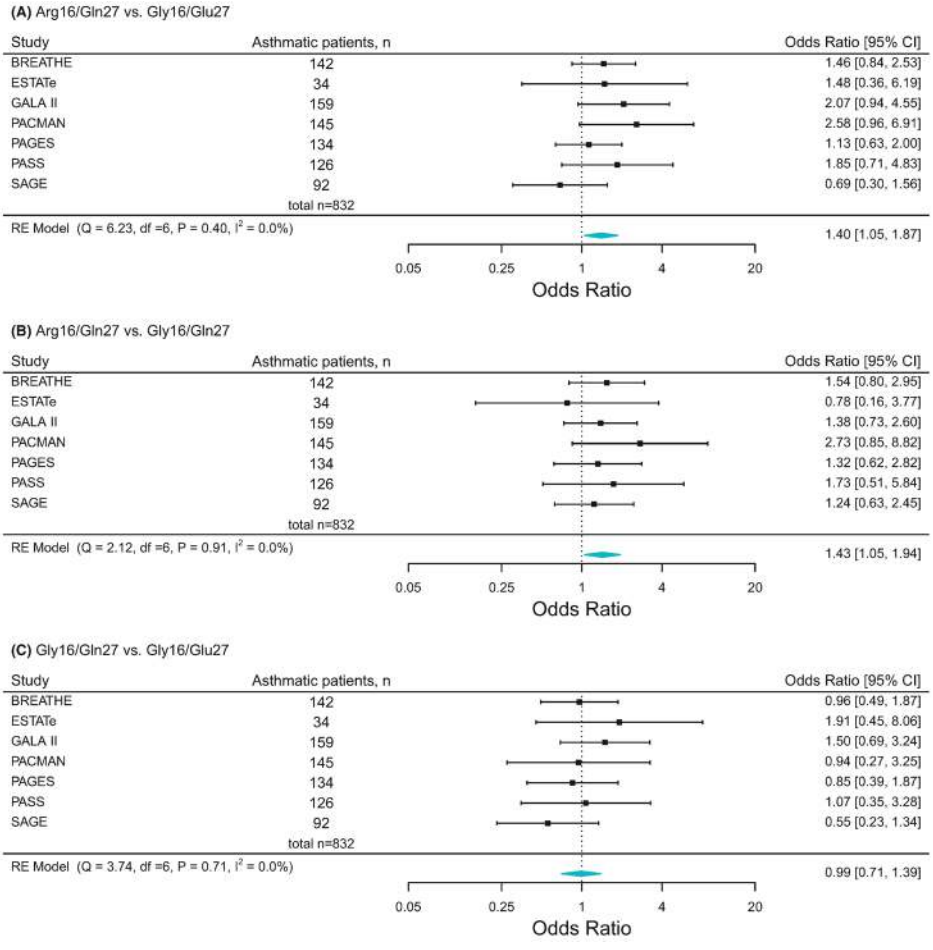


Figure 1. Forest plots of the association between the *ADRB2* haplotypes and the risk of asthma exacerbations (asthma-related hospitalizations/emergency department visits or oral corticosteroids use) in ICS plus LABA treatment group across studies. These plots describe Odds Ratios (ORs) and corresponding 95% Confidence Intervals (CIs), adjusted for age and sex

Sensitivity analyses

In patients treated with ICS plus LABA, we repeated the haplotype analysis separately for asthma-related OCS use and for asthma-related hospitalizations/ED visits. We observed the similar trends as the main analysis (Figures 2 and 3). Furthermore, no association between the *ADRB2* haplotypes and the risk of asthma exacerbations was observed in any of the other treatment groups (Table 3).

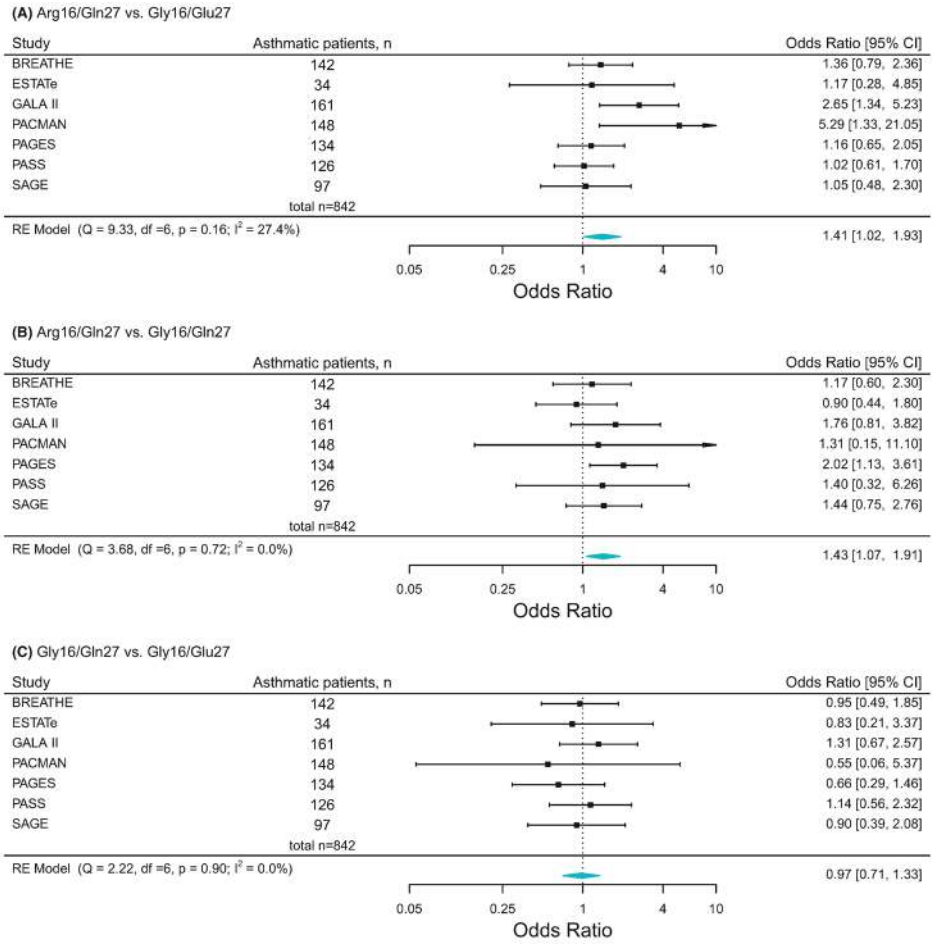


Figure 2. Forest plots of the association between the *ADRB2* haplotypes and the risk of asthma-related oral corticosteroids use in ICS plus LABA treatment group across studies. These plots describe Odds Ratios (ORs) and corresponding 95% Confidence Intervals (CIs), adjusted for age and sex

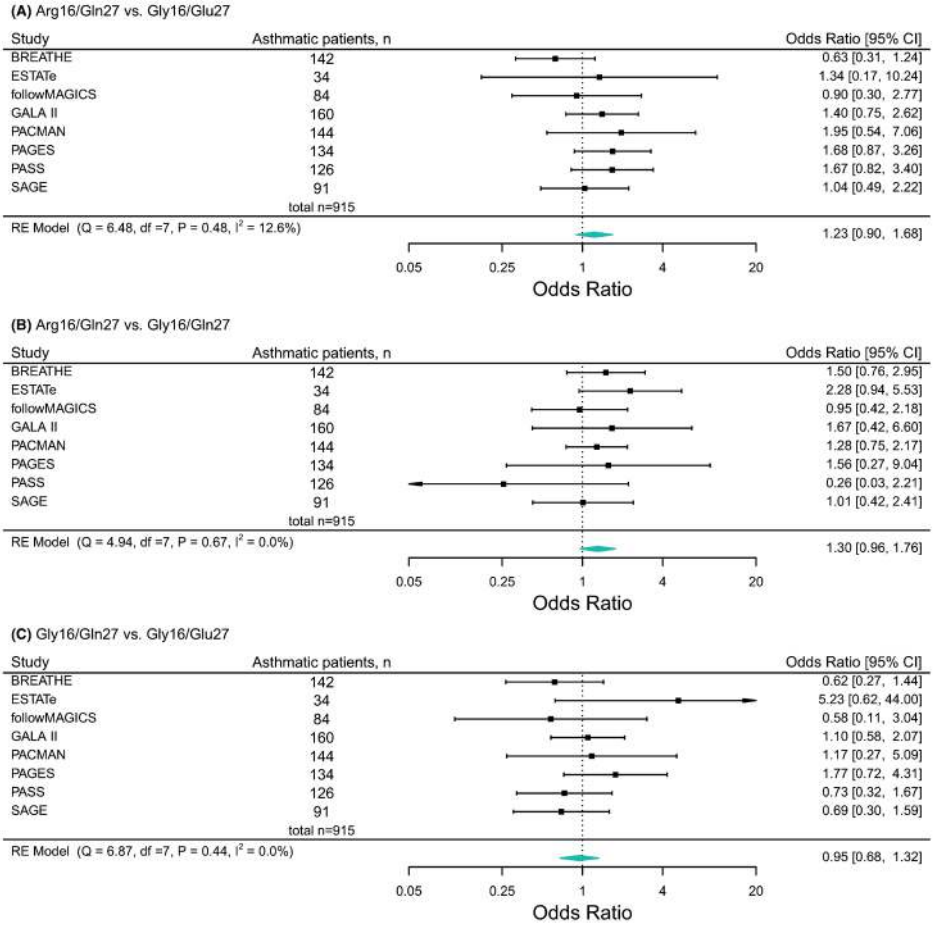


Figure 3. Forest plots of the association between the *ADRB2* haplotypes and the risk of asthma-related hospitalizations/ emergency department visits in ICS plus LABA treatment group across studies. These plots describe Odds Ratios (ORs) and corresponding 95% Confidence Intervals (CIs), adjusted for age and sex

Functional annotation and eQTL analysis of the *ADRB2* variants

Functional annotation, using Haploreg v4.1 data,[31] showed that rs1042713 and rs1042714 had several proxy variants in strong LD (D' =1 and $r^2 > 0.8$), but none of them was a non-synonymous proxy (Tables S1 and S2). Furthermore, the cis-eQTL data from Genenetwork showed that not only the Arg allele of rs1042713 but also the Gln allele of rs1042714 was associated with reduced levels expression of *ADRB2* in whole blood.[32] Therefore, these data indicated that the variants alter the *ADRB2* expression and function.

Table 3. Risk of asthma exacerbations across the other treatment groups

Haplotypes	BREATHE	ESTATe	follow-MAGICS	GALA II	PACMAN	PAGES	PASS	SAGE	SLOVENIA	SCSGES	Total combined results from all cohorts
OR (95% CI) for asthma exacerbations in patients treated with as-required SABA											
	n = 173	**N/A**	**N/A**	**n = 557**	**N/A**	**n = 51**	**N/A**	**n = 192**	**N/A**	**N/A**	**n = 973**
Arg16Gln27 vs. Gly16Glu27	1.28 (0.61, 2.70)	N/A	N/A	1.17 (0.84, 1.62)	N/A	0.54 (0.13, 2.31)	N/A	0.67 (0.36, 1.24)	N/A	N/A	1.00 (0.71, 1.40) I^2 = 21.50%
Arg16Gln27 vs. Gly16Gln27	0.92 (0.33, 2.60)	N/A	N/A	1.13 (0.80, 1.60)	N/A	2.10 (0.26, 17.03)	N/A	0.80 (0.42, 1.55)	N/A	N/A	1.05 (0.79, 1.41) I^2 = 0.00%
Gly16Gln27 vs. Gly16Glu27	1.40 (0.50, 3.97)	N/A	N/A	1.03 (0.73, 1.45)	N/A	0.26 (0.03, 2.09)	N/A	0.83 (0.43, 1.61)	N/A	N/A	0.99 (0.74, 1.32) I^2 = 0.00%
OR (95% CI) for asthma exacerbations in patients treated with ICS monotherapy											
	n = 562	**n = 65**	**N/A**	**n = 527**	**n = 484**	**n = 268**	**n = 29**	**n = 341**	**n = 178**	**n = 169**	**n = 2623**
Arg16Gln27 vs. Gly16Glu27	1.21 (0.88, 1.65)	0.74 (0.31, 1.76)	N/A	1.47 (1.00, 2.16)	0.74 (0.45, 1.21)	1.21 (0.80, 1.82)	N/A	0.70 (0.45, 1.09)	0.72 (0.44, 1.18)	1.00 (0.38, 2.62)	0.98 (0.78, 1.23) I^2 = 46.37%
Arg16Gln27 vs. Gly16Gln27	1.06 (0.72, 1.56)	1.58 (0.58, 4.30)	N/A	1.22 (0.88, 1.70)	0.96 (0.54, 1.71)	0.99 (0.61, 1.60)	N/A	1.01 (0.99, 1.02)	0.93 (0.50, 1.70)	0.67 (0.39, 1.15)	1.01 (0.99, 1.02) I^2 = 0.00%
Gly16Gln27 vs. Gly16Glu27	1.15 (0.78, 1.70)	0.47 (0.16, 1.37)	N/A	1.74 (1.15, 2.62)	0.77 (0.43, 1.36)	1.25 (0.78, 2.00)	0.67 (0.34, 4.97)	0.70 (0.43, 1.14)	0.78 (0.43, 1.42)	1.49 (0.55, 4.04)	1.01 (0.77, 1.33) I^2 = 46.05%
OR (95% CI) for asthma exacerbations in patients treated with ICS+LTRA											
	n = 37	**N/A**	**N/A**	**n = 203**	**N/A**	**n = 64**	**N/A**	**n = 34**	**N/A**	**N/A**	**n = 338**
Arg16Gln27 vs. Gly16Glu27	0.96 (0.29, 3.24)	N/A	N/A	1.33 (0.72, 2.45)	N/A	1.01 (0.44, 2.27)	N/A	1.11 (0.19, 6.55)	N/A	N/A	1.16 (0.75, 1.80) I^2 = 0.00%
Arg16Gln27 vs. Gly16Gln27	1.14 (0.32, 4.07)	N/A	N/A	0.96 (0.60, 1.52)	N/A	0.43 (0.14, 1.25)	N/A	1.50 (0.41, 5.54)	N/A	N/A	0.91 (0.62, 1.34) I^2 = 0.00%
Gly16Gln27 vs. Gly16Glu27	0.84 (0.25, 2.90)	N/A	N/A	1.39 (0.75, 2.60)	N/A	2.36 (0.72, 7.78)	N/A	0.74 (0.11, 5.04)	N/A	N/A	1.35 (0.83, 2.20) I^2 = 0.00%

Table 3. Continued

Haplotypes	BREATHE	ESTATe	follow-MAGICS	GALA II	PACMAN	PAGES	PASS	SAGE	SLOVENIA	SCSGES	Total combined results from all cohorts
OR (95% CI) for asthma exacerbations in patients treated with ICS+LABA+LTRA											
	n = 84	**N/A**	**N/A**	**n = 129**	**n = 43**	**n = 168**	**n = 229**	**n = 33**	**N/A**	**N/A**	**n = 686**
Arg16Gln27 vs. Gly16Glu27	0.81 (0.42, 1.58)	N/A	N/A	0.96 (0.38, 2.44)	0.41 (0.08, 2.14)	0.97 (0.56, 1.68)	1.58 (0.89, 2.83)	0.65 (0.10, 4.31)	N/A	N/A	1.03 (0.75, 1.41) I^2 = 2.57%
Arg16Gln27 vs. Gly16Gln27	1.53 (0.63, 3.72)	N/A	N/A	0.84 (0.40, 1.79)	0.27 (0.04, 1.63)	1.82 (0.93, 3.57)	1.40 (0.65, 3.01)	0.26 (0.02, 3.31)	N/A	N/A	1.22 (0.83, 1.79) I^2 = 5.91%
Gly16Gln27 vs. Gly16Glu27	0.53 (0.21, 1.35)	N/A	N/A	1.13 (0.45, 2.87)	1.52 (0.36, 6.47)	0.53 (0.28, 1.00)	1.13 (0.56, 2.29)	2.48 (0.18, 34.37)	N/A	N/A	0.83 (0.54, 1.26) I^2 = 17.70%

Abbreviations: Asthma exacerbations, asthma-related hospitalizations/emergency department visit or oral corticosteroids use; SABA, short-acting β_2-agonists; ICS, inhaled corticosteroids; LABA, long-acting β_2-agonists; LTRA, leukotriene receptor antagonists; Odds Ratios (ORs) and corresponding 95% Confidence Intervals (CIs) were reported, adjusted for age and sex; N/A, Not applicable

DISCUSSION

In this large multi-ethnic meta-analysis, we observed that the Arg16/Gln27 haplotype versus the Gly16/Glu27 haplotype and the Arg16/Gln27 haplotype versus the Gly16/Gln27 haplotype were associated with an increased risk of asthma exacerbations in children and young adults treated with ICS plus LABA. Considering that no statistically significant association was observed between the Gly16/Gln27 haplotype versus the Gly16/Glu27 haplotype and the risk of asthma exacerbations, we could conclude that the combined effect of two polymorphisms at codons 16 and 27 on asthma exacerbations is presumably mainly driven by the Arg16. Furthermore, we did not find an increased risk for exacerbations in asthmatic children carrying the Arg16 haplotype in any of the other treatment categories. The lack of association in the treatment category containing ICS, LABA, and LTRA might be due to both the bronchodilation and anti-inflammation effects of LTRA,[37] as well as to the relatively small sample size.

There was no heterogeneity (I^2 = 0.00%) in the main analysis between studies (Figure 1); however, the ORs were slightly different across the studies. The proportion of asthma exacerbations largely varied between the studies, lowest in PACMAN (recruiting from primary care and community pharmacies) and highest in PASS (recruiting from tertiary care), which might be due to the recruitment of patients from different health care settings (i.e. primary, secondary, and tertiary care, or community pharmacies) and thus reflect differences in asthma severity. Also, asthma treatment policy that affects doctors' underlying tendencies to prescribe OCS varies in different countries, which in turn could influence the proportion of asthma exacerbations.[38] In all studies, both SNPs were in complete linkage disequilibrium (D'~1) with each other; as a result, we determined three haplotypes of the four possible haplotypes (Arg16/Glu27 was not reported), which is in line with previous findings.[39,40] Furthermore, considering ethnicity variability in our study populations, we observed different minor allele frequencies in each SNP that resulted in considerable variations in r^2, which indicates the correlation coefficient of the allele frequencies. We also observed the highest risk allele frequencies (the Arg allele at rs1042713 and the Gln allele at rs1042714) in SCSGES, SAGE, and GALA II, whereas the Gly16/Glu27 haplotype frequency was substantially the lowest in these three studies, consistent with previous works.[41-46]

A recent systematic review[2] reported studies that investigated the association between the *ADRB2* variants and response to LABA in children and adults with asthma. In children, most studies reported an increased risk of asthma exacerbations in carriers of Arg16, whereas no association was found in adults.[4,7,8,10,47] So far, only two studies investigated the effect of rs1042714 on asthma exacerbations in children treated with ICS plus LABA and did not report significant associations.[4,9] Similarly, in adults, no association between rs1042714 and response to LABA concerning asthma exacerbations has been shown in a post hoc analysis from a randomized clinical trial.[8]

A few studies examined the association between these *ADRB2* haplotypes in subjects with asthma. However, they mainly focused on changes in forced expiratory volume in 1 s (FEV_1),[42] forced vital capacity (FVC), FEV_1/FVC ratio,[43] and overall mean changes in morning peak flow as primary outcomes.[48] To the best of our knowledge, this is the first large meta-analysis investigating the association between the *ADRB2* haplotypes and the risk of asthma exacerbations in patients treated with ICS plus LABA to this date. We know from the literature that Arg16 at rs1042713 is associated with an increased risk for asthma exacerbations; however, this association has not yet been investigated in the Arg haplotype carriers.[4,5,10]

The exact mechanism by which *ADRB2* polymorphisms confer risk for asthma exacerbations in patients treated with ICS plus LABA is still unknown. The mechanism(s) underlying the association between the Arg16 allele and an increased risk of exacerbations in asthmatic patients treated with ICS plus LABA might involve an enhanced agonist-induced down-regulation and uncoupling of airway β_2-receptor, resulting in sub-sensitivity of bronchoprotective response.[49] There is some evidence from the literature that *ADRB2* haplotypes regulate receptor transcript and protein expression.[42] Previous in-vitro findings indicated that the expression of the Arg16/Gln27 haplotype was significantly lower than the Gly16/Glu27 haplotype.[42] The latter results[42] are in line with eQTL data,[32] demonstrating decreased expression levels of *ADRB2* in the carriers of Arg16 and Gln27. Another possible explanation, based on the dynamic baseline receptor model proposed by Liggett,[50] could be that the Arg16 genotype would be slightly more resistant than the Gly16 genotype to endogenous down-regulation and desensitization. Thus, the Arg 16 genotype would remain more susceptible to further sub-sensitivity to the chronic use of exogenous agonists.[50] Hence, the observed weakened response to LABA in carriers of the Arg16/Gln27 haplotype is plausible.

As for all observational research, our study has strengths and limitations. This study is to be the largest meta-analysis investigating the combined effect of the *ADRB2* variants in asthmatic patients treated with ICS plus LABA. Also, we used quality-controlled genotyping data, physician diagnosed-asthma, and relevant clinical outcomes (asthma exacerbations). As the first limitation, we did not determine haplotype frequency using gene-counting estimates based on phase-known data. Instead, we obtained haplotype frequency estimates using the expectation-maximization (E-M) algorithm that previous studies have demonstrated the usefulness of this approach (E-M method)[51] and the validity of the statistical technique of this method.[52] Second, although the *ADRB2* rare variants could affect treatment response to LABA therapy,[53] our study was not powered to conduct rare variant analysis. Third, as we lacked information on treatment adherence and dosing in some of the PiCA cohorts, we could not adjust for these factors in our analyses. Fourth, as gene expression and eQTL are tissue-specific, ideally, they should be examined in the lung tissue of patients with asthma treated with ICS plus LABA. Finally, in our meta-analysis, we observed a significant OR (1.40), 95% CI (1.05–1.87) with a

P = 0.022, applying a multiple testing correction ($p < 0.025$) to define statistically significant results. We also calculated a prediction interval (PI); the PI in a random-effects model contains a highly probable effect estimate (OR) for a future observation if a new setting is similar to those included in the meta-analysis.[54,55] In this case, the 95% PI is (0.96–2.04) and thus indeed broader than the 95% CI.

In conclusion, we found that the Arg16 haplotype in *ADRB2*, presumably mainly driven by the Arg16, increased the risk of asthma exacerbations among users of ICS and LABA. The clinical benefits and risks associated with the use of LABA in patients with the Arg16 haplotype and genotypes need to be evaluated in randomized clinical trials such as the ongoing precision medicine clinical trial (the PUFFIN trial) investigating *ADRB2* genotype-guided (the Arg16 genotype) treatment in children with asthma.[56]

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SUPPLEMENTARY METHODS

Study Population

Data of ten independent studies participating in the Pharmacogenomics in Childhood of Asthma (PiCA) consortium[S1] were analyzed. All the studies have been approved by their local medical ethics committees and parents and/or participants provided written consent.

BREATHE is an observational study that includes children and young adults (age: 3-22 years)[S2] with physician-diagnosed asthma recruited from primary and secondary care units in Tayside, Scotland, and Brighton, United Kingdom. A detailed history including information on symptoms, treatment, asthma exacerbations, demographics, and anthropometric information was obtained from the participants. From 1307 individuals, mouthwash samples were collected for DNA analysis and the patients were considered to be non-Hispanic whites.

The Effectiveness and Safety of Treatment with Asthma Therapy in children (ESTATe), www.estate-studie.nl, is a case-control study that includes children and young adults (age: 4-19 years) with physician-diagnosed asthma recruited from primary care units in the Netherlands. Patients were selected from either the IPCI (Interdisciplinary Processing of Clinical Information) database or the PHARMO Database Network. Both databases contain the complete electronic medical records of more than one million patients throughout the Netherlands with detailed information on patient diagnosis, patient prescription (IPCI), or patient dispensing (PHARMO). During the study period (2000-2012), all children with asthma, 4 years or older and treated with asthma controller therapy were selected. Within this cohort, asthma exacerbation cases were defined as the use of systemic corticosteroids, asthma-related emergency department visits, or hospitalization. Each case was matched to four controls on age, sex, general practice (GP), and type of asthma controller therapy. Next, all potential cases and controls were invited to participate via their respective GP. If patients agreed to participate, they completed a research questionnaire, including questions on asthma control, and provided a saliva sample (for DNA extraction).

The followMAGICS study is the follow-up study of the observational Multicenter Asthma Genetics in Childhood Study (MAGICS), which includes physician-diagnosed asthmatic children and young adults (age: 7-25 years)[S3] recruited from secondary and tertiary centers in Germany and Austria. The ethnicity of patients was non-Hispanic whites, which was validated through principal component analysis.

The Genes-Environment and Admixture in Latino Americans (GALA II) and the Study of African Americans, Asthma, Genes, and Environments (SAGE) studies are two independent case-control asthma cohorts that focus on two different racial/ethnic groups based on the self-identified

ethnicity of the four grandparents of each subject: Hispanics/Latinos (GALA II) and African Americans (SAGE). Both studies recruited unrelated children and young adults (age: 8-21 years) using the same protocol and questionnaires from different areas in the United States and Puerto Rico (Chicago, Illinois; New York City, New York; Houston, Texas; San Francisco, California; and San Juan, Puerto Rico) for GALA.SAGE only recruited participants from the San Francisco Bay Area and California.[S4,S5] Cases were defined as subjects with physician-diagnosed asthma. Exclusion criteria were established as any of the following: ≥10 pack-years of smoking; any smoking within one year of the recruitment date; in the third trimester of pregnancy; or a history of any one of the following: sickle cell disease, cystic fibrosis, sarcoidosis, cerebral palsy, or heart or chest surgery. Exacerbations were defined as a patient-reported occurrence of at least one of the following asthma-related events during the last 12 months preceding study enrolment: emergency care, hospitalization, and oral corticosteroids use.

The Pharmacogenetics of Asthma Medication in Children Medication with Anti-inflammatory effects (PACMAN) study in the Netherlands,[S6] is an observational cohort study that included children (age: 4-12 years) with self-reported regular use of asthma medication recruited through community pharmacies. Children were selected from community pharmacies in the Netherlands that belonged to the Utrecht Pharmacy Practice Network for Education and Research (UPPER).[S7] During visits to community pharmacies, detailed information on exacerbations and medication over the last 12 months was collected. Dutch, Moroccan, and Turkish ethnicities were considered Caucasian.

The Pediatric Asthma Gene Environment Study (PAGES) was a cross-sectional observational study designed to relate asthma outcomes to environmental end genetic factors. Children with physician-diagnosed asthma were recruited from primary care (age: 5-16 years) and secondary care (ages 2-16 years) centers across Scotland.[S8] In a single assessment, participants completed questionnaires with questions on demographic characteristics, asthma control, treatment, quality of life, and diet. Physiological testing was completed in a subgroup of participants and saliva was obtained for DNA extraction.[S8]

The Pharmacogenetics of Adrenal Suppression Study (PASS) from the United Kingdom (age: 5-18 years) is a multicenter cohort of asthmatic children. The study initially aimed to explore the association between the use of corticosteroids and adrenal suppression. Children with asthma diagnosed by a secondary care pediatrician requiring inhaled corticosteroid therapy with clinical concern about adrenal suppression were included. The ethnicity of patients was self-reported as non-Hispanic whites.[S9,S10]

The Singapore Cross Sectional Genetic Epidemiology Study (SCSGES) (age: 6-31 years)[S11] is an ongoing cross-sectional genetic epidemiology study on allergic diseases among Singapore Chi-

nese individuals. Volunteers were of Chinese ethnicity and resident in Singapore, and their DNA was extracted from mouthwash and blood. The ethnicity of subjects was self-reported Chinese and confirmed by principal component analysis between previous genome-wide genotyping data and Han Chinese (CHB) population from the HapMap project.[S11,S12] Asthma was defined as having a physician diagnosis of asthma symptoms prior to recruitment. The SLOVENIA study is a case-control cohort (age: 5-18 years) and includes asthmatic children and young adults recruited from tertiary health centers from Murska Sobota, Slovenia. Asthma was defined by physician diagnosis and hospital records. All patients were self-identified Caucasians of Slovenian origin.[S13]

SUPPLEMENTARY RESULTS

Table S1. Functional annotation of rs1042713 using the HaploRegv4.1[S14]

Chr	pos (hg38)	LD (r^2)	LD (D')	variant	Ref	Alt	EUR freq	Enhancer histone marks	DNAse	Motifs changed	Selected eQTL hits	GENCODE genes
5	148819704	0.9	0.95	rs35283004	A	G	0.38	BLD, MUS		GR,Maf	2 hits	6.9kb 5' of *ADRB2*
5	148820281	0.81	0.92	rs71582318	T	C	0.37	BLD, SKIN		Pou1f1,TATA		6.3kb 5' of *ADRB2*
5	148821442	0.94	0.97	rs12189018	T	C	0.38	BLD		RXRA	2 hits	5.2kb 5' of *ADRB2*
5	148822166	0.94	0.97	rs35019280	AG	A	0.38	BLD		CIZ,GATA,HNF1	2 hits	4.4kb 5' of *ADRB2*
5	148822926	0.93	0.97	rs33910799	AG	A	0.38	BLD	BD	CEBPB,DMRT2	1 hit	3.7kb 5' of *ADRB2*
5	148825014	0.97	0.99	rs17778257	A	T	0.38	9 tissues	SKIN	5 altered motifs	4 hits	1.6kb 5' of *ADRB2*
5	148826178	0.96	0.98	rs12654778	G	A	0.38		38 tissues	Foxp3,p53	4 hits	414bp 5' of *ADRB2*
5	148826877	1	1	rs1042713	G	A	0.38		28 tissues	4 altered motifs	3 hits	*ADRB2*

Pos; position, LD; Linkage disequilibrium, Ref; reference, Alt; alternative, EUR freq; European frequency, eQTL; expression quantitative trait loci

Table S2: Functional annotation of rs1042714 using the HaploRegv4.1[514]

chr	pos (hg38)	LD (r^2)	LD (D')	variant	Ref	Alt	EUR freq	Enhancer histone marks	DNAse	Motifs changed	Selected eQTL hits	GENCODE genes
5	148819436	0.88	0.94	rs4705059	C	T	0.59	BLD, HRT, MUS	HRT	5 altered motifs		7.2kb 5' of *ADRB2*
5	148819441	0.88	0.94	rs4705060	G	A	0.59	BLD, MUS		4 altered motifs		7.2kb 5' of *ADRB2*
5	148819679	0.9	0.96	rs10078004	G	A	0.60			Mrg,NRSF		6.9kb 5' of *ADRB2*
5	148819882	0.9	0.96	rs67339154	A	G	0.60	BLD		Brachyury,TBX5		6.7kb 5' of *ADRB2*
5	148820448	0.94	0.97	rs56330463	T	C	0.59	BLD, SKIN		PPAR		6.1kb 5' of *ADRB2*
5	148820990	0.94	0.98	rs2082382	G	A	0.60	BLD	38 tissues	Foxo,Rad21	2 hits	5.6kb 5' of *ADRB2*
5	148821037	0.97	0.99	rs2082395	A	G	0.59	BLD	25 tissues	5 altered motifs	2 hits	5.6kb 5' of *ADRB2*
5	148821395	0.95	0.99	rs9325120	C	A	0.58	BLD		4 altered motifs		5.2kb 5' of *ADRB2*
5	148821692	0.97	0.99	rs11168066	C	A	0.59	BLD		Dmbx1,Otx2	2 hits	4.9kb 5' of *ADRB2*
5	148821753	0.96	0.99	rs11959615	T	A	0.59	BLD			2 hits	4.8kb 5' of *ADRB2*
5	148821910	0.97	0.99	rs35875547	AT	A	0.59	BLD, BRN		10 altered motifs		4.7kb 5' of *ADRB2*
5	148821922	0.97	0.99	rs11958940	A	T	0.59	BLD, BRN		NRSF,Zbtb3		4.7kb 5' of *ADRB2*
5	148822006	0.97	0.99	rs34064454	A	G	0.59	BLD, BRN		AIRE,Pax-4		4.6kb 5' of *ADRB2*
5	148823105	0.97	0.99	rs11746634	C	G	0.59	ESC, BLD		LUN-1,RORalpha1		3.5kb 5' of *ADRB2*
5	148823238	0.97	0.99	rs11168067	A	G	0.59	BLD		NRSF,Pitx2,SETDB1		3.4kb 5' of *ADRB2*
5	148823373	0.95	0.99	rs9325122	C	T	0.60	BLD		HDAC2,Pou2f2,Pou3f3		3.2kb 5' of *ADRB2*
5	148824199	0.97	0.99	rs1432622	T	C	0.59	BLD		7 altered motifs	2 hits	2.4kb 5' of *ADRB2*
5	148824445	0.97	0.99	rs1432623	C	T	0.59	BLD, SKIN		Nkx2		2.1kb 5' of *ADRB2*
5	148824558	0.97	0.99	rs11168068	C	T	0.59	BLD, SKIN		8 altered motifs		2kb 5' of *ADRB2*
5	148825489	0.97	0.99	rs2400707	A	G	0.59	12 tissues	SKIN,SKIN	HLF	2 hits	1.1kb 5' of *ADRB2*
5	148825809	0.97	0.99	rs2053044	A	G	0.59	5 tissues	35 tissues	8 altered motifs		783bp 5' of *ADRB2*
5	148826364	0.99	0.99	rs11168070	G	C	0.59		51 tissues	GR		228bp 5' of *ADRB2*
5	148826465	0.99	1	rs11959427	C	T	0.59	BRN	52 tissues	11 altered motifs		127bp 5' of *ADRB2*
5	148826785	0.98	1	rs1042711	C	T	0.59		35 tissues	6 altered motifs		5'-UTR of *ADRB2*
5	148826812	0.98	1	rs1801704	C	T	0.59	BRN	37 tissues	E2A,Sin3Ak-20,ZEB1		5'-UTR of *ADRB2*
5	148826910	1	1	**rs1042714**	G	C	0.59		21 tissues	GATA,PU.1		*ADRB2*

Pos; position, LD; Linkage disequilibrium, Ref; reference, Alt; alternative, EUR freq; European frequency, eQTL; expression quantitative trait loci

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2.3

Genome-wide association study of inhaled corticosteroid response in admixed children with asthma

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Clin Exp Allergy. 2019;00:1–10. https://doi.org/10.1111/ cea.13354

ABSTRACT

Background: Inhaled corticosteroids (ICS) are the most widely prescribed and effective medication to control asthma symptoms and exacerbations. However, many children still have asthma exacerbations despite treatment, particularly in admixed populations, such as Puerto Ricans and African Americans. A few genome-wide association studies (GWAS) have been performed in European and Asian populations, and they have demonstrated the importance of the genetic component in ICS response.

Objective: We aimed to identify genetic variants associated with asthma exacerbations in admixed children treated with ICS and to validate previous GWAS findings.

Methods: A meta-analysis of two GWAS of asthma exacerbations was performed in 1347 admixed children treated with ICS (Hispanics/Latinos and African Americans), analyzing 8.7 million genetic variants. Those with $P \leq 5 \times 10^{-6}$ were followed up for replication in 1697 asthmatic patients from six European studies. Associations of ICS response described in published GWAS were followed up for replication in the admixed populations.

Results: A total of 15 independent variants were suggestively associated with asthma exacerbations in admixed populations ($P \leq 5 \times 10^{-6}$). One of them, located in the intergenic region of *APOBEC3B* and *APOBEC3C*, showed evidence of replication in Europeans (rs5995653, $P = 7.52 \times 10^{-3}$) and was also associated with change in lung function after treatment with ICS ($P = 4.91 \times 10^{-3}$). Additionally, the reported association of the *L3MBTL4-ARHGAP28* genomic region was confirmed in admixed populations, although a different variant was identified.

Conclusions and clinical relevance: This study revealed the novel association of APOBEC3B and APOBEC3C with asthma exacerbations in children treated with ICS and replicated previously identified genomic regions. This contributes to the current knowledge about the multiple genetic markers determining responsiveness to ICS which could lead in the future the clinical identification of those asthma patients who are not able to respond to such treatment.

Keywords

African American, childhood asthma, exacerbations, Latino, pharmacogenomics

INTRODUCTION

Asthma is the most common chronic condition in children and young adults. In addition to the direct impact of the illness on the individual, severe exacerbations of asthma generate considerable economic costs to healthcare systems, as well as work and/or school absenteeism.[1]

Inhaled corticosteroids (ICS) are the most effective and commonly prescribed medications for symptom control and prevention of severe asthma exacerbations.[1] While most children using ICS experience a decrease in their asthma symptoms, 30%-40% will continue to experience exacerbations, and of these non-responders, 10%-15% may even have an increase in their exacerbations.[2] High variability in ICS response has been described also among ethnicities.[3] In addition to high asthma morbidity, exacerbations rates, and mortality, admixed populations have reduced ICS response.[4] These strong ethnic differences suggest a substantial hereditary component in the ICS response.[5] In fact, approximately 40%-60% of the variation in ICS response may be due to genetic factors.[6]

For several decades, pharmacogenetic studies have utilized candidate-gene approaches, which only evaluate a small portion of the genetic variation. More recently, these have evolved towards hypothesis-free approaches by implementing genome-wide association studies (GWAS).[7] Eight GWAS of ICS response have been performed to date,[8-15] revealing an association between 14 genomic regions and this trait.

However, the polymorphisms identified by GWAS to date only represent a small proportion of the heritability of ICS response, and hence, it is not possible to predict an individual's response to this treatment.[16] The design of the GWAS performed to date may be the main reason, where analyses are statistically underpowered to detect genetic associations. Most GWAS of ICS response have included a relatively small number of individuals (N < 1000) of primarily European and, to a lesser extent, Asian ancestry, with poor representation of admixed populations,[4] which include Hispanics/Latinos and African Americans. However, the increased asthma prevalence among admixed individuals with African ancestry, such as Puerto Ricans and African Americans, and the greater genetic diversity and specific genetic background of these populations present a unique opportunity to study the response to ICS treatment in asthma.[3,4]

We hypothesized that a large pharmacogenetic study of ICS response in admixed individuals with asthma that exhaustively explores the association of genetic variants across the genome could reveal novel genes associated with this trait. We also attempted to evaluate whether the associations described in GWAS performed in European and Asian populations could be generalized to admixed populations.

METHODS

Study populations

A total of eight independent studies participating in the Pharmacogenomics in Childhood of Asthma (PiCA) consortium[17] were analyzed as part of discovery and replication phases of this meta-GWAS. Individuals from two admixed populations were included in the discovery phase: the Genes-environments & Admixture in Latino Americans Study (GALA II) and the Study of African Americans, Asthma, Genes and Environments (SAGE). Samples from six European PiCA studies were used for replication. All studies have been approved by their local institutional review boards, and all participants/ parents provided written informed assent and consent, respectively. GALA II and SAGE were approved by the Human Research Protection Program Institutional Review Board of the University of California, San Francisco (San Francisco, United States) (ethics approval numbers: 217802 and 210362, respectively). PACMAN was approved by the Medical Ethics Committee of the University Medical Centre Utrecht (Utrecht, the Netherlands). The Tayside Committee on Medical Research Ethics (Dundee, United Kingdom) approved BREATHE. PASS was approved by the Liverpool Paediatric Research Ethics Committee (Liverpool, United Kingdom) (reference number: 08/H1002/56). SLOVENIA was approved by the Slovenian National Medical Ethics Committee (Ljubljana, Slovenia). ESTATe was approved by the Medische Ethische Toetsings Commissie, Erasmus Medical Center (Rotterdam, the Netherlands) (ethics approval number: MEC-2011-474). followMAGICS was approved by the Ethik- Kommission der Bayerischen Landesärztekammer (Munich, Germany) (ethics reference number: 01218).

Discovery phase

Patients from the GALA II and SAGE studies with a physician diagnosis of asthma who reported having active symptoms and asthma medication use within the last year were analyzed in the discovery phase. These are two independent studies focused on two different racial/ethnic groups based on the self-identified ethnicity of the four grandparents of each subject: Hispanics/ Latinos (GALA II) and African Americans (SAGE). Both studies recruited unrelated children and young adults, aged 8 to 21 years old, using the same protocol and questionnaires from different areas in the United States. GALA II also recruited individuals in Puerto Rico.[18]

Analyses were performed for a subset of 854 subjects from GALA II and 493 individuals from SAGE. Specifically, we assessed self-reported ICS use, age, gender, genome-wide genotypic data,[19,20] and information regarding presence or absence of severe asthma exacerbations, as defined by the European Respiratory Society (ERS) and the American Thoracic Society (ATS).[21] We examined exacerbations that occurred during the 12 months preceding the study enrolment (need to seek emergency asthma care, hospitalizations or the administration of oral corticosteroids).

Replication phase

Validation was carried out in European individuals from six independent studies participating in the PiCA consortium: the follow-up stage of the Multicenter Asthma Genetics in Childhood Study (followMAGICS); the Pharmacogenetics of Adrenal Suppression study (PASS); Pharmacogenetics of Asthma Medication in Children: Medication with Anti-inflammatory effects (PACMAN); Effectiveness and Safety of Treatment with Asthma Therapy in Children (ESTATe); BREATHE; and SLOVENIA studies. Details for each study are described in the Supporting Information.

The use of ICS and availability of data related to the presence/absence of asthma exacerbations during the previous 12 or 6 months were also applied as inclusion criteria for the individuals from these studies analyzed in the current study, whereas non-availability of data related to ICS use, asthma exacerbations, age, gender, and genotype data were considered as exclusion criteria. For those studies without data related to the events included in the ATS/ERS definition of asthma exacerbations, information regarding school absences, unscheduled general practitioner (GP) or respiratory system specialist visits was also considered.

Genome-wide genotyping, genetic ancestry assessment and imputation

Both GALA II and SAGE samples were genotyped using the Axiom® LAT1 array (Affymetrix Inc.), and quality control (QC) procedures were performed as described elsewhere.[19,20] Genotyping of the subjects included in the replication phase was performed on different genotyping platforms, as described in previous publications (see Supporting Information) (Table S1). In addition, four of the studies were genotyped for the purposes of the PiCA consortium and their QC is described in the Supporting Information.

Genetic ancestry was assessed by means of principal component (PC) analysis with EIGENSOFT 6.14 for the studies included in both discovery and replication phases.[22] Quantitative global genetic ancestry estimates were also obtained for the populations included in the discovery phase. An unsupervised model was applied using ADMIXTURE,[23] assuming the European (CEU), African (YRI), and Native American (NAM) as the parental populations for the Hispanics/Latinos, and YRI and CEU for African Americans. For that, reference haplotypes from CEU and YRI populations from the HapMap Project Phase III[24] were used. Moreover, haplotypes from individuals genotyped with Axiom® LAT1 array (Affymetrix Inc.) were considered as reference for NAM population, as described elsewhere.[19,25]

In all the studies, imputation was carried out by means of the Michigan Imputation Server (https://imputationserver.sph.umich.edu) using the second release of the Haplotype Reference Consortium (HRC) (r1.1 2016) as reference panel.[26] Haplotype reconstruction and imputation were performed with SHAPEIT v2.r790[27] and Minimac3,[28] respectively.

Association testing and meta-analysis in the discovery phase

GWAS analyses were carried out separately for GALA II and SAGE. Logistic regressions were used to evaluate the association between genetic variants and ICS response by means of the binary Wald test implemented in EPACTS 3.2.6.[29] The presence or absence of any asthma exacerbations during the last 12 or 6 months in patients treated with ICS was considered as a measure of ICS response, which was evaluated as a binary variable. Age, gender, and the first two PCs, obtained with EIGENSOFT 6.14,[22] were included as covariates in the regression models. The number of PCs included as covariates was chosen based on the comparison of different models that included up to 10 PCs, showing that results based on 2 PCs had the best fit with the expected values under the null hypothesis of no association.

Single nucleotide polymorphisms (SNPs) with a minor allele frequency (MAF) ≥ 1% and with imputation quality (Rsq) ≥ 0.3 in GALA II and SAGE, and shared among both populations, were meta-analyzed using METASOFT.[30] Fixed-effects or random-effects models were selected for each variant depending on absence or presence of heterogeneity, respectively, which was assessed by means of the Cochran Q test. A threshold of P-value ≤ 5×10^{-6} was arbitrarily set to select variants suggestively associated with asthma exacerbations, since this threshold is commonly adopted in GWAS.[31-35] Among those variants, independent associations were detected by means of logistic regression analyses conditioned on the most significant SNP of each locus using R 3.4.3.[36] This analysis provided a list of independent variants that were followed up for replication.

Association testing and meta-analysis in the replication phase

Statistical analyses were performed following the same methodology as in the discovery phase, except for the definition of asthma exacerbations available in each study and the number of PCs included as covariates in the association analyses (Table S1). Evidence of replication was considered for those SNPs that showed a combined *P*-value ≤ 0.05 in a meta-analysis of all the European studies and consistent directions of effects in both discovery and validation populations.

Association with ICS response measured as change in FEV_1

SNPs significantly associated with asthma exacerbations in both admixed and European populations were evaluated for association with the change in the forced expiratory volume in 1 second (FEV_1) after 6 weeks of treatment with ICS in 166 ICS users from the SLOVENIA study, the only cohort included in the analyses with this outcome measured. This variable was dichotomized to define responders and non-responders to ICS treatment using a cut-off of ≥8% improvement of FEV_1, which has been established as a good predictor of asthma severity in children.[37] Logistic regression models were applied including age, gender, and the first two PCs as covariates.

Functional evaluation of variants associated with ICS response

Functional annotation and evidence of significant expression quantitative trait loci (eQTL) were searched with HaploReg v4.1[38] based on data provided by the Encyclopedia of DNA Elements (ENCODE) project.[39] This was performed for the SNP associated with ICS response in admixed and European populations and those in high linkage disequilibrium (LD) ($r^2 > 0.9$) according to African populations from the 1000 Genomes Project (1KGP) data incorporated by HaploReg v4.1. Gene expression was inspected using the Portal for the Genotype-Tissue Expression (GTEx)[40] and the Gene Expression Atlas.[41] Moreover, evidence of association with enhancers was searched using the multiple sources available from GeneHancer.[42]

Validation of previous associations in admixed populations

Since previous GWAS of ICS response have focused on European and Asian populations,[8-15] we attempted to validate their results in admixed populations. A total of 25 SNPs near or within 14 genes declared as associated with ICS response[8-14] were followed up for replication in GALA II and SAGE.

Replication was attempted at the SNP level and also as genomic region, the latter considering variants located within 100 kilobases (kb) upstream or downstream from the gene where the variant was located or from the two closest genes in case the variant was intergenic. Evidence of replication was considered for SNPs nominally associated with ICS response ($P \leq 0.05$) that had the same direction of the effect as the published GWAS. For the replication at level of genomic region, a Bonferroni-like correction was applied to account for the number of independent variants tested within each genomic region, as estimated with empirical autocorrelations based on Markov chain Monte Carlo (MCMC) simulations. For this, an autocorrelation matrix was obtained based on the $-\log_{10}$ P-value of each SNP analyzed using the *effectiveSize* function from the R package *coda*,[43] as described elsewhere.[44] According to this, a Bonferroni-corrected significance threshold was estimated for each genomic region with $\alpha = 0.05/$ number of independent variants.

RESULTS

Characteristics of the study populations

The characteristics of the 1347 admixed asthmatic patients from GALA II and SAGE analyzed in the discovery phase and the 1697 Europeans subjects included in the replication are shown in Table 1 and Table S1, respectively. In terms of estimates of global ancestry in the admixed populations, Hispanics/Latinos had 13.6% African ancestry, 51.5% European ancestry, and 34.9% Native American ancestry. In contrast, African Americans had 79.4% African admixture and 20.6% European ancestry. Hispanics/Latinos reported a higher proportion of asthma exacerba-

tions in the 12 months preceding study enrolment (66.4%) than African Americans (51.9%). Although asthma exacerbations were differentially defined in the validation populations, similar proportions were found across the discovery and replication studies, except for PACMAN and SLOVENIA, with values of 11.0% and 34.1%, respectively (Table S1).

Table 1. Clinical and demographic characteristics of the ad mixed populations analyzed in the discovery phase

	GALA II (n = 854)	**SAGE (n = 493)**
Gender (% male)	57.3	54.2
Mean age ± SD (years)	12.1 ± 3.2	13.5 ± 3.4
Ethnicity	Hispanic/Latino	African American
Mean genetic ancestry (%)		
African	13.6	79.4
European	51.5	20.6
Native-American	34.9	NA
Asthma exacerbations in the last 12 months (%)	66.4	51.9
Emergency asthma care (%)	56.6	43.2
OCS use (%)	40.2	29.4
Hospitalizations (%)	12.6	5.7

NA, not available; OCS, oral corticosteroids; SD, standard deviation

Discovery phase

The meta-analysis of the GALA II and SAGE GWAS included 8.7 million SNPs that were shared among Hispanics/Latinos and African Americans and had MAF ≥ 1% and Rsq ≥ 0.3. The Q-Q plots of the association results for each individual study (Figure S1A and Figure S1B) and those obtained after combining both admixed populations did not reveal major genomic inflation due to population stratification (λ_{GC} = 1.04, Figure S1C). Although the genome-wide significant threshold *(P*-value ≤ 5×10^{-8}) was not reached by any of the variants, 27 SNPs with Rsq values ranging from 0.59 to 1.00 and located near or within 13 loci were suggestively associated with asthma exacerbations despite the use of ICS (*P*-value ≤ 5×10^{-6}) in admixed children and young adults (Figure 1 and Table S2).

After performing pairwise regression models conditioned on the most significant variant for each locus with at least two suggestive associations, one independent variant was detected per locus, except for *APOBEC3B-APOBEC3C* and *ANKRD30B*, where two SNPs remained significant after conditioning on each gene's most significant variant (Table S3). As a result, 15 SNPs were identified as independently associated with ICS response in admixed populations (Table S3) and were followed up for replication.

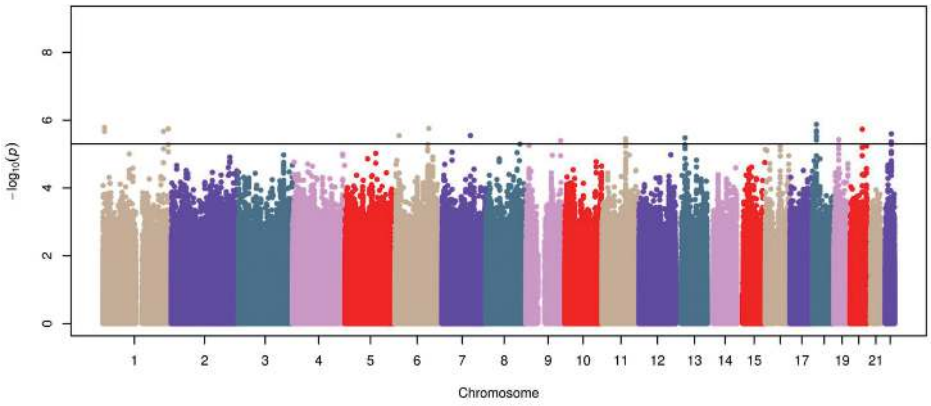

Figure 1. Manhattan plot of association results of ICS response in the discovery phase. Association results are represented as $-\log_{10}$ *p*-value on the *y*-axis along the chromosomes (*x*-axis). The suggestive significance threshold for replication is indicated by the black line ($p \leq 5 \times 10^{-6}$)

Replication phase

Of the 15 SNPs selected for replication in Europeans, 11 SNPs had a MAF ≥ 1% and Rsq ≥ 0.3 (ranging from 0.36 to 1.00) in Europeans and were forwarded for replication (Table 2). Of those, rs5995653, located within the intergenic region of *APOBEC3B* and *APOBEC3C* (Figure 2), showed evidence of nominal replication after combining the European studies. To check that the association of this SNP in the admixed populations was not confounded by unaccounted components of ancestry, different regression models were tested including estimates of genetic ancestry, different number of PCs or following the method described by Conomos et al,[45] which provided similar results (Table S4). The direction of effect for this SNP was the same in Europeans (OR for A allele: 0.76, 95% CI: 0.62-0.93, $P = 7.52 \times 10^{-3}$) as in the admixed samples (OR for A allele = 0.66, 95% CI: 0.56-0.79, $P = 4.80 \times 10^{-6}$) (Table 2). A meta-analysis of this SNP across the two phases resulted in a suggestive genome-wide significant association (OR for A allele = 0.70, 95% CI: 0.61-0.81, $P = 3.31 \times 10^{-7}$, Figure 3).

Association of rs5995653 with ICS response measured as change in FEV_1

The SNP rs5995653 was significantly associated with a positive response to the ICS treatment in SLOVENIA, measured as an increase in FEV_1 (OR for A allele = 2.16, 95% CI: 1.26-3.70, $P = 4.91 \times 10^{-3}$), which is concordant with the protective effect of this SNP with asthma exacerbations in both discovery and validation studies.

Table 2. Association results for the suggestive SNPs followed up for replication in European populations

					Admixed populations (n=1347)			European populations (n=1697)		
SNP	Chr.[a]	Position[b]	Nearest gene(s)	A1/A2	Freq.[c]	OR (95% CI)[d]	*p*-value	Freq.[c]	OR (95% CI)[d]	*p*-value
rs11121611	1	6367219	*ACOT7*	G/T	0.201	0.55 (0.43-0.70)	1.65×10^{-6}	0.062	0.97 (0.61-1.56)	0.247 [e]
rs35514893	6	15909525	*DTNBP1-MYLIP*	T/C	0.020	0.36 (0.23-0.55)	2.86×10^{-6}	0.082	0.73 (0.22-2.46)	0.613
rs4897302	6	123886231	*TRDN*	T/C	0.505	1.58 (1.31-1.91)	1.75×10^{-6}	0.221	0.96 (0.81-1.13)	0.637
rs61585310	7	104006510	*LHFPL3*	G/T	0.796	0.61 (0.49-0.75)	2.85×10^{-6}	0.763	0.91 (0.74-1.11)	0.352
rs7851998	9	126828514	*LHX2-NEK6*	A/G	0.191	0.56 (0.44-0.72)	3.97×10^{-6}	0.046	0.83 (0.65-1.06)	0.132
rs2125362	11	86167136	*ME3*	A/G	0.684	1.31 (0.68-2.56)	3.53×10^{-6} [e]	0.750	0.97 (0.82-1.16)	0.764
rs450789	13	33578233	*KL*	G/A	0.334	0.64 (0.53-0.77)	3.33×10^{-6}	0.271	0.97 (0.83-1.15)	0.756
rs12959468	18	15182381	*ANKRD30B-ROCK1*	A/G	0.039	0.39 (0.26-0.58)	2.99×10^{-6}	0.077	1.39 (0.74-2.62)	0.309
rs2278992	19	18095769	*KCNN1*	C/T	0.176	0.59 (0.47-0.74)	3.76×10^{-6}	0.151	1.00 (0.81-1.24)	0.991
rs6001366	22	39399941	*APOBEC3B-APOBEC3C*	T/C	0.079	0.47 (0.35-0.65)	2.53×10^{-6}	0.064	1.00 (0.72-1.38)	0.995
rs5995653	22	39404249	*APOBEC3B-APOBEC3C*	A/G	0.285	0.66 (0.56-0.79)	4.80×10^{-6}	0.508	0.76 (0.62-0.93)	**7.52×10^{-3}**

A1: Effect allele; A2: Non-effect allele; CI: Confidence Interval. [a]Chromosome; [b]Positions based on GRCh37/hg19 build; [c]Frequency of the effect allele; [d]Odds ratio for the effect alleles (additive model); [e]Random-effect model was applied since heterogeneity was found between admixed/European populations

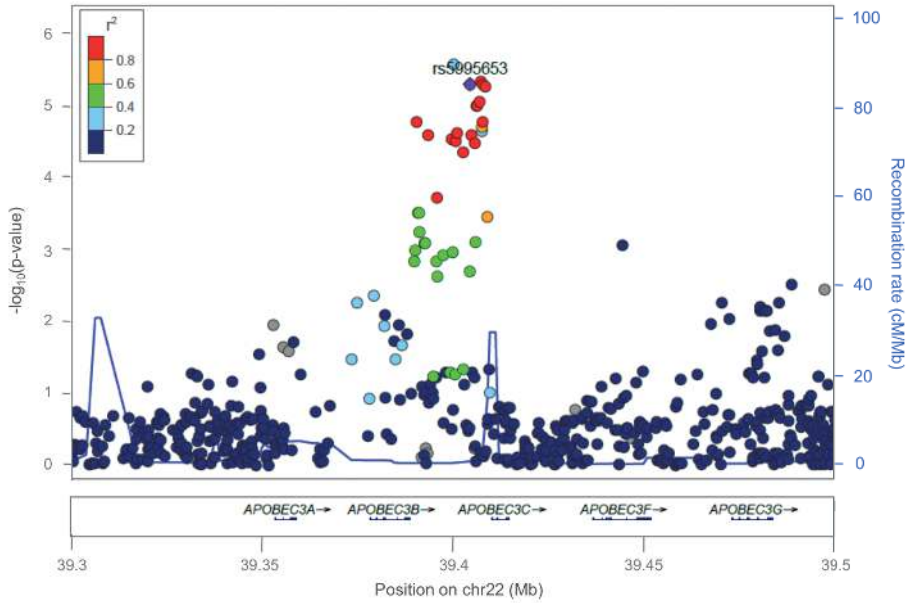

Figure 2. Regional plot of association results in the discovery phase for the *APOBEC3B-APOBEC3C* intergenic region, which represents a novel association with ICS response. Statistical significance of association results ($-\log_{10}$ *p*-value) (*y*-axis) is represented by chromosome position (*x*-axis) for each SNP as a dot. A diamond represents the independent association signal with evidence of replication in Europeans (rs5995653) and the remaining SNPs are color-coded based on their LD with this SNP, indicated by pairwise r^2 values for American populations from the 1KGP.

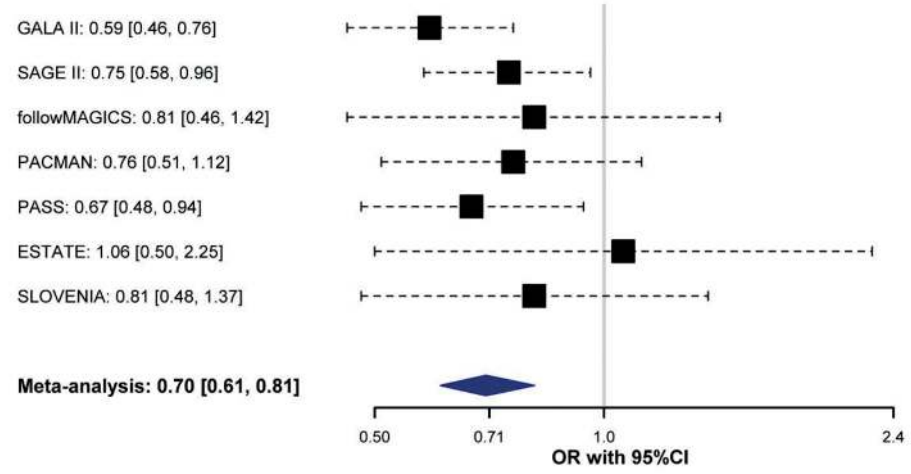

Figure 3. Forest plot of association effect of rs5995653 across studies. Odds ratio (OR) for the effect allele (A) is shown for each study and after combining them by black boxes and a blue diamond. Black dash lines indicate the corresponding 95% Confidence Intervals (95% CI) for each individual study.

In silico functional role of the novel association detected

The experimental data provided by the ENCODE project show that the SNP rs5995653 is located within a histone H3 lysine 4 mono methylation (H3K4me1) mark of an active gene enhancer and a DNase hypersensitivity site in blood cells.[39] This is concordant with the GeneHancer evidence that *APOBEC3B* has been associated with enhancers that regulate multiple transcription factor binding sites, indicating its involvement in the regulation of gene expression in different cell types, including lung fibroblasts.[42] Moreover, this variant is also in high LD with several eQTL in blood cells associated with the expression of *APOBEC3A* (rs9607601: $P = 1.80 \times 10^{-13}$ and rs5995654: $P = 9.10 \times 10^{-14}$), *APOBEC3G* (rs9607601: $P = 0.003$) and *CBX6* (rs9607601: $P = 3.94 \times 10^{-4}$ and rs5995654: $P = 4.00 \times 10^{-4}$).[38,39,46] In addition, previous functional studies have evidenced high levels of gene expression of both *APOBEC3B* and *APOBEC3C* in pulmonary cells (GTEx).[40,41]

Validation of previous associations of ICS response

None of the 25 SNPs previously associated with ICS response was consistently associated with asthma exacerbations in admixed populations (Table S5). To assess whether the lack of replication of previous GWAS hits could be due to the association of alternative genetic variants among different populations, a replication analysis was also performed at genomic region level. A total of 36,261 variants located within 100 kb upstream and downstream from 14 loci previously associated with ICS response were evaluated. After applying a Bonferroni-like correction for the number of variants analyzed within each genomic region, suggestive associations were observed for nine SNPs near three genomic regions: *ALLC* (min *P*-value = 4.69×10^{-4} for the SNP rs113903375), *L3MBTL4-ARHGAP28* (min *P*-value = 1.57×10^{-5} for the SNP rs62081416), and *ELMO2-ZNF334* (min *P*-value = 3.56×10^{-4} for the SNP rs2425845) (Table S6). However, applying a more restrictive correction for the total number of independent variants across all genomic regions ($P \leq 1.71 \times 10^{-5}$ for 2916 independent variants tested), only the association of rs62081416, located within the intergenic region of *L3MBTL4* and *ARHGAP28*, was significantly associated with ICS response in admixed individuals (OR for A allele = 2.44, 95% CI: 1.63-3.65, $P = 1.57 \times 10^{-5}$).

DISCUSSION

In this study, we carried out the first GWAS of ICS response in Hispanic/Latino and African American children and young adults with asthma. After combining the association results from these two populations, 15 independent suggestive association signals were associated with asthma exacerbations despite use of ICS, and one of them showed evidence of nominal replication in Europeans. This SNP was also significantly associated with an increase in FEV_1 after 6 weeks of treatment with ICS in one of the European studies where this outcome was measured.

These results revealed for the first time the association of *APOBEC3B* and *APOBEC3C* genes with ICS response in asthmatic children and young adults. Additionally, we validated the association of the *L3MBTL4-ARHGAP28* genomic region in admixed populations, which was previously described in a GWAS of ICS response in subjects of European descent.

The *APOBEC3B* and *APOBEC3C* genes encode two members of the apolipoprotein B mRNA-editing catalytic polypeptide 3 (*APOBEC3*) family. *APOBEC3* proteins are involved in RNA editing through the deamination of cytidine to uracil.[47] Their main function is related to innate immunity and is considered important restriction factors against a broad range of viruses.[48] However, *APOBEC3* proteins are also involved in cellular processes related to mutagenic activity,[49] including the development of several types of cancer, while *APOBEC3B* specifically has been associated with an increased risk of lung cancer.[50]

We found that the A allele of rs5995653, located 5.8 kb from the 3'UTR of *APOBEC3C*, showed a protective effect against asthma exacerbations and was associated with improvement on FEV_1 in patients treated with ICS. While no asthma-related functions have been attributed to any of the *APOBEC3* flanking genes, evidence of high levels of RNA expression has been found in pulmonary fibroblasts for both genes.[40,41] Furthermore, the functional evidence found for rs5995653 suggests that this SNP plays a key role in regulating the expression of genes involved in several cellular processes in the lung. Interestingly, respiratory viral infections are important risk factors for exacerbations in asthmatic children.[51] This fact is concordant with the consistent function of *APOBEC3B* and *APOBEC3C* as restrictors of viral infections, suggesting that the expression of these genes in pulmonary tissues could be involved in fighting against viral-induced asthma exacerbations in patients treated with ICS.

Our study has several strengths. First, this is the largest meta-GWAS of ICS response with a discovery phase specifically focused on Hispanic/Latino and African American asthma patients, the minority ethnic groups most affected by asthma in the United States.[4] Admixed populations with African and Native American have been underrepresented in the asthma pharmacogenomic studies of ICS response.[4] Secondly, we identified a novel association shared among admixed and European populations, which could be also influential in other populations. Third, we validated the association of three genomic regions previously described in GWAS of ICS response in European and Asian populations[11,13] and one of them was associated with an improvement in FEV_1 after treatment with ICS in adults.[11] This evidence reinforces the validity of asthma exacerbations as a good measure of response to the asthma treatment with ICS. Finally, the fact that the intergenic region of *L3MBTL4* and *ARHGAP28* has been previously identified in adults could suggest the existence of common genetic markers of ICS response among adulthood and childhood asthma.[13]

We recognize some limitations of our study. First, the most significant variant associated with ICS response in admixed and European populations did not reach genome-wide significance. This result was replicated in independent samples at nominal level, although it would not still be significant after a multiple comparison correction. Second, this study did not include a considerable larger sample size compared to the largest GWAS of ICS response published to date.[17] Third, even though the HRC reference panel is the largest catalogue of variants from the whole genome available to date,[26] admixed populations with African and Native American ancestries are not well represented. Fourth, asthma exacerbations were differentially defined in the European populations included in the replication phase. Nevertheless, this outcome was homogeneously defined in the studies included in the discovery phase, suggesting that the identified locus is robustly associated with asthma exacerbation across a range of definitions. Fifth, ICS response was evaluated as the presence or absence of asthma exacerbations in asthmatic patients with a self-reported use of ICS, which might not correspond to compliance or changes with the asthma control therapy. For this reason, the association signal detected was followed up for replication using a quantitative measurement of ICS response, which was only available in one of the European populations. Additional studies should seek to validate the association signal when using change in FEV_1 after the treatment with ICS as the response variable. Finally, functional evidence relating the intergenic region of *APOBEC3B* and *APOBEC3C* with ICS response in asthma patients was not directly assessed in this current study, since only experimental data available in public databases were queried. Therefore, in vitro experiments in relevant tissues and cell types for ICS response are needed to evaluate the functional roles of these loci in order to confirm their implication in this trait.

In summary, our meta-GWAS in admixed children and young adults identified a novel association of genetic variants from the intergenic region of *APOBEC3B* and *APOBEC3C* as with ICS response in subjects with asthma. We also validated the association of one genomic region previously associated with ICS response. Our study demonstrates the advantages of including admixed populations in asthma pharmacogenomic studies of ICS response.

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SUPPLEMENTARY METHODS

Studies included in the replication phase

followMAGICS (n = 147)

FollowMAGICS is the follow-up phase of the observational Multicenter Asthma Genetics in Childhood Study (MAGICS). All children were initially recruited at secondary and tertiary centers from Germany and Austria after physician's diagnosis of asthma. During the follow-up phase of the same children and young adults (followMAGICS), now aged 7 to 25 years, persistence of asthma symptoms was queried using a patient questionnaire. A description of the genome-wide genotyping with the Illumina Sentrix HumanHap300 BeadChip array (Illumina, Inc.) and quality control (QC) procedures is provided elsewhere.[S1-S4]

PASS (n = 402)

The Pharmacogenetics of Adrenal Suppression study (PASS) is a multicenter cohort that was initially conceived to explore the clinical and pharmacogenomic associations between the use of corticosteroids and the adrenal suppression. Children and young adults aged 5 to 18 years old with clinical diagnosis of asthma, inhaled corticosteroids (ICS) therapy under pediatric supervision, and clinical concern about adrenal suppression were recruited from the United Kingdom. Detailed description about the study design, data collection, characteristics of participants, genotyping with the Illumina Omni Express 8v1 array (Illumina, Inc.) and QC procedures is described in previous publications.[S5-S6]

PACMAN (n = 654)

The Pharmacogenetics of Asthma Medication in Children: Medication with Anti-inflammatory effects (PACMAN) study is an observational cohort that includes children aged 4 to 12 years old with a self-reported use of any asthma medication recruited through records of community pharmacies in the Netherlands. Detailed information on asthma symptoms, exacerbations and medication over the last 12 months was collected during visits to community pharmacies is available elsewhere.[S7]

ESTATe (n = 102)

The Effectiveness and Safety of Treatment with Asthma Therapy in children (ESTATe) is a case-control study that includes children and young adults (4-19 years) with a physician diagnosis of asthma recruited from primary care units in the Netherlands. Patients were selected from either Interdisciplinary Processing of Clinical Information (IPCI) database or the PHARMO Database Network. Both databases contain the electronic medical records of more than one million patients throughout the Netherlands with detailed information on patient diagnosis, patient prescription (IPCI) or patient dispensing (PHARMO). During the study period (2000

- 2012) all children with asthma, aged 5 years and older and treated with asthma controller therapy were selected. Within this study, cases with asthma exacerbations based on use of systemic corticosteroids, emergency room (ER) visits or hospitalizations because of asthma were selected. Each case was matched to four controls based on similarity in age, gender, general practitioner (GP) and type of asthma controller therapy. Next, all potential cases and controls were invited to participate via their respective GP. If patients agreed to participate, they provided written consent, completed a research questionnaire including questions on asthma control and provided a saliva sample (for DNA extraction).

BREATHE (n = 210)

BREATHE is a study that includes children and young adults aged 3 to 22 years old with physician diagnosis of asthma recruited at primary and secondary care units from the United Kingdom. A detailed clinical history and, demographic and anthropometric information was obtained from all participants.[S8-S10]

SLOVENIA (n = 182)

SLOVENIA is a case-control study including children and young adults with mild and moderate persistent asthma aged 5 to 18 years old of Slovenian origin recruited from tertiary health centers. Asthma was defined by physician diagnosis and hospital records according to American Thoracic Society (ATS) criteria. Forced expiratory volume in 1 second (FEV_1) expressed as a percentage of predicted value for sex, height and age was measured before therapy and 6 weeks after treatment with the use of a Vitalograph 2150 spirometer (Compact, Buckingham, UK) according to ERS/ATS guidelines. ICS was regularly administered to part of the asthmatic patients included in the study. Patients with other chronic inflammatory diseases except for those with asthma and atopic diseases and asthmatics treated with other asthma medications were excluded from the study.[S11]

Genotyping and quality control analyses in the validation studies genotyped for the current study

Four of the validation studies (PACMAN, ESTATe, BREATHE, and SLOVENIA) were specifically genotyped for the purposes of the Pharmacogenomics in Childhood of Asthma (PiCA) consortium. The Illumina Infinium CoreExome-24 BeadChip (Illumina, Inc.) was used to genotype samples from the PACMAN, ESTATe, and BREATHE studies, whereas genotyping was performed with the Illumina Global Screening Array-24 v1.0 BeadChip (Illumina, Inc.) for subjects included in SLOVENIA.

QC analyses were performed in these studies using PLINK 1.09.[S12] Several QC steps were performed at individual level. Firstly, concordance between the reported and the genetic gender assessed by means of the genotype data from the X chromosome was inspected and individu-

als with discordances in gender information were discarded from further analyses. Secondly, subjects with a genotyping completion rate (CR)<95% were discarded, as well as those with heterozygosity rates higher or lower than 4 standard deviations of the population mean. Thirdly, cryptic relatedness of individuals and population stratification were assessed. For that, single nucleotide polymorphisms (SNPs) and regions of extended linkage disequilibrium were pruned out keeping approximately 100000 SNPs for each study. An identity-by-descent matrix was estimated to remove those duplicated or related individuals. Evidence of relatedness was considered for second-degree relatives or higher evidenced by values of PIHAT ≥0.2. Then, a Principal Component (PC) analysis was performed with EIGENSOFT 6.14[S13] in order to detect population stratification due to existence of individuals with large differences in ancestry. Additionally, this analysis provided PC estimations that were included as covariates in the association testing. Finally, those individuals with a reported use of ICS and available information regarding the presence or absence of asthma exacerbations were selected for association analyses.

Moreover, genetic markers were filtered in order to exclude those with >5% missing genotypes. However, deviations from Hardy-Weinberg Equilibrium were not inspected since the datasets analyzed only included patients with asthma.

From a total of 893 genome-wide genotyped samples from PACMAN, 23 individuals had CR<95%. In addition, 20 subjects were discarded because of excessive or reduced heterozygosity rates. Furthermore, ten individuals with discordance in gender information were discarded from further analyses. Fifty-three pairs of related subjects were detected and one individual from each pair was selected based on availability of information related to the presence/absence of severe asthma exacerbations and medication use. After QC, a total of 487050 autosomal markers and 654 asthma patients treated with ICS were selected for the analyses.

From the 111 samples that were genotyped in ESTATe, those with a CR<99% and excessive autosomal heterozygosity were discarded. Furthermore, three pairs of related individuals were identified and one subject from each pair was excluded. A total of 526121 SNPs located at autosomal chromosomes remained after QC analyses.

A total of 288 samples from BREATHE were genotyped for the purposes of the PiCA consortium. During QC procedures, five individuals were discarded due to excessive or reduced heterozygosity rates, in addition to three subjects that showed large differences in ancestry based on a PC analysis. Moreover, eight pairs of related individuals were detected, and only one participant was selected from each pair based on availability of clinical information. Furthermore, a total of 176412 SNPs accomplished the QC criteria.

From the 336 samples from SLOVENIA that were genotyped, ten subjects with discordances in gender information were removed. Moreover, 13 subjects with a genotyping CR <95% and two with an excessive or reduced proportion of heterozygote genotypes were discarded for association analyses. After QC analyses, 182 individuals with a reported use of ICS and availability of data related to the presence/absence of asthma exacerbations during the previous 12 months were kept for the analyses. The number of autosomal genetic variants that passed the QC was 560996.

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Table S1. Clinical and demographic characteristics of the European populations analyzed in the replication phase

	followMAGICS (n = 147)	PACMAN (n = 654)	PASS (n = 402)	ESTATe (n = 102)	BREATHE (n = 210)	SLOVENIA (n = 182)
Gender (% male)	59.9	61.6	55.0	58.8	60.5	57.1
Mean age ± SD (years)	17.2 ± 3.0	8.7 ± 2.3	12.0 ± 2.0	10.6 ± 4.2	9.1 ± 4.0	10.8 ± 3.4
Recruitment country	Germany/Austria	Netherlands	United Kingdom	Netherlands	United Kingdom	Slovenia
Ethnicity	European	European	European	European	European	European
Asthma exacerbations in the last 12 months (%)	53.1	11.0	51.7[a]	48	51.4[a]	34.1
Definition	ER visits/ hospitalizations/ GP visits/specialist visits	ER visits/OCS use	OCS use	ER visits/ hospitalizations/ OCS use	OCS use/ hospitalizations/ school absences	ER visits/ hospitalizations/ OCS use
ER visits (%)	7.5	6.1	NA	NA	NA	28.0
OCS use (%)	NA	6.7	51.7	35.3	47.6	12.6
Hospitalizations (%)	3.4	NA	NA	12.7[b]	46.2	9.9
GP visits (%)	49.0	NA	NA	NA	NA	NA
Specialist visits (%)	21.8	NA	NA	NA	NA	NA
School absences (%)	NA	NA	NA	NA	46.2	NA
Genotyping platform	Illumina Sentrix HumanHap300 BeadChip (Illumina)	Illumina Infinium CoreExome-24 BeadChip (Illumina)	Illumina Omni Express 8v1 (Illumina)	Illumina Infinium CoreExome-24 BeadChip (Illumina)	Illumina Infinium CoreExome-24 BeadChip (Illumina)	Illumina Global Screening Array-24 v1.0 BeadChip (Illumina)

[a]Asthma exacerbations-related data was available for the 6 precedent months of the study enrollment; [b]ER visits and hospitalizations were considered as a single variable.
SD: standard deviation; ER: emergency room; OCS: systemic corticosteroids; GP: general practitioner; NA: not available

Table S2. Summary of the SNPs suggestively associated with asthma exacerbations in admixed individuals treated with ICS

					GALA II (n=854)				SAGE II (n=493)			
SNP	**Chr.[a]**	**Position[b]**	**Nearest gene(s)**	**A1/A2**	**Freq.[c]**	**Rsq[d]**	**OR (95% CI)[e]**	**p-value**	**Freq.[c]**	**Rsq[d]**	**OR (95% CI)[e]**	**p-value**
rs11121611	1	6367219	*ACOT7*	G/T	0.201	0.595	0.58 (0.44-0.75)	6.29×10^{-5}	0.062	0.708	0.42 (0.23-0.77)	5.08×10^{-3}
rs3789494	1	6370476	*ACOT7*	G/A	0.201	0.594	0.58 (0.44-0.76)	7.98×10^{-5}	0.062	0.706	0.42 (0.23-0.77)	5.08×10^{-3}
rs116561422	1	221136237	*HLX*	T/G	0.011	0.879	0.31 (0.13-0.72)	6.76×10^{-3}	0.059	0.866	0.31 (0.17-0.56)	1.00×10^{-4}
rs606572	1	238746080	*ZP4*	G/A	0.884	0.939	0.51 (0.34-0.76)	8.75×10^{-4}	0.644	0.906	0.62 (0.47-0.81)	4.44×10^{-4}
rs35514893	6	15909525	*DTNBP1-MYLIP*	T/C	0.020	0.935	0.51 (0.25-1.05)	6.67×10^{-2}	0.082	0.972	0.29 (0.17-0.50)	7.46×10^{-6}
rs4897302	6	123886231	*TRDN*	T/C	0.505	0.997	1.58 (1.25-2.00)	1.28×10^{-4}	0.221	0.988	1.59 (1.16-2.18)	4.26×10^{-3}
rs61585310	7	104006510	*LHFPL3*	G/T	0.796	1.000	0.59 (0.44-0.78)	3.27×10^{-4}	0.763	0.999	0.63 (0.46-0.85)	2.54×10^{-3}
rs7851998	9	126828514	*LHX2*	A/G	0.191	0.983	0.52 (0.40-0.69)	2.68×10^{-6}	0.046	0.937	0.78 (0.43-1.43)	0.421
rs7122239	11	86165109	*ME3*	T/C	0.623	0.998	1.76 (1.41-2.19)	4.29×10^{-7}	0.570	0.995	1.11 (0.86-1.43)	0.437
rs2125362	11	86167136	*ME3*	A/G	0.684	0.996	1.84 (1.46-2.30)	1.57×10^{-7}	0.750	0.991	0.93 (0.70-1.24)	0.624
rs2125363	11	86167202	*ME3*	G/C	0.684	0.995	1.84 (1.46-2.30)	1.57×10^{-7}	0.750	0.990	0.93 (0.70-1.24)	0.624
rs450789	13	33578233	*KL*	G/A	0.334	0.997	0.64 (0.50-0.81)	2.82×10^{-4}	0.271	0.986	0.64 (0.47-0.86)	3.69×10^{-3}
rs140275688	18	15096270	*ANKRD30B*	C/G	0.020	0.829	0.34 (0.17-0.70)	3.19×10^{-3}	0.118	0.913	0.46 (0.30-0.69)	1.71×10^{-4}

Table S2. Continued

							GALA II (n=854)				SAGE II (n=493)	
rs540731596	18	15097277	*ANKRD30B*	T/A	0.021	0.827	0.34 (0.17-0.70)	3.19×10^{-3}	0.119	0.910	0.44 (0.29-0.67)	1.07×10^{-4}
rs142954031	18	15112933	*ANKRD30B*	T/C	0.021	0.831	0.32 (0.16-0.67)	2.53×10^{-3}	0.115	0.916	0.45 (0.29-0.68)	1.83×10^{-4}
rs147911586	18	15115442	*ANKRD30B*	G/T	0.021	0.825	0.34 (0.17-0.70)	3.19×10^{-3}	0.119	0.902	0.44 (0.29-0.67)	1.07×10^{-4}
rs141514992	18	15116537	*ANKRD30B*	T/C	0.021	0.825	0.34 (0.17-0.70)	3.19×10^{-3}	0.119	0.900	0.44 (0.29-0.67)	1.07×10^{-4}
rs570126373	18	15151837	*ANKRD30B*	G/A	0.021	0.826	0.38 (0.19-0.77)	6.73×10^{-3}	0.118	0.888	0.45 (0.30-0.68)	1.69×10^{-4}
rs12959468	18	15182381	*ANKRD30B*	A/G	0.039	0.838	0.48 (0.27-0.86)	1.46×10^{-2}	0.077	0.823	0.33 (0.19-0.56)	4.28×10^{-5}
rs2278992	19	18095769	*KCNN1*	C/T	0.176	0.995	0.59 (0.44-0.79)	2.92×10^{-4}	0.151	0.994	0.59 (0.41-0.85)	4.04×10^{-3}
rs2278993	19	18096073	*KCNN1*	A/G	0.176	0.999	0.59 (0.44-0.79)	2.92×10^{-4}	0.151	0.998	0.59 (0.41-0.85)	4.04×10^{-3}
rs76657538	19	18098215	*KCNN1*	A/G	0.177	0.988	0.59 (0.44-0.79)	2.92×10^{-4}	0.152	0.977	0.59 (0.41-0.85)	2.46×10^{-3}
rs113480515	20	44461764	*SNX21*	G/C	0.010	0.994	0.15 (0.06-0.38)	6.64×10^{-5}	0.046	0.982	0.37 (0.19-0.70)	2.46×10^{-3}
rs6001366	22	39399941	*APOBEC3B-APOBEC3C*	T/C	0.079	0.845	0.50 (0.34-0.72)	2.27×10^{-4}	0.064	0.840	0.42 (0.24-0.75)	3.04×10^{-3}
rs5995653	22	39404249	*APOBEC3B-APOBEC3C*	A/G	0.285	0.958	0.59 (0.46-0.76)	2.82×10^{-5}	0.508	0.896	0.75 (0.58-0.96)	0.024
rs6001375	22	39407116	*APOBEC3B-APOBEC3C*	A/G	0.235	0.978	0.58 (0.45-0.75)	2.25×10^{-5}	0.256	0.949	0.73 (0.55-0.97)	0.033
rs4299420	22	39407685	*APOBEC3B-APOBEC3C*	G/T	0.236	0.975	0.58 (0.45-0.74)	2.13×10^{-5}	0.254	0.940	0.74 (0.55-0.98)	0.036

[a]Chromosome; [b]Positions based on GRCh37/hg19 build; [c]Frequency of the effect allele; [d]Imputation quality score; [e]Odds ratio for the effect alleles (additive model)
A1: Effect allele; A2: Non-effect allele; CI: Confidence Interval

Table S3. Results from the conditional regression models for each genomic region with suggestive associations in the discovery phase

Nearest gene(s)	SNP	Chr.[a]	Position[b]	Meta-analysis		Conditional regression model	
				OR (95% CI)[c]	*p*-value	Conditioned on	*p*-value
ACOT7	**rs11121611**	1	6367219	0.55 (0.43-0.70)	1.65×10^{-6}	rs11121611	NA
	rs3789494	1	6370476	0.55 (0.43-0.70)	2.14×10^{-6}		0.279
HLX	**rs116561422**	1	221136237	0.31 (0.19-0.50)	2.14×10^{-6}	NA	NA
ZP4	**rs606572**	1	238746080	0.58 (0.46-0.72)	1.80×10^{-6}	NA	NA
DTNBP1-MYLIP	**rs35514893**	6	15909525	0.36 (0.23-0.55)	2.86×10^{-6}	NA	NA
TRDN	**rs4897302**	6	123886231	1.58 (1.31-1.91)	1.75×10^{-6}	NA	NA
LHFPL3	**rs61585310**	7	104006510	0.61 (0.49-0.75)	2.85×10^{-6}	NA	NA
LHX2	**rs7851998**	9	126828514	0.56 (0.44-0.72)	3.97×10^{-6}	NA	NA
ME3	rs7122239	11	86165109	1.40 (0.89-2.21)	4.27×10^{-6} [d]	rs2125362	0.315
	rs2125362	11	86167136	1.31 (0.68-2.56)	3.53×10^{-6} [d]		NA
	rs2125363	11	86167202	1.31 (0.68-2.56)	3.53×10^{-6} [d]		0.278
KL	**rs450789**	13	33578233	0.64 (0.53-0.77)	3.33×10^{-6}	NA	NA
ANKRD30B	rs140275688	18	15096270	0.42 (0.30-0.61)	2.26×10^{-6}	rs540731596, rs12959468	0.263
	rs540731596	18	15097277	0.41 (0.29-0.59)	1.34×10^{-6}		NA
	rs142954031	18	15112933	0.41 (0.29-0.60)	2.05×10^{-6}		0.485
	rs147911586	18	15115442	0.41 (0.29-0.59)	1.34×10^{-6}		0.939
	rs141514992	18	15116537	0.41 (0.29-0.59)	1.34×10^{-6}		0.904
	rs570126373	18	15151837	0.43 (0.30-0.62)	3.84×10^{-6}		0.927
	rs12959468	18	15182381	0.39 (0.26-0.58)	2.99×10^{-6}		NA
KCNN1	**rs2278992**	19	18095769	0.59 (0.47-0.74)	3.76×10^{-6}	rs2278992	NA
	rs2278993	19	18096073	0.59 (0.47-0.74)	3.76×10^{-6}		0.589
	rs76657538	19	18098215	0.59 (0.47-0.74)	3.76×10^{-6}		0.336
SNX21	**rs113480515**	20	44461764	0.28 (0.16-0.47)	1.86×10^{-6}	NA	NA
APOBEC3B-APOBEC3C	**rs6001366**	22	39399941	0.47 (0.35-0.65)	2.53×10^{-6}	rs6001366, rs5995653	NA
	rs5995653	22	39404249	0.66 (0.56-0.79)	4.80×10^{-6}		NA
	rs6001375	22	39407116	0.64 (0.53-0.77)	4.36×10^{-6}		0.361
	rs4299420	22	39407685	0.64 (0.53-0.78)	4.81×10^{-6}		0.335

[a]Chromosome; [b]Positions based on GRCh37/hg19 build; [c]Odds ratio for the effect alleles (additive model); [d]Random-effect model was applied since heterogeneity was found between Latinos/Hispanics and African Americans. Independent SNPs of each gene region are in boldface

Table S4. Association results for rs5995653 in admixed populations after applying different methods to account for population stratification

Method	Ancestry covariates[a]	GALA II (n = 854)	SAGE (n = 493)	Meta-analysis (n = 1347)
		p-value	*p*-value	*p*-value
Regression models adjusted by PCs estimated with EIGENSOFT	PC1 + PC2	2.82×10^{-5}	0.024	4.80×10^{-6}
	PC1 +...+ PC3	7.72×10^{-5}	0.014	5.76×10^{-6}
	PC1 +...+ PC4	8.21×10^{-5}	0.014	4.57×10^{-6}
	PC1 +...+ PC5	1.48×10^{-4}	0.010	4.92×10^{-6}
	PC1 +...+ PC6	1.51×10^{-4}	0.011	4.92×10^{-6}
	PC1 +...+ PC7	1.79×10^{-4}	0.014	8.07×10^{-6}
	PC1 +...+ PC8	1.76×10^{-4}	0.016	1.01×10^{-5}
	PC1 +...+ PC9	1.75×10^{-4}	0.016	1.01×10^{-5}
	PC1 +...+ PC10	1.10×10^{-4}	0.014	4.74×10^{-6}
PC-KC method (Conomos *et al.*)[b]	PC1 + PC2[c]	3.45×10^{-5}	0.021	1.10×10^{-5}
	PC1 + PC2[d]	2.47×10^{-5}	0.021	8.18×10^{-6}
	PC1 + PC2[e]	2.36×10^{-5}	0.022	7.88×10^{-6}
	PC1 +...+ PC5[e]	4.35×10^{-5}	0.022	1.45×10^{-5}
	PC1 +...+ PC6[e]	2.91×10^{-5}	0.023	1.03×10^{-5}
Regression models adjusted by genetic ancestry	AFR + NAM for GALA II and AFR for SAGE	2.20×10^{-5}	0.015	1.87×10^{-6}

[a]All models also included age and gender as covariates; [b]Principal Components were estimated based on a kinship matrix using Principal Components Analysis in Related Samples (PC-AiR), which were used to calculate adjusted kinship coefficients by means of PC-Relate. A genetic relationship matrix was included as random effects, whereas covariates were included as fixed effects, as described in Conomos et al. Am J Hum Genet 2016; 98:165-84; [c]Relatedness estimated adjusting by the first two PCs; [d]Relatedness estimated adjusting by the first five PCs; [e]Relatedness estimated adjusting by the first six PCs. AFR: African ancestry; NAM: Native American ancestry; PC: Principal Component

Table S5. Results for SNPs previously associated with ICS response. Evidence found in admixed individuals

					GALA II (n=854)			SAGE (n=493)			Meta-analysis (n=1,347)		
Nearest gene(s)	**SNP**	**Chr.[a]**	**Position[b]**	**A1/A2**	**Freq.[c]**	**OR (95% CI)[d]**	**p-value**	**Freq.[c]**	**OR (95% CI)[d]**	**p-value**	**OR (95% CI)[d]**	**p-value**	**Citation**
ALLC	rs17445240	2	3703041	G/A	0.067	1.10 (0.70-1.73)	0.687	0.020	0.96 (0.39-2.38)	0.936	1.07 (0.71-1.60)	0.746	
	rs13418767	2	3704830	T/G	0.111	0.98 (0.69-1.4)	0.915	0.226	0.95 (0.70-1.29)	0.728	0.96 (0.76-1.21)	0.739	
	rs6754459	2	3707423	T/C	0.298	1.06 (0.84-1.35)	0.605	0.675	0.79 (0.59-1.05)	0.108	0.94 (0.78-1.13)	0.528	
	rs17017879	2	3713658	C/G	0.038	1.05 (0.61-1.79)	0.867	0.017	1.15 (0.40-3.27)	0.795	1.07 (0.66-1.72)	0.789	1
	rs7558370	2	3714261	C/A	0.066	1.28 (0.81-2.01)	0.293	0.107	0.88 (0.60-1.30)	0.526	1.03 (0.77-1.38)	0.842	
	rs11123610	2	3723026	A/G	0.704	0.84 (0.66-1.07)	0.164	0.487	1.02 (0.78-1.33)	0.895	0.92 (0.77-1.10)	0.348	
FBXL7	rs10044254	5	15783596	G/A	0.240	1.01 (0.78-1.32)	0.920	0.349	1.31 (0.99-1.73)	0.057	1.14 (0.94-1.38)	0.170	2
FTSJD2	rs2395672	6	37428577	A/G	0.170	1.16 (0.86-1.56)	0.342	0.045	0.65 (0.34-1.21)	0.175	1.04 (0.79-1.36)	0.781	3
MMS22L-FBXL4	rs6924808	6	98358575	A/G	0.530	1.00 (0.81-1.23)	0.974	0.574	1.06 (0.82-1.39)	0.643	1.02 (0.87-1.21)	0.792	4
PDE10A-T	rs6456042	6	166534742	C/A	0.746	0.94 (0.73-1.22)	0.657	0.639	0.99 (0.76-1.29)	0.964	0.97 (0.81-1.16)	0.725	5
	rs3127412	6	166535561	T/C	0.746	0.94 (0.73-1.22)	0.657	0.639	0.99 (0.76-1.29)	0.964	0.97 (0.81-1.16)	0.725	
	rs1134481	6	166571164	G/T	0.686	0.83 (0.66-1.05)	0.119	0.786	1.34 (0.98-1.82)	0.067	1.04 (0.66-1.66)	0.233	
	rs2305089	6	166579270	T/C	0.495	0.87 (0.70-1.09)	0.226	0.263	1.17 (0.87-1.58)	0.295	0.97 (0.81-1.15)	0.718	5
	rs3099266	6	166581147	C/T	0.658	0.88 (0.70-1.11)	0.276	0.802	1.33 (0.97-1.83)	0.075	1.07 (0.71-1.6)	0.435	

Table S5. Continued

Nearest gene(s)	SNP	Chr.[a]	Position[b]	A1/A2	GALA II (n=854)			SAGE (n=493)			Meta-analysis (n=1,347)		Citation
					Freq.[c]	OR (95% CI)[d]	p-value	Freq.[c]	OR (95% CI)[d]	p-value	OR (95% CI)[d]	p-value	
UMAD1-GLCCI1	rs37972	7	8007509	C/T	0.613	1.20 (0.97-1.49)	0.095	0.788	1.10 (0.81-1.50)	0.523	1.17 (0.98-1.39)	0.084	6
MAGI2	rs2691529	7	77803275	T/C	0.743	1.07 (0.85-1.36)	0.562	0.734	1.16 (0.87-1.55)	0.302	1.11 (0.92-1.33)	0.271	3
TRIM24	rs6467778	7	138178222	G/A	0.759	1.06 (0.83-1.35)	0.651	0.831	0.98 (0.69-1.39)	0.890	1.03 (0.84-1.26)	0.770	3
SHB-ALDH1B1	rs4271056	9	38232043	C/T	0.139	0.85 (0.62-1.15)	0.295	0.200	1.00 (0.73-1.37)	0.988	0.92 (0.73-1.15)	0.446	3
NAV2-HTATIP2	rs1353649	11	20253599	G/A	0.635	0.98 (0.79-1.22)	0.851	0.577	1.14 (0.89-1.45)	0.290	1.05 (0.89-1.23)	0.572	4
L3MBTL4-ARHGAP28	rs9303988	18	6667583	C/T	0.617	0.99 (0.80-1.22)	0.910	0.585	1.13 (0.87-1.46)	0.372	1.04 (0.88-1.23)	0.631	3
HRH4-ZNF521	rs9955411	18	22074720	T/A	0.238	1.19 (0.93-1.53)	0.175	0.273	0.96 (0.73-1.27)	0.792	1.08 (0.90-1.30)	0.409	5
ZNF432-ZNF841	rs3752120	19	52552021	T/C	0.200	0.85 (0.66-1.09)	0.196	0.055	1.37 (0.79-2.36)	0.262	0.92 (0.73-1.16)	0.483	7
	rs3450	19	52552999	C/T	0.232	0.86 (0.68-1.10)	0.227	0.142	1.25 (0.87-1.78)	0.232	0.97 (0.79-1.18)	0.739	
	rs12460587	19	52586919	G/T	0.203	0.86 (0.66-1.10)	0.224	0.060	1.23 (0.73-2.07)	0.447	0.91 (0.73-1.15)	0.443	7
ELMO2-ZNF334	rs279728	20	45080421	T/C	0.104	0.65 (0.46-0.91)	0.013	0.214	0.92 (0.66-1.28)	0.613	0.77 (0.61-0.98)	**0.037**	3

[a]Chromosome; [b]Positions based on GRCh37/hg19 build; [c]Frequency of the effect allele; [d]Odds ratio for the effect alleles. A1: Effect allele; A2: Non-effect allele; CI: Confidence Interval. Citations: 1. Park TJ, Park JS, Cheong HS, Park BL, Kim LH, Heo JS et al. Genome-wide association study identifies ALLC polymorphisms correlated with FEV_1 change by corticosteroid. Clin Chim Acta 2014; 436:20-26. 2. Park HW, Dahlin A, Tse S, Duan QL, Schuemann B, Martinez FD et al. Genetic predictors associated with improvement of asthma symptoms in response to inhaled corticosteroids. J Allergy Clin Immunol 2014; 133:664-9 e5. 3. Dahlin A, Denny J, Roden DM, Brilliant MH, Ingram C, Kitchner TE et al. CMTR1 is associated with increased asthma exacerbations in patients taking inhaled corticosteroids. Immun Inflamm Dis 2015; 3:350-359. 4. Wang Y, Tong C, Wang Z, Mauger D, Tantisira KG, Israel E et al. Pharmacodynamic genome-wide association study identifies new responsive loci for glucocorticoid intervention in asthma. Pharmacogenomics J 2015; 15:422-429. 5. Tantisira KG, Damask A, Szefler SJ, Schuemann B, Markezich A, Su J et al. Genome-wide association identifies the T gene as a novel asthma pharmacogenetic locus. Am J Respir Crit Care Med 2012; 185:1286-1291. 6. Tantisira KG, Lasky-Su J, Harada M, Murphy A, Litonjua AA, Himes BE et al. Genomewide association between GLCCI1 and response to glucocorticoid therapy in asthma. N Engl J Med 2011; 365:1173-1183. 7. Wu AC, Himes BE, Lasky-Su J, Litonjua A, Peters SP, Lima J, et al. Inhaled corticosteroid treatment modulates ZNF432 gene variant's effect on bronchodilator response in asthmatics. J Allergy Clin Immunol 2014; 133:723-728 e3

Table S6. Genomic region association results in the discovery phase of previously reported loci

Gene	# SNPs tested	# Independent signals	Bonferroni p-value threshold	Significant SNPs after Bonferroni-like correction	SNP min p-value	A1/A2	OR (95% CI)[a]	p-value
ALLC	1197	61	8.23×10^{-4}	rs113903375 rs73140873	rs113903375	G/A	2.55 (1.51-4.31)	**4.69×10^{-4}**
FBXL7	1406	53	9.52×10^{-4}	NA	rs80016637	G/A	2.13 (1.34-3.37)	1.33×10^{-3}
FTSJD2	819	156	3.20×10^{-4}	NA	rs72855423	A/G	0.63 (0.47-0.83)	1.12×10^{-3}
MMS22L-FBXL4	4069	158	3.17×10^{-4}	NA	rs77248643	A/G	2.10 (1.39-3.17)	4.28×10^{-4}
PDE10A-T	4173	265	1.88×10^{-4}	NA	rs519368	C/A	0.67 (0.54-0.84)	5.16×10^{-4}
UMAD1-GLCCI1	2941	292	1.71×10^{-4}	NA	rs11978146	C/T	0.73 (0.60-0.88)	1.40×10^{-3}
MAGI2	6171	196	2.55×10^{-4}	NA	rs75174008	T/C	0.53 (0.37-0.78)	1.07×10^{-3}
TRIM24	891	479	1.04×10^{-4}	NA	rs79076168	G/A	1.88 (1.15-3.10)	0.013
SHB-ALDH1B1	2205	254	1.97×10^{-4}	NA	rs113593997	C/T	0.43 (0.25-0.74)	2.31×10^{-3}
NAV2-HTATIP2	3088	291	1.72×10^{-4}	NA	rs7126277	G/A	1.37 (1.14-1.64)	9.09×10^{-4}
L3MBTL4-ARHGAP28	4181	150	3.33×10^{-4}	rs62081416 rs61481914 rs9789132 rs4337383 rs12604117	rs62081416	C/T	2.44 (1.63-3.65)	**1.57×10^{-5}**
HRH4-ZNF521	3301	404	1.24×10^{-4}	NA	rs8094894	T/A	1.73 (1.30-2.29)	1.77×10^{-4}
ZNF432-ZNF841	993	129	3.86×10^{-4}	NA	rs8107315	T/C	0.80 (0.68-0.95)	0.011
ELMO2-ZNF334	826	28	1.79×10^{-3}	rs2425845 rs2425846	rs2425845	T/C	1.08 (0.34-3.44)	**3.56×10^{-4}**

[a]Odds ratio for the effect alleles. A1: Effect allele; A2: Non-effect allele; CI: Confidence Interval; NA: not available. Significant *p*-values after multiple comparison adjustment are in boldface

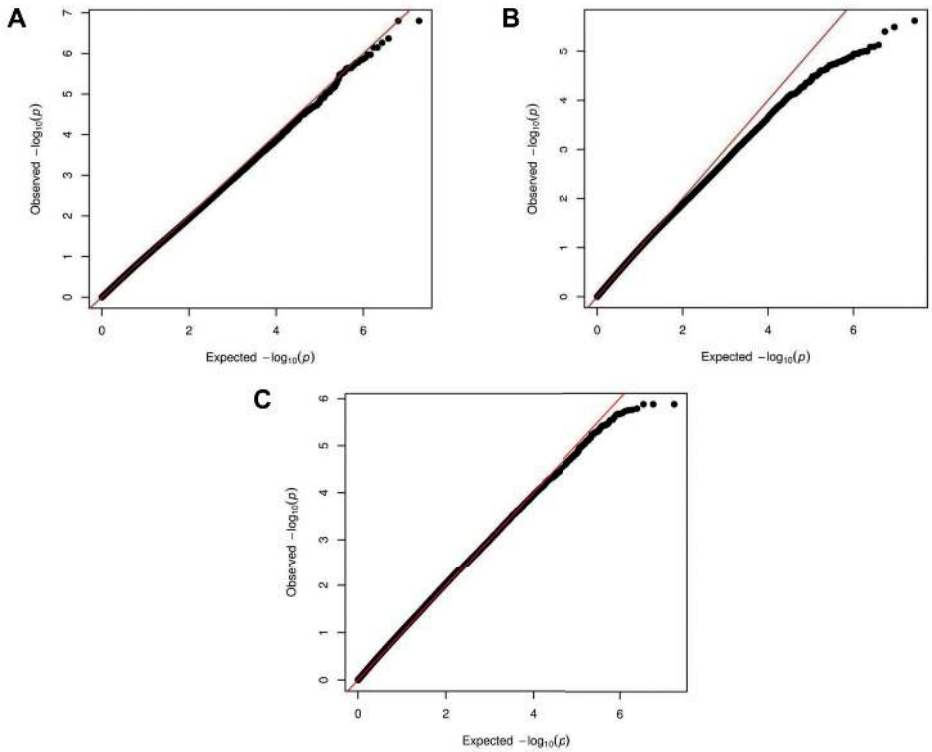

Figure S1. Quantile-quantile plots of association results of ICS response in the discovery phase. Observed and expected association results are represented as -log10 p-value on the y-axis and x-axis, respectively. A) Q-Q plot of association results in Hispanics/Latinos (GALA II) (λGC = 1.03); B) Q-Q plot of association results in African Americans (SAGE) (λGC = 0.96); C) Q-Q plot of association results after combining both admixed populations (λGC = 1.04)

2.4

Genome-wide association study of asthma exacerbations despite inhaled corticosteroids use

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Eur Respir J. 2021 May 13;57(5):2003388. doi: 10.1183/13993003.03388-2020.

ABSTRACT

Rationale: Substantial variability in response to asthma treatment with inhaled corticosteroids (ICS) has been described among individuals and populations, suggesting the contribution of genetic factors. Nonetheless, only a few genes have been identified to date. We aimed to identify genetic variants associated with asthma exacerbations despite ICS use in European children and young adults and to validate the findings in non-Europeans. Moreover, we explored whether a gene-set enrichment analysis could suggest potential novel asthma therapies.

Methods: A genome-wide association study (GWAS) of asthma exacerbations was tested in 2,681 European-descent children treated with ICS from eight studies. Suggestive association signals were followed up for replication in 538 European asthma patients. Further evaluation was performed in 1773 non-Europeans. Variants revealed by published GWAS were assessed for replication. Additionally, gene-set enrichment analysis focused on drugs was performed.

Results: 10 independent variants were associated with asthma exacerbations despite ICS treatment in the discovery phase ($p \leq 5x10^{-6}$). Of those, one variant at the *CACNA2D3-WNT5A* locus was nominally replicated in Europeans (rs67026078, p = 0.010), but this was not validated in non-European populations. Five other genes associated with ICS response in previous studies were replicated. Additionally, an enrichment of associations in genes regulated by trichostatin A treatment was found.

Conclusions: The intergenic region of *CACNA2D3* and *WNT5A* was revealed as a novel locus for asthma exacerbations despite ICS treatment in European populations. Genes associated were related to trichostatin A, suggesting that this drug could regulate the molecular mechanisms involved in treatment response.

INTRODUCTION

Asthma is the most common chronic condition in children and young adults.[1] Inhaled corticosteroids (ICS) are the first-line treatment recommended by current international guidelines to control and prevent asthma symptoms.[1] Although ICS are the most effective medication for improving symptoms and preventing severe exacerbations,[2] high inter-individual variability in ICS response has been described.[3] Studies have shown that 30–40% of the asthmatic children treated with ICS do not show an improvement of their symptoms and that 10–15% of them may even experience worsening of asthma exacerbations despite the regular use of this medication.[3] Moreover, marked variation in ICS response has been described among populations.[4]

The contribution of genetic factors in asthma-related traits has been widely suggested.[5] Specifically, the variation in ICS response has been suggested to be the result of the interaction of several factors such as the specific asthma endotype, comorbidities, ancestry, the environment, and the individual's genetic composition.[6] Approximately 40–60% of the total variation in ICS response may be explained by genetic factors.[7] Pharmacogenetic studies of ICS response have focused mostly on a few genes with known biological implications in the mechanisms of action of ICS.[5] More recently, genome-wide association studies (GWAS) have explored the role of genetic variation in ICS response.[8-10] Overall, these GWAS have identified 13 genes associated with different definitions of ICS response, most of which were not previously associated with asthma-related phenotypes, except for *PDE10A*.[11] However, it is expected that more genes are involved in the response to this asthma treatment. Moreover, the genetic architecture of clinical markers of disease severity, such as asthma exacerbations or lung function measurements, is not completely disentangled.[12,13] The studies performed to date have been limited by the relatively small number of study participants. Therefore, there is a need for studies including a large number of individuals to increase the power to detect significant associations with asthma severity and ICS response.[5] Increasing the knowledge about the genetic markers involved in asthma progression and therapeutic response would be of special importance in clinical practice since current international guidelines for the management of asthma propose pharmacological stepwise approaches based on the occurrence and persistence of clinical outcomes as indicators of disease severity.[1]

In the present study, we aimed to replicate suggested associations in a candidate gene approach and to identify novel genetic variants involved in the occurrence of asthma exacerbations despite ICS treatment by performing a large GWAS in Europeans and to examine whether this genetic variation is shared with other populations. In addition, we explored whether a gene-set enrichment analysis of the GWAS results could suggest treatments that could be potential therapeutic alternatives in patients who do not respond to ICS therapy.

METHODS

Ethics statement

All studies included were approved by their local institutional review boards and written informed consent was provided by participants or their parents/caregivers. All methods were carried out following guidelines and regulations for human subject research under the principles of the Declaration of Helsinki.

Study populations

A total of 14 independent studies participating in the Pharmacogenomics in Childhood of Asthma (PiCA) consortium[14] were included in this study. Eight available studies in populations of European descent at the time of data collection were included in the discovery phase, whereas replication of association results was evaluated in three additional independent European studies. Further validation was performed in three non-European studies from Hispanic/Latino, African American, and Asian populations.

Discovery phase

Asthma patients from eight independent European studies were analyzed in the discovery phase: the Pharmacogenetics of Asthma Medication in Children: Medication with Anti-inflammatory Effects (PACMAN); the Pediatric Asthma Gene–Environment Study (PAGES); BREATHE; the Genetics of the Scottish Health Research Register (GoSHARE); the Pharmacogenetics of Adrenal Suppression with Inhaled Steroids study (PASS); SLOVENIA; the follow-up stage of the Multicenter Asthma Genetics in Childhood Study (followMAGICS); and Effectiveness and Safety of Treatment with Asthma Therapy in Children (ESTATe). All these studies included children and young adults aged 2–25 years recruited in five different European countries. Among the participants, only individuals with reported use of ICS, information about asthma exacerbations, and genome-wide genotyping data were included. ICS use was based on declared use of any type of ICS and/or combination with long-acting β_2-agonists at least once in the previous 12 months based on self-reports, pharmacy or medical records.[15] A period of the past 6 months was considered for those studies without data available related to the previous year. A detailed description of each study is provided in the supplementary material.

The presence or absence of at least one asthma exacerbation episode during the 6 or 12 months preceding the study enrolment was assessed. Severe asthma exacerbations were defined by a need for emergency care, hospitalizations or administration of systemic corticosteroids because of asthma for PACMAN, GoSHARE, PASS, SLOVENIA, and ESTATe (Table 1).[16] Definitions of moderate asthma exacerbations were used in BREATHE-PAGES, BREATHE, and followMAGICS (Table 1), since no information was available for any of the previous variables.[16] Therefore, data related to unscheduled general practitioner or respiratory system specialist visits and school

absence were also considered in the definition of asthma exacerbations for BREATHE-PAGES, BREATHE, and follow MAGICS (Table 1), as described elsewhere.[15]

Replication phase

Validation of the results found in the discovery phase was carried out in three independent European studies: the Avon Longitudinal Study of Parents and Children (ALSPAC), the Childhood Asthma Management Program (CAMP), and the Children Allergy Milieu Stockholm an Epidemiological Study (BAMSE). Definitions of ICS use and asthma exacerbations were based on retrospective information about the 12 months prior to study enrolment adopting the same criteria applied in the discovery phase, except for prospective data from CAMP. Further details about these studies are described in the supplementary material.

Assessment of ICS associations in non-European populations

Association signals with evidence of replication ($p \leqslant 0.05$) among Europeans were evaluated in Latino/ Hispanic subjects from the Genes-Environment and Admixture in Latino Americans (GALA II) study, African American subjects included from the Study of African Americans, Asthma, Genes, and Environments (SAGE), and Asian subjects from the Singapore Cross-Sectional Genetic Epidemiology Study (SCSGES). Information about the presence or absence of asthma exacerbations despite ICS use in the 12 months prior to study enrolment was considered. The details on these studies are described in the Supplementary material.

Genotyping, genetic ancestry estimation and imputation

Samples from the studies included in the discovery phase were genotyped using different platforms for previous studies (Table 1),[15] except for PAGES, GoSHARE, and part of the samples from BREATHE. These studies were genotyped using the Axiom Precision Medicine Research Array (Affymetrix, Santa Clara, CA, USA) by Centro Nacional de Genotipado (CeGen; www.cegen.org). The same quality control procedures described in *HERNANDEZ-PACHECO et al.*[15] were applied to all the studies. Further details are available in the supplementary material.

Details about the genotyping of the replication samples are provided in the supplementary material and summarized in Supplementary Table S1. Similarly, the genotyping methods used for the non-European studies are described in Supplementary Table S2.

Assessment of the genetic ancestry was carried out through principal component (PC) analyses or by model-based assessments of the proportions of genetic ancestry (GALA II and SAGE).[15] For SCSGES, estimation of ancestry was not performed, since genome-wide genotyping was not available. The second release of the Haplotype Reference Consortium (r1.1 2016) was used as reference panel for imputation,[17] except for CAMP and ALSPAC, where phase three of the 1000 Genomes Project was used.[18]

Association analysis in the discovery phase

GWAS analyses were carried out separately for each study, except for PAGES and a subset of individuals from BREATHE that were genotyped together with PAGES. These two studies were analyzed together since the similarities of the study design, type of biological samples, demographic and clinical characteristics, and genotyping platform used, and are denoted as BREATHE-PAGES. Association between genetic variants and the binary variable of asthma exacerbations was tested employing the binary Wald logistic regression model implemented in EPACTS 3.2.6.[19] Regression models included as covariates age, sex, and the PCs needed to control for population stratification within each study.

Results for single nucleotide polymorphisms (SNPs) with a minor allele frequency (MAF) $\geqslant$1% and imputation quality (r^2) $\geqslant$0.3 obtained for each study included in the discovery phase were meta-analyzed. Fixed-effects or random-effects models were applied using METASOFT,[20] depending on the significance of the Cochran Q-test evidencing heterogeneity among the studies analyzed. Association with asthma exacerbations despite the use of ICS treatment was considered at suggestive significance level ($p \leqslant 5 \times 10^{-6}$), which was arbitrarily set based on the criteria commonly adopted in GWAS studies.[15]

Independent association signals were detected from these results through conditional and joint multiple-SNP analyses, as implemented in Genome-wide Complex Trait Analysis 1.92.0.[21] Stepwise model selection was carried out to select independently associated SNPs within each genomic region with a suggestive association signal through a linkage disequilibrium correlation matrix obtained with the data from PACMAN, the largest study included in the discovery phase. Independent SNPs associated ($p \leqslant 5 \times 10^{-6}$) with asthma exacerbations were followed-up for replication.

Association analysis in the replication phase

Association analyses were performed in three different PiCA studies of European descent. The definition of asthma exacerbations used for each replication population is described in Supplementary Table S1. Association testing in BAMSE was performed following the same methodology as in the discovery phase. Logistic regressions were carried out in CAMP and ALSPAC using PLINK 1.9,[22] and SNPTEST 2.5.2,[23] respectively. Association results obtained from the European replication studies for variants associated with asthma exacerbations despite ICS use at nominal level ($p \leqslant 0.05$), and with the same direction of the effects as in the discovery phase were meta-analyzed following the same methodology as described earlier.

Association analysis in non-European populations

The association of the variant with evidence of replication was further assessed in GALA II and SAGE using the same statistical methodology applied for the studies included in the discovery

phase. In SCSGES (Supplementary Table S2), association with asthma exacerbations was evaluated using logistic regressions adjusted by age and sex using PLINK 1.9.[22]

Evidence of validation was considered if the variant assessed showed a p-value $\leqslant$0.05 and the same direction of the effect as the one found in European populations.

Association analysis accounting for ICS dosage and asthma severity

Several sensitivity analyses were performed to ascertain whether the effect of the associations found in different populations was driven by potential confounders of the response to asthma medication or disease severity. Specifically, association analyses with asthma exacerbations were performed for the variant with evidence of replication. First, logistic regressions were carried out evaluating the association with the presence/absence of asthma exacerbations accounting for the daily ICS dosage in PACMAN, the largest study with available information for this variable, as described in the supplementary material. Additionally, association analyses were carried out accounting for asthma severity based on the classification into treatment steps based on a modification of the guidelines established by the British Thoracic Society (BTS) and the Scottish Intercollegiate Guidelines Network (SIGN).[24] Only those individuals with available information about the use of the medications included in the classification into treatment steps were selected and they were classified as described in the supplementary material.

In silico functional evaluation of variants associated with asthma exacerbations despite ICS use

Functional evaluation of the variant with evidence of replication was carried out using publicly available databases. Evaluation of functional evidence described in the Encyclopedia of DNA Elements (ENCODE) was used to assess the role as expression quantitative trait loci (eQTL), DNase hypersensitivity sites and histone marks using HaploReg v4.1,[25] and the Portal for the Genotype-Tissue Expression was also queried.[26] Previous significant evidence as protein quantitative trait loci (pQTL) or methylation quantitative trait loci (meQTL) was also explored using publicly available information by means of the PhenoScanner v2 tool.[27, 28]

Validation of previously reported ICS genes in European populations

Previous studies identified a total of 26 SNPs located near or within 15 genes associated with ICS response in different populations (Supplementary Table S3). These variants were analyzed in the present dataset using the meta-analysis results of the discovery phase of the current GWAS.

Validation of previous associations was performed at the SNP level, searching for consistent association at the nominal level ($p\leqslant0.05$). Additionally, replication was also assessed as genomic regions, analyzing variants located within 100 kb upstream and downstream from the gene limits. A Bonferroni-corrected significance threshold was estimated for each genomic region as

α=0.05 per number of independent variants analyzed, using the same methodology as described elsewhere.[15]

Enrichment analysis of drug targets

A gene-set enrichment analysis focused on drugs was performed using the summary association results from the discovery phase of this GWAS. An overlap between the genes associated with asthma exacerbations in the discovery phase and gene sets with previous evidence of expression inhibition or induction after exposure to drugs or small molecules was inspected. For that, variants were first assigned to the nearest gene using the UCSC Table Browser tool.[29] Not only were SNPs associated ($p \leqslant 5 \times 10^{-6}$) with asthma exacerbations despite ICS treatment in the discovery phase included, but those significant at $p \leqslant 1 \times 10^{-4}$ were also analyzed to increase the statistical power to detect genes previously identified to show drug-induced changes in expression levels. This threshold was arbitrarily set as it is commonly carried out in gene-set enrichment approaches.[30, 31] For this analysis, the information available at the Drug Signatures Database and DrugMatrix was used utilizing the Enrichr tool.[32] Evidence of significant enrichment at drugs was considered for those genes with significant drug-related expression changes after accounting for the multiple comparisons tested (false discovery rate (FDR) $\leqslant$0.05).

RESULTS

Characteristics of the study populations

2681 children and young adults with asthma from eight studies were analyzed in the discovery phase (Table 1), whereas 538 patients from different populations were included in the replication stage of this GWAS in Europeans (supplementary Table S1). Individuals from the studies analyzed in the discovery phase showed a similar mean age, except for followMAGICS, which included individuals with older ages (17.2±3.0 years) (Table 1). Although different definitions of asthma exacerbations were used, similar proportions of exacerbations were found across European populations included in the discovery phase, except for PACMAN and GoSHARE, which showed the lowest asthma exacerbations rates (11.0% and 13.8%, respectively) (Table 1). Among the non-European samples, Latino/Hispanic subjects from GALA II had the highest proportion of asthma exacerbations occurrence despite the treatment with ICS (66.4%) (Supplementary Table S2).

Association results in European populations

Association results for a total of 8.1 million common SNPs (MAF $\geqslant$1%) with $r^2 \geqslant 0.3$ and shared among the eight European populations included in the discovery phase were meta-analyzed. No major evidence of genomic inflation due to population stratification was found when each study was individually analyzed (Supplementary Figure S1a–h), nor after combining them in a

Table 1. Clinical and demographic characteristics of the European populations included in the discovery phase

	PACMAN	BREATHE-PAGES	GoSHARE	PASS	SLOVENIA	BREATHE	followMAGICS	ESTATe
Sample size n	654	540	472	402	182	182	147	102
Male	61.6	60.4	24.8	55.0	57.1	59.3	59.9	58.8
Age years	8.7 ± 2.3	10.2 ± 3.5	11.3 ± 5.7	12.0 ± 2.0	10.8 ± 3.4	8.9 ± 4.0	17.2 ± 3.0	10.6 ± 4.2
Recruitment country	Netherlands	United Kingdom	United Kingdom	United Kingdom	Slovenia	United Kingdom	Germany/Austria	Netherlands
Asthma exacerbations in the last 12 months (%)	11.0	54.1[####]	13.8	51.7[####]	34.1	52.7[####]	53.1	48.0
Definition	ER visits/ OCS use	hospitalizations/ OCS use/ school absences	hospitalizations/ OCS use	OCS use	ER visits/ hospitalizations/ OCS use	OCS use/ hospitalizations/ school absences	ER visits/ hospitalizations/ GP visits/ specialist visits	ER visits/ hospitalizations/ OCS use
ER visits (%) [#]	6.1	NA	NA	NA	28.0	NA	7.5	NA
OCS use (%) [¶]	6.7	35.0	13.8	51.7	12.6	48.4	NA	35.3
Hospitalizations (%) [+]	NA	13.5	0.21	NA	9.9	46.7	3.4	12.7 [¶¶]
GP visits (%) [§]	NA	NA	NA	NA	NA	NA	49.0	NA
Specialist visits (%) [ƒ]	NA	NA	NA	NA	NA	NA	21.8	NA
School absences (%) [##]	NA	43.1	NA	NA	NA	47.2	NA	NA

Table 1. Continued

	PACMAN	BREATHE-PAGES	GoSHARE	PASS	SLOVENIA	BREATHE	followMAGICS	ESTATe
Treatment steps [¶¶]								
Step 2 (%) [++]	70.6	37.6	97.3	7.5	NA	61.0	29.3	63.7
Step 3 (%) [§§]	20.8	32.6 [+++,§§§]	2.5 [+++,§§§]	32.1 [§§§]	NA	29.1 [+++,§§§]	59.8 [§§§]	33.3 [§§§]
Step 4 (%) [fff]	5.4	29.8 [fff]	0.2 [fff]	57.2	NA	9.9 [fff]	10.9	2.0
No classification	3.2	NA	NA	3.2	NA	NA	NA	1.0
Genotyping platform	Illumina Infinium CoreExome-24 BeadChip (Illumina)	Axiom Precision Medicine Research Array (Affymetrix)	Axiom Precision Medicine Research Array (Affymetrix)	Illumina Omni Express 8v1 (Illumina)	Illumina Global Screening Array-24 v1.0 BeadChip (Illumina)	Illumina Infinium CoreExome-24 BeadChip (Illumina)	Illumina Sentrix HumanHap300 BeadChip (Illumina)	Illumina Infinium CoreExome-24 BeadChip (Illumina)

Data are presented as mean±SD or %, unless otherwise stated. PACMAN: Pharmacogenetics of Asthma Medication in Children: Medication with Anti-inflammatory Effects; PAGES: Pediatric Asthma Gene–Environment Study; GoSHARE: Genetics of the Scottish Health Research Register; PASS: Pharmacogenetics of Adrenal Suppression with Inhaled Steroids study; followMAGICS: the follow-up stage of the Multicenter Asthma Genetics in Childhood Study; ESTATe: Effectiveness and Safety of Treatment with Asthma Therapy in Children; ER: emergency room; OCS: systemic corticosteroids; GP: general practitioner; NA: not available. #: proportion of patients with any exacerbations who sought emergency care due to asthma; [¶]: proportion of patients with any exacerbations who needed to use oral corticosteroids because of asthma; [+]: proportion of patients with any exacerbations who needed to be hospitalized because of asthma; [§]: proportion of patients with any exacerbations who needed any unscheduled GP visits because of asthma; [f]: proportion of patients with any exacerbations who needed any respiratory system specialist visits because of asthma; [##]: proportion of patients with any exacerbations who were absent from school because of asthma; [¶¶]: adapted from British Thoracic Society/Scottish Intercollegiate Guidelines Network guidelines; [++]: as-needed short-acting β_2-agonists (SABA) plus regular inhaled corticosteroids (ICS); [§§]: as-needed SABA plus regular ICS and long-acting β_2-agonists (LABA); [ff]: as-needed SABA plus regular ICS, LABA and leukotriene receptor antagonists (LTRA); [###]: asthma exacerbations-related data were available for the six precedent months of the study enrolment; [¶¶¶]: ER visits and hospitalizations were considered as a single variable; [+++]: as-needed SABA plus combinations of ICS and LABA; as-needed SABA plus ICS and combinations of ICS and LABA; [§§§]: as-needed SABA plus ICS and LTRA was also considered; [fff]: as-needed SABA plus LABA, combinations of ICS and LABA, and LTRA; as-needed SABA plus ICS, combinations of ICS and LABA, and LTRA; or as-needed SABA plus combinations of ICS and LABA, and LTRA was also considered.

meta-analysis (λ_{GC}=1.04) (Supplementary Figure S1i). Although no associations were detected at the genome-wide significance level ($p \leqslant 5\times10^{-8}$), a total of 19 variants near or within 10 loci showed $p \leqslant 5\times10^{-6}$ in European children and young adults (Supplementary Table S4; Figure 1). Among those polymorphisms, one independent variant per locus was found after performing pairwise regressions conditioned on the most significant variant for each locus with more than one association signal. Thus, a total of 10 independent signals were detected (Table 2), which were followed-up for replication.

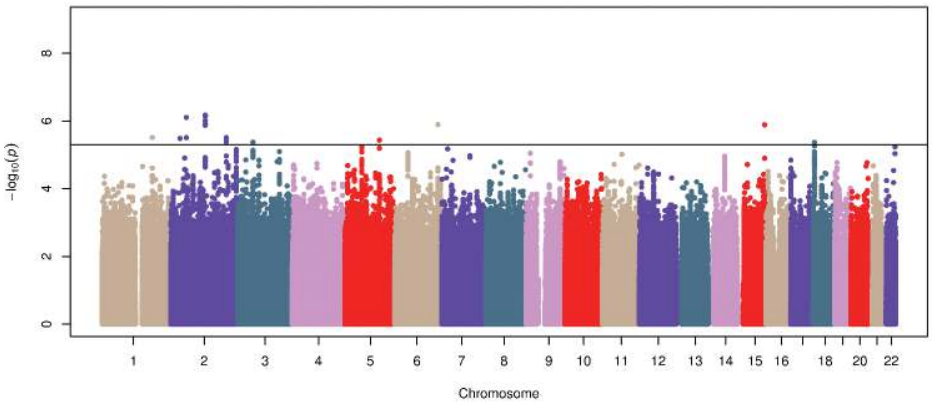

Figure 1. Manhattan plot of association results of asthma exacerbations in ICS users included in the discovery phase. Association results are represented as -$\log_{10}$ *p*-value on the *y*-axis along the chromosomes (*x*-axis). The horizontal black line shows the suggestive significance threshold for replication ($p \leq 5\times10^{-6}$).

Of the 10 variants associated with asthma exacerbations despite ICS treatment in the discovery phase ($p \leqslant 5\times10^{-6}$), only the SNP rs67026078, located within the intergenic region of *CACNA2D3* and *WNT5A* (Figure 2), showed nominal replication after meta-analyzing the European studies included in the replication (OR for C allele 1.83, 95% CI 1.16–2.90; p=0.010) (Table 3). The association had a consistent effect as in the discovery phase (OR for C allele 1.50, 95% CI 0.93–2.43; $p=4.22\times10^{-6}$) (Table 3). Suggestive genome-wide association was found for this SNP after performing a meta-analysis across the European studies analyzed in both phases (OR for C allele 1.58, 95% CI 1.11–2.26; $p=4.34\times10^{-7}$) (Figure 3). Nonetheless, the association effect of this variant was mostly driven by the studies with information about the occurrence of asthma exacerbations available for a 12-month period. This could be explained by the fact that a wider timeframe makes exacerbation events likely to occur, but also by the larger sample size analyzed compared to the studies with information based on the previous 6 months (n=1557 versus n=1124).

Table 2. Summary of the conditional regression models for each locus suggestively associated with asthma exacerbations in patients treated with inhaled corticosteroids (ICS) in the discovery phase

						Meta-analysis (n=2681)		Conditional regression model	
Nearest gene(s)	**SNP**	**Chr.** [a]	**Position** [b]	**E/NE**	**Freq.** [c]	**OR (95% CI)** [d]	**p-value**	**Conditioned on**	**p-value**
ZNF648-GLUL	**rs71632139**	1	182326506	C/G	0.109	1.60 (1.31-1.94)	3.07×10^{-6}	NA	NA
LTBP1	**rs11681246**	2	33466620	G/A	0.436	0.72 (0.63-0.83)	3.28×10^{-6}	NA	NA
CCDC85A-VRK2	**rs113364932**	2	56668971	A/G	0.063	2.20 (1.61-3.01)	7.86×10^{-7}	rs113364932	NA
	rs72805125	2	56684554	T/C	0.063	2.09 (1.53-2.85)	3.11×10^{-6}		0.888
CNTNAP5	rs76496334	2	125427606	T/C	0.022	2.29 (1.64-3.19)	9.69×10^{-7}	rs144289311	0.491
	rs146921813	2	125432412	C/G	0.022	2.26 (1.63-3.16)	1.34×10^{-6}		0.534
	rs141194780	2	125432413	A/G	0.022	2.26 (1.63-3.16)	1.34×10^{-6}		0.534
	rs144289311	2	125432440	A/G	0.022	2.33 (1.67-3.25)	6.73×10^{-7}		NA
	rs145694710	2	125434780	T/C	0.022	2.28 (1.63-3.17)	1.21×10^{-6}		0.515
	rs17011852	2	125440426	G/A	0.022	2.32 (1.66-3.24)	7.27×10^{-7}		NA
AOX1	rs2465662	2	201501145	C/T	0.283	1.13 (0.77-1.66)	4.08×10^{-6} §	rs7587871	0.847
	rs7587871	2	201505269	A/C	0.318	1.09 (0.75-1.58)	3.10×10^{-6} §		NA
	rs7420798	2	201506713	G/A	0.318	1.09 (0.75-1.58)	3.24×10^{-6} §		NA
	rs12988162	2	201507154	A/T	0.318	1.08 (0.75-1.57)	4.14×10^{-6} §		NA
CACNA2D3-WNT5A	**rs67026078**	3	55162698	C/T	0.085	1.50 (0.93-2.43)	4.22×10^{-6} §	NA	NA
ZNF608-GRAMD3	**rs444610**	5	125315286	A/T	0.398	1.36 (1.09-1.69)	3.68×10^{-6} §	NA	NA
NOX3-ARID1B	**rs2493700**	6	156826363	G/C	0.677	0.71 (0.62-0.82)	1.28×10^{-6}	NA	NA
SPATA8-ARRDC4	**rs72759231**	15	97550165	G/A	0.058	1.97 (1.50-2.59)	1.30×10^{-6}	NA	NA
DLGAP1-ZBTB14	**rs28761328**	18	4746271	A/T	0.148	1.56 (1.29-1.89)	4.26×10^{-6}	NA	NA

Bold type represents independent single nucleotide polymorphisms (SNPs) of each gene region. E: effect allele; NE: non-effect allele; NA: not available. [a] Chromosome; [b] Positions based on GRCh37/hg19 build; [c] Frequency of the effect allele in European populations from the 1,000 Genomes Project phase 3; [d] Odds ratio for the effect alleles (additive model); § Random-effect model was applied since heterogeneity was found between European studies.

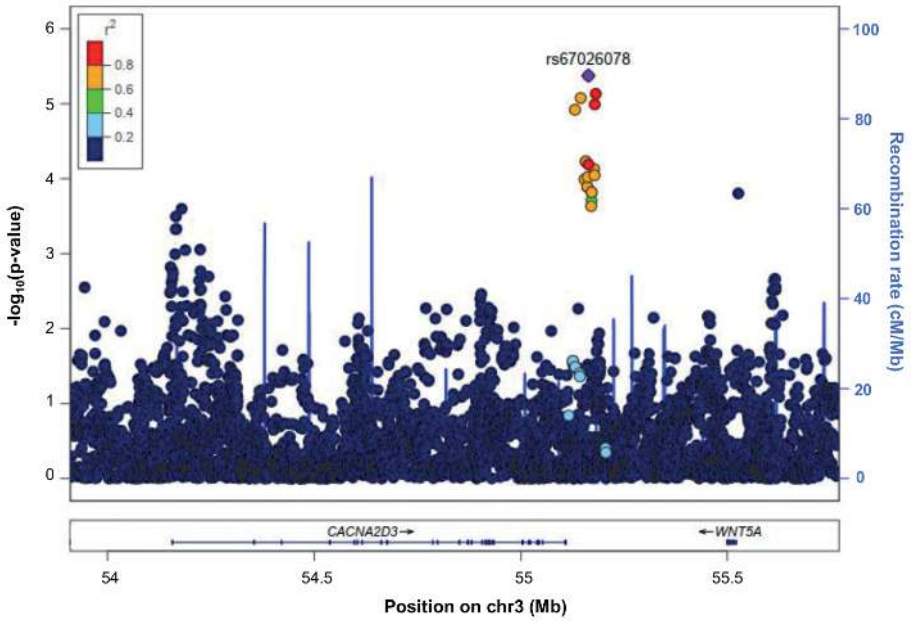

Figure 2. Regional plot of association results for the *CACNA2D3-WNT5A* locus for the European populations included in the discovery phase. Logarithmic transformation of the association results ($-\log_{10}$ *p*-value) is represented in the *y*-axis by chromosome position (*x*-axis) for each SNP as a dot. The SNP rs67026078 with evidence of replication in the European populations included in the replication phase is represented by a diamond. The remaining variants are color-coded based on pairwise r^2 values with that SNP for European populations from the 1000 Genomes Project.

Assessment of ICS associations in non-European populations

The SNP rs67026078 with evidence of replication in independent European populations was not associated with asthma exacerbations in patients treated with ICS from Hispanic/Latino nor African American populations (Supplementary Table S5). In Asian subjects, this variant was not consistently associated with asthma exacerbations in SCSGES (Supplementary Table S5). Differences in the effect allele frequency of this variant were found among the populations evaluated, being higher in the studies of European ancestry included in the discovery (6.1–9.3%) and replication (5.7–9.4%) phases, compared to the non-European populations. Specifically, this variant had a frequency of 4.7%, 4.9%, and 1.4% in Hispanic/Latino, African American, and Asian subjects, respectively.

Association analysis accounting for ICS dosage and asthma severity

Sensitivity analyses of asthma exacerbations despite ICS use including daily medication dosages as a covariate in 521 asthma patients of European descent from the PACMAN study revealed that the association effect of rs67026078 adjusted by the ICS did not account for the association with the occurrence of asthma exacerbations (OR for C allele 1.24, 95% CI 1.14–1.34; $p=2.30\times10^{-7}$). These results were equivalent in terms of significance to those obtained applying

the original association model for the same individuals with complete data, but the effect sizes were smaller (OR for C allele 4.30, 95% CI 2.33– 7.92; $p=2.98\times10^{-6}$ in the model not adjusted by ICS dose). Similar results were found adjusting by a categorical variable related to ICS dose based on age groups (OR for C allele 1.23, 95% CI 1.14–1.34; $p=2.02\times10^{-7}$) (Supplementary Table S6).

Association analyses adjusted by asthma severity based on treatment steps classification were performed in 2282 asthma patients from the discovery phase with available data related to the medication use (Table 1). The SNP rs67026078 was suggestively associated with asthma exacerbations after accounting for disease severity (OR for C allele 1.43, 95% CI 0.88–2.33; $p=1.05\times10^{-5}$). These results are equivalent to those obtained applying the original association models to the individuals with available classification into treatment steps (OR for C allele 1.45, 95% CI 0.91–2.33; $p=1.03\times10^{-5}$).

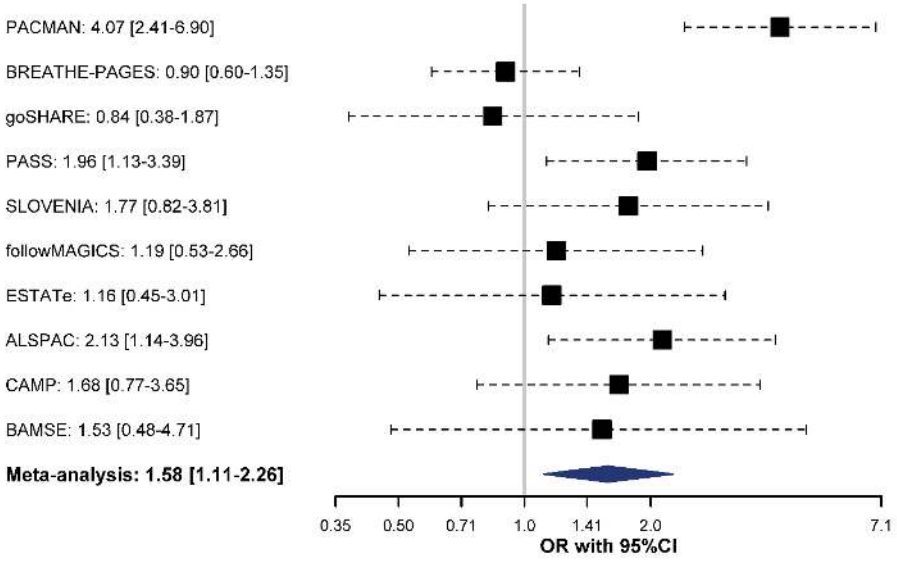

Figure 3. Forest plot of association effect of rs67026078 across European studies included in the genome-wide association study of asthma exacerbations despite inhaled corticosteroid treatment. Association effects are shown in terms of odds ratio for the effect allele for each study and after meta-analyzing the results from both phases by black boxes and a diamond, respectively. Effect of association results is not given for BREATHE, since rs67026078 did not pass quality control checks. PACMAN: Pharmacogenetics of Asthma Medication in Children: Medication with Anti-inflammatory Effects; PAGES: Pediatric Asthma Gene– Environment Study; GoSHARE: Genetics of the Scottish Health Research Register; PASS: Pharmacogenetics of Adrenal Suppression with Inhaled Steroids study; followMAGICS: the follow-up stage of the Multicenter Asthma Genetics in Childhood Study; ESTATe: Effectiveness and Safety of Treatment with Asthma Therapy in Children; ALSPAC: Avon Longitudinal Study of Parents and Children; CAMP: Childhood Asthma Management Program; BAMSE: Children Allergy Milieu Stockholm an Epidemiological Study

Table 3. Association results for the independent suggestive associations followed-up for replication in populations of European descent

					Discovery phase		Replication phase							
					Meta-analysis (n=2681)		ALSPAC (n=258)		CAMP (n=175)		BAMSE (n=105)		Meta-analysis (n=538)	
SNP	Chr.[a]	Position[b]	Nearest gene(s)	E/NE	OR (95% CI) [d]	p-value	OR (95% CI)[d]	p-value	OR (95% CI)[d]	p-value	OR (95% CI)[d]	p-value	OR (95% CI)[d]	p-value
rs71632139	1	182326506	*ZNF648-GLUL*	C/G	1.60 (1.31-1.94)	3.07×10^{-6}	1.36 (0.76-2.43)	0.315	1.16 (0.60-2.22)	0.665	1.31 (0.48-3.47)	0.604	1.27 (0.85-1.89)	0.243
rs11681246	2	33466620	*LTBP1*	G/A	0.72 (0.63-0.83)	3.28×10^{-6}	1.50 (1.00-2.27)	0.051	1.12 (0.69-1.80)	0.649	1.12 (0.59-2.08)	0.738	1.28 (0.97-1.70)	0.082
rs113364932	2	56668971	*CCDC85A-VRK2*	A/G	2.20 (1.61-3.01)	7.86×10^{-7}	0.58 (0.14-2.39)	0.424	1.87 (0.69-5.08)	0.222	0.47 (0.11-1.92)	0.305	1.00 (0.49-2.03)	0.991
rs144289311	2	125432440	*CNTNAP5*	A/G	2.33 (1.67-3.25)	6.73×10^{-7}	1.71 (0.72-4.07)	0.234	0.51 (0.14-1.88)	0.314	0.88 (0.13-5.41)	0.892	1.14 (0.58-2.23)	0.703
rs7587871	2	201505269	*AOX1*	A/C	1.09 (0.75-1.58)	3.10×10^{-6} [e]	0.70 (0.44-1.10)	0.117	1.40 (0.89-2.19)	0.146	0.98 (0.53-1.78)	0.949	0.99 (0.75-1.32)	0.942
rs67026078	3	55162698	*CACNA2D3-WNT5A*	C/T	1.50 (0.93-2.43)	4.22×10^{-6} [e]	2.06 (1.07-3.97)	0.032	1.68 (0.77-3.65)	0.193	1.53 (0.48-4.71)	0.471	1.83 (1.16-2.90)	**0.010**
rs444610	5	125315286	*ZNF608-GRAMD3*	A/T	1.36 (1.09-1.69)	3.68×10^{-6} [e]	0.76 (0.51-1.15)	0.189	0.83 (0.53-1.29)	0.409	0.79 (0.43-1.43)	0.455	0.79 (0.61-1.04)	0.091
rs2493700	6	156826363	*NOX3-ARID1B*	G/C	0.71 (0.62-0.82)	1.28×10^{-6}	1.10 (0.68-1.76)	0.697	1.05 (0.67-1.64)	0.827	0.65 (0.35-1.17)	0.162	0.96 (0.72-1.28)	0.573
rs72759231	15	97550165	*SPATA8-ARRDC4*	G/A	1.97 (1.50-2.59)	1.30×10^{-6}	0.91 (0.39-2.14)	0.829	0.60 (0.28-1.27)	0.180	0.83 (0.22-3.05)	0.785	0.74 (0.44-1.24)	0.247
rs28761328	18	4746271	*DLGAP1-ZBTB14*	A/T	1.56 (1.29-1.89)	4.26×10^{-6}	0.40 (0.19-0.82)	0.007	1.23 (0.65-2.36)	0.524	0.70 (0.28-1.68)	0.433	0.74 (0.48-1.13)	0.164

Bold type represents the independent single nucleotide polymorphism (SNP) with evidence of replication in independent European populations. E: effect allele; NE: non-effect allele; ALSPAC: Avon Longitudinal Study of Parents and Children; CAMP: Childhood Asthma Management Program; BAMSE: Children Allergy Milieu Stockholm an Epidemiological Study; NA: not available. [a] Chromosome; [b] based on GRCh37/hg19 build; [d] Odds ratio for the effect alleles (additive model); [e] Random-effect model was applied since heterogeneity was found between European studies.

Functional evaluation of the variant associated with asthma exacerbations despite ICS use

According to the ENCODE project, the SNP with evidence of replication among Europeans, rs67026078, is located within a histone H3 lysine 4 mono-methylation (H3K4me1) mark in several tissues, including fetal lung fibroblasts and other fetal pulmonary cells. Its suggestive role in regulating gene expression is also shown by the fact that this is a DNAse hypersensitivity site in lung fibroblast primary cells.[33] However, no evidence of significant eQTL was found for this SNP. Nonetheless, previously the SNP rs67026078 had been significantly identified ($p \leqslant 0.01$) as pQTL and meQTL. Specifically, B.B. Sun and coworkers found this variant to be associated with protein expression levels for 16 different proteins in plasma (Supplementary Table S7).[27, 28, 34] Some of these have been related to molecular and cellular processes related to asthma pathophysiology (ADAMTS5) and involved directly or indirectly in the Wnt pathway (PSMA2, ADAMTS5, ATAD2, CHST3, TEAD3).[35] Moreover, rs67026078 was found to regulate the methylation patterns of a CpG site (cg16278514) at the intergenic region of *CACNA2D3* and *WNT5A* in whole blood by M.J. Bonder and co-workers.[27, 28, 36] Interestingly, both *CACNA2D3* and *WNT5A* are expressed in pulmonary tissues.[26]

Validation of genes previously associated with ICS response

Among the 26 SNPs associated in previous GWAS of ICS response, one variant intergenic to *UMAD1* and *GLCCI1* (rs37972) showed evidence of replication in European populations included in the PiCA consortium (OR for C allele 1.20, 95% CI 1.05–1.37; $p=6.58\times10^{-3}$) (Supplementary Table S8). Considering the genomic regions where these genes reside, 33096 variants located within 100 kb upstream and downstream from the 15 genes of ICS response previously described were evaluated. Accounting for the number of independent association signals within each genomic region, evidence of replication was found for 40 SNPs near five genomic regions: *PDE10A-T* (SNP with min p-value: rs57042153, OR for T allele 1.43, 95% CI 1.20–1.70; $p=5.97\times10^{-5}$), *UMAD1-GLCCI1* (rs13235500, OR for G allele 0.71, 95% CI 0.60–0.85; $p=2.44\times10^{-4}$), *SHB-ALDH1B1* (SNP with min p-value: rs341488, OR for A allele 2.24, 95% CI 1.48–3.40; $p=1.44\times10^{-4}$), *ZNF432-ZNF841* (SNP with min p-value: rs67834224, OR for A allele 0.65, 95% CI 0.52–0.82; $p=2.86\times10^{-4}$), *ELMO2-ZNF334* (SNP with min p-value: rs11087003, OR for C allele 0.77, 95% CI 0.66–0.89; $p=5.84\times10^{-4}$) (Supplementary Table S9). However, none of these associations were significant after correction for the total number of SNPs tested across all genomic regions (1799 independent SNPs: Bonferroni-like correction significance threshold of $p \leqslant 2.78\times10^{-5}$).

Enrichment analysis in European asthmatic children and young adults treated with ICS

Enrichment analysis of associations from the GWAS results focused on drugs was carried out, including 782 SNPs associated with asthma exacerbations despite ICS treatment ($p \leqslant 1\times10^{-4}$) in the discovery phase. A total of 49 different drugs and small molecules that had been found

to regulate expression levels of the genes associated with asthma exacerbations in the GWAS were revealed (Supplementary Table S10). Of those, trichostatin A (TSA) remained statistically significant after adjusting for multiple comparisons (FDR=0.035) (Supplementary Table S10). Specifically, 30 of the 83 genes associated at $p \leqslant 1 \times 10^{-4}$ in our GWAS had been previously proposed as targets of TSA, since changes in expression levels were found to be triggered by the exposure to this drug (Supplementary Table S11). These genes included several loci previously associated with asthma-related traits and allergic diseases (e.g. *RERE*, *NEGR1*, *ROBO2*, *LAMA2*, *SLC11A2*, *JMJD1C*) or involved in drug metabolism (e.g. *AOX1*) (Supplementary Table S12).[35, 37]

DISCUSSION

To our knowledge, this study describes the results of the largest GWAS of asthma exacerbations in children and young adults treated with ICS to date. After combining eight different studies of European ancestry, 10 independent variants were found to be suggestively associated with asthma exacerbations despite ICS treatment. One SNP within the intergenic region of *CACNA2D3* and *WNT5A* showed evidence of replication at nominal level in three independent European populations. However, this was not validated in Latino/Hispanic, African American or Asian subjects, which could be due to ancestry-specific effects. Additionally, we found evidence of replication for five different genes associated with ICS response by previous GWAS studies at SNP or genomic-region level. Furthermore, an enrichment analysis of association signals with asthma exacerbations revealed TSA as a potential regulator of the molecular mechanisms involved in asthma pathogenesis.

CACNA2D3 encodes a member of the α-2/δ subunit family, which are voltage-dependent calcium channels consisting of a complex of α-1, α-2/δ, β and γ subunits. Specifically, *CACNA2D3* modulates the calcium current density through the regulation of the influx of calcium ions into the cell upon membrane polarisation.[38] *CACNA2D3* has important functions given the fact that calcium is a secondary messenger involved in multiple cellular processes such as cell proliferation, apoptosis, adhesion, and migration.[39] This gene could have a role in respiratory diseases, since variants located near to *CACNA2D3* have been recently associated with different lung function measurements, which are important predictors of asthma severity and progression.[40,41] Specifically, these associations include forced expiratory volume in 1 s (FEV_1), forced vital capacity (FVC), and the FEV_1/FVC ratio in COPD patients from the large cohort of European descent UK Biobank,[42,43] and the change in lung function after administration of bronchodilators in smokers.[44] It is well known that pulmonary function is an important predictor of asthma severity and progression.[40,41] Additionally, an intronic *CACNA2D3* variant (rs1820616) has been associated with the fractional concentration of nitric oxide in exhaled air,[45] which is a good indicator of inflammatory patterns in the airways and a powerful approach to support asthma

diagnosis in children[46] and to monitor the adherence and response to medications.[47] These findings suggest that *CACNA2D3* could be involved in asthma progression, including the risk of asthma exacerbations, even in patients under ICS therapy.

WNT5A encodes for the WNT family member 5A, a lipid-modified glycoprotein that activates diverse signaling pathways.[48] This protein has been evidenced to play a crucial role in development during embryogenesis, oncogenesis, and regulation of inflammatory processes in infectious disorders.[49] Moreover, other genes encoding for ligands involved in the WNT signaling pathway are associated with impaired lung function in asthmatic children.[50] This suggests that *WNT5A* could be also involved in the pulmonary capacity in asthma. Interestingly, genes associated with asthma susceptibility have been linked to WNT signaling through a gene-set enrichment analysis.[30] Specifically, this biological process seems to play regulatory and suppressive roles through the modulation of inflammation and structural changes in airways. WNT ligands have been proposed to act on the major players implicated on inflammatory processes such as dendritic and T-helper type 2 (Th2) cells and macrophages.[51] Indeed, WNT molecules regulate the homeostasis of these cells, avoiding dysregulated immune responses, which could trigger several diseases, including allergic asthma.[51]

Specifically, expression of *WNT5A* has been positively associated with Th2-mediated airway inflammation in asthmatic patients.[52] Additionally, eosinophils derived from asthma patients have been found to enhance expression levels of this gene in airway smooth muscle (ASM) cells, triggering cell proliferation, inflammatory processes, and airway remodelling.[53] It is well known that eosinophilia at blood and tissue levels is one of the most important phenotypes in asthma patients,[54] triggered by high levels of chemokines and cytokines. Specifically, eosinophils migrate from lymph nodes to the airway in asthma, where they adhere to the ASM, releasing transforming growth factor (TGF)- β_1 molecules.[55] Increased levels of TGF-β_1 have been related to the overexpression of *WNT5A* in ASM cells at gene and protein levels compared to healthy individuals. Therefore, production of extracellular matrix proteins is induced, increasing ASM mass and contractility and hence airway remodeling by means of hypertrophy and hyperplasia.[53] These findings suggest the important role of the *WNT5A* and the *WNT* signaling pathway in asthma pathogenesis, making it a promising therapeutic target in asthma,[56] throughout inhibition of WNT ligands biogenesis, secretion, and blocking their ligand–receptor interactions through small pharmacological molecules.[49] Nonetheless, further research is needed to explore the potential side-effects of drugs targeting this pathway, since tumorigenesis-related functions have been also widely attributed to WNT molecules.[57]

The C allele of the SNP rs67026078, which is located 54.1 kb from the 3′ untranslated region of *CACNA2D3*, was found to be associated with an increased risk of asthma exacerbations despite the ICS treatment across the European studies analyzed in the discovery and replication

phases. Sensitivity analyses accounting for baseline asthma severity suggested that the effect of this association is related to the response to asthma medications or to the biological drivers of asthma exacerbations. Nonetheless, this was not shown to be significantly associated with asthma exacerbations in patients treated with ICS from Hispanic/Latino, African American or Asian populations. This result could be explained by ancestry-driven effects evidenced by the lower frequency of the effect allele of this variant in non-European populations. This polymorphism had not been previously associated with asthma treatment response, although functional evidence suggests that this variant could be actively involved in the regulation of gene expression in cells from lung tissue.[33]

We also performed a gene-set enrichment analysis focusing on drugs, finding evidence of enrichment of TSA, which had been proposed to target several genes previously associated with asthma-related traits and drug metabolism, suggesting that TSA could be involved in the molecular mechanisms underlying the occurrence of asthma exacerbations despite ICS treatment. These findings demonstrate that GWAS approaches in combination with gene-set enrichment analyses seem to be a powerful strategy to explore potential novel therapeutic interventions, even in the absence of genome-wide associations.[58, 59]

TSA is a hydroxamic acid extracted from the bacterial genus *Streptomyces* with a wide range of histone deacetylase (HDAC) inhibitor activities in mammalian cells.[60] Specifically, TSA belongs to a family of compounds acting on metal-dependent HDACs, inhibiting histone deacetylation and causing hyperacetylation of core histones, which is one of the major regulators of the chromatin structure.[61] Nonetheless, HDAC inhibitors have been demonstrated to act on diverse nonhistone substrates involved in several functions, such as cell signaling, chromatin structure, and DNA repair, among others.[62]

Interestingly, the potential clinical utility of HDAC inhibitors in asthma has been investigated.[62] Several studies in animal models[62–64] have suggested that the inhibition of HDACs by TSA could play an important role in the reduction of asthma development by decreasing airway inflammation and hyperresponsiveness.[65] These findings, together with evidence that HDACs regulate sensitivity to glucocorticosteroids,[62] suggest that histone acetylation may play a key role in asthma development,[66] and seems to be a promising target for alternatives to the standard medications currently used in the management of asthma. Specifically, in vivo experiments in allergen-challenged mice have demonstrated that treatment with TSA decreases eosinophils and lymphocytes levels in bronchial alveolar lavage. Reduced expression levels of inflammatory mediators such as Th2 cytokines were also detected.[66] Moreover, it has been found that TSA shows additive effects in combination with glucocorticosteroids, suggesting that it might target the main pathological processes in asthma through mechanisms of action different from the classical asthma anti-inflammatory medications.[63] Additionally, BANERJEE *et al*.[63] found that TSA

could have important functions in the inhibition of bronchoconstriction by inducing remodeling changes. It has been demonstrated that TSA treatment might inhibit the release of intracellular calcium, reducing ASM contraction in human lung slices and ASM cells *in vitro* expose to contractile agonists.[63]

Although the effects of TSA on chromatin structure and regulation of gene expression in pulmonary tissues are still unclear,[63] these findings suggest that TSA could potentially play an important role in asthma through epigenetic modifications and regulate the molecular mechanisms involved in response to ICS. Nonetheless, to the best of our knowledge, the effect of TSA on asthma patients has not been tested in clinical trials yet and little is known about the potential side-effects of this drug. For this reason, there is still a long way for the potential introduction of TSA as controller therapy in clinical practice.

The current study has some limitations that need to be acknowledged. First, the genome-wide significance level was reached neither in the discovery phase nor after combining the results with independent European studies. Although to our knowledge our study includes the largest sample size analyzed in any GWAS of exacerbations despite ICS use performed in children and young adults with asthma to date, the lack of genome-wide associations could be explained by reduced statistical power given by differences in patient recruitment and definition of asthma exacerbations tested in association in both discovery and replication phases. Additionally, no covariates related to the etiology of asthma exacerbations and exposure to potential environmental triggers were considered in the association analyses. Second, retrospective information about the occurrence or absence of asthma exacerbations partly based on self-reports was used, which could not be fully informative of the real ICS response. Moreover, a period of 6 or 12 months preceding the study enrolment was considered, which could have introduced substantial heterogeneity in the interpretation of treatment response, since more exacerbations are possible in additional 6 months and nonresponse might be more likely to occur in 12 months. Third, although the standard definition of severe asthma exacerbations established by the European Respiratory Society and the American Thoracic Society considering them as the need for unscheduled medical care because of asthma[16] was used, this information was incomplete for some of the European studies included in the discovery or replication phases. Therefore, data regarding unscheduled visits to general practitioners or respiratory disease specialists and school absences due to asthma were considered instead, which captures moderate asthma exacerbations. Additionally, no variables indicating whether ICS therapy had been initiated before or after exacerbations episodes were available. Altogether, this heterogeneity in data availability could represent a potential interpretation bias in terms of response to asthma treatment. Fourth, specific ICS dose and type or any index of treatment adherence were not included as covariates in the association analyses, since information related to these variables was not available for most of the studies included in this GWAS. Fifth, although in *silico* evaluation of the functional implication of *CACNA2D3* and *WNT5A* on asthma exacerbations was carried out,

in vitro experiments, pharmacogenomic research of pre-existing randomized controlled trials, and longitudinal asthma studies are needed to confirm their role in asthma treatment response.

In summary, our GWAS of asthma exacerbations in children and young adults treated with ICS revealed a novel association in Europeans. We also found evidence of replication of variants previously associated with different definitions of ICS response in asthma patients of European descent and suggested TSA as a potential novel therapy that could be implicated in mechanisms controlling asthma symptoms and moderate-to-severe exacerbations in patients treated with ICS. These findings suggest that the integration of different analytical methods could be a powerful strategy providing new insights into the molecular mechanisms underlying ICS response and suggesting alternative asthma therapies.

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SUPPLEMENTARY METHODS

Studies included in the discovery phase

PACMAN (n = 654)

The Pharmacogenetics of Asthma Medication in Children: Medication with Anti-inflammatory effects (PACMAN) study is an observational cohort including children (4-12 years old) who reported the use of any asthma medication. This information was obtained through records of community pharmacies in the Netherlands. Further details about the study design have been extensively described elsewhere.[S1]

PAGES (n = 437)

The Pediatric Asthma Gene-Environment Study (PAGES) is a cross-sectional study that recruited children and young adults (2-16 years old) with a pediatrician's diagnosis of asthma attending secondary care clinics at five different centers across the United Kingdom: Aberdeen, Edinburg, Glasgow, Kilmarnock, and Brighton. Participants were invited to attend a clinical assessment where questionnaires about dietary and quality of life were complimented, and saliva samples were collected. Any coexisting respiratory disease or specific significant health problems were used as exclusion criteria.[S2]

BREATHE (n = 288)

The BREATHE study recruited children and young adults aged 3 to 22 years old with a physician diagnosis of asthma at primary and secondary care units from the United Kingdom. Detailed information about the eligibility criteria and study design has been described elsewhere.[S3-S5] From the total number of BREATHE samples included in the discovery phase of this genome-wide association study (GWAS), 182 had been genotyped using the Illumina Infinium CoreExome-24 BeadChip (Illumina) array, whereas genotypes of samples from 103 patients were obtained using the Axiom™ Precision Medicine Research Array (Affymetrix Inc.). The latter were tested in association together with PAGES samples due to similarities of study design and sample characteristics and were denoted as BREATHE-PAGES.

GoSHARE (n = 472)

As part of the Genetic of Scottish Health Research Register (GoSHARE) study, children and young adults aged 3 to 18 years old were recruited from National Health Service databases containing complete electronic medical records (EMR), prescription information, hospital, and emergency room records from Tayside (Scotland). A detailed description is available in McKinstry *et al*.[S6]

PASS (n = 402)

The Pharmacogenetics of Adrenal Suppression study (PASS) is a multicenter cohort including children and young adults aged 5 to 18 years old from the United Kingdom with a physician diagnosis of asthma and on inhaled corticosteroids (ICS) therapy under pediatric supervision. Clinical concern about adrenal suppression was also considered as eligibility criterion since this study was initially designed to explore the clinical and pharmacogenomic associations between the use of corticosteroids and adrenal suppression. A detailed description is available in previous publications.[S7, S8]

SLOVENIA (n = 182)

SLOVENIA recruited children and young adults (5-18 years old) with mild and moderate persistent asthma from tertiary health centers in Slovenia. Asthma was defined by physician diagnosis and hospital records according to the American Thoracic Society (ATS) criteria. Forced expiratory volume in 1 second (FEV_1) expressed as a percentage of predicted was measured before and after 6 weeks after treatment with ICS using the Vitalograph 2150 spirometer (Compact, Buckingham, UK) according to ERS/ATS guidelines. ICS was regularly administered to part of the asthmatic patients included in the study.[S9]

followMAGICS (n = 147)

FollowMAGICS is the follow-up phase of the observational Multicenter Asthma Genetics in Childhood Study (MAGICS). Children with a physician's diagnosis of asthma were initially recruited at secondary and tertiary centers from Germany and Austria. Persistence of asthma symptoms was used as an inclusion criterion for the follow-up phase of the same patients (followMAGICS), now aged from 7 to 25 years.[S10-S13]

ESTATe (n = 102)

The Effectiveness and Safety of Treatment with Asthma Therapy in children (ESTATe) is a case-control study including children and young adults aged 4 to 19 years old with a physician diagnosis of asthma. Patients were selected at primary care units from the Netherlands based on electronic medical records. The use of asthma controller therapy was used as an eligibility criterion. A more detailed description of the study design was provided elsewhere.[S14]

Studies included in the replication phase

ALSPAC (n = 258)

The Avon Longitudinal Study of Parents and Children (ALSPAC) is a birth cohort that recruited pregnant women in Avon (United Kingdom). Data from parents and children were regularly collected since the child was born during research clinic assessments. The main purpose of the follow-up phase of this cohort is to study the transition from childhood into adulthood of those

children. This study includes a wide variety of phenotypic, environmental, genetic, and epigenetic information from children. Further details about the data available, recruitment criteria and strategy are available elsewhere.[S15-S17]

Pregnant women residents in Avon (United Kingdom) with expected dates of delivery 1st April 1991 to 31st December 1992 were invited to take part in the study. The initial number of pregnancies enrolled is 14541 (for these at least one questionnaire has been returned or a "Children in Focus" clinic had been attended by 19/07/99). Of these initial pregnancies, there was a total of 14676 fetuses, resulting in 14062 live births and 13988 children who were alive at 1 year of age.

When the oldest children were approximately 7 years of age, an attempt was made to bolster the initial sample with eligible cases who had failed to join the study originally. As a result, when considering variables collected from the age of seven onwards (and potentially abstracted from obstetric notes) there are data available for more than the 14541 pregnancies mentioned above. The number of new pregnancies not in the initial sample (known as Phase I enrolment) that are currently represented on the built files and reflecting enrolment status at the age of 24 is 913 (456, 262 and 195 recruited during Phases II, III and IV respectively), resulting in an additional 913 children being enrolled. The phases of enrolment are described in more detail in the cohort profile paper and its update. The total sample size for analyses using any data collected after the age of seven is therefore 15454 pregnancies, resulting in 15589 fetuses. Of these 14901 were alive at 1 year of age.

A 10% sample of the ALSPAC cohort, known as the Children in Focus (CiF) group, attended clinics at the University of Bristol at various time intervals between 4 to 61 months of age. The CiF group was chosen at random from the last 6 months of ALSPAC births (1432 families attended at least one clinic). Excluded were those mothers who had moved out of the area or were lost to follow-up, and those partaking in another study of infant development in Avon.

Ethical approval for the study was obtained from the ALSPAC Ethics and Law Committee and the Local Research Ethics Committees. Further details are available in the cohort profile article[S15-S17] and the study website contains details of all the data that is available through a fully searchable data dictionary and variable search tool: http://www.bristol.ac.uk/alspac/researchers/our-data/. Informed consent for the use of data collected via questionnaires and clinics was obtained from participants following the recommendations of the ALSPAC Ethics and Law Committee at the time.

The ALSPAC children were genotyped on the Illumina HumanHap550-Quad platform, by the Wellcome Trust Sanger Institute, Cambridge (United Kingdom) and the Laboratory Corporation of America, Burlington, NC, and using support from 23andMe.

The UK Medical Research Council and Welcome (Grant ref: 102215/2/13/2) and the University of Bristol provide core support for ALSPAC. This publication is the work of the authors and Raquel Granell will serve as a guarantor for the contents of this paper.

CAMP (n = 175)

The Childhood Asthma Management Program (CAMP) study was initially conceived as a clinical trial based on the concerns of the multiple side effects of the long-term use of steroids. Children aged 5 to 12 years at the time of study enrolment with a clinical diagnosis of chronic asthma were included. Evidence of severe asthma or other respiratory diseases was used as exclusion criteria, among others.[S18-S20]

BAMSE (n = 105)

The Children Allergy Milieu Stockholm an Epidemiological Study (BAMSE) is a prospective population-based birth cohort initially conceived for the study of the relation of breast-feeding and risk factors for allergic diseases and asthma in childhood. Follow-up questionnaires about environmental exposures and allergy-related symptoms during the first years of life were obtained from parents. Blood samples and lung function measures were collected from children at the age of 8 years. Reaction to common inhalant and food allergens was also evaluated. Asthma was defined as episodes of wheeze and bronchial hypersensitivity, whereas allergic sensitization was considered with positive evidence of reaction to common allergens.[S21-S23]

Assessment of ICS associations in non-European populations

GALA II (n = 854)

Genes-Environment and Admixture in Latino Americans (GALA II) is a case-control study of asthma including children and young adults aged 8 to 21 years with four Latino grandparents. Participants were recruited from five different centres in the United States and Puerto Rico (Chicago, Illinois; New York City, New York; Houston, Texas; San Francisco, California; and San Juan, Puerto Rico). Subjects with a physician diagnosis of asthma were defined as cases. A detailed description of the eligibility and exclusion criteria has been previously described.[S24, S25]

SAGE (n = 493)

The Study of African Americans, Asthma, Genes and Environments (SAGE) is a cross-sectional asthma study with similar characteristics to GALA II but focused on individuals with four grandparents of African American ancestry. Subjects were recruited in the San Francisco Bay

Area, California, and United States. Further details about the study design have been published elsewhere.[S24, S25]

SCSGES (n = 425)

The Singapore Cross-Sectional Genetic Epidemiology Study (SCSGES) is an ongoing case-control and cross-sectional genetic epidemiology study on allergic diseases among Singapore individuals aged 7 to 20 years. [S26] Recruitment was carried out at the National University of Singapore (NUS) and the KK Women's and Children's Hospital in Singapore. Mouthwash and blood samples were collected from each participant. Asthma was defined as a physician diagnosis of asthma symptoms before recruitment.[S26-S28]

A variant with evidence of replication in Europeans and selected for further validation in non-European populations was genotyped in SCSGES using the MassARRAY® iPLEX® Gold (Agena Bioscience Inc.) through genotyping services provided by CeGen. QC procedures were applied using PLINK 1.9,[S29] which included ensuring call rates above 95% for the samples and the SNP analysed, and a Hardy-Weinberg equilibrium *p*-value>0.05.

Quality control analyses in the studies included in the discovery phase genotyped for the current study

Samples from PAGES, goSHARE, and part of BREATHE were genotyped for the current study with the Axiom™ Precision Medicine Research Array (Affymetrix Inc.) by Centro Nacional de Genotipado (CeGen; www.cegen.org). Genotyping assays were successfully performed for 1233 samples (PAGES, n=589; goSHARE, n=511; BREATHE, n=135). Preliminary quality control (QC) analyses were performed on raw genotype data using the *Best Practices workflow* for human samples implemented in Axiom™ Analysis Suite (Affymetrix Inc.) to detect variants and samples with very low quality. Moreover, variants with misclassification of genotype clusters were discarded, keeping those with ≤5% missing genotypes, minor allele frequency (MAF) ≥1% and Fisher's Linear Discriminant values ≥4.65. Genetic markers located at sexual chromosomes and the pseudoautosomal region and those corresponding to insertions and deletions were discarded.

Additionally, standard QC procedures applied in GWAS approaches were carried out, as described in Hernandez-Pacheco *et al.*[S14] After QC, 398634 autosomal variants and 1012 samples were selected for association analyses with asthma exacerbations despite ICS use.

Association analysis accounting for ICS dosage and asthma severity

Sensitivity analyses were performed for the variant with evidence of replication to ascertain whether the effect of the associations with asthma exacerbations despite ICS use was driven by the specific medication dosage. Logistic regressions were carried out evaluating the association

with a binary variable of the presence/absence of asthma exacerbations, which was defined as the need for emergency care/and or use of systemic corticosteroids because of asthma in the 12 months prior to the study enrolment, through general linear models implemented in R 3.4.4.[S30] Patients treated with ICS from PACMAN, the only study with information available about daily ICS dosage, were included in the analyses. This information was based on the daily dosages of equivalents to budesonide described in the last prescription for ICS inhaler refilling before study enrolment that was recorded in pharmacy electronic systems.[S31] The association model applied included the information about the occurrence of asthma exacerbations as a dependent variable, and allele dosages of the SNP rs67026078 as an independent variable plus age, gender, principal components, and a quantitative variable related to daily ICS dose as covariates. This analysis was also carried out adjusting by a categorical variable derived from the daily ICS dosage taking into account that different ICS dosages are recommended by international guidelines based on the age group and asthma severity of the patients. Therefore, ICS dosage was categorized into low, medium or high depending on whether the individuals were <12 years old (100-200 mcg, 200-400 mcg, >400 mcg) or ≥12 years old (200-400 mcg, 400-800 mcg, >800 mcg).[S32]

Additionally, association analyses were carried out for the SNP rs67026078 accounting for asthma severity based on the classification into treatment steps based on a modification of the guidelines established by the British Thoracic Society and the Scottish Intercollegiate Guidelines Network (BTS/SIGN).[S33] Only those individuals from the studies included in the discovery stage with available information about the use of the medications included in the classification into treatment steps were selected. SLOVENIA was not included since information about any of the medications included in the definition of treatment steps was not available. BREATHE was also excluded because rs67026078 did not pass quality control checks. Therefore, individuals were classified as follows: *Step 1*, as-needed use of short-acting β_2-agonists (SABA); *Step 2*, as-needed use of SABA plus regular ICS; *Step 3*, as-needed use of SABA plus regular ICS and long-acting β_2-agonists (LABA), *Step 4*, as-needed use of SABA plus regular ICS, LABA, and leukotriene receptor antagonists (LTRA). Alternatively, patients with reported use of SABA as needed plus combinations of ICS and LABA; as-needed SABA plus ICS and combinations of ICS and LABA; or as-needed SABA plus ICS and LTRA were also classified into *Step 3*. *Step 4* was also defined as the use of SABA as needed plus LABA, combinations of ICS and LABA, and LTRA; as-needed SABA plus ICS, combinations of ICS and LABA, and LTRA; or as-needed SABA plus combinations of ICS and LABA, and LTRA. All the patients were classified into *Step 2* or above since ICS use was considered as one of the inclusion criteria in our study. Association testing was individually carried out for each study through logistic regressions using R 3.4.4,[S31] applying the same regression models used in the discovery phase but also adjusted by treatment steps. Association results were combined in a meta-analysis using METASOFT.[S34]

Ethical approval of each study included

The Medical Ethics Committee of the University Medical Centre Utrecht (Utrecht, the Netherlands) approved PACMAN (protocol number: 08/023). PAGES was approved by the Cornwall and Plymouth Research Ethics Committee (Plymouth, United Kingdom). GoSHARE and BREATHE were approved by the Tayside Committee on Medical Research Ethics (Dundee, United Kingdom). The Liverpool Paediatric Research Ethics Committee (Liverpool, United Kingdom) (reference number: 08/H1002/56) approved PASS. The Slovenian National Medical Ethics Committee (Ljubljana, Slovenia) approved SLOVENIA (reference number: 0120-569/2017/4). followMAGICS was approved by the Ethik-Kommission der Bayerischen Landesärztekammer (Munich, Germany) (ethics reference number: 01218). The Medische Ethische Toetsings Commissie, Erasmus Medical Centre (Rotterdam, the Netherlands) (ethics approval number: MEC-2011-474) approved ESTATe. ALSPAC was approved by Bristol Research Ethics Committees and the ALSPAC Ethics and Law Committee (Bristol, United Kingdom). The clinic's institutional review board (IRB) approved CAMP (Boston, United States) (ethics approval number: 1999-P-001549). BAMSE was approved by the Regional ethical committee in Stockholm (Stockholm, Sweden) (ethics approval numbers: 02-420 and 2010/1474-31/3). The Human Research Protection Program Institutional Review Board of the University of California, San Francisco (San Francisco, United States) approved GALA II and SAGE (ethics approval numbers: 10-00889 and 10-02877, respectively). SCSGES was approved by the Institutional Review Board at the National University of Singapore (Singapore) (ethics approval number: B-14-150, 07–023, 09–256, 10–445, and 13–075).

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Table S1. Clinical and demographic characteristics of the studies analyzed in the replication phase

	ALSPAC (n = 258)	CAMP (n = 175)	BAMSE (n = 105)
Gender (% male)	59.3	56.6	65.7
Mean age ± SD (years)	13.9 ± 0.12	8.82 ± 2.1	8.3 ± 0.4
Recruitment country	United Kingdom	United States	Sweden
Asthma exacerbations in the last 12 months (%)	26.0	12.6	45.7
Definition	hospitalizations/ school absences	ER visits/ hospitalizations/ OCS use	hospitalizations/ ER visits/school absences
ER visits (%) [a]	NA	12.6	11.4
OCS use (%) [b]	NA	NA	NA
Hospitalizations (%) [c]	14.9	12.6	0.9
School absences (%) [d]	95.4	NA	40.9
Genotyping platform	HumanHap550 Quad+ BeadChip (Illumina)	Illumina HumMap 550k v3 (Illumina)	Human610-Quad BeadChip (Illumina)

[a] Proportion of patients with any exacerbations who sought emergency care due to asthma; [b] Proportion of patients with any exacerbations who used oral corticosteroids because of asthma; [c] Proportion of patients with any exacerbations who were hospitalized because of asthma; [d] Proportion of patients with any exacerbations who were absent from school because of asthma. SD: standard deviation; ER: emergency room; OCS: systemic corticosteroids; NA: not available

Table S2. Clinical and demographic characteristics of the non-European studies

	GALA II (n = 854)	SAGE (n = 493)	SCSGES (n = 426)
Gender (% male)	57.3	54.2	60.1
Mean age ± SD (years)	12.1 ± 3.2	13.5 ± 3.4	13.56 ± 6.20
Recruitment country	United States	United States	Singapore
Ancestry	Latino/Hispanic	African American	Asian
Asthma exacerbations in the last 12 months (%)	66.4	51.9	36.6
Definition	ER visits/ hospitalizations/ OCS use	ER visits/ hospitalizations/ OCS use	ER visits/ hospitalizations/ OCS use
ER visits (%) [a]	56.6	43.2	22.8
OCS use (%) [b]	40.2	29.4	18.3
Hospitalizations (%) [c]	12.6	5.7	5.9
Genotyping platform	Axiom LAT1 array (ThermoFisher)	Axiom LAT1 array (ThermoFisher)	MassARRAY iPLEX Gold (Agena Bioscience)

[a] Proportion of patients with any exacerbations who sought emergency care due to asthma; [b] Proportion of patients with any exacerbations who used oral corticosteroids because of asthma; [c] Proportion of patients with any exacerbations who were hospitalized because of asthma. SD: standard deviation; ER: emergency room; OCS: systemic corticosteroids

Table S3. Genes identified by genome-wide association studies of ICS response published to date

Genes associated	Population	Sample size	Age group	Definition of ICS response	Reference
UMAD1-GLCCI1	European	118	Children	% ΔFEV_1	1
PDE10A-T, HRH4-ZNF521	European	418	Children + adults	% ΔFEV_1	2
ALLC	Asian	189	Adults	% ΔFEV_1	3
ZNF432-ZNF841	European	581	Children	BDR	4
FBXL7	European	124	Children	Asthma symptoms	5
CMTR1, MAGI2, TRIM24, SHB-ALDH1B1, L3MBTL4-ARHGAP28, ELMO2-ZNF334	European	369	Children + adults	Asthma exacerbations	6
MMS22L-FBXL4, NAV2-HTATIP2	European	120	Adults	% ΔFEV_1	7
NA	European	110	Children	% ΔFEV_1, AHR	8
NA	Multiple (European, admixed, Asian)	2,672	Adults	% ΔFEV_1	9
EDDM3B	Admixed	244	Children + adults	ACT	10

ACT: asthma control test; AHR: airway hyperresponsiveness; BDR: bronchodilator response; ICS: inhaled corticosteroids; ΔFEV_1: change in forced expiratory volume in one second

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Table S4. Summary of the SNPs suggestively associated with asthma exacerbations despite ICS treatment in European populations included in the discovery phase

					PACMAN (n=654)		BREATHE-PAGES (n=540)		GoSHARE (n=472)	
SNP	**Chr.** [a]	**Position** [b]	**Nearest gene(s)**	**E/NE**	**OR (95% CI)** [c]	**p-value**	**OR (95% CI)** [c]	**p-value**	**OR (95% CI)** [c]	**p-value**
rs71632139	1	182326506	*ZNF648-GLUL*	C/G	1.22 (1.76-1.97)	0.413	2.06 (1.40-3.02)	2.40×10^{-4}	1.60 (0.94-2.73)	0.084
rs11681246	2	33466620	*LTBP1*	G/A	0.55 (0.37-0.81)	2.50×10^{-3}	0.74 (0.57-0.96)	0.025	0.73 (0.49-1.09)	0.124
rs113364932	2	56668971	*CCDC85A-VRK2*	A/G	1.68 (0.78-3.61)	0.184	1.76 (0.91-3.40)	0.095	4.13 (2.22-7.68)	7.13×10^{-6}
rs72805125	2	56684554	*CCDC85A-VRK2*	T/C	1.85 (0.86-4.00)	0.116	1.45 (0.78-2.71)	0.244	3.95 (2.11-7.40)	1.70×10^{-5}
rs76496334	2	125427606	*CNTNAP5*	T/C	1.25 (0.53-2.92)	0.609	2.70 (1.36-5.34)	4.38×10^{-3}	2.87 (1.43-5.76)	3.05×10^{-3}
rs146921813	2	125432412	*CNTNAP5*	C/G	1.25 (0.53-2.92)	0.609	2.70 (1.36-5.34)	4.38×10^{-3}	2.87 (1.43-5.76)	3.05×10^{-3}
rs141194780	2	125432413	*CNTNAP5*	A/G	1.25 (0.53-2.92)	0.609	2.70 (1.36-5.34)	4.38×10^{-3}	2.87 (1.43-5.76)	3.05×10^{-3}
rs144289311	2	125432440	*CNTNAP5*	A/G	1.45 (0.60-3.47)	0.411	2.70 (1.36-5.34)	4.38×10^{-3}	2.87 (1.43-5.76)	3.05×10^{-3}
rs145694710	2	125434780	*CNTNAP5*	T/C	1.25 (0.53-2.92)	0.609	2.70 (1.36-5.34)	4.38×10^{-3}	2.96 (1.47-5.95)	2.40×10^{-3}
rs17011852	2	125440426	*CNTNAP5*	G/A	1.39 (0.58-3.33)	0.465	2.70 (1.36-5.34)	4.38×10^{-3}	2.96 (1.47-5.95)	2.40×10^{-3}
rs2465662	2	201501145	*AOX1*	C/T	1.58 (1.09-2.28)	0.016	0.97 (0.75-1.27)	0.844	0.81 (0.53-1.24)	0.322
rs7587871	2	201505269	*AOX1*	A/C	1.70 (1.20-2.42)	3.08×10^{-3}	0.85 (0.66-1.10)	0.222	0.85 (0.56-1.29)	0.455
rs7420798	2	201506713	*AOX1*	G/A	1.70 (1.19-2.42)	3.16×10^{-3}	0.85 (0.66-1.10)	0.222	0.85 (0.56-1.29)	0.455
rs12988162	2	201507154	*AOX1*	A/T	1.66 (1.17-2.36)	4.52×10^{-3}	0.85 (0.65-1.10)	0.215	0.85 (0.56-1.28)	0.428
rs67026078	3	55162698	*CACNA2D3-WNT5A*	C/T	4.07 (2.41-6.90)	1.72×10^{-7}	0.90 (0.60-1.35)	0.622	0.84 (0.38-1.87)	0.671
rs444610	5	125315286	*ZNF608-GRAMD3*	A/T	1.12 (0.79-1.59)	0.515	1.77 (1.37-2.30)	1.50×10^{-5}	1.32 (0.91-1.92)	0.149
rs2493700	6	156826363	*NOX3-ARID1B*	G/C	0.68 (0.48-0.96)	0.030	0.82 (0.63-1.08)	0.154	0.64 (0.43-0.96)	0.029
rs72759231	15	97550165	*SPATA8-ARRDC4*	G/A	2.57 (1.45-4.55)	1.18×10^{-3}	2.20 (1.31-3.72)	3.07×10^{-3}	1.27 (0.58-2.78)	0.545
rs28761328	18	4746271	*DLGAP1-ZBTB14*	A/T	1.31 (0.82-2.10)	0.259	1.34 (0.93-1.94)	0.113	1.94 (1.24-3.03)	3.55×10^{-3}

Table S4. Continued

					PASS (n=402)		SLOVENIA (n=182)		BREATHE (n=182)	
SNP	**Chr.** [a]	**Position** [b]	**Nearest gene(s)**	**E/NE**	**OR (95% CI)** [c]	**p-value**	**OR (95% CI)** [c]	**p-value**	**OR (95% CI)** [c]	**p-value**
rs71632139	1	182326506	*ZNF648-GLUL*	C/G	1.40 (0.92-2.15)	0.119	2.22 (1.09-4.54)	0.028	1.28(0.61-2.71)	0.514
rs11681246	2	33466620	*LTBP1*	G/A	0.80 (0.61-1.06)	0.123	0.87 (0.56-1.34)	0.529	NA	NA
rs113364932	2	56668971	*CCDC85A-VRK2*	A/G	2.08 (1.03-4.19)	0.041	1.76 (0.52-6.00)	0.365	NA	NA
rs72805125	2	56684554	*CCDC85A-VRK2*	T/C	2.08 (1.03-4.19)	0.041	1.76 (0.52-6.00)	0.365	NA	NA
rs76496334	2	125427606	*CNTNAP5*	T/C	2.37 (1.18-4.75)	0.015	1.99 (0.47-8.33)	0.348	NA	NA
rs146921813	2	125432412	*CNTNAP5*	C/G	2.27 (1.13-4.56)	0.022	1.99 (0.47-8.33)	0.348	NA	NA
rs141194780	2	125432413	*CNTNAP5*	A/G	2.27 (1.13-4.56)	0.022	1.99 (0.47-8.33)	0.348	NA	NA
rs144289311	2	125432440	*CNTNAP5*	A/G	2.27 (1.13-4.56)	0.022	1.99 (0.47-8.33)	0.348	NA	NA
rs145694710	2	125434780	*CNTNAP5*	T/C	2.37 (1.18-4.75)	0.015	1.99 (0.47-8.33)	0.348	NA	NA
rs17011852	2	125440426	*CNTNAP5*	G/A	2.37 (1.18-4.75)	0.015	1.99 (0.47-8.33)	0.348	NA	NA
rs2465662	2	201501145	*AOX1*	C/T	0.45 (0.32-0.63)	4.90×10^{-6}	1.69 (1.01-2.80)	0.044	1.26 (0.71-2.23)	0.422
rs7587871	2	201505269	*AOX1*	A/C	0.46 (0.33-0.64)	6.01×10^{-6}	1.57 (0.94-2.64)	0.084	1.31 (0.77-2.26)	0.321
rs7420798	2	201506713	*AOX1*	G/A	0.46 (0.33-0.64)	6.01×10^{-6}	1.57 (0.94-2.64)	0.084	1.30 (0.76-2.24)	0.334
rs12988162	2	201507154	*AOX1*	A/T	0.46 (0.33-0.64)	6.01×10^{-6}	1.57 (0.94-2.64)	0.084	1.30 (0.76-2.24)	0.334
rs67026078	3	55162698	*CACNA2D3-WNT5A*	C/T	1.96 (1.13-3.39)	0.017	1.77 (0.82-3.81)	0.147	NA	NA
rs444610	5	125315286	*ZNF608-GRAMD3*	A/T	0.95 (0.70-1.28)	0.717	1.93 (1.24-3.02)	3.85×10^{-3}	2.26 (1.11-4.58)	0.024
rs2493700	6	156826363	*NOX3-ARID1B*	G/C	0.63 (0.46-0.85)	2.83×10^{-3}	0.66 (0.40-1.08)	0.097	0.74 (0.46-1.19)	0.218
rs72759231	15	97550165	*SPATA8-ARRDC4*	G/A	2.39 (1.18-4.84)	0.016	1.64 (0.64-4.23)	0.307	NA	NA
rs28761328	18	4746271	*DLGAP1-ZBTB14*	A/T	1.74 (1.14-2.67)	0.010	1.96 (1.00-3.83)	0.051	NA	NA

Table S4. Continued

					followMAGICS (n=147)		ESTATe (n=102)		Meta-analysis (n=2,681)	
SNP	**Chr.** [a]	**Position** [b]	**Nearest gene(s)**	**E/NE**	**OR (95% CI)** [c]	**p-value**	**OR (95% CI)** [c]	**p-value**	**OR (95% CI)** [c]	**p-value**
rs71632139	1	182326506	*ZNF648-GLUL*	C/G	1.19 (0.50-2.83)	0.692	2.38 (0.72-7.80)	0.154	1.60 (1.31-1.94)	3.07×10^{-6}
rs11681246	2	33466620	*LTBP1*	G/A	0.57 (0.35-0.92)	0.023	0.68 (0.35-1.31)	0.248	0.72 (0.63-0.83)	3.28×10^{-6}
rs113364932	2	56668971	*CCDC85A-VRK2*	A/G	3.11(0.80-12.12)	0.101	0.45 (0.09-2.32)	0.339	2.20 (1.61-3.01)	7.86×10^{-7}
rs72805125	2	56684554	*CCDC85A-VRK2*	T/C	3.11(0.80-12.12)	0.101	0.45 (0.09-2.32)	0.339	2.09 (1.53-2.85)	3.11×10^{-6}
rs76496334	2	125427606	*CNTNAP5*	T/C	1.90 (0.58-6.23)	0.290	3.96 (0.58-26.83)	0.159	2.29 (1.64-3.19)	9.69×10^{-7}
rs146921813	2	125432412	*CNTNAP5*	C/G	1.90 (0.58-6.23)	0.290	3.96 (0.58-26.83)	0.159	2.26 (1.63-3.16)	1.34×10^{-6}
rs141194780	2	125432413	*CNTNAP5*	A/G	1.90 (0.58-6.23)	0.290	3.96 (0.58-26.83)	0.159	2.26 (1.63-3.16)	1.34×10^{-6}
rs144289311	2	125432440	*CNTNAP5*	A/G	1.90 (0.58-6.23)	0.290	3.96 (0.58-26.83)	0.159	2.33 (1.67-3.25)	6.73×10^{-7}
rs145694710	2	125434780	*CNTNAP5*	T/C	1.90 (0.58-6.23)	0.290	2.75 (0.36-21.07)	0.331	2.28 (1.63-3.17)	1.21×10^{-6}
rs17011852	2	125440426	*CNTNAP5*	G/A	1.90 (0.58-6.23)	0.290	2.75 (0.36-21.07)	0.331	2.32 (1.66-3.24)	7.27×10^{-7}
rs2465662	2	201501145	*AOX1*	C/T	2.78 (1.50-5.15)	1.14×10^{-3}	0.99 (0.47-2.07)	0.973	1.13 (0.77-1.66)	4.08×10^{-6} [d]
rs7587871	2	201505269	*AOX1*	A/C	2.33 (1.28-4.22)	5.32×10^{-3}	0.85 (0.42-1.73)	0.660	1.09 (0.75-1.58)	3.10×10^{-6} [d]
rs7420798	2	201506713	*AOX1*	G/A	2.33 (1.28-4.22)	5.32×10^{-3}	0.85 (0.42-1.73)	0.660	1.09 (0.75-1.58)	3.24×10^{-6} [d]
rs12988162	2	201507154	*AOX1*	A/T	2.33 (1.28-4.22)	5.32×10^{-3}	0.85 (0.42-1.73)	0.660	1.08 (0.75-1.57)	4.14×10^{-6} [d]
rs67026078	3	55162698	*CACNA2D3-WNT5A*	C/T	1.19 (0.53-2.66)	0.670	1.16 (0.45-3.01)	0.764	1.50 (0.93-2.43)	4.22×10^{-6} [d]
rs444610	5	125315286	*ZNF608-GRAMD3*	A/T	1.06 (0.66-1.72)	0.809	1.23 (0.62-2.45)	0.558	1.36 (1.09-1.69)	3.68×10^{-6} [d]
rs2493700	6	156826363	*NOX3-ARID1B*	G/C	1.01 (0.61-1.66)	0.983	0.50 (0.25-0.99)	0.047	0.71 (0.62-0.82)	1.28×10^{-6}
rs72759231	15	97550165	*SPATA8-ARRDC4*	G/A	1.60 (0.68-3.77)	0.286	0.78 (0.18-3.46)	0.746	1.97 (1.50-2.59)	1.30×10^{-6}
rs28761328	18	4746271	*DLGAP1-ZBTB14*	A/T	1.77 (0.80-3.90)	0.157	1.09 (0.47-2.52)	0.836	1.56 (1.29-1.89)	4.26×10^{-6}

[a] Chromosome; [b] Positions based on GRCh37/hg19 build; [c] Odds ratio for the effect alleles (additive model); [d] Random-effect model was applied since heterogeneity was found between European studies. CI: Confidence Interval; E: Effect allele; NE: Non-effect allele; SNP: single-nucleotide polymorphism

Table S5. Association results with asthma exacerbations in patients treated with ICS for the SNP rs67026078 in non-European populations

Study	Ancestry	Sample size	Freq. [a]	OR (95% CI) [b]	*p*-value
GALA II	Hispanic/Latino	854	0.047	0.94 (0.57-1.54)	0.800
SAGE	African American	493	0.049	1.12 (0.61-2.04)	0.712
SCSGES	Asian	426	0.014	0.33 (0.07-1.54)	0.160

[a] Frequency of the effect allele (C); [b] Odds ratio for the effect alleles (additive model).
CI: Confidence Interval; ICS: inhaled corticosteroids; SNP: single-nucleotide polymorphism.

Table S6. Association results for rs67026078 with asthma exacerbations despite ICS use adjusting by the daily ICS dosage in PACMAN

Association model	OR (95% CI) [d]	*p*-value
Original association model [a]	4.30 (2.33-7.92)	2.98×10^{-6}
Association model accounting for daily ICS dosage (quantitative) [b]	1.24 (1.14-1.34)	2.30×10^{-7}
Association model accounting for daily ICS dosage (categorical) [b,c]	1.23 (1.14-1.34)	2.02×10^{-7}

[a] Asthma exacerbations ~ SNP + Age + Gender; [b] Asthma exacerbations ~ SNP + Age + Gender + ICS dosage; [c] Meta-analysis of association results adjusted by ICS dosage categorized into low, medium and high depending on whether the patients were <12 years old (100-200 mcg, 200-400 mcg, >400 mcg) or ≥12 years old (200-400 mcg, 400-800 mcg, >800 mcg); [d] Odds ratio for the effect allele (C) (additive model). Only asthma patients treated with ICS from PACMAN with available information about daily ICS dosage were included in all the analyses (n=521). CI: Confidence Interval; ICS: inhaled corticosteroids; SNP: single-nucleotide polymorphism

Table S7. Proteins with expression levels affected by the SNP rs67026078

Protein	Beta	p-value	Function(s)	Participation in the Wnt signaling pathway[a]
PSMA2	-0.139	2.40×10^{-3}	Degradation of proteins	Yes
EDN2	0.135	3.09×10^{-3}	Vasoconstriction	NA
SLC26A5	0.126	5.62×10^{-3}	Anions transport	NA
ADAMTS5	0.126	5.89×10^{-3}	Connective tissue organization, development, inflammation; important role in lymphocyte T migration	Yes
IER3IP1	0.124	6.46×10^{-3}	Cell differentiation and migration	NA
ANXA9	-0.124	6.61×10^{-3}	Binding phospholipids and extracellular matrix proteins	NA
NECTIN3	0.123	6.76×10^{-3}	Cellular adhesion	NA
ARHGAP1	-0.123	7.08×10^{-3}	GTPase activator for Rho/Rac proteins	NA
ATAD2	0.123	7.24×10^{-3}	Transcription factor	Yes
SCAMP5	0.122	7.59×10^{-3}	Calcium-dependent exocytosis, blood pressure	NA
SGTA	0.120	8.71×10^{-3}	Protein binding	NA
IFNL2	0.119	8.91×10^{-3}	Regulation of antiviral, antitumor, immunomodulatory activities	NA
IQCF1	0.119	9.12×10^{-3}	Sperm capacitation and acrosome reaction	NA
MPIG6B	-0.119	9.12×10^{-3}	Hematopoietic lineage differentiation	NA
CHST3	0.118	9.77×10^{-3}	Cell migration and differentiation	Yes
TEAD3	0.117	0.010	Tumor suppression and control of organ size	Yes

[a] Proteins without available evidence of direct or indirect implications in the Wnt pathway are denoted by NA. Information provided by PhenoScanner v2

Table S8. Results of SNP-level replication of previous associations of ICS response in the GWAS results from the discovery phase

					Published GWAS of ICS response			European populations (n = 2,681) [d]	
Nearest gene(s)	SNP	Chr. [a]	Position [b]	E/NE	Definition of ICS response	OR (95% CI) [c]	p-value	OR (95% CI) [c]	p-value
ALLC	rs17445240	2	3703041	G/A	% ΔFEV_1	1.43 (1.25-1.65)	5.01×10^{-7}	0.94 (0.75-1.17)	0.558
	rs13418767	2	3704830	T/G	% ΔFEV_1	1.40 (1.22-1.62)	2.77×10^{-6}	0.95 (0.76-1.18)	0.639
	rs6754459	2	3707423	T/C	% ΔFEV_1	1.43 (1.24-1.65)	5.73×10^{-7}	0.92 (0.80-1.07)	0.272
	rs17017879	2	3713658	C/G	% ΔFEV_1	1.40 (1.22-1.61)	2.49×10^{-6}	1.10 (0.83-1.44)	0.509
	rs7558370	2	3714261	C/A	% ΔFEV_1	1.39 (1.21-1.60)	3.73×10^{-6}	1.09 (0.74-1.60)	0.377
	rs11123610	2	3723026	A/G	% ΔFEV_1	0.69 (0.60-0.80)	3.57×10^{-7}	0.94 (0.82-1.07)	0.339
FBXL7	rs10044254	5	15783596	G/A	Asthma symptoms	3.29 (1.94-5.58)	1.02×10^{-5}	0.93 (0.79-1.09)	0.376
CMTR1	rs2395672	6	37428577	G/A	Asthma exacerbations	1.08 (1.04-1.12)	1.86×10^{-5}	1.09 (0.92-1.27)	0.320
MMS22L-FBXL4	rs6924808	6	98358575	A/G	% ΔFEV_1	NA	5.31×10^{-7}	1.09 (0.96-1.25)	0.194
PDE10A-T	rs6456042	6	166534742	C/A	% ΔFEV_1	NA	6.67×10^{-6}	1.02 (0.89-1.16)	0.770
	rs3127412	6	166535561	T/C	% ΔFEV_1	NA	9.68×10^{-6}	1.02 (0.90-1.17)	0.742
	rs1134481	6	166571164	G/T	% ΔFEV_1	NA	NA	0.96 (0.84-1.10)	0.571
	rs2305089	6	166579270	T/C	% ΔFEV_1	NA	NA	1.01 (0.89-1.15)	0.887
	rs3099266	6	166581147	C/T	% ΔFEV_1	NA	NA	1.01 (0.89-1.15)	0.849
UMAD1-GLCCI1	rs37972	7	8007509	C/T	% ΔFEV_1	NA	0.010	1.20 (1.05-1.37)	**6.58×10^{-3}**
MAGI2	rs2691529	7	77803275	T/C	Asthma exacerbations	0.97 (0.94-1.00)	0.051	1.11 (0.95-1.29)	0.207
TRIM24	rs6467778	7	138178222	G/A	Asthma exacerbations	1.01 (1.00-1.03)	0.021	0.88 (0.75-1.04)	0.125
SHB-ALDH1B1	rs4271056	9	38232043	C/T	Asthma exacerbations	0.96 (0.93-0.99)	6.71×10^{-3}	0.97 (0.82-1.15)	0.702
NAV2-HTATIP2	rs1353649	11	20253599	G/A	% ΔFEV_1	NA	3.92×10^{-9}	0.93 (0.79-1.09)	0.353

Table S8. Continued

					Published GWAS of ICS response				European populations (n = 2,681) [d]	
Nearest gene(s)	**SNP**	**Chr.** [a]	**Position** [b]	**E/NE**	**Definition of ICS response**	**OR (95% CI)** [c]	**p-value**	**Citation**	**OR (95% CI)** [c]	**p-value**
EDDM3B	rs3827907	14	21238798	C/T	ACT	0.00 (0.00-0.00)	7.79×10^{-8}	7	0.93 (0.81-1.06)	0.285
L3MBTL4-ARHGAP28	rs9303988	18	6667583	C/T	Asthma exacerbations	1.03 (1.00-1.05)	0.012	3	0.97 (0.84-1.12)	0.668
HRH4-ZNF521	rs9955411	18	22074720	T/A	% ΔFEV_1	NA	1.28×10^{-4}	5	1.13 (0.96-1.31)	0.133
ZNF432-ZNF841	rs3752120	19	52552021	T/C	BDR	1.03 (1.02-1.05)	4.58×10^{-6}	8	1.10 (0.93-1.29)	0.283
	rs3450	19	52552999	C/T	BDR	1.03 (1.02-1.04)	1.93×10^{-6}		1.11 (0.94-1.30)	0.218
	rs12460587	19	52586919	G/T	BDR	1.04 (1.02-1.05)	5.69×10^{-7}		1.08 (0.84-1.40)	0.065
ELMO2-ZNF334	rs279728	20	45080421	T/C	Asthma exacerbations	1.02 (1.01-1.03)	6.45×10^{-3}	3	1.11 (0.88-1.40)	0.392

[a] Chromosome; [b] Positions based on GRCh37/hg19 build; [c] Odds ratio for the effect alleles. ACT: asthma control test; BDR: bronchodilator response; CI: Confidence Interval; E: Effect allele; GWAS: genome-wide association study; ICS: inhaled corticosteroids; NA: not available; NE: Non-effect allele; SNP: single-nucleotide polymorphism; ΔFEV_1: change in forced expiratory volume in one second. SNPs with evidence of replication in European populations are in boldface.

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3. Dahlin A, *et al. CMTR1* is associated with increased asthma exacerbations in patients taking inhaled corticosteroids. *Immun Inflamm Dis* 2015; 3:350-359.

4. Wang Y, *et al.* Pharmacodynamic genome-wide association study identifies new responsive loci for glucocorticoid intervention in asthma. *Pharmacogenomics J* 2015; 15:422-429.

5. Tantisira KG, *et al.* Genome-wide association identifies the *T* gene as a novel asthma pharmacogenetic locus. *Am J Respir Crit Care Med* 2012; 185:1286-1291.

6. Tantisira KG, *et al.* Genome-wide association between *GLCCI1* and response to glucocorticoid therapy in asthma. *N Engl J Med* 2011; 365:1173-1183.

7. Levin AM, *et al.* Integrative approach identifies corticosteroid response variants in diverse populations with asthma. *J Allergy Clin Immunol* 2019; 143:1791-1802.

8. Wu AC, *et al.* Inhaled corticosteroid treatment modulates *ZNF432* gene variant's effect on bronchodilator response in asthmatics. *J Allergy Clin Immunol* 2014; 133:723-8 e3.

Table S9. Genomic-region replication of previous associations of ICS response. Evidence in European asthmatic patients treated with ICS

Gene	# SNPs tested	# Independent signals	Bonferroni p-value threshold	Significant SNPs after Bonferroni-like correction	SNP min p-value	E/NE	OR (95% CI) [a]	p-value
ALLC	916	40	1.25×10^{-3}	NA	rs11538545	C/G	1.24 (1.07-1.43)	4.72×10^{-3}
FBXL7	1321	224	2.23×10^{-4}	NA	rs496319	C/A	0.66 (0.50-0.87)	3.28×10^{-3}
CMTR1	596	115	4.36×10^{-4}	NA	rs115615046	A/G	0.63 (0.45-0.88)	7.16×10^{-3}
MMS22L-FBXL4	4060	123	4.05×10^{-4}	NA	rs7356837	G/A	0.78 (0.68-0.91)	1.11×10^{-3}
PDE10A-T	3841	155	3.22×10^{-4}	rs6921718 rs57042153 rs16898014 rs57105633 rs10485104 rs61410629 rs73022152 rs1328379 rs1328381 rs73022170	rs57042153	T/G	1.43 (1.20-1.70)	**5.97×10^{-5}**
PDE10A-T	3841	155	3.22×10^{-4}	rs73022172 rs3008005 rs2987296 rs2987297 rs2987298 rs2987299 rs6902596 rs6917844 rs828559 rs828560	rs57042153	T/G	1.43 (1.20-1.70)	**5.97×10^{-5}**
UMAD1-GLCCI1	2506	155	3.22×10^{-4}	rs13235500	rs13235500	G/A	0.71 (0.60-0.85)	**2.44×10^{-4}**
MAGI2	5649	260	1.92×10^{-4}	NA	rs7777283	A/G	1.22 (0.94-1.57)	2.62×10^{-4}
TRIM24	800	81	6.20×10^{-4}	NA	rs12008128	G/T	1.46 (1.16-1.86)	1.62×10^{-3}

Table S9. Continued

Gene	# SNPs tested	# Independent signals	Bonferroni p-value threshold	Significant SNPs after Bonferroni-like correction	SNP min p-value	E/NE	OR (95% CI) [a]	p-value
SHB-ALDH1B1	1845	127	3.93×10^{-4}	rs7032491 rs78364831 rs76532500 rs77634759 rs79377925 rs341488	rs341488	A/G	2.24 (1.48-3.40)	**1.44×10^{-4}**
NAV2-HTATIP2	2730	86	5.80×10^{-4}	NA	rs80132255	C/G	2.37 (1.37-4.09)	2.09×10^{-3}
EDDM3B	904	45	1.12×10^{-3}	NA	rs8020322	A/T	1.53 (1.13-2.07)	6.42×10^{-3}
L3MBTL4-ARHGAP28	3337	103	4.85×10^{-4}	NA	rs400243	A/G	0.65 (0.49-0.87)	3.18×10^{-3}
HRH4-ZNF521	2983	169	2.95×10^{-4}	NA	rs12608210	A/G	1.28 (1.12-1.48)	4.95×10^{-4}
ZNF432-ZNF841	873	85	5.90×10^{-4}	rs73056004 rs67834224	rs67834224	A/C	0.65 (0.52-0.82)	**2.86×10^{-4}**
ELMO2-ZNF334	735	31	1.62×10^{-3}	rs11087003 rs9941764 rs6032764 rs4239703 rs4239704 rs4813018 rs6032769 rs6032770 rs6032771 rs6032772 rs4810494	rs11087003	C/T	0.77 (0.66-0.89)	**5.84×10^{-4}**

[a] Odds ratio for the effect alleles.

CI: Confidence Interval; E: Effect allele; NA: not available; NE: effect allele; SNP: single-nucleotide polymorphism. Significant *p*-values after multiple comparison adjustments are in boldface

Table S10. Results of the gene-set enrichment analysis in European populations

Drug	p-value	Adjusted p-value (FDR)	# Enriched genes	Use [a]
Trichostatin A	6.00×10^{-5}	**0.035**	30	Proposed as novel asthma treatment.
Pantothenic acid (vitamin B5)	4.80×10^{-3}	0.714	2	Vitamin supplement.
Daunorubicin	5.06×10^{-3}	0.714	6	Leukemia and other neoplasms.
Retinoic acid	0.011	0.714	27	Acne, photo-damaged skin, keratinization disorders, acute promyelocytic leukemia.
Osimertinib	0.017	0.714	2	Metastatic non-small cell lung cancer.
Methotrexate	0.022	0.787	4	Arthritis, severe psoriasis, breast cancer, non-Hodgkin's lymphoma.
Etoposide	0.023	0.787	4	Several types of cancer (e.g.: testicular cancer, lung cancer, lymphomas, leukemia, neuroblastomas, ovarian cancer).
Tioguanine	0.024	0.787	4	Acute leukemia.
Diethylstilbestrol	0.025	0.787	4	Menopausal and postmenopausal disorders.
Cisplatin 1.17 mg	0.025	0.787	4	Several types of cancer (e.g.: small cell lung cancer, ovarian cancer, lymphomas, germ cell tumors).
Carmustine 4 mg	0.025	0.787	4	Brain tumors and other malignant neoplasms.
Ethosuximide	0.025	0.787	4	Absence seizures.
Amantadine	0.025	0.787	4	Influenza A infection, Parkinson's disease, extrapyramidal reactions, post herpetic neuralgia.
HG-9-91-01 (SIK inhibitor 1)	0.026	0.714	3	Research use (inhibition of salt-inducible kinases (SIKs)).
Cisplatin 2 mg	0.026	0.787	4	Several types of cancer (e.g.: sarcomas, small cell lung cancer, ovarian cancer, lymphomas, germ cell tumors).
Busulfan	0.027	0.787	4	Chronic myeloid leukemia.
Ibuprofen	0.027	0.787	4	Pain reliever (e.g.: several mild pains, arthritis).
Leflunomide	0.028	0.787	4	Rheum.
Bromfenac	0.029	0.787	4	Ocular pain and inflammation.
Carmustine 16 mg	0.029	0.787	4	Brain tumors and other malignant neoplasms.
Clarithromycin 56 mg	0.029	0.787	4	Bacterial infections.
Rofecoxib 3 mg	0.029	0.787	4	Osteoarthritis, rheumatoid arthritis, primary dysmenorrhea, migraine attacks.
Sumatriptan	0.029	0.787	4	Migraines and cluster headaches.
Rofecoxib 775 mg	0.030	0.787	4	Osteoarthritis, rheumatoid arthritis, primary dysmenorrhea, migraine attacks.
Arbutin	0.030	0.714	4	Urinary infections, several skin diseases with cutaneous hyperpigmentation or hyperactive melanocyte function.

Table S10. Continued

Drug	p-value	Adjusted p-value (FDR)	# Enriched genes	Use [a]
Foscarnet	0.030	0.787	4	Cytomegalovirus retinitis, human herpes virus infection and human immunodeficiency virus infection (HIV).
Fomepizole	0.031	0.787	4	Methanol or ethylene glycol poisoning.
Phenelzine	0.033	0.787	4	Panic disorder, social anxiety disorder.
Ajmaline	0.034	0.714	4	Wolff–Parkinson–White syndrome, monomorphic ventricular tachycardia, bundle branch block and syncope.
Azathioprine	0.034	0.787	4	Rejection after organ transplantation, autoimmune diseases, Crohn's disease, ulcerative colitis, multiple sclerosis.
Indomethacin	0.034	0.787	4	Migraines, rheumatoid arthritis, ankylosing spondylitis, osteoarthritis, acute shoulder pains, acute gouty arthritis, postoperative ocular inflammation, and pain.
Dicoumarol	0.035	0.787	4	Oral anticoagulant agent.
Ciclosporin	0.035	0.787	4	Rejection after organ transplantation, rheumatoid arthritis, psoriasis, persistent nummular keratitis, severe ulcerative colitis.
Neomycin	0.035	0.787	4	Bacterial infections.
Tenidap	0.036	0.787	4	Rheumatoid arthritis.
Carmustine 16 mg	0.037	0.787	4	Brain tumors and other malignant neoplasms.
Daunorubicin	0.037	0.787	4	Leukemia and other neoplasms.
Letrozole	0.038	0.787	4	Breast cancer.
Calcium	0.038	0.714	3	Nutritional supplement.
Clarithromycin 476 mg	0.040	0.787	4	Bacterial infections.
Phenelzine	0.033	0.787	4	Panic disorder, social anxiety disorder.
Phentolamine	0.041	0.787	4	Hypertension, pheochromocytoma, vasospasm of Raynaud disease and frostbite, clonidine
Ethylene Glycol	0.043	0.787	4	Several.
Naproxen	0.043	0.787	4	Rheumatic diseases, migraines, acute pain.
Fenofibrate	0.045	0.787	4	Hypercholesterolemia, hypertriglyceridemia.
Silver	0.049	0.714	2	Bacterial skin and central nervous system infections, ventilator-associated pneumonia, and other infections.
Flavin adenine dinucleotide	0.049	0.714	1	Ophthalmic treatment for vitamin B2 deficiency, multiple acyl-CoA dehydrogenase deficiency, riboflavin deficiency.
CHEMBL380598	0.049	0.714	1	Unknown.
GSK690693	0.049	0.714	1	Research use (tumors, cancer, lymphomas).

[a] Source: DrugBank (https://www.drugbank.ca) FDR: false discovery rate. Drugs with significant evidence to be enriched at genes associated with asthma exacerbations in Europeans are in boldfac

Table S11. Genes enriched at trichostatin A in European children with asthma

				GWAS of asthma exacerbations despite ICS use in Europeans (n=2681)		
Gene	**Chr.** [a]	**Position begin 5'** [b]	**Position end 3'** [b]	**SNP min p-value**	**OR (95% CI)** [c]	***p*-value**
RERE	1	8412457	8877702	rs149875147	1.75 (1.34-2.30)	4.32×10^{-5}
NEGR1	1	71861623	72748417	rs517762	1.39 (1.18-1.63)	6.59×10^{-5}
DPYD	1	97543299	98386615	rs115051546	4.54 (2.14-9.62)	8.06×10^{-5}
LTBP1	2	33172039	33624576	rs11681246	0.72 (0.63-0.83)	3.28×10^{-6}
PRKCE	2	45878454	46415129	rs6738524	1.29 (0.92-1.82)	5.04×10^{-5}
NRXN1	2	50145643	51259674	rs7569775	0.72 (0.62-0.83)	1.24×10^{-5}
MYO3B	2	171034655	171511681	rs6756607	0.76 (0.66-0.86)	3.10×10^{-5}
AOX1	2	201450591	201541787	rs7587871	1.09 (0.75-1.58)	3.10×10^{-6}
PLEKHM3	2	208686012	208890284	rs10208193	1.37 (1.18-1.58)	2.05×10^{-5}
RBMS3	3	29322473	30051886	rs6549930	1.34 (1.18-1.53)	1.42×10^{-5}
FHIT	3	59735036	61237133	rs12489758	2.38 (1.56-3.63)	5.31×10^{-5}
ROBO2	3	75955845	77699115	rs72891545	4.79 (2.36-9.73)	1.44×10^{-5}
ARHGAP24	4	86396267	86923823	rs62315626	3.15 (1.90-5.21)	8.19×10^{-6}
BANK1	4	102332443	102995969	rs74934013	2.66 (0.75-9.44)	9.45×10^{-5}
SEMA5A	5	9035138	9546233	rs707637	1.44 (1.21-1.72)	4.96×10^{-5}
CDH10	5	24487209	24645087	rs17459974	1.35 (1.17-1.55)	3.63×10^{-5}
LAMA2	6	129204286	129837714	rs12527452	0.73 (0.59-0.90)	3.37×10^{-5}
PDE10A	6	165740776	166400091	rs57042153	1.43 (1.20-1.70)	5.97×10^{-5}
HERPUD2	7	35672269	35735181	rs79634971	1.29 (1.14-1.47)	7.84×10^{-5}
GSN	9	123970072	124095121	rs113561738	2.10 (1.49-2.96)	2.84×10^{-5}
JMJD1C	10	64926981	65225722	rs12780983	1.33 (1.15-1.53)	9.03×10^{-5}
KCNMA1	10	78629359	79398353	rs571396	1.16 (0.83-1.61)	8.16×10^{-5}
OPCML	11	132284871	133402414	rs514075	1.63 (1.30-2.03)	2.02×10^{-5}
TMTC1	12	29653746	29937692	rs78501135	1.56 (1.27-1.92)	2.44×10^{-5}
SLC11A2	12	51373184	51422349	rs440595	1.38 (1.18-1.60)	3.20×10^{-5}
CPM	12	69235977	69365350	rs1695154	0.75 (0.65-0.86)	3.71×10^{-5}
RTN1	14	60062694	60337684	rs1952032	1.37 (1.19-1.58)	1.10×10^{-5}
COLEC12	18	319355	500729	rs71352938	1.60 (1.27-2.03)	7.91×10^{-5}
ASXL3	18	31158541	31331156	rs10164193	1.65 (1.30-2.10)	4.77×10^{-5}
ADAMTS5	21	28290231	28339439	rs233900	1.28 (1.02-1.61)	4.05×10^{-5}

[a] Chromosome; [b] Positions based on GRCh37/hg19 build; [c] Odds ratio for the effect alleles. CI: Confidence Interval; GWAS: genome-wide association study; ICS: inhaled corticosteroids; NA: not available; SNP: single-nucleotide polymorphism

Table S12. Genes enriched at trichostatin A in European children with previous evidence of potential implication in asthma-related traits or treatment response

Gene	Main(s) function(s) of protein encoded	Asthma-related traits with evidence of association	Reference
RERE	Regulation of transcriptional activity, apoptosis	Asthma susceptibility	Ferreira *et al. Am J Hum Genet* 2019; 104:665-684 Zhu *et al. Eur Respir J* 2019; 54:1901507
		Allergic diseases	Pickrell *et al. Nat Genet* 2016; 48:709-717 Ferreira et al. *Nat Genet* 2017; 49:1752-1757
		Lung function measurements	Kichaev *et al. Am J Hum Genet* 2019; 104:65-75 Shrine *et al. Nat Genet* 2019; 51:481-493
NEGR1	Axon regeneration	Allergic rhinitis	Waage *et al. Nat Genet* 2018; 50:1072-1080
		Asthma susceptibility	Zhu *et al. Eur Respir J* 2019; 54:1901507
		Lung function measurements	Kichaev *et al. Am J Hum Genet* 2019; 104:65-75
LTBP1	Regulation of TGF-β1 activity, organogenesis, airways structural changes	Lung function measurements	Kichaev *et al. Am J Hum Genet* 2019; 104:65-75
MYO3B	Protein kinase activity, cochlear hair bundle morphogenesis	Lung function measurements	Kichaev *et al. Am J Hum Genet* 2019; 104:65-75
			Shrine *et al. Nat Genet* 2019; 51:481-493
AOX1	Metabolism of xenobiotics and drugs, regulation of reactive oxygen species homeostasis	Lung function measurements	Kichaev *et al. Am J Hum Genet* 2019; 104:65-75
			Shrine *et al. Nat Genet* 2019; 51:481-493
ROBO2	Axon guidance and cell migration	Lung development	Anselmo *et al. Gene Expr Patterns* 2003; 3:13-19
		Eosinophils migration	Ye *et al. J Immunol* 2010; 185:6294-6305
		Lung function measurements	Lutz *et al. BMC Genet* 2015; 16:138
		Asthma susceptibility	Ding *et al. Hum Genomics* 2013; 7:16
ARHGAP24	Cell polarity, cell morphology and cytoskeletal organization	Lung function measurements	Kichaev *et al. Am J Hum Genet* 2019; 104:65-75
			Shrine *et al. Nat Genet* 2019; 51:481-493
BANK1	B-cell receptor-induced calcium mobilization	Eczema	Kichaev *et al. Am J Hum Genet* 2019; 104:65-75
		Allergic diseases	Shrine *et al. Nat Genet* 2019; 51:481-493
		Lung function measurements	Kichaev *et al. Am J Hum Genet* 2019; 104:65-75
LAMA2	Cell attachment, migration and organization into tissues	Lung function measurements	Kichaev *et al. Am J Hum Genet* 2019; 104:65-75 Shrine *et al. Nat Genet* 2019; 51:481-493
GSN	Assembly and disassembly of actin filaments	Allergic diseases	Shrine *et al. Nat Genet* 2019; 51:481-493
JMJD1C	Thyroid hormone-dependent regulation of transcriptional activity	Asthma susceptibility	Almoguera *et al. Am J Respir Crit Care Med* 2017; 195:456-463
		Lung function measurements	Wyss *et al. Nat Commun* 2018; 9:2976

Table S12. Continued

Gene	Main(s) function(s) of protein encoded	Asthma-related traits with evidence of association	Reference
KCNMA1	Repolarization of cell membrane potential, contraction of smooth muscle	Lung function measurements	Wain *et al. Nat Genet* 2017; 49:416-425
			Shrine *et al. Nat Genet* 2019; 51:481-493
			Kichaev *et al. Am J Hum Genet* 2019; 104:65-75
OPCML	Protein metabolism	Lung function measurements	Kichaev *et al. Am J Hum Genet* 2019; 104:65-75
TMTC1	Transference of mannosyl residues, ossification of spine ligament	Lung function measurements	Kichaev *et al. Am J Hum Genet* 2019; 104:65-75
			Shrine *et al. Nat Genet* 2019; 51:481-493
SLC11A2	Metal transport; hepatic iron accumulation and tissue distribution	Lung function measurements	Kichaev *et al. Am J Hum Genet* 2019; 104:65-75
CPM	Monocyte differentiation, control of peptide hormone, growth factor activity, degradation of extracellular proteins	Lung function measurements	Kichaev *et al. Am J Hum Genet* 2019; 104:65-75
			Shrine *et al. Nat Genet* 2019; 51:481-493
RTN1	Neuroendocrine secretion; membrane trafficking in neuroendocrine cells	Lung function measurements	Kichaev *et al. Am J Hum Genet* 2019; 104:65-75
			Shrine *et al. Nat Genet* 2019; 51:481-493
COLEC12	Host defense	Lung function measurements	Kichaev *et al. Am J Hum Genet* 2019; 104:65-75
			Shrine *et al. Nat Genet* 2019; 51:481-493
ADAMTS5	Connective tissue organization, development, inflammation, cell migration	Lung function measurements	Kichaev *et al. Am J Hum Genet* 2019; 104:65-75
			Shrine *et al. Nat Genet* 2019; 51:481-493

TGF-β1: transforming growth factor β1

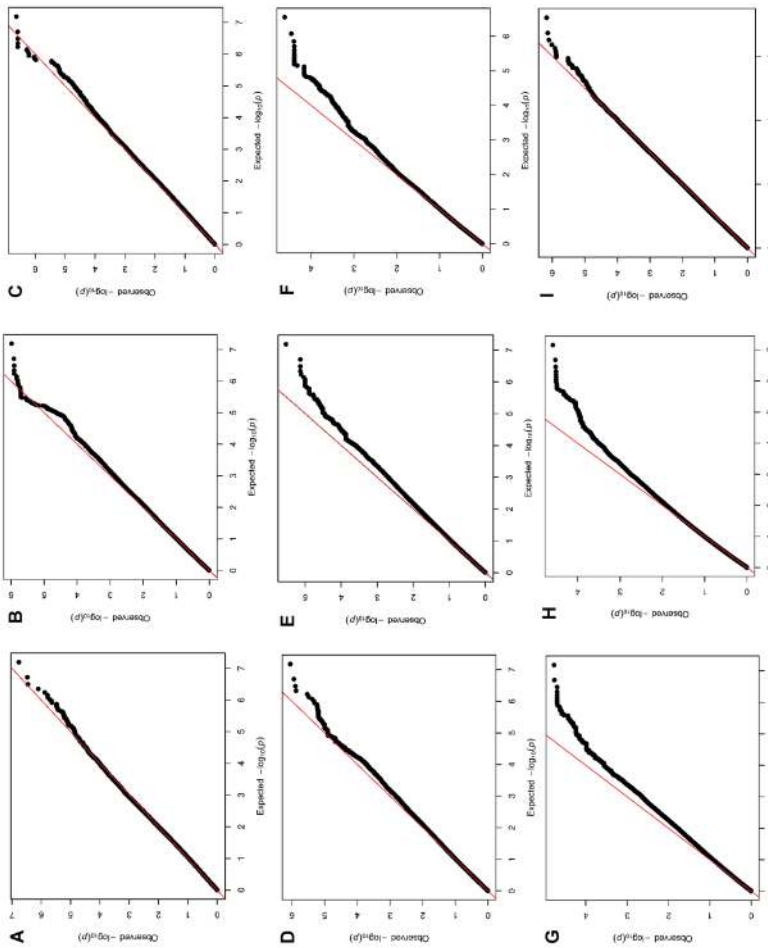

Figure S1. Quantile-quantile plots of association results of asthma exacerbations in patients treated with ICS from the European studies analyzed in the discovery phase. Observed and expected association results are represented as -log10 p-value on the y-axis and x-axis, respectively. Figures S1A-H represent the Q-Q plots of association results for each individual study:A) PACMAN (λGC = 0.98); B) BREATHE-PAGES (λGC = 1.02); C) GoSHARE (λGC = 0.91); D) PASS (λGC = 0.96); E) SLOVENIA (λGC = 0.95); F) BREATHE (λGC = 1.03); G) followMAGICS (λGC = 0.88); H) ESTATe (λGC = 1.06). Figure S1I corresponds to the Q-Q plot of association results after combining those eight European populations in a meta-analysis (λGC = 1.04).

3

Risk of COPD exacerbations

3.1

Effect of β-blockers on the risk of COPD exacerbations according to indication of use: the Rotterdam Study

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ERJ Open Res. 2021 Jun 28;7(2):00624-2020. doi: 10.1183/23120541.00624-2020.

ABSTRACT

Backgrounds: Observational studies report a reduction of COPD exacerbations in patients treated with β-blockers. In contrast, the Beta-Blockers for the Prevention of Acute Exacerbations of Chronic Obstructive Pulmonary Disease (BLOCK COPD) randomized controlled trial which excluded COPD patients with cardiovascular conditions showed an increase in COPD exacerbations. It is unclear whether this discrepancy could be explained by underlying cardiovascular comorbidity.

Objective: We examined whether the association between use of β-blockers and risk of COPD exacerbations differed between patients with and without a cardiovascular indication for β-blockers use.

Methods: Within the Rotterdam Study, we followed COPD subjects until the first COPD exacerbation, or end of follow-up. Cardiovascular indication for β-blockers use was defined as a history of hypertension, coronary heart disease, atrial fibrillation, and/or heart failure at baseline. The association between β-blockers use and COPD exacerbations was assessed using Cox proportional hazards models adjusted for age, sex, smoking, incident cardiovascular disease (i.e. heart failure, hypertension, atrial fibrillation, and/or coronary heart disease during follow-up), respiratory drugs, and nitrates.

Results: In total, 1312 COPD patients with a mean age of 69.7±9.2 years were included. In patients with a cardiovascular indication (n=755, mean age of 70.4±8.8 years), current use of cardioselective β-blockers was significantly associated with a reduced risk of COPD exacerbations (HR 0.69, 95% CI 0.57–0.85). In contrast, in subjects without a cardiovascular indication (n=557, mean age of 68.8±9.7 years), current use of cardioselective β-blockers was not associated with an altered risk of COPD exacerbations (HR 0.94, 95% CI 0.55–1.62).

Conclusion: Use of cardioselective β-blockers reduced the risk of exacerbations in COPD patients with concomitant cardiovascular disease. Therefore, the potential benefits of β-blockers might be confined to COPD patients with cardiovascular disease.

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is a leading cause of death worldwide.[1,2] COPD exacerbations that are related to poor prognosis and severe COPD exacerbations requiring hospital admission increase the total costs due to COPD management.[2,3] Cardiovascular comorbidities encompassing arterial hypertension, ischemic heart disease, atrial fibrillation, and heart failure are common in patients with COPD.[4] Based on the recommendations of the Global Initiative for Chronic Obstructive Lung Disease (GOLD) guidelines, comorbidities in COPD patients should be treated according to the standard strategies irrespective of the presence of COPD.[2] β-blockers are recommended for the first-line treatment of several cardiovascular conditions, including coronary artery disease, heart failure, atrial fibrillation, and hypertension (in case of concomitant with heart failure, angina pectoris, or recent myocardial infarction).[5–7]

Observational studies have already investigated the beneficial effect of the use of β-blockers in patients with COPD, but not stratified by the presence of cardiovascular disease.[8,9] The effect of β-blockers use on survival and exacerbations has also been examined in patients with COPD and hypertension.[10,11]

While observational studies and large meta-analysis reported that the use of β-blockers is associated with reductions in mortality, hospital admissions, and exacerbations in COPD patients,[8,9,11–15] physicians are still reluctant to prescribe β-blockers in patients with cardiovascular disease and concomitant COPD due to concerns about the potential risk of β-blockers induced bronchoconstriction.[16–18] Recently, the Beta-Blockers for the Prevention of Acute Exacerbations of Chronic Obstructive Pulmonary Disease (BLOCK COPD) trial, a double-blind placebo-controlled randomized clinical trial (RCT), reported an increased risk of severe (leading to hospitalization), and very severe (leading to intubation and mechanical ventilation) COPD exacerbations in metoprolol treated COPD patients without an indication for the use of β-blockers. However, the time until the first COPD exacerbation was similar in the metoprolol group and the placebo group.[19] It is still unclear whether the current discrepancy between findings from observational studies and the BLOCK COPD RCT could be explained by underlying cardiovascular comorbidity.

Therefore, we aimed to examine whether the association between the use of β-blockers and the risk of COPD exacerbations differed between patients with and without a cardiovascular indication for the use of β-blockers.

METHODS

Setting and study population

The present study was performed within the Rotterdam Study, an ongoing prospective population-based cohort study in the well-defined Ommoord district in the city of Rotterdam in the Netherlands.[20] The Rotterdam Study comprises approximately 15000 participants, aged ⩾45 years, and includes four sub-cohorts (RS-I, RS-II, RS-III, and RS-IV). Baseline data were collected from 1989 to 1992 in RS-I (n=7983), from 2000 to 2003 in RS-II (n=3011), from 2006 to 2009 in RS-III (n=3932), and RS-IV (n=4000) was established in 2016. Follow-up examinations were conducted periodically, which consisted of a home interview and an extensive set of tests at the research facility. In addition, the relevant data were retrieved from the medical records of the general practitioners, nursing homes, and hospitals. The Medical Ethics Committee of the Erasmus Medical Center approved the Rotterdam Study. All participants provided written informed consent.

The study population for the analyses consisted of COPD patients who gave informed consent for follow-up monitoring and had pharmacy and co-variables data available until January 1, 2011.

COPD and COPD exacerbation

The diagnosis of COPD was confirmed by pre-bronchodilator obstructive spirometry (forced expiratory volume in 1 s (FEV_1)/forced vital capacity (FVC) <0.7). If an interpretable spirometry was not available in the Rotterdam Study, the use of respiratory drugs (Anatomical Therapeutic Chemical Classification codes: R03) was exclusively used for potential case finding; each potential case was validated through evaluation of all medical records, specialist letters, and hospitalization. COPD cases were then identified as having a clear and well-founded physician diagnosis of COPD base on clinical presentation and/or lung function assessed by the general practitioner or respiratory physician.[21] Prevalent COPD was defined as COPD diagnosed before the study start, and incident COPD was defined as the first diagnosis of COPD during follow-up. The start date of follow-up was defined as the date of study enrolment for prevalent COPD or the date of diagnosis for incident COPD. We followed COPD patients until the first COPD exacerbation, death, lost to follow-up, or the end of the follow-up (i.e. January 1, 2011), whichever came first. COPD exacerbations were defined as acute episodes of worsening of respiratory symptoms requiring use of either systemic corticosteroids and/or antibiotics (moderate exacerbations), or requiring hospitalization (severe exacerbations).[22, 23] The outcome was defined as the first moderate-to-severe COPD exacerbation during follow-up and the date of outcome was taken as the index date.

Drug exposure

We obtained medication dispensing data from the computerized pharmacies in the study district. Records of all filled prescriptions from January 1, 1991 onwards were available and included information on the product name, the Anatomical Therapeutic Chemical Classification codes, the dispensing date, the prescribed dosing regimen, and the amount dispensed. The exposure of interest was β-blockers (C07) and these were categorized into non-cardioselective β-blockers (C07AA) and cardioselective β-blockers (C07AB). Patients were considered as "current users" if they used a β-blocker on the day of the first exacerbation (i.e. the index date) or when the last day of use of β-blockers fell within 30 days prior to the index date. If the last day of use of β-blockers was more than 30 days prior to the index date, subjects were considered as "past users". Patients were considered as "non-users", if they did not use β-blockers prior to the first exacerbation during the study period.

Co-variables

Several co-variables were considered as potential confounding factors such as: age at index date, sex, smoking, body mass index (BMI), use of respiratory drugs (R03), and use of cardiovascular drugs (i.e., diuretics (C03), calcium antagonists (C08), agents acting on the renin-angiotensin system (C09), antiarrhythmic (C01), nitrates (C01DA), and lipid-lowering drugs (C10)). Incident cardiovascular disease (i.e. arterial hypertension, coronary heart disease, atrial fibrillation, and/or heart failure during follow-up), as a time-varying determinant, was also considered as a confounder. BMI was calculated as weight divided by height squared ($kg \cdot m^{-2}$). Hypertension was defined as a resting blood pressure above 140/90 mmHg or the use of blood pressure-lowering medication. The diagnosis of heart failure was during follow-up using the medical records of the participants.[24] Coronary heart disease (CHD) was defined as a compound outcome including fatal or nonfatal myocardial infarction or CHD mortality.[24] Data on smoking were obtained from questionnaires and were categorized into "never" or "ever-smokers".

Statistical analyses

Continuous variables were presented as means with standard deviations (SD) and as medians and interquartile ranges, where appropriate. Categorical variables were described as counts (n) and proportions (%). Quantitative variables were statistically compared with a Student's t-test (parametric) or Wilcoxon signed rank sum test (non-parametric, when necessary). Categorical variables were statistically compared with Chi-squared test.

The use of β-blockers, other medications, age at index date, and incident cardiovascular disease were included in the models as time-varying determinants.[25] Individuals were considered to have a cardiovascular indication for treatment with β-blockers in case they were diagnosed with arterial hypertension, coronary heart disease, atrial fibrillation, and/or heart failure at baseline.

In the main analysis, the total population was stratified into participants with or without a cardiovascular indication for the use of β-blockers at baseline.

The association between the use of β-blockers and COPD exacerbations was assessed using Cox proportional hazards models, adjusted for age, sex, smoking, and other factors that changed the crude estimate for current use of β-blockers by more than 10% (i.e. incident cardiovascular disease during follow-up, use of respiratory drugs, and nitrates). β-blockers are currently no longer considered as first-line treatment for hypertension in COPD.[26] Therefore, in a sensitivity analysis, the patients with a cardiovascular indication for the use of β-blockers were stratified into two strata: 1) only hypertension; and 2) coronary heart disease, atrial fibrillation, and/or heart failure with or without hypertension (strict cardiovascular indication). Furthermore, to assess the effect of long-term use of β-blockers, we repeated the analysis for long term use of β-blockers defined as the use of β-blockers for at least 270 days during one year prior to the index date. A p-value <0.05 was considered statistically significant. All statistical analyses were conducted using the statistical software package SPSS/24.0 and package R (version 3.3.3).

RESULTS

COPD patients characteristics

The study flow of participants is described in Figure 1. The COPD patients characteristics are shown in Table 1. A total of 1312 COPD patients with a mean ± SD age of 69.7±9.2 years were included. Males comprised 57.2% (n=750) of the cohort and 84.1% (n=1104) were ever smokers. The median (interquartile range) follow-up time was 426 (155–1037) days. At the end of follow-up, 1055 (80.4%) first COPD exacerbations were recorded. Patients with a cardiovascular indication for the use of β-blockers (n=755, age=70.4±8.8 years, sex male=58.0%) were significantly (p=0.002) older than those (n=557, age=68.8±9.7 years. sex male=56.0%) without a cardiovascular indication for the use of β-blockers. During follow-up, several subjects developed cardiovascular events in both categories with and without a cardiovascular indication for the use of β-blockers (116 (15.4%) and 128 (23.0%), respectively).

β-blocker use and risk of COPD exacerbation

In the total population, current use of cardioselective β-blockers, compared with non-use, was associated with a reduced risk of exacerbations (HR 0.69, 95% CI 0.58–0.83). In patients with a cardiovascular indication for the use of β-blockers (Table 2), current use of cardioselective β-blockers reduced the risk of COPD exacerbations by 31% (HR 0.69, 95% CI 0.57–0.85). In contrast, in subjects without a cardiovascular indication for the use of β-blockers, current use of cardioselective β-blockers did not alter the risk of COPD exacerbations (HR 0.94, 95% CI 0.55–1.62).

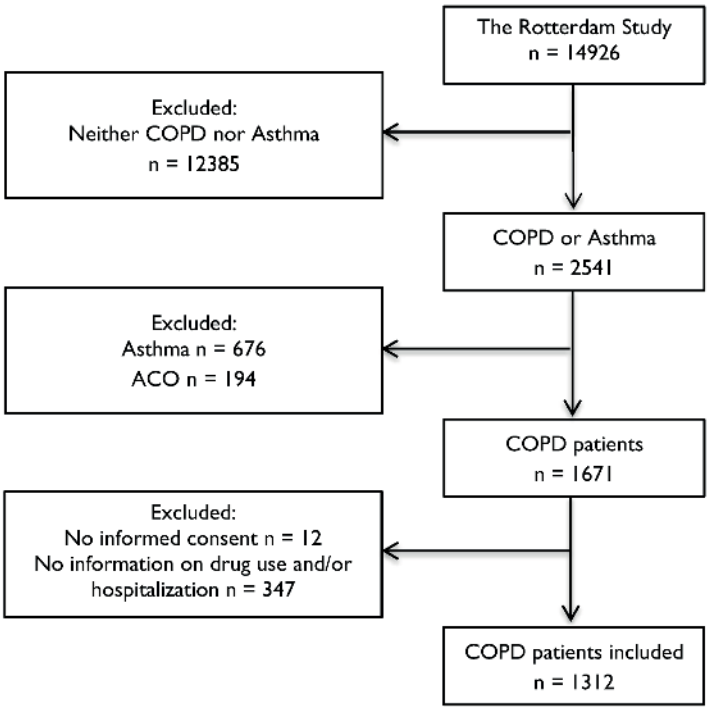

Figure 1. Flowchart of participants. ACO: asthma-COPD overlap; COPD patients were included until January 1, 2011

Table 1. Patients characteristics at baseline

Characteristics	Total COPD patients	Cardiovascular Indication for ß-blockers	No cardiovascular Indication for ß-blockers	P
Patients n	1312	755	557	
Age years	69.7±9.2	70.4 (8.8)	68.8 (9.7)	0.002
Male sex	750 (57.2)	438 (58.0)	312 (56.0)	0.470
Ever smoker	1,104 (84.1)	632 (83.7)	472 (84.7)	0.450
BMI kg/m^2	26.0 (23.7-28.9)	26.5 (24.1-29.4)	25.5 (23.0-28.0)	<0.001
Heart Failure	94 (7.2)	94 (12.5)	NA	NA
Coronary heart diseases	156 (11.9)	156 (20.7)	NA	NA
Atrial fibrillation	31 (2.4)	31(4.1)	NA	NA
Hypertension[#]	685 (52.2)	685 (90.7)	NA	NA
Diabetes[¶]	105 (8)	81 (10.7)	24 (4.3)	<0.001

Data are presented as mean±SD, n (%) or median (interquartile range), unless otherwise stated. Cardiovascular indication for β-blockers use was defined as a history of hypertension, coronary heart disease, atrial fibrillation, and/or heart failure at baseline. BMI: body mass index; NA: not applicable. [#]: data were missing on hypertension in 218 subjects; [¶]: diabetes mellitus was defined as a fasting serum glucose concentration of ⩾7.0 mmol·L^{-1} or a non-fasting serum glucose concentration of ⩾11.1 mmol·L^{-1} or the use of blood glucose-lowering medications.[40]

Table 2. Use of β-blockers and risk of COPD exacerbations

ß-blockers	No. of Exacerbations	Crude hazard ratio (95%CI)	p	Adjusted[#] hazard ratio (95%CI)	p
Total COPD (n = 1312)					
No use	731	**Reference**		**Reference**	
Cardioselective ß-blocker					
Current use	161	0.74 (0.62-0.88)	0.001	0.69 (0.58-0.83)	0.00005
Past use	95	1.02 (0.83-1.27)	0.822	1.01 (0.81-1.26)	0.915
Non-cardioselective ß-blocker					
Current use	25	1.09 (0.73-1.62)	0.674	1.19 (0.79-1.79)	0.395
Past use	34	0.91 (0.64-1.28)	0.583	0.76 (0.53-1.08)	0.123
No cardiovascular indication for ß-blockers use (n = 557)					
No use	400	**Reference**		**Reference**	
Cardioselective ß-blockers					
Current use	18	1.38 (0.85-2.23)	0.193	0.94 (0.55-1.62)	0.835
Past use	21	1.02 (0.66-1.58)	0.934	0.98 (0.62-1.54)	0.918
Non-cardioselective ß-blockers					
Current use	2	NA	NA	NA	NA
Past use	8	1.17 (0.58-2.37)	0.653	1.01 (0.49-2.06)	0.979
Cardiovascular indication for ß-blockers use (n = 755)					
No use	331	**Reference**		**Reference**	
Cardioselective ß-blockers					
Current use	143	0.71 (0.58-0.86)	0.001	0.69 (0.57-0.85)	0.0004
Past use	74	1.03 (0.80-1.33)	0.788	1.08 (0.83-1.40)	0.560
Non-cardioselective ß-blockers					
Current use	23	1.15 (0.75-1.75)	0.528	1.38 (0.89-2.12)	0.147
Past use	26	0.87 (0.58-1.30)	0.498	0.71 (0.47-1.07)	0.106

[#]: Adjusted for age, sex, smoking, incident cardiovascular disease (i.e., heart failure, hypertension, atrial fibrillation, and/or coronary heart disease) during follow-up, use of respiratory drugs (R03), and nitrates (C01DA). NA: not applicable

The results of sensitivity analysis showed that current use of cardioselective β-blockers significantly reduced the risk of COPD exacerbations across two strata of cardiovascular indication of β-blockers use, HR 0.64 (95% CI 0.44–0.91) in subjects with a strict cardiovascular indication for the use β-blockers (i.e., ischemic heart disease, atrial fibrillation, and/or heart failure) and HR 0.69 (95% CI 0.54–0.89) in subjects with only arterial hypertension as a cardiovascular indication (Figure 2). We have also observed that long-term use of cardioselective β-blockers was significantly associated with a reduction in the risk of COPD exacerbations (HR 0.69, 95% CI 0.57–0.85; P=0.001) in COPD patients with a cardiovascular indication for the use of β-blockers (data not shown).

Furthermore, current use of non-cardioselective β-blockers was significantly associated with an increased risk of COPD exacerbations (HR 2.91, 95% CI 1.65–5.13) in patients with only hypertension as cardiovascular indication, but the numbers were low (Figure 2).

DISCUSSION

In this prospective cohort study, we found that current use of cardioselective β-blockers was associated with a reduced risk of COPD exacerbations in patients with a cardiovascular indication for β-blockers use. The sensitivity analysis also indicated that the reduced risk associated with the current use of cardioselective β-blockers in COPD patients with a cardiovascular indication for β-blockers was almost similar in patients with only hypertension as a cardiovascular indication versus patients with ischemic heart disease, atrial fibrillation, and/or heart failure as a cardiovascular indication.

The use of β-blockers is frequently withheld in patients with COPD and concurrent cardiovascular disease due to concerns about potential adverse pulmonary effects such as bronchospasm. In fact, the non-cardioselective β-blocker, such as propranolol, may deter the bronchodilator response to β_2-agonists in COPD patients.[27] However, clinical trials and meta-analyses have indicated that the use of cardioselective β-blockers did not have a significant effect on FEV_1, response to β_2-agonists or respiratory symptoms in COPD patients.[14, 28, 29] The results of a murine model indicated that chronic use of β-blockers could reduce airway inflammation and mucus production.[30] Furthermore, some cardioselective β-blockers (e.g. nebivolol), might modify nitric oxide production, resulting in vasodilation and cardioprotective activity in hypertensive subjects with COPD.[31, 32] Additionally, β-blockers have been reported to reduce the release and synthesis of endothelin-1, a bronchoconstrictor peptide that mediated airway inflammation and may be involved in COPD exacerbations.[33–35] Due to the cardioprotective effects of β-blockers, the use of such drugs may reduce the risk of COPD exacerbations triggered by cardiovascular causes. β-blockers may reduce heart rate,[36] relieve arrhythmias which can lead to cardiac and respiratory decompensation,[37] and moderate the risk of acute coronary syndromes associated with the use of β-agonists.[38]

A previous study by RUTTEN et al.[11] reported that the use of β-blockers reduced the risk of exacerbations in patients with COPD. This association remained in patients with COPD but without overt cardiovascular disease (i.e. only hypertension as the main indication for the prescription of β-blockers). Also, AU et al.[10] assessed the association between the type of antihypertensive medication and all-cause mortality as well as COPD exacerbations among patients with COPD and concomitant hypertension, in particular receiving single-agent antihypertensive therapy. They found a significant reduction in the risk of mortality associated with the β-blockers use, compared to calcium channel blockers and all other antihypertensive agents, among COPD patients with hypertension and cardiac disease, but not in the category of COPD patients with hypertension and without cardiac disease. However, the association between the use of β-blockers and the risk COPD exacerbations was not statistically significant.[10] In the current study, we add to the literature by investigating the effect of β-blockers on the risk of

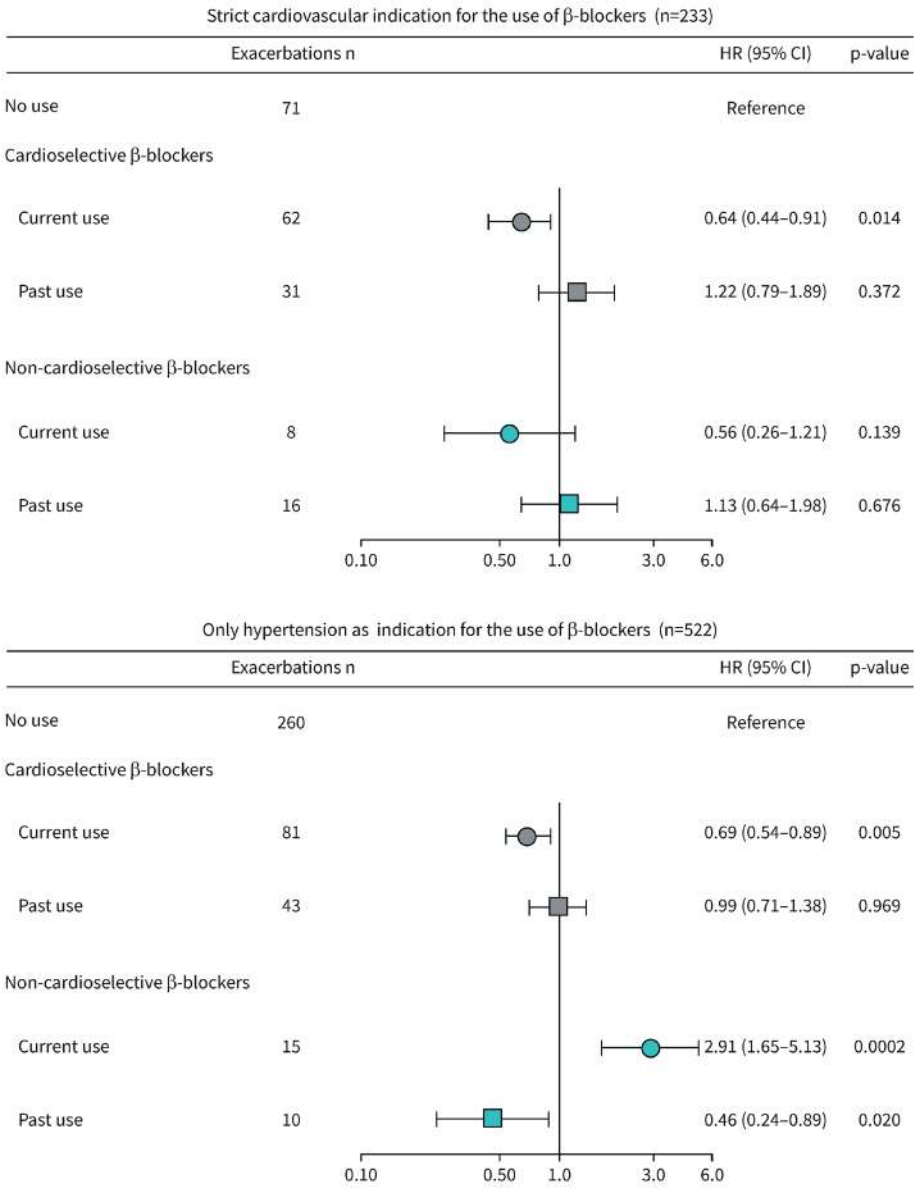

Figure 2. Use of β-blockers and risk of COPD exacerbations (sensitivity analyses). Strict cardiovascular indication was defined as a history of coronary heart disease, atrial fibrillation, and/or heart failure (with or without hypertension) at baseline. The analyses were adjusted for age, sex, smoking, incident cardiovascular disease (i.e., heart failure, hypertension, atrial fibrillation, and/or coronary heart disease) during follow-up, use of respiratory drugs (R03), and nitrates (C01DA). HR: hazard ratio

COPD exacerbations in COPD patients, stratified by the presence of cardiovascular conditions (defined as a history of hypertension, coronary heart disease, atrial fibrillation, and/or heart failure) or absence of any cardiovascular conditions at baseline. Furthermore, we also adjusted

for cardiovascular disease occurring during follow-up. A meta-analysis of 15 observational cohort studies confirmed that the use of β-blockers in patients with COPD might decrease the risk of overall mortality and reduce the risk of COPD exacerbations.[13] Furthermore, the results of a large multicenter cohort study (COPDGene) indicated that β-blockers are associated with a significant reduction of the risk of COPD exacerbations regardless of the severity of airflow obstruction.[8] Although observational studies using real-world data play an essential role in providing evidence on the effects of medications, they could be subject to methodological limitations, such as residual confounding.[31] Therefore, randomized clinical trials are essential to enhance the knowledge about the potential beneficial effects of the use of β-blockers in COPD patients.

Recently, DRANSFIELD et al.,[19] in the BLOCK COPD trial, assigned COPD patients randomly to metoprolol or placebo, with risk of first COPD exacerbation as primary endpoint; however, they excluded COPD patients with an obvious cardiovascular disease, and thus, enrolled only COPD patients without an indication for treatment with a β-blocker. Although the FEV_1 was similar in the two groups, there was a higher risk of severe exacerbation (hospitalization) and very severe exacerbation (intubation and mechanical ventilation) among the patients who received metoprolol. Furthermore, there was no significant difference in the median time until the first exacerbation in the metoprolol group versus the placebo group.[19] Our results indicated that in subjects without a cardiovascular indication, the use of cardioselective β-blockers did not significantly alter the risk of COPD exacerbations; however, due to the limited sample size of β-blockers users in this category, we might be underpowered to find a significant association.

We found that in patients with a cardiovascular indication for the use of β-blockers, current use of cardioselective β-blockers was associated with a reduced risk of COPD exacerbations. Our study reconciles the results of previous observational studies[8, 12, 13] with the results of the BLOCK COPD RCT.[19] Previous observational studies included COPD patients regardless of concomitant cardiovascular disease, whereas the BLOCK COPD RCT excluded COPD patients with an established indication for the use of β-blockers. Therefore, the findings of the BLOCK COPD trial are relevant only to COPD patients without an indication for the use of β-blockers.

As for all observational research, our study has strengths and limitations. An important strength of our study is the fact that we used data from the Rotterdam Study which is an ongoing prospective population-based cohort with a prolonged follow-up of more than 20 years. Data were prospectively collected for all participants, independent of research questions or forthcoming diseases, which makes it less prone to information and selection bias. Furthermore, in this study we used a cohort with complete coverage of all filled prescriptions. We also analyzed exposures as time-dependent variables in a Cox regression model.[25]

A potential limitation of our study is that spirometry data were only available after 2002. Hence, it could lead to an underestimation of asymptomatic COPD in the Rotterdam Study before 2002. In addition, the use of pre-bronchodilator spirometry implies the possibility of misclassification of some asthma patients as COPD patients in the Rotterdam Study. To control this limitation, we additionally identified and validated patients with physician-diagnosed asthma and excluded them (Figure 1). In the Rotterdam Study, COPD exacerbations (moderate and severe) were recorded based on pharmacy-filled prescription data and a national hospitalization register, which prevents recall bias compared to self-reporting of the COPD exacerbations.[22] However, there is the potential of misdiagnosis of COPD exacerbations (especially in patients with heart failure) as differentiating between worsening of heart failure symptoms and COPD exacerbations is not easy in daily life.[39] Also, for prevalent COPD, we did not have information on COPD exacerbations prior to the start of the Rotterdam Study and thus could not account for this in the analysis. Besides, lung function data at baseline were not available for all COPD patients; therefore, we were not able to consider the severity of COPD at baseline in the analysis. Moreover, smoking status was assessed at four-yearly intervals through questionnaires explaining why we categorized smoking into ever and never-smoking. Also, the use of β-blockers was based on pharmacy dispensing data and not on actual intake which might result in an overestimation of actual use. Furthermore, 23% of patients without a cardiovascular indication for the use of β-blockers at baseline later developed incident cardiovascular disease which was controlled for in the analysis. Moreover, we acknowledge that the limited sample size of β-blockers users in the category without a cardiovascular indication is the main limitation of our study. Finally, although we adjusted for potential confounding factors, residual confounding might remain.

In conclusion, we observed that the use of cardioselective β-blockers decreased the risk of exacerbations in COPD patients with a cardiovascular indication for β-blockers use. Therefore, the potential benefits of β-blockers might be confined to COPD patients with cardiovascular disease.

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3.2

β_2-Adrenergic receptor (*ADRB2*) gene polymorphisms and risk of COPD exacerbations: the Rotterdam Study

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J. Clin. Med. 2019, 8, 1835; doi: 10.3390/jcm8111835

ABSTRACT

Background: The role of the β_2-adrenergic receptor (*ADRB2*) gene in patients with chronic obstructive pulmonary disease (COPD) is unclear.

Objective: We investigated the association between *ADRB2* variants and the risk of exacerbations in COPD patients treated with inhaled β_2-agonists.

Methods: Within the Rotterdam Study, a population-based cohort study, we followed 1053 COPD patients until the first COPD exacerbation or end of follow-up and extracted rs1042713 (16Arg > Gly) and rs1042714 (27Gln > Glu) in *ADRB2*. Exposure to inhaled β_2-agonists was categorized into current, past, or non-use on the index date (date of COPD exacerbation for cases and on the same day of follow-up for controls). COPD exacerbations were defined as acute episodes of worsening symptoms requiring systemic corticosteroids and/or antibiotics (moderate exacerbations), or hospitalization (severe exacerbations). The associations between *ADRB2* variants and COPD exacerbations were assessed using Cox proportional hazards models, adjusting for age, sex, use of inhaled corticosteroids, daily dose of β_2-agonists, and smoking.

Results: In current users of β_2-agonists, the risk of COPD exacerbation decreased by 30% (hazard ratio (HR); 0.70, 95% CI: 0.59–0.84) for each copy of the Arg allele of rs1042713 and by 20% (HR; 0.80, 95% CI: 0.69–0.94) for each copy of the Gln allele of rs1042714. Furthermore, current users carrying the Arg16/Gln27 haplotype had a significantly lower risk (HR; 0.70, 95% CI: 0.59–0.85) of COPD exacerbation compared to the Gly16/Glu27 haplotype.

Conclusion: We observed that the Arg16/Gln27 haplotype in *ADRB2* was associated with a reduced risk of COPD exacerbation in current users of inhaled β_2-agonists.

Keywords: chronic obstructive pulmonary disease; inhaled β_2-agonists; exacerbations; *ADRB2* gene

INTRODUCTION

Chronic Obstructive Pulmonary Disease (COPD) is a common disease, which is characterized by a persistent expiratory airflow limitation that is usually progressive.[1] Exacerbations of respiratory symptoms frequently occur in COPD patients and are triggered by environmental pollutants, respiratory infections with bacteria or viruses, and unknown factors.[1] Inhaled β_2-receptor agonists are one of the main classes of bronchodilators used to treat airflow obstruction.[1] The β_2-adrenergic receptor is a member of the G protein-coupled transmembrane receptors widely located on airway smooth muscle cells that mediate relaxation and thus bronchodilation,[2,3] and therefore is an important drug target in COPD treatment. The gene encoding the β_2-adrenergic receptor, *ADRB2*, is a small intron-less gene on chromosome 5q31-32.[2] Multiple single nucleotide polymorphisms (SNPs) in this gene have been described.[2] Two of these SNPs code for amino acid changes at positions 16 [arginine to glycine (16Arg > Gly); rs1042713] and 27 [glutamine to glutamic acid (27Gln > Glu); rs1042714], both of which are common variants and have previously been studied.[4,5]

There is inconsistent evidence from previous studies on the association between *ADRB2* polymorphisms and treatment response to inhaled β_2-agonists on COPD exacerbations,[6–8] short-term bronchodilator response (BDRs),[9,10] and long-term changes in forced expiratory volume in 1 s (FEV_1) in patients with COPD.[10] In addition, most studies assessed the effect of each SNP in isolation but not the combined effect of their haplotypes.

In this study, our main objective was to investigate whether two functional SNPs of the *ADRB2* gene, rs1042713 (16Arg > Gly) and rs1042714 (27Gln > Glu), and their haplotypes were associated with risk of exacerbations in COPD patients treated with inhaled β_2-agonists.

METHODS

Setting and study population

The current study was conducted using data from the Rotterdam Study, an ongoing prospective population-based cohort study among inhabitants of the Ommoord district of Rotterdam, the Netherlands. The rationale and design of the Rotterdam Study have been described elsewhere.[11] The Rotterdam Study (RS) includes three sub-cohorts RS-I, RS-II, and RS-III. Baseline data were collected from 1989 to 1992 in RS-I (n = 7983), from 2000 to 2003 in RS-II (n = 3011), and from 2006 to 2009 in RS-III (n = 3932). Follow-up examinations were conducted periodically, which consisted of a home interview and an extensive set of tests at the research facility. In addition, the data from the medical records of the general practitioners (GPs), nursing homes, and hospitals were collected. The Medical Ethics Committee of the Erasmus Medical Center

approved the Rotterdam Study, and written consent was obtained from all participants. The study population for our analysis consisted of all participants with COPD who gave informed consent for follow-up monitoring and had pharmacy, genetic, and co-variables data available until 1 January 2011.

COPD and COPD exacerbations

The diagnosis of COPD was confirmed by pre-bronchodilator obstructive spirometry (forced expiratory volume in 1 s (FEV_1)/forced vital capacity (FVC) < 0.7).[12] In case spirometry was uninterpretable, COPD cases were diagnosed by a physician based on clinical history, physical examination, and spirometry.[12] COPD diagnosed prior to study start was defined as prevalent COPD, and incident COPD was defined as the first diagnosis of COPD during follow-up.

Subjects were followed from cohort entry or the date of COPD diagnosis (incident COPD) until the first COPD exacerbation, death, lost to follow-up, or the end of the study period (i.e., 1 January 2011), whichever came first. A moderate COPD exacerbation was defined as an acute episode of worsening of COPD symptoms requiring a course of systemic corticosteroid and/or antibiotics.[13] If a patient was hospitalized because of COPD exacerbation, it was classified as a severe COPD exacerbation.[13] The first COPD exacerbation was defined as the outcome of interest and the date of outcome was taken as the index date.

Drug exposure

Medication dispensing data were obtained from the computerized pharmacies in the study district. Records of all filled prescriptions from 1 January 1991 onwards were available and included information on the product name, the Anatomical Therapeutic Chemical Classification (ATC) codes,[14] the dispensing date, the prescribed dosing regimen, and the amount dispensed. The studied β_2-agonists inhalers comprised of (i) short-acting β_2-agonists (SABA): salbutamol either in monotherapy (R03AC02) or as a fixed-dose combination with ipratropium bromide (R03AL02), terbutaline (R03AC03), fenoterol either in monotherapy (R03AC04) or as a fixed-dose combination with ipratropium bromide (R03AL01), and (ii) long-acting β_2-agonists (LABA): salmeterol either in monotherapy (R03AC12) or as a fixed-dose combination with fluticasone (R03AK06), formoterol either in monotherapy (R03AC13) or as a fixed-dose combination with budesonide (R03AK07) or with beclometasone (R03AK08). The newer β_2-agonists inhalers like indacaterol or olodaterol either in monotherapy or as a fixed-dose combination with inhaled corticosteroid (ICS) were not yet available on the Dutch market at the time the study was conducted. To investigate a dose-response relationship, the prescribed daily dose of each β_2-agonist was expressed in standardized defined daily doses according to the ATC/DDD-stem of the World Health Organization (DDDs).[14] Patients were considered as "current users" if they used a β_2-agonist on the index date or when the last use of β_2-agonists fell within 14 days prior to the index date. If the date of last use of β_2-agonists was more than 14 days prior to the index date, subjects were considered as

"past users". Patients were considered as "non-users" if they had never used β_2-agonists prior to the index date during the study period. Data on ICS use, as monotherapy and/or fixed-dose combination with LABA, were extracted from pharmacy records with ATC codes (R03BA, R03AK06, R03AK07, and R03AK08). ICS users were compared to non-users as a reference group.

Genotyping

Subjects in RS were genotyped with Illumina 500 (+duo) and Illumina Human 610-Quad BeadChips. The quality control (QC) procedures were applied. The genotype data were imputed with the 1000-Genomes reference panel (phase 1, V.3) using MACH V.1.0.15/1.0.16. We extracted genotype dosages for two SNPs rs1042713 (16Arg > Gly) and rs1042714 (27Gln > Glu) within the *ADRB2* gene. Imputation quality for both SNPs was high (>0.99).

Functional annotation of variants and expression quantitative trait loci (eQTL) analysis

We retrieved all proxy SNPs in high linkage disequilibrium (LD) (r^2 threshold > 0.8, limit distance 100 kb, and population panel CEU) with the *ADRB2* variants; rs1042713 and rs1042714. For the functional annotation of the variants, we checked their predicted functions, including effects on gene regulation, protein structure, and splicing by using the HaploRegv4.1 (https://www.broadinstitute.org/mammals/haploreg/haploreg.php).[15] The correlation of the SNPs and its proxies in high LD with the expression level of the *ADRB2* gene in whole blood was checked using expression quantitative trait loci (eQTL) data from GeneNetwork.[16]

Co-variables

Co-variables consisted of age, sex, smoking, use of ICS, and the daily dose of β_2-agonists. Data on smoking were obtained from questionnaires and were categorized into "never" or "ever-smokers". Further details are described in the Supplementary Methods.

Systematic review

We conducted an extensive electronic literature search of Embase, Medline Ovid, and Cochrane Central using multiple search terms (Supplementary Table S1) to identify all articles investigating the association between the *ADRB2* polymorphisms of interest, namely rs1042713 and/or rs1042714 and the risk of COPD exacerbation in patients treated with inhaled β_2-agonists. Our literature search was restricted to studies published in English from inception until 30 September 2019. Further details are described in the Supplementary Methods.

Statistical analyses

Cox proportional hazards models were used to calculate hazard ratios (HRs) and their 95% confidence intervals (CIs) to analyze the association between each polymorphism of the *ADRB2* gene (as well as their haplotypes) and time to first COPD exacerbation. The exposure status to inhaled

β_2-agonists was analyzed as a time-dependent variable.[17] The model estimates the exposure status of the case to inhaled β_2-agonists on the event date (index date) and the exposure status of all other participants in the cohort on the same date of follow-up.[17] Thus, each stratum consisted of one case and all other cohort participants who were event-free on the index date and still in follow-up.[17] To account for potential confounding by indication, we stratified the study population into three categories, namely current users, past users, and non-users as defined in the methods section. An additive genetic model was assumed for the analysis. For SNPs analyses, we included rs1042713 and rs1042714 separately in the models and adjusted for age, sex, and smoking in the total cohort of COPD patients. In the categories of non-users and past users of β_2-agonists, we adjusted for age, sex, ICS use, and smoking. The model was further adjusted for the daily dose of β_2-agonists as a continuous variable in the category of current users.

The Haploview 4.2[18] was used to estimate haplotypes frequencies and linkage disequilibrium (LD) between two SNPs. The haplo.stats package[19] (version 1.7.7) for R was applied to analyze the association between haplotypes and COPD exacerbations. The statistical methods of the haplo.stats package assume that all subjects are unrelated and linkage phase of the genetic markers is unknown.[19] The haplo.design function[19] was used to calculate haplotype effects for the haplotypes: Arg16/Gln27 and Gly16/Gln27 in reference to the baseline effect of the most frequent haplotype (Gly16/Glu27).

Most studies evaluated the effect of polymorphisms of the *ADRB2* gene among COPD patients with a smoking history. Hence, we investigated the association in ever-smokers. Sensitivity analyses were performed to evaluate the effect of *ADRB2* polymorphisms in the strata of current users of SABA only and LABA only. Because two SNPs (rs1042713 and rs1042714) were investigated, a Bonferroni-corrected P-value lower than 2.5×10^{-2} (0.05/2) was considered statistically significant. The data were analyzed using the SPSS statistical software version 24 (IBM SPSS Statistics for Windows; IBM Corp, Armonk, NY, USA) and R package (version 3.3.3) for haplotype analysis using the haplo.stats.

RESULTS

Characteristics of the study population

The study flow of participants is described in the Supplementary Figure S1. Table 1 shows the baseline characteristics of the study population. The mean age (± SD) was 69.6 ± 9.0 years and 57.1% of subjects were male. At the end of follow-up, 80.0% of the study population (n = 842) had at least one COPD exacerbation. The minor allele frequencies for rs1042713 (Arg) and rs1042714 (Glu) were 0.35 and 0.47, respectively. Both SNPs were in Hardy-Weinberg equilibrium and they showed an LD with $r^2 = 0.47$ ($D^{\wedge} = 1$). Three haplotypes were determined at positions 16 and 27, and haplotype frequencies were as follows: Gly16/Glu27 (0.48), Arg16/Gln27 (0.35), and Gly16/Gln27 (0.17).

Table 1. Baseline characteristics of COPD subjects

Characteristics	COPD Subjects
n	1053
Age (years), mean (SD)	69.6 ± 9.0
Sex (Male), no. (%)	601 (57.1)
Ever smoker*, no.(%)	891 (84.6)
Status at the end of follow up, no. (%)	
Individuals with COPD exacerbation	842 (80.0)
Individuals without COPD exacerbation	211 (20.0)
BMI kg/m^2, median (IQR)	25.9 (4.7)
Heart failure, no. (%)	82 (7.8)
Coronary heart diseases, no. (%)	132 (12.5)
Hypertension*, no. (%)	575 (54.6)
Diabetes mellitus, no. (%)	83 (7.9)
Minor allele (A) frequency (rs1042713)	0.35
rs1042713 genotype, no. (%)	
Arg/Arg (AA)	134 (12.7)
Arg/Gly (AG)	473 (44.9)
Gly/Gly (GG)	446 (42.4)
Minor allele (G) frequency (rs1042714)	0.47
rs1042714 genotype, no. (%)	
Glu/Glu (GG)	232 (22.0)
Glu/Gln (GC)	536 (50.9)
Gln/Gln (CC)	285 (27.1)
Haplotypes frequency	
Gly16/Glu27	0.48
Arg16/Gln27	0.35
Gly16/Gln27	0.17

SD, standard deviation; BMI: body mass index; IQR, Interquartile Range (the difference between 75th and 25th percentiles).*Data were missing on smoking in two subjects and on hypertension in 146 subjects

Association of *ADRB2* polymorphisms and COPD exacerbations

In current β_2-agonist users, the risk of COPD exacerbation decreased by 30% (HR: 0.70, 95% CI; 0.59–0.84) for each copy of the Arg allele of rs1042713 and by 20% (HR: 0.80, 95% CI; 0.69–0.94) for each copy of the Gln allele of rs1042714 in the adjusted models (Table 2). The rs1042713 and rs1042714 polymorphisms were not associated with the risk of COPD exacerbation in the total cohort of COPD patients (irrespective of β_2-agonists use) as well as in non-users and past users of inhaled β_2-agonists (Table 2).

Table 2. *ADRB2* polymorphisms (per copy of the effect allele) and the risk of COPD exacerbations

Db SNP No.*	Effect allele	Events [1]	Crude Model		Adjusted Model	
			HR (95% CI)	P	HR (95% CI)	P
Total COPD Population (irrespective of inhaled $ß_2$-agonist use)						
rs1042713	Arg[2]	n = 842	0.93 (0.84 - 1.02)	NS	0.93 (0.84 - 1.02)	NS
rs1042714	Gln[3]	n = 842	0.97 (0.88 - 1.06)	NS	0.97 (0.89 - 1.07)	NS
Non-users of inhaled $ß_2$-agonist						
rs1042713	Arg[2]	n = 375	1.02 (0.88 - 1.18)	NS	0.98 (0.85 - 1.13)	NS
rs1042714	Gln[3]	n = 375	1.05 (0.91 - 1.21)	NS	1.05 (0.91 - 1.21)	NS
Past users of inhaled $ß_2$-agonists						
rs1042713	Arg[2]	n = 154	0.96 (0.76 - 1.22)	NS	1.03 (0.81 - 1.31)	NS
rs1042714	Gln[3]	n = 154	0.88 (0.70 - 1.11)	NS	0.97 (0.76 - 1.23)	NS
Current users of inhaled $ß_2$-agonists						
rs1042713	Arg[2]	n = 313	0.70 (0.59 - 0.82)	3.1×10^{-5}	0.70 (0.59 - 0.84)	9.2×10^{-5}
rs1042714	Gln[3]	n = 313	0.80 (0.69 - 0.94)	5.9×10^{-3}	0.80 (0.69 - 0.94)	7.2×10^{-3}

*Seattle SNP database number.[1] Events, COPD exacerbations; HR, Hazard ratio; [2]Arg (A) allele frequency: 0.35; [3]Gln (C) allele frequency: 0.53. NS. Non-significant

Additive genetic model was used for analyses. In total COPD population; adjusted for age, sex, and smoking. In non- and past-users of β_2-agonist; adjusted for age, sex, smoking, and use of inhaled corticosteroids. In current-users, adjusted for age, sex, smoking, use of inhaled corticosteroids, and the daily dosage of β_2-agonists.

To explore the combined effect of the two SNPs, we performed haplotype analysis (Figure 1). In the adjusted model, current β_2-agonist users carrying the Arg16/Gln27 haplotype had a reduced risk of COPD exacerbation (HR: 0.70, 95% CI; 0.59–0.85) compared to the Gly16/Glu27 haplotype. No protective effect of the Gly16/Gln27 haplotype on COPD exacerbation could be observed (Figure 1).

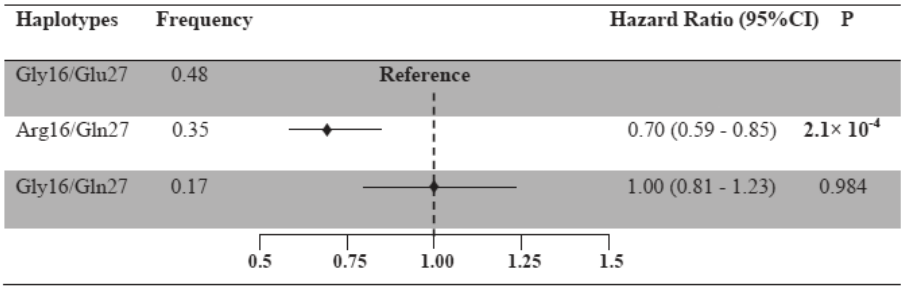

Figure 1. The *ADRB2* haplotypes and the risk of COPD exacerbations in current users of β_2- agonists. The effect of Arg16/Gln27 and Gly16/Gln27 haplotypes compared to the effect of Gly16/Glu27 haplotype. The analyses were adjusted for age and sex, smoking, use of inhaled corticosteroids, and the daily dosage of β_2-agonists.

Haploreg v4.1 data showed that rs1042713 and rs1042714 have no non-synonymous proxy variants in strong LD ($r^2 > 0.8$) (Supplementary Tables S2 and S3). Moreover, the cis-eQTL

data form GeneNetwork showed that the Arg allele (A) of rs1042713 and the Gln allele (C) of rs1042714 are associated with reduced levels of the *ADRB2* gene in whole blood.[16]

Sensitivity analyses

We repeated the analysis by excluding never-smokers from our cohort of current users of β_2-agonists (Table 3 and Figure 2). The results of SNPs and haplotypes analyses remained statistically significant and with similar risk estimates as for the main analyses. When we performed the analysis in strata of current users of SABA only and LABA only, we observed a statistically significantly reduced risk of COPD exacerbations per copy of the Arg allele of rs1042713 among current users of SABA (Table 4). In the LABA only treatment category, we observed a similar trend as in the main analysis; however, the estimates lacked statistical significance (Table 4).

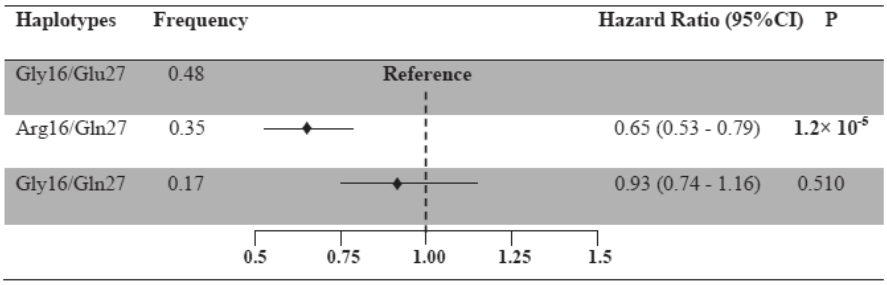

Figure 2. The *ADRB2* haplotypes and the risk of COPD exacerbations in current users of β_2- agonists (smokers only). The effect of Arg16/Gln27 and Gly16/Gln27 haplotypes compared to the effect of Gly16/Glu27 haplotype. The analyses were adjusted for age and sex, use of inhaled corticosteroids, and the daily dosage of β_2-agonists.

Table 3. *ADRB2* polymorphisms (per copy of the effect allele) and the risk of COPD exacerbations in COPD population in current-users of β_2-agonists (smokers only)

Db SNP No.*	Effect allele	Events [1]	Crude Model		Adjusted Model	
			HR (95% CI)	P	HR (95% CI)	P
rs1042713	Arg[2]	n = 277	0.64 (0.53 - 0.77)	1.9×10^{-6}	0.66 (0.55 - 0.80)	1.2×10^{-5}
rs1042714	Gln[3]	n = 277	0.73 (0.62 - 0.86)	2.1×10^{-4}	0.74 (0.63 - 0.87)	3.8×10^{-4}

*Seattle SNP database number.[1] Events, COPD exacerbations; HR, hazard ratio [2]Arg (A) allele frequency: 0.35 [3]Gln (C) allele frequency: 0.53. Additive genetic model was used for analyses. The analyses were adjusted for age, sex, use of inhaled corticosteroids, and the daily dosage of β_2-agonists.

Systematic review

A flow chart (Supplementary Figure S2) describes study identification, screening, and inclusion. Three clinical trials, as well as four observational studies that investigated the association of interest, met the inclusion criteria. Due to differences in assessments and definitions of the outcome, data could not be pooled (Table 5). Details of the results of the systematic review are provided in the Supplementary Materials.

Table 4. *ADRB2* polymorphisms (per copy of the effect allele) and the risk of COPD exacerbations in current-users of SABA only or LABA only

Db SNP No.*	Effect allele	Events[1]	Crude Models		Adjusted Models	
			HR (95% CI)	P	HR (95% CI)	P
SABA only						
rs1042713	Arg[2]	n = 205	0.73 (0.59 - 0.90)	2.9×10^{-3}	0.72 (0.58 - 0.90)	3.0×10^{-3}
rs1042714	Gln[3]	n = 205	0.81 (0.67 – 0.99)	3.6×10^{-2}	0.80 (0.66 - 0.98)	3.0×10^{-2}
LABA only						
rs1042713	Arg[2]	n = 85	0.73 (0.53 - 1.03)	7.1×10^{-2}	0.70 (0.48 – 0.98)	4.0×10^{-2}
rs1042714	Gln[3]	n = 85	0.91 (0.67 - 1.22)	0.525	0.92 (0.67 - 1.27)	0.631

*Seattle SNP database number.[1] Events, COPD exacerbations; HR, Hazard ratio
[2]Arg (A) allele frequency: 0.35[3].Gln (C) allele frequency: 0.53. Additive genetic model was used for analyses adjusted model: adjusted for age, sex, use of inhaled corticosteroids, the daily dosage of β_2-agonists, and smoking.

DISCUSSION

In this population-based cohort study, we observed that *ADRB2* polymorphisms: rs1042713 and rs1042714 were associated with a reduced risk of COPD exacerbation in current users of inhaled β_2-agonists. Also, among current users of β_2-agonist, carriers of the Arg16/Gln27 haplotype had a significantly lower risk of COPD exacerbation compared to those with the Gly16/Glu27 haplotype.

To the best of our knowledge, this is the first population-based study assessing the association between *ADRB2* polymorphisms and COPD exacerbations in patients with COPD treated with inhaled β_2-agonists. In a substudy of the POET-COPD trial[7] a one year randomized, double-blind, and double-dummy trial found that amongst patients treated with salmeterol, those with the Arg/Arg genotype of rs1042713 had a reduced risk of COPD exacerbations compared to patients with the Arg/Gly and Gly/Gly genotypes which is in line with our findings.[7] However, the findings of other clinical trials[5,8] showed no significant associations between *ADRB2* polymorphisms and the number of COPD exacerbations in LABA users.[5,8] The clinical trials which were included in our systematic review[5,7,8] (Table 5) investigated the effect of *ADRB2* polymorphism and the risk of COPD exacerbations in patients exposed to LABA whereas we assessed the effect of *ADRB2* polymorphisms among inhaled β_2-agonists users irrespective whether this was a SABA or a LABA. In a sensitivity analysis, we investigated this association in LABA users only and similar findings as for the main analysis were observed, although these results were no longer statistically significant; this, in turn, can be explained by the small sample size in this particular treatment category. A recent observational study, in spirometry-confirmed COPD patients, examined the associations between *ADRB2* polymorphisms (Arg16Gly and Gln27Glu) and risk of severe COPD exacerbations.[20] The results of the study showed an increased risk of COPD exacerbations in carriers of Arg16 and Gln27.[20] However, the proportion of COPD patients treated with LABA from the Copenhagen General Population Study was low (9.8%)[20] particularly in comparison to our finding that revealed a protective effect in the category of current users of inhaled β_2-agonists. So far,

Table 5. Overview of the studies included in the review

Study (year)	Design	Study population	Country	Treatment	Outcome	Definition of COPD exacerbation	SNP(s)	Estimate/Association
All participants were on $ß_2$-agonists treatment								
Rabe. et al. (2014)[7]	Randomized controlled trial	2,561 COPD patients with a history of smoking	Multicentre in 25 countries	Salmeterol plus inhaled corticosteroids	Time to first COPD exacerbation Kaplan-Meier curves were produced and the log-rank test was used for comparison.	Need of antibiotics or systemic glucocorticoids or admission to hospital	rs1042713 rs1042714	**rs1042713:** Arg16Arg genotype was associated with reduced risk of exacerbation compared to Gly16Gly and Arg16Gly genotypes **rs1042714:** no association
Bleeker et al. (2012)[8]	Two randomized controlled trials	Study1, 1,456 Study2, 1,383 COPD patients with a history of smoking	Multicentre (US, Europe and Mexico)	Formoterol only or in combination with budesonide	Number of COPD exacerbations per patient-treatment year	Need of oral corticosteroid treatment or hospitalization	rs1042713	No association between **rs1042713** genotypes and number of COPD exacerbations per patient-treatment year
Yelensky et al (2012)[5]	Retrospective analysis of phase III clinical trials	565 COPD patients with a history of smoking	USA	Patients treated with Indacaterol for 26 weeks	Number of COPD exacerbations during the 26-week of treatment; using Poisson regression	Need of systemic glucocorticoid therapy, antibiotics, oxygen treatment and/or hospitalization or emergency room visit.	rs1042711 rs1042713 rs1042714 rs1800888	No association between the SNPs and number of COPD exacerbations.

Table 5. Continued

Study (year)	Design	Study population	Country	Treatment	Outcome	Definition of COPD exacerbation	SNP(s)	Estimate/Association
Not all participants were on ß$_2$-agonists treatment								
Ingebrigtsen et al (2019)[20]	Prospective cohort	5219 COPD patients and 85.3% of them had a history of smoking (Copenhagen General Population Study)	Denmark	Only 9.8 % of COPD patients were on LABA treatment	Time to first exacerbation. By using univariable competing risks regression analyses	As acute admissions with a discharge diagnosis of COPD	rs1042713 rs1042714	Both SNPs were associated with an increased risk of COPD exacerbations
Hussein et al (2017)[21]	Case-control study	61 COPD patients with a history of smoking, (recruited from three hospitals)	Egypt	88% of patients were on ß$_2$-agonists treatment	Number of exacerbations	No definition for COPD exacerbation	rs1042713 rs1042714	**rs1042713:** Arg16 genotypes and haplotypes were associated with more frequent exacerbations.
Emeryk-Mksymiuk et al. (2017)[6]	Retrospective study	92 COPD patients with a history of smoking, (recruited from outpatient clinic)	Poland	83% of patients were on ß$_2$-agonists treatment	Self-reported exacerbations	Need of antibiotic therapy, systemic glucocorticoid therapy or hospitalization	rs1042713 rs1042714	**rs1042713:** patients with Arg/Arg genotype required more frequent treatment with antibiotics, as well as systemic corticosteroid therapy. **rs1042714:** no association
Vacca et al (2009)[22]	Case-control study	190 COPD patients with a history of smoking (recruited from two centres)	Germany	No information on ß$_2$-agonist treatment	≥3 exacerbations within the last 3 year vs no exacerbation within the last 2 years	Need of hospitalization	rs1042713 rs1042714	**rs1042713:** No association reported **rs1042714:** No association reported

a few studies have examined the association between *ADRB2* haplotypes and response to β_2-agonist.[9,21,23] A study in Egypt[21] of patients with COPD (n = 61), assessed the association between *ADRB2* haplotypes and COPD exacerbations. In contrast to our findings, they showed that the Arg16 genotypes and haplotype were associated with frequent COPD exacerbations. However, not all of COPD patients in this study were on regular β_2-agonist treatment (88% exposed), and the definition used for COPD exacerbations was not provided.[21]

To summarize, a number of studies have assessed the effect of *ADRB2* polymorphisms on treatment response to β_2-agonists with inconsistent results.[5–9,20–25] Variation in the results might be related to differences in the study populations, study designs, ethnicity, outcome definitions, treatment classifications, concomitant drugs, as well as power-related issues due to different sample sizes.

The mechanism by which *ADRB2* polymorphisms confer risk for COPD exacerbations in patients treated with inhaled β_2-agonists is still unknown. Green et al. conducted in-vitro experiments in human airway smooth muscle cells and showed that cells expressing Arg allele at rs1042713 in *ADRB2* underwent less downregulation in response to long-term β_2-agonist exposure compared to cells expressing Gly allele at this position in *ADRB2*.[26] This is in line with our findings showing a reduced risk of COPD exacerbations in carriers of the Arg allele treated with β_2-agonist.

In contrast to COPD, previous studies in asthmatic patients suggested that the Arg allele (A) of rs1042713 was associated with an increased risk of asthma exacerbations in children and young adults.[27,28] Indeed, COPD and asthma have been defined as two distinct diseases. COPD is characterized by persistent respiratory symptoms while in asthma, respiratory symptoms vary over time and also in intensity.[1,29] Furthermore, exacerbations are typically triggered by allergens and infections in patients with asthma and COPD, respectively.[1,29] However, it is still unclear how the SNP would be differently associated with exacerbations in patients with COPD compared to asthmatic patients.

The strengths of the Rotterdam Study are the prospective, population-based cohort design with an extended follow-up. Data were prospectively collected through consistent procedures for all subjects, independent of research questions or upcoming diseases, which made it less prone to selection and information bias.

A potential limitation of our study is the fact that spirometry data were only available from 2002 onwards. Therefore, it could result in an underestimation of asymptomatic COPD in the Rotterdam Study before January 2002. In addition, reversibility tests were not performed which might lead to an overestimation of the prevalence of COPD.[30,31] To overcome this limitation, patients with asthma diagnosis were identified and excluded.[12] Furthermore, smoking status was assessed at the time of visiting the center and not at the index date, implying potential misclassification of smoking status; however, smoking status was categorized into ever and never-smokers. Misclas-

sification would only occur if non-smokers start to smoke during follow-up, which is unlikely in COPD patients. Also, we might have overestimated the use of β_2-agonists as the exposure was based on dispensing data and not on actual intake. We obtained haplotype frequency estimates using the expectation-maximization (E-M) algorithm. Despite some concerns regarding the accuracy of the methods using phase-unknown data, previous studies have confirmed the usefulness of the haplotype approach[32] and the validity of the statistical technique[33] based on phase-unknown data of unrelated individuals. Moreover, as gene expression and eQTL are tissue-specific, in an optimal setting, they should be examined in lung tissue of COPD patients treated with inhaled β_2-agonists.

In conclusion, we demonstrated that the Arg16/Gln27 haplotype in *ADRB2* was associated with a reduced risk of exacerbation in COPD patients treated with inhaled β_2-agonists. However, further research is needed to confirm these findings.

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SUPPLEMENTARY METHODS

Baseline characteristics

BMI was calculated as weight divided by height squared (kg/m^2). Diabetes mellitus was defined as a fasting serum glucose concentration of ≥7.0 mmol/L or a non-fasting serum glucose concentration of ≥11.1 mmol/L or the use of blood glucose-lowering medications.[S1] Hypertension was defined as a resting blood pressure above 140/90 mmHg or the use of blood pressure-lowering medication. The diagnosis of heart failure was based on follow-up using the medical records of the participants.[S2] Coronary Heart Diseases (CHD) was defined as a compound outcome including fatal or nonfatal myocardial infarction or CHD mortality.[S2]

Systematic review

We conducted an extensive electronic literature search of Embase, Medline Ovid, and Cochrane Central using multiple search terms (Table S1) to identify all articles investigating *ADRB2* polymorphisms; rs1042713 and/or rs1042714 and/or their haplotypes and COPD exacerbations in patients exposed to β_2-agonists. Our literature search was restricted to studies published in English from inception up until 30 September 2019. Additional potentially relevant articles were searched through article reference lists.

Review criteria and data extraction

We considered all original articles, excluding conference abstracts, editorials, short surveys, and animal studies. We did not set any limits on study design, sample size, location, or follow-up. Studies were included if they met the following three criteria;

(1) COPD patients exposed to inhaled short-acting (SABA) and/or long-acting β_2-agonists (LABA) were eligible to be included in the review.
(2) The exposure variable of interest was *ADRB2* polymorphisms; rs1042713 and/or rs1042714 and/or their haplotypes.
(3) The outcome of interest was COPD exacerbations. COPD exacerbation was defined as acute episodes of worsening symptoms requiring a course of systemic corticosteroid and/or antibiotics and/or hospitalization and/or emergency room visit.

The first author (LK) screened all studies from their titles and abstracts and excluded those that were not relevant. The full texts of potential papers were assessed independently by two authors (LK and KV). In case of heterogeneity across studies, the results of each study were reported individually.

SUPPLEMENTARY RESULTS

The literature search yielded 369 hits, of which 270 unique articles remained after excluding duplicates. Of these 270 articles, the title and abstract were reviewed and 236 articles were excluded (conference abstracts (26), editorials (10), experimental studies (5), short surveys (5) and as they were unrelated to the association between *ADRB2* polymorphisms and treatment response to inhaled β_2-agonist in COPD (190). We reviewed 34 full-text articles and 27 of these were excluded for the following reasons; review article (13), letter (1), focus on other SNPs in *ADRB2* (3), focus on different outcomes (10). In total, three clinical trials and four observational studies were withheld, but in the latter, not all of the included patients were on treatment with inhaled β_2-agonist (Figure S2).

Briefly, the three clinical trials that met inclusion criteria[S3-S5] were published between 2012 and 2014. The sample size ranged from 565 to 2561. Two studies were multicentre, and another one was from the United States. One assessed the association of two SNPs with time to first COPD exacerbation using Kaplan-Meier curves and the log-rank test. Rabe et al.[S4] found that patients with the Arg16Arg genotype and using salmeterol and ICS had a significantly lower risk of COPD exacerbations compared with Gly16Gly (P=0.0018) and Arg16Gly (p=0.0130) genotypes.[S4] They found no significant differences in exacerbation risk between the genotypes of rs1042714.[S4] Two other studies[S3,S5] assessed the association of the SNP(s) with the number of COPD exacerbations. One of them used Poisson regression to asses this association and while the other study described the distribution of the number of COPD exacerbation across the genotype categories of rs104213. They found no significant association between SNPs and COPD exacerbations in COPD patients using LABA.[S3,S5]

In our search, four observational studies[S6-S9] also evaluated the association of the SNP(s) with the number of COPD exacerbations. They were published between 2009 and 2019 and included patients from hospitals, medical centres, outpatient clinics, and the general population. Their sample size ranged from 61 to 5219. However, not all of the included patients in these four observational studies were on treatment with inhaled β_2-agonist. The results of a recent observational study showed an increased risk of COPD exacerbations in carriers of Arg16 and Gln27.[S9] However, the proportion of COPD patients treated with LABA from the Copenhagen General Population Study was low (9.8 %).[S9] Due to differences in assessments and definitions of the outcome, this precluded a meta-analysis with pooling of results. Therefore, we reported the findings separately for each study in Table 5 in the main text.

Table S1. Search strategy per library

Embase.com
('adrb2 gene'/de OR 'adrb2 protein human'/de OR (adrb2 OR adrb-2):ab,ti OR (('beta 2 adrenergic receptor'/de OR 'beta adrenergic receptor'/de OR (((beta OR ß OR beta2 OR ß2) NEAR/3 adrenerg* NEAR/3 receptor*) OR ((beta OR ß OR beta2 OR ß2) NEAR/3 (adrenorecept* OR adrenocept* OR agonist*))):ab,ti) AND ('genetics'/exp OR 'genetic parameters'/exp OR 'genetic polymorphism'/exp OR genotype/exp OR 'genetic marker'/exp OR 'genetic association'/de OR 'genome-wide association study'/de OR (haplotype* OR polymorph* OR genetic* OR pharmacogenetic* OR snp OR genom* OR gwas):ab,ti))) AND ('chronic obstructive lung disease'/de OR (copd OR (chronic* NEAR/3 obstruct* NEAR/3 (lung OR pulmonar*))):ab,ti) AND [english]/lim
Medline Ovid
(ADRB2 protein, human.nm. OR (adrb2 OR adrb-2).ab,ti. OR ((Receptors, Adrenergic, beta-2/ OR Receptors, Adrenergic, beta/ OR (((beta OR beta2) ADJ3 adrenerg* ADJ3 receptor*) OR ((beta OR beta2) ADJ3 (adrenorecept* OR adrenocept* OR agonist*))).ab,ti.) AND (exp Genetics/ OR Genetics.fs. OR exp Genetic Phenomena/ OR exp Genetic Association Studies/ OR (haplotype* OR polymorph* OR genetic* OR pharmacogenetic* OR snp OR genom* OR gwas).ab,ti.))) AND (Pulmonary Disease, Chronic Obstructive/ OR (copd OR (chronic* ADJ3 obstruct* ADJ3 (lung OR pulmonar*))).ab,ti.) AND english.la.
Cochrane CENTRAL
((adrb2 OR adrb-2):ab,ti OR (((((beta OR ß OR beta2 OR ß2) NEAR/3 adrenerg* NEAR/3 receptor*) OR ((beta OR ß OR beta2 OR ß2) NEAR/3 (adrenorecept* OR adrenocept* OR agonist*))):ab,ti) AND ((haplotype* OR polymorph* OR genetic* OR pharmacogenetic* OR snp OR genom* OR gwas):ab,ti))) AND ((copd OR (chronic* NEAR/3 obstruct* NEAR/3 (lung OR pulmonar*))):ab,ti)

Table S2. Functional annotation of rs1042713 using the HaploRegv4.1

Chr	pos (hg38)	LD (r^2)	LD (D')	variant	Ref	Alt	EUR freq	Enhancer histone marks	DNAse	Motifs changed	Selected eQTL hits	GENCODE genes
5	148819704	0.9	0.95	rs35283004	A	G	0.38	BLD, MUS		GR,Maf	2 hits	6.9kb 5' of *ADRB2*
5	148820281	0.81	0.92	rs71582318	T	C	0.37	BLD, SKIN		Pou1f1,TATA		6.3kb 5' of *ADRB2*
5	148821442	0.94	0.97	rs12189018	T	C	0.38	BLD		RXRA	2 hits	5.2kb 5' of *ADRB2*
5	148822166	0.94	0.97	rs35019280	AG	A	0.38	BLD		CIZ,GATA,HNF1	2 hits	4.4kb 5' of *ADRB2*
5	148822926	0.93	0.97	rs33910799	AG	A	0.38	BLD	BD	CEBPB,DMRT2	1 hit	3.7kb 5' of *ADRB2*
5	148825014	0.97	0.99	rs17778257	A	T	0.38	9 tissues	SKIN	5 altered motifs	4 hits	1.6kb 5' of *ADRB2*
5	148826178	0.96	0.98	rs12654778	G	A	0.38		38 tissues	Foxp3,p53	4 hits	414bp 5' of *ADRB2*
5	148826877	1	1	**rs1042713**	G	A	0.38		28 tissues	4 altered motifs	3 hits	*ADRB2*

Pos, position; LD, Linkage disequilibrium; Ref, reference; Alt, alternative; EUR freq, European frequency; eQTL, expression quantitative trait loci

Table S3. Functional annotation of rs1042714 using the HaploRegv4.1

chr	pos (hg38)	LD (r^2)	LD (D')	variant	Ref	Alt	EUR freq	Enhancer histone marks	DNAse	Motifs changed	Selected eQTL hits	GENCODE genes
5	148819436	0.88	0.94	rs4705059	C	T	0.59	BLD, HRT, MUS	HRT	5 altered motifs		7.2kb 5' of *ADRB2*
5	148819441	0.88	0.94	rs4705060	G	A	0.59	BLD, MUS		4 altered motifs		7.2kb 5' of *ADRB2*
5	148819679	0.9	0.96	rs10078004	G	A	0.60			Mrg,NRSF		6.9kb 5' of *ADRB2*
5	148819882	0.9	0.96	rs67339154	A	G	0.60	BLD		Brachyury,TBX5		6.7kb 5' of *ADRB2*
5	148820448	0.94	0.97	rs56330463	T	C	0.59	BLD, SKIN		PPAR		6.1kb 5' of *ADRB2*
5	148820990	0.94	0.98	rs2082382	G	A	0.60	BLD	38 tissues	Foxo,Rad21	2 hits	5.6kb 5' of *ADRB2*
5	148821037	0.97	0.99	rs2082395	A	G	0.59	BLD	25 tissues	5 altered motifs	2 hits	5.6kb 5' of *ADRB2*
5	148821395	0.95	0.99	rs9325120	C	A	0.58	BLD		4 altered motifs		5.2kb 5' of *ADRB2*
5	148821692	0.97	0.99	rs11168066	C	A	0.59	BLD		Dmbx1,Otx2	2 hits	4.9kb 5' of *ADRB2*
5	148821753	0.96	0.99	rs11959615	T	A	0.59	BLD			2 hits	4.8kb 5' of *ADRB2*
5	148821910	0.97	0.99	rs35875547	AT	A	0.59	BLD, BRN		10 altered motifs		4.7kb 5' of *ADRB2*
5	148821922	0.97	0.99	rs11958940	A	T	0.59	BLD, BRN		NRSF,Zbtb3		4.7kb 5' of *ADRB2*
5	148822006	0.97	0.99	rs34064454	A	G	0.59	BLD, BRN		AIRE,Pax-4		4.6kb 5' of *ADRB2*
5	148823105	0.97	0.99	rs11746634	C	G	0.59	ESC, BLD		LUN-1,RORalpha1		3.5kb 5' of *ADRB2*
5	148823238	0.97	0.99	rs11168067	A	G	0.59	BLD		NRSF,Pitx2,SETDB1		3.4kb 5' of *ADRB2*
5	148823373	0.95	0.99	rs9325122	C	T	0.60	BLD		HDAC2,Pou2f2,Pou3f3		3.2kb 5' of *ADRB2*
5	148824199	0.97	0.99	rs1432622	T	C	0.59	BLD		7 altered motifs	2 hits	2.4kb 5' of *ADRB2*
5	148824445	0.97	0.99	rs1432623	C	T	0.59	BLD, SKIN		Nkx2		2.1kb 5' of *ADRB2*
5	148824558	0.97	0.99	rs11168068	C	T	0.59	BLD, SKIN		8 altered motifs		2kb 5' of *ADRB2*
5	148825489	0.97	0.99	rs2400707	A	G	0.59	12 tissues	SKIN,SKIN	HLF	2 hits	1.1kb 5' of *ADRB2*
5	148825809	0.97	0.99	rs2053044	A	G	0.59	5 tissues	35 tissues	8 altered motifs		783bp 5' of *ADRB2*
5	148826364	0.99	0.99	rs11168070	G	C	0.59		51 tissues	GR		228bp 5' of *ADRB2*
5	148826465	0.99	1	rs11959427	C	T	0.59	BRN	52 tissues	11 altered motifs		127bp 5' of *ADRB2*
5	148826785	0.98	1	rs1042711	C	T	0.59		35 tissues	6 altered motifs		5'-UTR of *ADRB2*
5	148826812	0.98	1	rs1801704	C	T	0.59	BRN	37 tissues	E2A,Sin3Ak-20,ZEB1		5'-UTR of *ADRB2*
5	148826910	1	1	**rs1042714**	G	C	0.59		21 tissues	GATA,PU.1		*ADRB2*

Pos, position; LD, Linkage disequilibrium; Ref, reference; Alt, alternative; EUR freq, European frequency; eQTL, expression quantitative trait loci

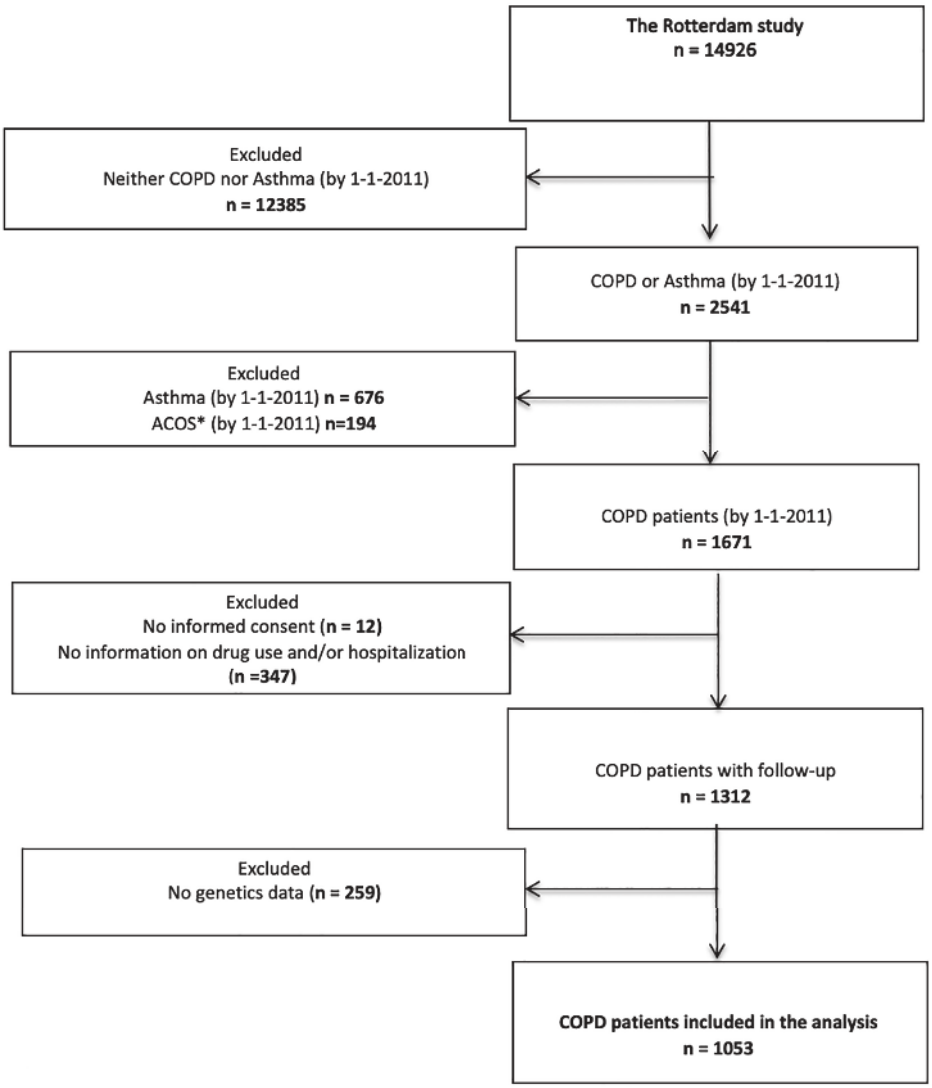

Figure S1. Flowchart of participants
* Asthma and COPD overlap syndrome

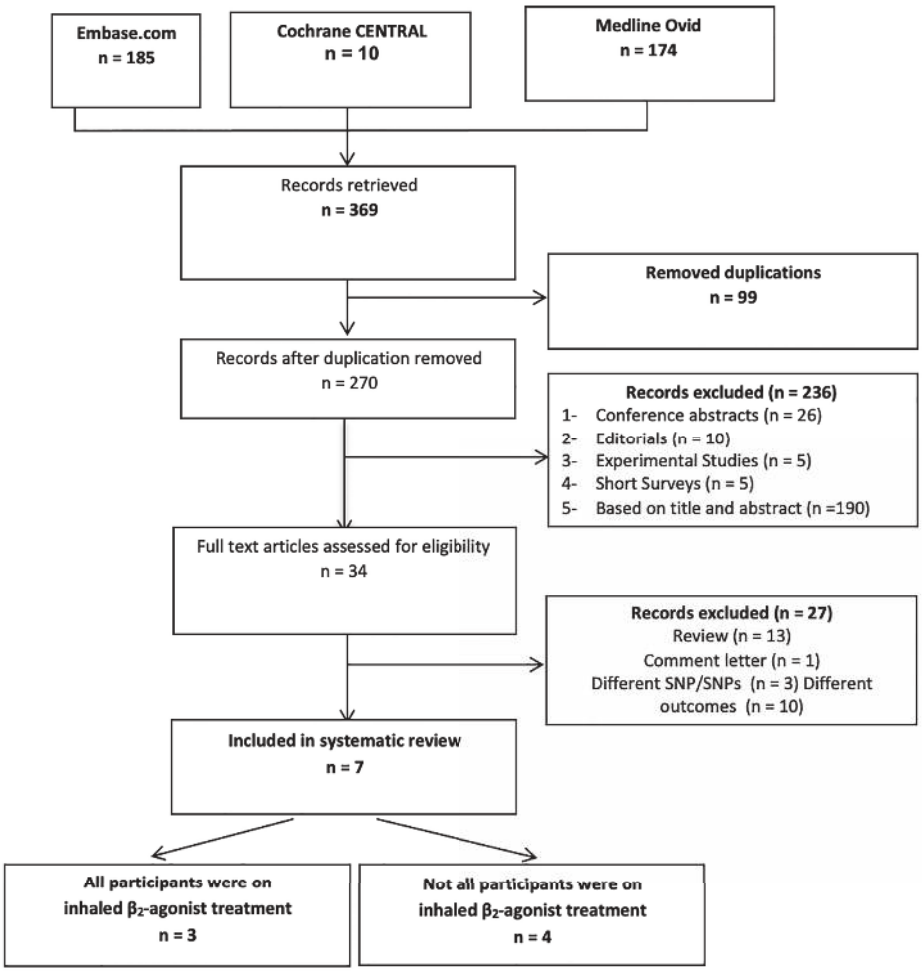

Figure S2. A flow chart describing the steps for including studies in the review

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3.3

DNA methylation sites associated with rate of COPD exacerbations

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4

Real-life adherence to COPD maintenance therapy

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To be submitted

5

General discussion

Worldwide, asthma and chronic obstructive pulmonary disease (COPD) are the most prevalent chronic obstructive respiratory disorders. About 300 million people worldwide suffer from asthma, and 100 million more may suffer by 2025.[1,2] Prevalence of COPD ranged from 7.8% to 19.7% across the world.[3] As a major public health burden, COPD is responsible for over 3 million deaths in 2019, making it the third most common cause of death worldwide.[3,4] Both disorders are characterized by airway obstruction and chronic inflammation of the lungs. Asthma and COPD have distinctly different inflammation patterns, which accounts for their various symptoms, clinical presentations, and responses to treatment. In COPD the predominant inflammatory cells are neutrophils, macrophages, and CD8 (Tc1) lymphocytes, whereas eosinophils, mast cells, and CD4 (T helper 2 cells) lymphocytes are more prevalent in asthma. A slow progressive airflow limitation results from this inflammatory pattern in COPD. In contrast, bronchoconstriction and hyperresponsiveness result from asthma's inflammatory pattern.[5-7]

Asthma treatment aims to control symptoms and prevent asthma-related mortality, exacerbations, and persistent airflow limitations.[8] However, the response to treatment varies widely. Despite the fact that some of this heterogeneity of response can be attributed to adherence, correct inhalation technique, and environmental factors, genetic variations play a crucial role in influencing response to treatment.[9-14] Therefore, genetic markers may be useful in guiding treatment decisions. In addition to utilizing genetic markers to improve asthma guided-treatment, there is a growing interest in using biomarkers such as blood eosinophils, blood IgE, septum eosinophils, and fractional exhaled nitric oxide (FENO) to refine asthma management .[15]

Although there is no cure for COPD, pharmacological treatment, particularly maintenance therapy, can reduce symptoms, decrease the severity and frequency of exacerbations, improve health status, and treat comorbidities in COPD patients.[3,16,17] Genetic variations, such as functional single nucleotide polymorphisms (SNPs) in the *ADRB2* gene, may also play a role in COPD treatment with long-acting β_2-agonist (LABA).[18] Moreover, co-morbidities may affect mortality, hospitalization, and the need for specific treatment.[3] Therefore, patients with COPD should be routinely screened for the presence of co-morbidities such as cardiovascular disorders and treated accordingly.[3]

The findings of this thesis contribute to the body of knowledge concerning asthma and COPD management, specifically focusing on the role of genetics in risk of COPD and asthma exacerbations. Furthermore, this thesis investigated adherence to maintenance therapy in COPD patients.

In this chapter, we discuss the key findings of our study and the methodological challenges that we encountered while conducting our research. In addition, we discuss the potential im-

plications of our findings in a broader context. Finally, future research directions regarding the management of COPD and asthma are discussed.

MAIN FINDINGS

FCER2 T2206C variant and FENO levels

As a biomarker of airway type 2 inflammation, FENO is recommended as a supplementary tool in diagnosing asthma, as well as managing and monitoring the condition.[8,19] We used data from the Pharmacogenetics of Asthma Medication in Children: Medication with Anti-inflammatory effects (PACMAN) study, an observational cohort study that included children (age: 4-12 years) with self-reported regular use of asthma medication recruited through community pharmacies in the Netherlands.[20] Our study analyzed a dataset comprising 593 children, with data on FENO levels and genetic variants. We found a significant association between the *FCER2* T2206C variant, the minor allele of rs28364072, and lower FENO levels in the overall population. Upon dividing the total population into two groups - those with "well-controlled" asthma and those with "not well-controlled" asthma - we observed a significant association between the *FCER2* T2206C variant and lower FENO levels among patients with well-controlled asthma. However, this association was not significant among patients with not well-controlled asthma.[21] A study among Vietnamese children (n = 95) found higher FENO levels among carriers of the minor allele of rs28364072 but the results were not statistically significant.[22] Notably, all children in our study reported using inhaled corticosteroids (ICS), and more than 90% were Caucasians.[21] In contrast, in the Vietnamese study, none of the patients with uncontrolled asthma were receiving ICS for at least one month at the time of inclusion.[22] The cis-eQTL data obtained from the GTEx portal and GeneNetwork demonstrated a significant association between the minor allele G of rs28364072 and reduced levels of *FCER2* expression in whole blood.[23] It is reasonable to speculate that this could result in reduced NO production and, consequently, lower levels of FENO among carriers of the variant. Previous studies observed that the minor allele of rs28364072 was shown to be associated with asthma exacerbation in children treated with ICS.[24,25] FENO is shown to be correlated with total blood IgE.[24,25] It also appears that the *FCER2* gene plays a role in the regulation of IgE production (Figure 1).[26]

ADRB2 haplotypes and asthma exacerbations

Several genetic polymorphisms have been identified in the β_2-Adrenergic receptor (*ADRB2*) gene, of which the A46G (rs1042713, Arg16Gly) variant has been associated with an increased risk of exacerbations in asthmatic children treated with LABA.[11] In the light of the need to provide better insight into the relationship between genetic variations, at codons 16 (Arg16Gly) and 27 (Gln27Glu), in *ADRB2* and asthma exacerbations, we meta-analyzed data from ten independent studies, participating in the multi-ethnic Pharmacogenomics in Childhood Asthma (PiCA)

consortium.[28] The PiCA consortium was established in 2013 in order to enhance international collaborations and conduct large-scale pharmacogenomics studies in children with well-defined asthma across different ethnic groups.[28] The results of the meta-analysis (n = 832) showed that the haplotype Arg16/Gln27, presumably driven by the Arg16, was significantly associated with increased risk of asthma exacerbations among subjects treated with ICS and LABA.[29]

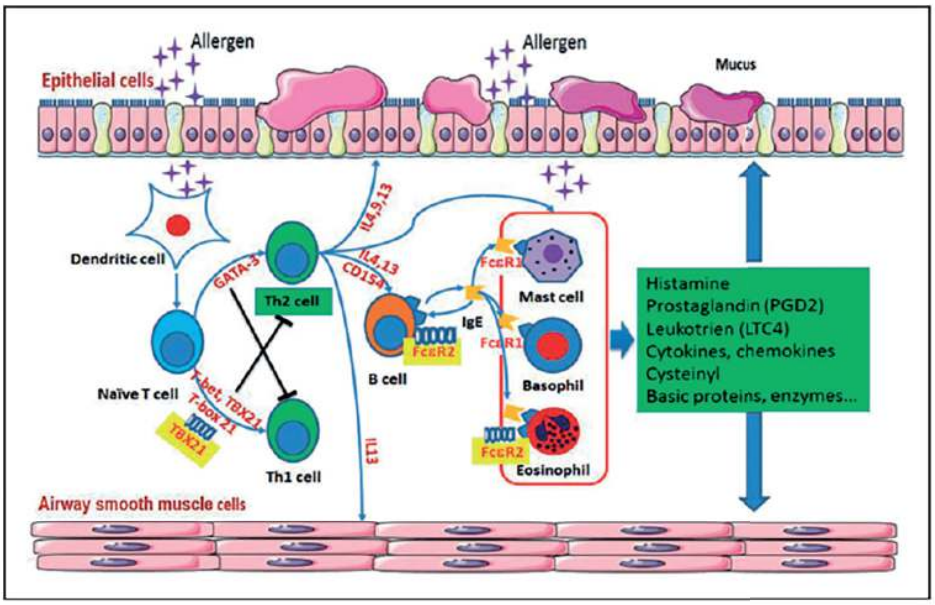

Figure 1: Mechanism of airway inflammation in asthma. Reproduced from Duong-Thi-Ly, H et al., 2017, Effects of genetic factors to inhaled corticosteroid response in children with asthma: a literature review; The Journal of International Medical Research. Pages, 1818-1830, Vol.45, Issue 6, Fig.1.[27] Abbreviations: GATA-3, GATA binding protein 3; IL, interleukin; CD, cluster of differentiation; TBX21, T-box 21; Th, T helper; FceR, Fc fragment of IgE receptor

The rs1042713 (Arg16Gly) in *ADRB2* has been shown to influence LABA response in children in observational studies.[30,31] In a recent study, the testing of *ADRB2* rs1042713 prior to the use of LABA was found to be a cost-effective (the costs of medications and exacerbation management were included) approach compared with not genotyping for asthma in children.[32] In a previous randomized clinical trial (RCT), 62 asthmatic children with the homozygous Arg16 genotype were randomized to either salmeterol or montelukast.[13] In asthmatic children expressing Arg16, leukotriene receptor antagonists (LTRAs) were effective as tailored second-line controller therapy instead of use of salmeterol.[13] However, two post hoc RCT analyses found no significant association between *ADRB2* polymorphisms and asthma exacerbations in individuals treated with LABA.[33] There is still a need for an RCT to evaluate the impact of *ADRB2* rs1042713 genotyping in children before initiating a LABA. A clinical trial, known as PUFFIN, is currently

being conducted to investigate the effects of *ADRB2* genotype-guided (Arg16 genotype) treatment in children with asthma.[34]

Pharmacogenomics of asthma exacerbations despite ICS use

As of to date, it is still impossible to predict an individual's response to ICS treatment based on the polymorphisms identified by genome-wide association studies (GWAS), as only a small portion of the heritability of ICS response has been identified. A possible explanation may be that this is due to the design of the GWAS performed to date, where statistical tests have been underpowered to detect genetic associations. The PiCA consortium provides a valuable platform for investigating large-scale pharmacogenomics studies within a multi-ethnic pediatric asthma population.[28] Based on two meta-analyses of GWAS on asthma exacerbations, conducted using data from independent studies participating in the PiCA consortium, we explored potential associations between genetic variants across the genome and ICS treatment.[35,36]

In the first GWAS of response to ICS among admixed populations (**Chapter 2.3**), a single marker (rs5995653) located within the intergenic region of *APOBEC3B* and *APOBEC3C* showed a suggestive protective association against asthma exacerbations in Hispanic/Latino and African American children and young adolescents treated with ICS, with evidence of replication in European studies. Also, the same variant was associated with change in lung function after treatment with ICS. As part of the innate immune response to virus infection, *APOBEC3* (A3) cytidine deaminases inhibit the replication of retrovirus.[37] Although it has been demonstrated that the flanking *APOBEC3* genes do not exhibit any functions related to asthma; high levels of RNA are expressed by both genes in pulmonary fibroblasts.[38,39] It has also been demonstrated that rs5995653 plays a key role in regulating the expression of genes involved in several cellular processes in the lung.[38,39] A recent work indicated that the ICS-response-associated SNPs including rs5995653 (*APOBEC3B- APOBEC3C*), were identified as microbiome quantitative trait loci of Streptococcus, Tannerella, and Campylobacter in the upper-airway ($0.027 \leq FDR \leq 0.050$).[40] Previous GWASs, focused on European and Asian populations, reported 25 SNPs near or within 14 genes to be associated with ICS response in children.[14,41-47] However, none of them were significantly associated with ICS response among admixed populations in our first GWAS.[35]

In the second GWAS (**Chapter 2.4**) of response to ICS among European-descent children treated with ICS in eight independent studies, during the discovery phase, ten independent variants were associated with asthma exacerbations despite ICS treatment ($p \leq 5 \times 10^{-6}$). There was one variant (rs67026078) at the *CACNA2D3-WNT5A* locus that was nominally replicated in Europeans but this was not validated in non-European populations. Recent studies found that variants near or within *CACNA2D3* are associated with several lung function measurements, including forced expiratory volume in one second (FEV_1), forced vital capacity (FVC), and the FEV_1/FVC ratio, in COPD patients from the United Kingdom (UK) Biobank.[48,49] Furthermore,

these variants have been linked to changes in lung function after bronchodilator use in smokers.[50] In addition, an intronic *CACNA2D3* variation (rs1820616) also affects the FENO levels.[51] It has previously been demonstrated that *WNT5A* encodes a protein that regulates infection-related inflammation.[52] Furthermore, genes encoding *WNT* signaling ligands are associated with impaired lung function in asthmatic children.[53] Through a gene set enrichment analysis, genes associated with asthma susceptibility have been linked to *WNT* signaling.[54] There is evidence that *WNT* ligands act on the major players involved in inflammatory processes, including dendritic cells, T-helper type 2 (Th2) cells, and macrophages.[55] Several diseases, including allergic asthma, can be triggered by dysregulated immune responses caused by WNT molecules.[55]

Further, in **Chapter 2.4**, an enrichment analysis focusing on drugs revealed that trichostatin A (TSA), a histone deacetylase (HDAC) inhibitor, was also associated with asthma exacerbations. Several studies indicate that TSA can reduce asthma development by decreasing airway inflammation and hyperresponsiveness, reducing the expression of inflammatory mediators, and inhibiting bronchoconstriction by causing remodeling changes in inflammation in the airways in a murine model of allergic asthma.[56-60] Also, TSA has been demonstrated to decrease eosinophils and lymphocytes in the bronchial alveolar lavage in allergic mice.[59] By combining publicly available data from different omics sources, Hernandez-Pacheco et.al, have indicated that variants of *LTBP1* are linked to asthma exacerbations, despite the use of ICS, in Europeans or admixed populations.[61] At the time of our second GWAS, 26 SNPs within or near 15 genes associated with different ICS response definitions were identified through earlier GWASs.[14,41-47,62] In our study, only five of these genes were validated in the European population.[41-43,46]

Effect of β-blockers on the risk of COPD exacerbations

Cardiovascular comorbidities are common among patients with COPD.[3] Previous studies reported that the prevalence of heart failure in COPD patients ranged from 20% to 70%, and COPD patients had a significantly higher prevalence of cardiovascular diseases than those without COPD (59.6% vs. 28.4%).[63,64] As per the guidelines of the Global Initiative for Chronic Obstructive Lung Disease (GOLD), comorbidities in patients suffering from COPD should be treated using standard strategies, regardless of the presentence of COPD.[3] Despite guidelines, β-blockers remain underused in COPD regardless of their effectiveness in treating cardiovascular diseases, e.g. heart failure and myocardial infarction, due to persistent concerns about potential bronchoconstriction.[65]

In the Rotterdam Study, an ongoing prospective population-based cohort study comprising inhabitants of the Ommoord district of Rotterdam, the Netherlands, we found that in COPD patients with cardiovascular comorbidity, use of cardioselective β-blockers was significantly associated with a reduced risk of COPD exacerbations. Contrary to this, in subjects without any cardiovascular disease, use of cardioselective β-blockers was not associated with an altered

risk of COPD exacerbations.[66] Results from previous observational studies indicated significant beneficial effect of β-blockers on COPD exacerbation and mortality among COPD patients.[67-70] However, in the BLOCK COPD multicenter RCT, COPD patients without any indication for β-blockers use who received metoprolol had a significantly increased risk of severe exacerbations (resulting in hospitalization) and very severe exacerbations (requiring mechanical ventilation and intubation).[71] In the UK, an ongoing pragmatic RCT, Bisoprolol in COPD Study (BICS), examines whether adding bisoprolol to routine COPD treatment reduces the rate of exacerbations. The hypothesis that bisoprolol exerts its beneficial effects only in those with undiagnosed heart disease will be tested in a sub-study.[72] Several significant differences exist between the BICS trial and the BLOCK COPD trial. In BICS, bisoprolol is being tested because, unlike metoprolol, it has a higher beta1:beta2 receptor selectivity ratio (14:1) than metoprolol (2:1). Further, participants in BLOCK COPD underwent electrocardiographic (ECG) recordings and were disqualified based on pre-specified abnormalities in their ECGs. In UK primary care settings, β-blockers are routinely prescribed without an ECG; therefore, BICS participants are likely to represent COPD patients with more unrecognized heart disease, and thus potentially a greater need for β-blockers. To date, there is no evidence supporting the use of β-blockers in COPD patients without a cardiovascular indication.[3]

ADRB2 gene and the risk of COPD exacerbations

A number of studies have reported inconsistent results regarding the association between *ADRB2* polymorphisms and treatment responses in COPD exacerbations. In the Rotterdam Study, we found that each copy of the Arg allele at rs1042713 and each copy of the Gln allele at rs1042714 in *ADRB2* were associated with a reduced risk of exacerbation in COPD patients treated with inhaled β_2-agonists.[73] Previously, the Prevention Of Exacerbations with Tiotropium in COPD. (POET-COPD) clinical trial found that amongst patients treated with salmeterol, those with the Arg/Arg genotype of rs1042713 had a reduced risk of COPD exacerbations compared to patients with the Arg/Gly and Gly/Gly genotypes which is in line with our findings.[18] It should be noted, however, that other previous clinical trials did not indicate a significant association between *ADRB2* polymorphisms and COPD exacerbations.[74,75] After our work, Wang et al examined clinical samples, bronchial epithelium, from patients with squamous cell lung carcinoma (SCC) with COPD from the PROGgene database.[76] Their results suggested that the pathogenesis of SCC caused by COPD is regulated by *ADRB2* among others.[76] Furthermore, a study that analyzed the genes associated with COPD progression and complications reported that several genes, including *ADRB2*, were associated with COPD aggravation.[77] In this study, these genes were demonstrated to be extensively involved in regulating COPD complications.[77] Moreover, recent research with a small sample size has shown that patients with Arg16Arg had a lower incidence of exacerbations. Each copy of Gly of rs1042713 was associated with an increased incidence of exacerbations, in line with our results. After LABA/LAMA treatment, however, the number of exacerbations was similar across groups.[78]

DNA methylation sites and the risk of COPD exacerbations

Several recent studies have investigated changes in DNA methylation in patients with COPD, providing insight into potential disease mechanisms.[79-83] However, there has been little research conducted on DNA methylation and COPD exacerbations.

In the Rotterdam Study, the meta-analysis of epigenome-wide association study (EWAS) of the discovery and the replication sets identified two CpG (Cytosine-phosphate-Guanine) sites, cg25545920 located in the aristaless-related homeobox (*ARX*) gene on chromosome X and cg08906631 located in the *ZNF616* gene on chromosome 19 significantly associated with COPD exacerbation rate.

Previously, it has been reported that the CpG site, Cg25545920, exhibited epigenome-wide methylation that increases with age.[84] In another study, using Illumina 450karray, the authors examined DNA methylation in proliferating myoblast cells, identifying cg25545920 as one of the significant CpG sites on chromosome X with significant differences in methylation between men and women.[85] Xia et al[86] conducted a correlation analysis between differential methylated positions (DMPs) and gene expression. A correlation test of 20450 DMPs with nearby genes' expression (10 kb) demonstrated that cg25545920 was significantly correlated with *ARX* gene expression (FDR < 0.05).[86] Moreover, an EWAS with age, gender, and smoking status in a family study of 123 individuals of Arab descent demonstrated that cg25545920 was among genome-wide gender-related differentially methylated CpG sites.[87] Furthermore, the *ARX* gene was shown among the distal-signature genes downregulated in small airway epithelium of basal stem cells in smokers.[88] According to the Melbourne Collaborative Cohort Study's results, there were 4496 cross-sectional associations at a significant level of $P < 10^{-7}$, including a couple of CpGs on the *ARX* gene, which were implicated in smoking-associated DNA methylation changes.[89] It has been shown that *ZNF616* is involved in cellular senescence.[90] As a result of cellular senescence induced by smoking exposure, senescence-associated secretory phenotypes contribute to COPD development.[91-93]

Adherence to COPD maintenance therapy

As with most chronic conditions, including COPD, adherence to therapy is a challenging issue. Adherence to inhaled maintenance therapy appears to have a significant impact on treatment goals in patients with COPD.[3] It has been demonstrated that non-adherence to COPD medications is associated with decreased symptom control, increased risk of exacerbations, increased healthcare utilization, and costs, as well as decreased health-related quality of life and a higher mortality rate.[94-98] The use of inhaled medication is a critical element of managing COPD, but adherence to inhaled medication is low, even in cases of very severe disease.[3] A systematic review investigated the rate of non-adherence among COPD patients prescribed maintenance medication.[99] Of the 38 studies

included, 37 reported non-adherence to COPD medication ranging from 22% to 93%.[99] Non-adherence rates varied among studies, possibly due to the types of measures that the studies used.[99]

Multiple factors influence adherence, including social/environmental, individual, and treatment-related factors.[100] Previous studies reported several factors associated with low adherence, including co-morbidities, smoking status, depression, education, disease severity, and factors related to drug regimens, such as polypharmacy, dosage complexity, and adverse effects.[96,99,101]

A recent review by Shah et al. described various methods to assess medication adherence, and the choice of method should be based on the research design, the availability of data, and the study objectives.[102] In brief, adherence can be measured retrospectively, prospectively, or through a report by the patient or clinician (Figure 2).[102] In retrospective studies, adherence is measured by filling or dispense dates and by the number of days supplied. Measures such as medication possession ratios (MPRs) and percentage of covered days (PDCs) as well as group-based trajectory models (GBTMs) fall into this category.[102]

In prospective studies, adherence can be determined through observations, such as pill counts and therapeutic drug monitoring, as well as customized pill packaging and containers.[102] For example, adherence to salmeterol/fluticasone inhaler therapy in patients with COPD can objectively be quantified with an electronic audio recording device (INCA).[103]

An assessment of medication adherence may as well be based on reports by patients and clinicians using diaries, interviews, and instruments.[102]

We conducted a retrospective cohort study using electronic primary care data from Integrated Primary Care Information (IPCI) in the Netherlands and The Health Improvement Network (THIN) in the United Kingdom to examine adherence to inhaled maintenance therapy in COPD patients from 2007-2016. We found that patients' adherence to inhaled maintenance therapy was suboptimal. In addition, we found that older age and past smokers had a better adherence to inhaled maintenance therapy, while underlying conditions in particular depression was associated with poor adherence.

Results from a systematic review show that improving adherence to COPD medications requires a better understanding of the disease and its treatment, as well as a greater level of trust of patients in healthcare professionals.[99] Another systematic review[104] assessing interventions intended to improve adherence to pharmacological therapy found that multi-component interventions, including educational, motivational, and behavioral components delivered by health professionals, may assist in improving adherence. In order to improve adherence, individuals should participate in the development of an individually tailored treatment plan.[104]

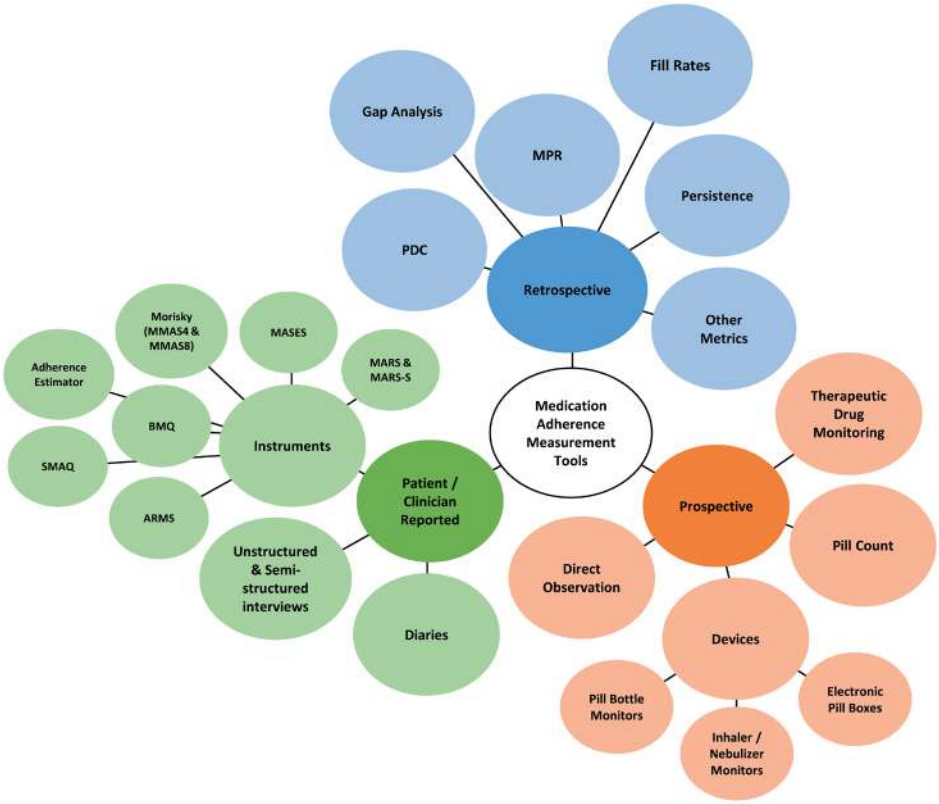

Figure 2. Summary of Medication Adherence Measurement Tools, Reproduced from Shah et al., 2023,[102] Research and scholarly methods: Measuring medication adherence; Journal of the American Collage of Clinical Pharmacy. Pages. 416–426, Vol. 6, Issue 4, Fig. 1. Abbreviations: SMAQ, Simplified medication adherence questionnaire; ARMS. Adherence to refills and medications scale; BMQ, Brief medication questionnaire; MARS, Medication adherence report scale; MARS-5, Five-item medication adherence report scale; MASES, Medication adherence self-efficacy scale; MPR, medication possession ratio; PDC, proportion of days covered

METHODOLOGICAL CONSIDERATIONS AND CHALLENGES

Population-based asthma research using different data sources

In this thesis, all asthma-related research was conducted using data from independent cohorts from the PICA consortium. In **Chapter 2.1,** we used only data from the PACAMAN study; in **Chapter 2.2**, **Chapter 2.3** and **Chapter 2.4** we used data from multiple cohorts from the PiCA consortium, containing genetic and clinical information from more than 14000 children and young adults with asthma, representing European, Hispanic/Latino, African American, and Asian ethnicities.[28]

In spite of the fact that population stratification may be a limitation of the PiCA consortium,[28] the heterogeneity resulting from different ancestries allowed us to analyze different genetic markers associated with treatment response among asthmatic patients of different ethnic

backgrounds. To control for population stratification, whenever appropriate, genome-wide association analyses were adjusted for principal components. Also, data was not polled, but analyses were conducted separately for each study, and the findings were meta-analyzed.

A further limitation might be the broad age range (4-21 years) of PiCA patients.[28] However, this reflects the general asthmatic population in clinical practice where children and young adults of all ages consult their physician for care. Furthermore, the diagnosis of asthma was based on physician diagnoses in 17 out of 21 PiCA studies and not necessarily supported by lung function tests.[28] It is true that physician diagnostic criteria may vary between countries, but these differences are a reflection of current clinical practice.[28] Also, the definition of asthma exacerbations varied between the studies participating in the PiCA consortium. Moreover, some asthma studies participating in PiCA considered a period of 6 or 12 months to assess treatment response, which introduced heterogeneity.[28] Additionally, questionnaires were used to collect information on asthma medications, asthma control, and asthma exacerbations in a number of studies participating in the projects in **Chapter 2.1**, **Chapter 2.2**, **Chapter 2.3**, and **Chapter 2.4**. Although previous research showed that parental questionnaires could provide reliable asthma medication information,[105-108] this type of approach is more prone to information bias.

COPD studies conducting in the Rotterdam Study

In this thesis, research on COPD (**Chapter 3.1**, **Chapter 3.2** and **Chapter 3.3**), was conducted using data from the Rotterdam Study, a population-based cohort study consisting of people of 45 years or older from Rotterdam. Age was the only criterion for inclusion, participants were recruited regardless of their health status, and most chronic diseases in elderly persons were diagnosed during follow-up. As a result, selection bias and information bias were minimized.[109]

A challenge in the Rotterdam Study was that spirometry was only introduced after 2002. In clinical settings, mild COPD is often underestimated because mild COPD cases rarely seek medical attention. It is possible that, prior to 2002, we may have underestimated the prevalence of mild COPD. Additionally, the absence of reversibility tests presents another limitation. This limitation was overcome by excluding asthmatic subjects from our analyses. Furthermore, the COPD diagnosis was based on the fixed FEV_1/FVC ratio (0.7) recommended by the GOLD guidelines,[3] rather than on the lower limit of normal (LLN). It should be noted that this fixed ratio approach may lead to over-diagnosis of COPD in healthy elderly individuals and under-diagnosis in young adults, particularly in those with mild COPD.[3,110,111]

COPD exacerbations were defined as acute episodes of worsening of symptoms requiring a course of steroids and/or antibiotics or hospitalization.[112] In **Chapter 3.3**, we had access to hospital admissions entered into the Dutch medical registry during the follow-up period for the discovery set but not for the replication set. As a result, we could only validate hospitalizations

due to COPD exacerbations based on physician notes for the replication set. It is necessary to acknowledge that physicians may not record this information consistently and comprehensively. In addition, both the discovery and replication sets had an exacerbation rate ranging from 0 to 4.

Moreover, self-administered questionnaires to determine smoking status as used in the Rotterdam Study may introduce information bias. There is evidence that patients with lung diseases understate their smoking status, as people tend to minimize undesirable behavior.[113] Due to this, smoking rates are often underestimated, and when used as a confounder in regression analysis, effect estimates may be overestimated or underestimated. This is particularly important in epigenetic studies of lung diseases since smoking affects DNA methylation and gene expression.[114,115]

In **Chapter 3.3**, Illumina 450K arrays were used as part of our methylation study, which could measure only 1.7% of the CpGs in the genome. In this array, most CpGs (41%) are located at gene promoters, 31% at gene bodies, 35% at 3'UTRs, and 3% at intergenic regions. On the Illumina 450k arrays, enhancer regions are not present in large numbers. Future studies in this area may benefit from the upgraded Illumina 850K EPIC array, which contains many new CpGs and high coverage of enhancer regions.[116] Furthermore, in EWAS studies, a large sample size is required to improve statistical power to detect subtle changes. The discovery and replication sets used for the methylation project **(Chapter 3.3**) were relatively small and underpowered. To combine results from the discovery and replication sets, we conducted a meta-analysis that confirmed two CpGs that reached the significance threshold. In this thesis, we utilized blood to measure DNA methylation levels and gene expression. Traditionally, COPD has been viewed as a respiratory disease primarily induced by tobacco use. However, COPD has significant manifestations beyond the lungs, known as systemic manifestations. Several potential health risks are associated with this condition, including unexpected weight loss, skeletal muscle dysfunction, cardiovascular disease, osteoporosis, and depression. A key mechanism underlying these systemic effects is chronic systemic inflammation.[117,118] Those systemic effects are detectable in plasma, for example, through the role of macrophages and neutrophils in COPD pathogenesis, so blood as the relevant tissue can be appropriate.[119,120] In whole blood DNA methylation studies, such as **Chapter 3.3**, different types of leukocytes are used (lymphocytes, monocytes, and granulocytes). Due to the possibility of cell-specific DNA methylation, it can be problematic to use a mixture of cell types that are subject to inter-individual variability. Therefore, these analyses should be adjusted for cell proportions which can be measured within the study, such as we used in this thesis, or predicted by using computational methods based on the DNA methylation data.[121]

Electronic health records

In **Chapter 4**, we assessed adherence to COPD maintenance therapy using electronic health records (EHRs) from primary care in two countries, IPCI in the Netherlands and THIN in the UK.[122,123] These databases allowed us to study large populations and provided valuable insight into COPD treatment in the real world, in opposite to the population as selected for RCTs. Various types of information can be captured in EHRs, including demographics, diagnoses, prescriptions, vital signs, laboratory results, vaccinations, and radiologic results. However, there are some pitfalls to the use of EHRs for pharmacoepidemiological research as described by the European Network of Centers for Pharmacoepidemiology and Pharmacovigilance (ENCePP) guideline,[124] such as:

- **Incompleteness of data capture:** Physicians do not systematically record all patient data, but instead record only data relevant to the care of a specific patient. This was demonstrated in our project by the missing information on smoking and spirometry data for many patients.
- **Bias in the assessment of drug exposure:** Primary care databases do not record over-the-counter medications or prescriptions from specialists. In addition, information on actual drug intake is often missing. We used data from IPCI and THIN which collects information on prescription data but not dispensing data.
- **Lack of harmonization of definition of conditions and outcomes:** Researchers often have to fall back on definitions customized to the information available in the databases they have access to, which may require validation when using other data sources. In our study on the adherence to COPD treatment, COPD diagnosis was based on a combination of prescription and diagnosis codes; however no free text validation was done. This might have induced misclassification of COPD.
- **Variability between healthcare systems**: The differences between healthcare systems make it difficult to compare results. For our research on treatment adherence, we had access to two primary care databases from the Netherlands (IPCI) and the UK (THIN). We know that UK healthcare is different from Dutch healthcare in terms of hospital referral, type of drugs (within a class) being added to the formulary, national guidelines, and more. Despite these differences, there are also some similarities, including that general practitioners serve as gatekeepers for all types of health care in both countries.

Confounding

As for all observational research, there is a risk of confounding which might lead to erroneous associations. If randomization is not an option, observational research tries to control for potential confounders either by restricting or matching the participants on important potential confounders, or through the analyses e.g. via stratification or by utilizing multivariate techniques (e.g. multivariate logistic regression or proportional hazard analysis) and by controlling for time-dependent co-variables, in order to achieve optimal results.[125] This thesis used several of

these techniques, including multivariate analysis and time-dependent analyses of variables. While we attempted to adjust for confounders when they were available, residual confounders that were unknown or unmeasured may still affect our findings.

External validity

An important consideration in epidemiological research is external validity, or the extent to which the results are generalizable to other situations and people. It is important to reflect on external validity when interpreting the results of **Chapter 2.1**, which identified the association between *FCER2* T2206C variant and FENO levels, which should be replicated in a larger sample size of Caucasian subjects. In **Chapter 3.3**, a significant association between two DNA methylation sites and COPD exacerbations was also found and replicated in a relatively small sample size. This should be interpreted with caution and would benefit from larger replication. Other results in this thesis have been replicated and confirmed either within these thesis or via other observational studies and/or clinical trials. Still additional replication is recommendable to further ensure external validity ideally in different population settings.

POTENTIAL IMPLICATIONS AND FUTURE DIRECTIONS

Asthma and COPD are prevalent conditions however cannot yet be cured, resulting in a significant burden on the healthcare system. The aim of this thesis was to explore factors that may improve the management of patients with COPD or asthma.

We identified some SNPs that are relevant in the management of patients with asthma and suggest that further research is required to identify the genetic markers underlying differential responsiveness to either ICS or LABA in patients with asthma. Future research should ideally include a large sample size of individuals from diverse populations but with a homogeneous asthma definition. It would also be beneficial to incorporate clinical measures of response to asthma (ICS or LABA) therapy, i.e., exacerbations, biomarkers, such as FENO, IgE, and eosinophils. In addition, the use of large reference panels for imputation that cover a significant number of genetic variants across the genome and provide adequate representation of the ancestry groups under study should also be taken into account. The recent release of the Trans-Omics for Precision Medicine (TOPMed)[126] reference panel is expected to make a substantial contribution to human genomics. Noncoding, structural, and low-frequency variants could be investigated in asthma treatment response. With the combination of these approaches and information from different omics sources, it is expected that a better understanding of the genetic factors that determine the response to asthma medications can be gained to tailor therapy to each patient's specific needs. Additionally, the results of the PUFFIN clinical trial[34] might open the door to implementing *ADRB2* genotype-guided treatment for childhood asthma in clinical practice.

With regard to the role of genetics in COPD management, this thesis found that Arg16 in *ADRB2* is associated with an increased risk of COPD exacerbations, which has also been observed through a post-hoc analysis of the POET-COPD trial.[18] Future prospective clinical trials could provide valuable information regarding the interaction between *ADRB2* genotype and LABA treatment. Moreover, it would be interesting to investigate in vitro why the association between the *ARDB2* variant and the risk of exacerbations differs among COPD and asthma patients. Specifically, while Arg16 is linked to a reduced risk of COPD exacerbations, the same SNP is associated with an increased risk of asthma exacerbations. Additionally, there is a critical need for more research in multi-omics studies, integrating genomics, epigenomics, transcriptomics, proteomics, and metabolomics of COPD and asthma using longitudinal designs, which measure multiple omics layers within the lung tissue of the same individual at different points in time. Although it has not yet been confirmed whether administering cardioselective β-blockers to COPD patients without a labeled indication is beneficial, further research is needed to establish the benefits of these medications in COPD patients with a labeled indication. In this thesis, we confirmed that adherence to maintenance therapy in COPD patients is suboptimal. We also explored risk factors of non-adherence. As for this type of research, societal and behavioral factors might be important. Future research on this topic should consider prospective, longitudinal studies that include patient characteristics, medication characteristics (e.g., dose, schedule, and formulation), and physician prescribing behavior, which may differ among healthcare systems.

CONCLUSION

In the past years, we have conducted research on the management of individuals with asthma or COPD. In this thesis, we demonstrated that FENO levels, as a biomarker for managing asthma, were associated with the *FCER2* T2206C variant and that treatment response to ICS or LABA was linked with some genetic variants in patients with asthma.

Furthermore, we described the association between genetic factors (in particular Arg 16 in *ADRB2),* DNA methylation, and the use of cardioselective β- blockers and COPD exacerbations. In addition, we confirmed that adherence to COPD maintenance therapy was suboptimal and identified risk factors of non-adherence.

In our thesis, we further elaborate on the methodological challenges of our research, potential implications, and future research directions. Further research on this topic will ultimately lead to a personalized approach to managing patients with asthma or COPD, where improved disease management and tailored treatments will reduce exacerbations and enhance quality of life.

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6

Summary

SUMMARY

Asthma and chronic obstructive pulmonary disease (COPD) are both chronic inflammatory lung diseases that affect breathing, but their characteristics and underlying mechanisms are quite different. COPD and asthma management require a multidisciplinary approach. Genetics is increasingly recognized as a factor influencing both the risk of developing these conditions and the response to treatment, providing the potential for more tailored treatment. This thesis aimed to enhance the understanding of asthma and COPD management, specifically focusing on genetic factors that influence these conditions. In **Chapter 1,** we introduced current knowledge and insights into the epidemiology, risk factors, burden, mechanisms, and management of asthma and COPD.

In **Chapter 2**, the role of genetic factors in asthma, with a particular focus on asthma exacerbations and fractional exhaled nitric oxide (FENO), was investigated. In **Chapter 2.1**, using data from the PACMAN study (Pharmacogenetics of Asthma Medication in Children: Medication with Anti-inflammatory effects), we found a significant association between the *FCER2* T2206C variant, the minor allele of rs28364072, and lower FENO levels in patients with asthma. After stratifying the asthma population into two groups, those with 'well-controlled' asthma and those with 'not well-controlled' asthma, we found that a significant association between the *FCER2* T2206C genotype and lower levels of FENO was present only in patients with well-controlled asthma. In **Chapter 2.2,** the results of a meta-analysis (n = 832) using data from 10 independent studies participating in the multi-ethnic Pharmacogenomics in Childhood Asthma (PiCA) consortium showed that the haplotype Arg16/Gln27 in *ADRB2*, presumably driven by the Arg16, was significantly associated with increased risk of asthma exacerbations among subjects treated with inhaled corticosteroids (ICS) and long-acting β_2-agonists (LABA). In **Chapter 2.3** and **Chapter 2.4,** we performed genome-wide association studies (GWAS) meta-analysis using databases participating in the PiCA consortium**.** In **Chapter 2.3,** based on the GWAS of response to ICS among admixed populations (n = 1347), a single marker (rs5995653) within the intergenic region of *APOBEC3B* and *APOBEC3C* was shown to have a protective effect against asthma exacerbations in children and adolescents of Hispanic/Latino and African descent treated with ICS. The finding was replicated in European populations (n = 1697) as well. In **Chapter 2.4,** we performed a GWAS meta-analysis on 2681 children of European descent treated with ICS across eight studies. The suggestive association signals identified were subsequently tested for replication in 538 European asthma patients. Further evaluation was conducted in a cohort of 1773 non-European asthma patients. One variant at the *CACNA2D3-WNT5A* locus was associated with asthma exacerbations despite ICS treatment and was nominally replicated in Europeans (rs67026078; p=0.010). However, this finding was not validated in non-European populations**.**

In **Chapter 3,** using data from the Rotterdam Study, an ongoing prospective population-based cohort study, we described the association between 1) the use of β- blockers; 2) *ADRB2* variants; 3) DNA methylation and COPD exacerbations. In **Chapter 3.1,** we found that the use of cardioselective β-blockers among COPD patients with cardiovascular comorbidity was significantly associated with a reduced risk of COPD exacerbations. However, in subjects without cardiovascular disease, use of cardioselective β-blockers was not associated with an altered risk of COPD exacerbations. In **Chapter 3.2,** we described the association between *ADRB2* variants and COPD exacerbations. We reported that the Arg16/Gln27 haplotype had a significantly lower risk of COPD exacerbation compared to the Gly16/Glu27 haplotype in patients treated with inhaled β_2-agonists. In **Chapter 3.3**, we meta-analyzed the epigenome-wide association study (EWAS) of the discovery and replication sets. We identified two CpG (Cytosine-phosphate-Guanine) sites as significantly associated with rate of COPD exacerbations, cg25545920 located on chromosome X in the *ARX* gene and cg08906631 located on chromosome 19 in the *ZNF616* gene.

In **Chapter 4**, we examined adherence to COPD maintenance therapy using group-based trajectory models (GBTMs). We conducted a retrospective cohort study using electronic primary care data from the Integrated Primary Care Information (IPCI) database in the Netherlands and The Health Improvement Network (THIN) database in the United Kingdom. We investigated adherence to inhaled maintenance therapy among incident COPD patients from 2007 to 2016. Our findings revealed that patients' adherence to inhaled COPD maintenance therapy was not optimal. Furthermore, we observed that older patients and past smokers demonstrated better adherence to inhaled maintenance therapy. In contrast, depression was associated with poor adherence.

In **Chapter 5,** we discussed the key findings of the studies included in this thesis. This chapter focused on methodological aspects, underlined the clinical implications of our results, and proposed potential areas for future research.

SAMENVATTING

Astma en chronische obstructieve longziekte (COPD; chronic obstructive pulmonary disease) zijn beide chronische ontstekingsziekten van de longen die de ademhaling beïnvloeden maar hun kenmerken en onderliggende mechanismen zijn aanzienlijk verschillend. De behandeling van COPD en astma vereist een multidisciplinaire aanpak. Genetica wordt steeds meer erkend als een factor die zowel het risico op het ontwikkelen van deze aandoeningen als de respons op behandeling beïnvloedt, wat de mogelijkheid biedt voor meer 'op maat' gekozen behandelingen. Dit proefschrift had als doel de kennis rond het management van astma en COPD te verbeteren, met specifieke aandacht voor genetische factoren die deze aandoeningen beïnvloeden. In **Hoofdstuk 1** introduceerden we de huidige kennis en inzichten met betrekking tot de epidemiologie, risicofactoren, ziektelast, mechanismen en behandeling van astma en COPD.

In **Hoofdstuk 2** werd de rol van genetische factoren bij astma onderzocht, met speciale aandacht voor astma-exacerbaties en fractionele uitademing van stikstofmonoxide (FENO; fractional exhaled nitric oxide). In **Hoofdstuk 2.1** vonden we, gebruikmakend van data uit de PACMAN (Pharmacogenetics of Asthma Medication in Children: Medication with Anti-inflammatory effects) studie, een significante associatie tussen de *FCER2* T2206C-variant, het minder voorkomende allel van rs28364072, en lagere FENO-niveaus in patiënten met astma. Toen de analyse herhaald werd in patiënten met 'goed gecontroleerd' astma en patiënten met "niet goed gecontroleerd" astma, vonden we enkel een significante associatie tussen het *FCER2* T2206C-genotype en lagere FENO-niveaus in patiënten met goed gecontroleerd astma. **Hoofdstuk 2.2** beschreef de resultaten van een meta-analyse (n = 832) met gegevens van 10 onafhankelijke studies die deelnemen aan het multi-etnische Pharmacogenomics in Childhood Asthma (PiCA) consortium. De resultaten van de meta-analyse lieten zien dat het haplotype Arg16/Gln27 in *ADRB2*, vermoedelijk gedreven door Arg16, significant geassocieerd was met een verhoogd risico op astma-exacerbaties bij deelnemers behandeld met inhalatiecorticosteroïden (ICS) en langwerkende β_2-agonisten (LABA). In **Hoofdstuk 2.3** en **Hoofdstuk 2.4** hebben we meta-analyses uitgevoerd voor genoom-brede associatiestudies (GWAS; genome-wide association studies) op gegevens van studies die deelnamen aan het PiCA-consortium. In **Hoofdstuk 2.3** bleek, op basis van de GWAS meta-analyse van respons op ICS bij gemengde populaties, een enkele marker (rs5995653) binnen het intergenische gebied van *APOBEC3B* en *APOBEC3C* een beschermend effect te hebben tegen astma-exacerbaties bij kinderen en adolescenten van Hispanic/Latino- en Afrikaanse afkomst die met ICS behandeld werden. Deze bevinding werd ook gerepliceerd bij Europeanen. In **Hoofdstuk 2.4** voerden we een GWAS meta-analyse uit bij 2681 kinderen van Europese afkomst die behandeld werden met ICS in acht studies. De geïdentificeerde suggestieve associatiesignalen werden vervolgens getest voor replicatie in een groep van 538 Europese astmapatiënten. Bovendien werd verdere evaluatie uitgevoerd in een cohort van 1773 niet-Europese astmapatiënten. Eén variant op de *CACNA2D3-WNT5A*-locus

was geassocieerd met astma-exacerbaties ondanks ICS-behandeling en werd nominaal gerepliceerd bij Europeanen (rs67026078; p=0,010). Deze bevinding werd echter niet bevestigd in niet-Europese populaties.

In **Hoofdstuk 3** beschreven we door middel van de gegevens van de Rotterdam Study, een prospectieve cohortstudie, de associatie tussen 1) het gebruik van β-blokkers; 2) *ADRB2*-varianten; 3) DNA-methylatie en COPD-exacerbaties. In **Hoofdstuk 3.1** vonden we een significante associatie tussen het gebruik van cardioselectieve β-blokkers en een gedaald risico op COPD exacerbaties bij COPD-patiënten met cardiovasculaire comorbiditeit. Bij deelnemers zonder hart- en vaatziekten was het gebruik van cardioselectieve β-blokkers echter niet geassocieerd met een veranderd risico op COPD-exacerbaties. In **Hoofdstuk 3.2** is de associatie tussen *ADRB2*-varianten en COPD-exacerbaties beschreven. We identificeerden dat deelnemers met het Arg16/Gln27-haplotype en behandeld met β_2-agonisten een aanzienlijk lager risico op COPD-exacerbaties hadden ten opzichte van deelnemers met het Gly16/Glu27-haplotype die ook behandeld werden met β_2-agonisten. In **Hoofdstuk 3.3** beschreven we de resultaten van de meta-analyse van de epigenoom-brede associatiestudie (EWAS; epigenome-wide association study) van de ontdekking- en replicatiesets. We identificeerden twee CpG-sites (Cytosine-fosfaat-Guanine) die significant geassocieerd waren met COPD-exacerbaties: cg25545920 op chromosoom X in het *ARX*-gen en cg08906631 op chromosoom 19 in het *ZNF616*-gen.

In **Hoofdstuk 4** onderzochten we de therapietrouw voor COPD-onderhoudsmedicatie middels groepsgebaseerde trajectmodellen (GBTMs; group-based trajectory models). We beschreven de resultaten van een retrospectieve cohortstudie, gebruikmakend van de elektronische gegevens van twee huisartsen-databases, namelijk de Integrated Primary Care Information (IPCI) database in Nederland en The Health Improvement Network (THIN) database in het Verenigd Koninkrijk gedurende de studieperiode 2007 en 2016. Uit de resultaten bleek dat patiënten met incident COPD niet optimaal therapietrouw waren aan de geïnhaleerde onderhoudstherapie voor COPD. Bovendien zagen we dat oudere patiënten en voormalige rokers een betere therapietrouw hadden.

Hoofdstuk 5 beschreef de belangrijkste bevindingen van de studies die in dit proefschrift zijn opgenomen. Dit hoofdstuk ging verder in op methodologische aspecten van het uitgevoerde onderzoek, beschreef de klinische implicaties van onze resultaten en stelde mogelijke onderzoeksvragen voor de toekomst voor.

ABBREVIATIONS

ATS	American Thoracic Society
ADRB2	Adrenoceptor Beta 2 gene
B2AR	Beta2-Adrenergic Receptor
COPD	Chronic Obstructive Pulmonary Disease
DNA	Deoxyribonucleic Acid
ERS	European Respiratory Society
ESTATe	Effectiveness and Safety of Treatment with Asthma Therapy
EWAS	Epigenome-Wide Association Study
FEV_1	Forced Expiratory Volume in 1 second
FENO	Fractional Exhaled of Nitric Oxide
FVC	Forced Vital Capacity
GBD	Global Burden of Diseases
GINA	Global Initiative for Asthma
GOLD	Global Initiative for Chronic Obstructive Lung Disease
GWAS	Genome-Wide Association Studies
HRQoL	Health-Related Quality of Life
ICS	Inhaled Corticosteroids
IPCI	Integrated Primary Care Information
LABA	Long-Acting $Beta_2$ Agonists
LAMA	Long-Acting Muscarinic Antagonists
LTRA	Leukotriene Receptor Antagonists
NO	Nitric Oxide
PACMAN	Pharmacogenetics of Asthma Medication in Children: Medication with Anti-inflammatory effects
PiCA	Pharmacogenomics in Childhood Asthma
SABA	Short-Acting $Beta_2$ Agonists
SAMA	Short-Acting Muscarinic Antagonists
SNPs	Single Nucleotide Polymorphisms
THIN	The Health Improvement Network

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PHD PORTFOLIO

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General courses and training

- Scientific Integrity Course 2018
- 34th International Conference on Pharmacoepidemiology & Therapeutic Risk Management, Prague, Czech Republic (2018) 2018
  - ISPE Newcomer/Early Stage Investigator Pharmacoepidemiology Workshop
  - Propensity Scores in Pharmacoepidemiology
  - Advanced Topics in PE: Advanced Pharmacoepidemiology
- 35th International Conference on Pharmacoepidemiology & Therapeutic Risk Management, Philadelphia, USA (2019) 2019
  - Adherence to Medication
  - Dr. Gopen's Writing Course
  - Intermediate Pharmacoepidemiology: Approaches to Unmeasured Confounders
  - Applied Sensitivity Analysis

Seminars, symposia, and workshops

- The annual symposium of the Netherlands Network Precision Medicine 2017
- Observational Health Data Sciences and Informatics (OHDSI) Europe Symposium, Rotterdam, Netherlands 2018
- PhD day, Erasmus MC 2018
- Observational Health Data Sciences and Informatics (OHDSI) Europe Symposium, Rotterdam, Netherlands 2019

International conferences

- 34th International Conference on Pharmacoepidemiology & Therapeutic Risk Management, Prague, Czech Republic (1 poster discussion) 2018
- 28th International Congress of the European Respiratory Society, Paris, France (1 poster presentation and 1 poster discussion) 2018
- 35th International Conference on Pharmacoepidemiology & Therapeutic Risk Management, Philadelphia, USA (1 poster spotlight presentation) 2019
- 29th International Congress of the European Respiratory Society, Madrid, Spain (2 poster presentations and 1 poster discussion) 2019
- 36th International Conference on Pharmacoepidemiology & Therapeutic Risk Management, Virtual (1 poster discussion) 2020
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Teaching activities

- Teaching assistant- Pharmacoepidemiology and drug safety Master of Science in Health Science, Netherlands Institute for Health Sciences, Rotterdam, the Netherlands 2019

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- ICPE scholarship to attend the 35th International Conference on Pharmacoepidemiology & Therapeutic Risk Management, Philadelphia, USA 2019
- Travel grant from The Lung Foundation Netherlands to attend 29th International Congress of the European Respiratory Society, Madrid, Spain 2019

ACKNOWLEDGEMENTS

I am filled with a deep sense of gratitude as I turn to the last page of this significant chapter in my academic journey. My heart is filled with deep gratitude to all those who provided me the possibility to complete this PhD journey.

Hereby few words to my promotors, Dr. Katia Verhamme and Prof. Bruno Stricker, and others who contributed to my research.

Dear Katia,
You have been more than a promotor. You gave me the freedom to work independently and the supervisory guidance at the same time. Your support, grounded in vast knowledge, has been nothing short of extraordinary. Your consistent availability, whether for a quick in person visit or a late-night email, has been priceless. Your modesty and kindness shined through every interaction. You keenly listened to my ideas and patiently helped me shape them into achievable steps. Our Tuesday and Thursday lunches will remain one of my fondest memories. Since my departure from EMC in December 2019, we remained in close touch. During the hard days, your encouraging words kept me on my feet. Thank you.

Dear Bruno,
Your knowledge has been a guiding light throughout my PhD, from the very beginning. Your timely responses contributed greatly to the smooth progression of my research. Celebrating your birthdays with your students and colleagues wherever we were, whether Prague or across the seas in Philadelphia, remains as a lasting joyful memory. I have learned so much from our collaboration and your unwavering support has been a cornerstone to my success. Thank you.

Dear Johan (Prof. van der Lei),
You trusted me and gave me the opportunity to start the PhD studies while I was still working as a staff member in the department. Despite your tight schedules, you took the time to meet me monthly. Thanks for all that.

Dear Guy (Prof. Brusselle),
Your supervision has been invaluable. Your ability to see the bigger picture about my research and providing me with insightful feedback enriched my work. Thank you for your time and dedication.

Dear Lies (Prof. Lahousse),
I am grateful for your attention to detail and your unwavering support. Your prompt feedbacks, always given within two days, has been invaluable. Thank you for your involvement as a co-author.

Dear Peter (Prof. Rijnbeek)
My special thanks for all your kindness and for bringing new opportunities to the Medical Informatics Department as the Chair.

Dear Miriam (Prof. Sturkenboom),
I am not able to find the words to express my thankfulness for giving me the opportunity to join your department in the beginning of my academic career path. Your vision and dedication to scientific work continues to inspire me. I am proud to have had the opportunity to work with you at Erasmus MC.

My heartfelt thanks go to all the co-authors and the PiCA members for their invaluable contributions to the work presented in this thesis. Your insightful suggestions, comments, and ideas were fundamental. With special thanks to Dr. Niloufar Farzan, Dr. Natalia Hernandez-Pacheco, Dr. Susanne Vijverberg, Dr. Maria Pino-Yanes, Prof. Anke H. Maitland-van der Zee, and Dr. Mohsen Ghanbari.

I would also like to extend my thankfulness to the members of the reading committee; Prof. Aerts, Prof. van den Berge, and Dr. Pijnenburg.

Sincere appreciation for the encouragement and support from former PhD candidates who greatly enriched my academic journey namely, Nathalie, Remy, Christel, Esme, Emmely, Sara, Ross, Kiki, Marlies, Marten, Sadaf, Linda, Johnmary, Ivana, Pooja, and Eliza. And special thanks to my wonderful friends from the NIHES Master program: Ruchika, Emanuela, and Leyla. How could I not mention Ruchika's generous food baskets that arrived at my door unannounced when I had totally forgotten to cook.

I would also like to acknowledge the indispensable support of the individuals and office staff of the Medical Informatics Department (MID) at Erasmus MC: Desiree de Jong, Tineke de Ben, Sander Woerdeman, and Ilse Schuemie. The entire MID had a significant positive impact on my PhD work. My gratitude is extended to Mees Mosseveld, Marcel de Wilde, Armando Leyba, and Maria de Rider too.

Furthermore, I am deeply grateful to the participants of the Rotterdam Study for their commitment and the general practitioners in the Ommoord district, whose collaboration and data collection made the Rotterdam Study possible. Here I must mention the invaluable contributions of the Rotterdam Study data management team, Frank, Jolande, and Nano.

Dear Zahra,
I am delighted to have such a wonderful friendship with you and your kind family. Thank you for our sweet Persian chats, meals, and the joyful moments we shared together.

Dear Fariba,
We first met at a symposium in Utrecht, and since then, we became close friends. I am proud to have such a warm and friendly relationship with you and your dear family.

Dear Natalie,
We have known each other for such a long time and have shared so many memorable moments together. You inspire me as a strong, intelligent, and ambitious peer, while being a caring mother at the same time. I am proud to call you my friend and honored to have you as my paranymph.

Dear Ali,
My son I am thrilled that you will stand by my side as my paranymph. This takes me back to 24 years ago when I held you in my arms for the first time as a newborn. You have continually challenged and inspired me to learn more, and I am incredibly proud to be not only your mother but also your close friend. We have created so many wonderful memories together, from watching cartoons and playing games to learning Dutch when we moved to the Netherlands. Now, you speak Dutch like a native, while I still struggle. I feel incredibly fortunate to be one of the rare PhD students who has the chance to have their own child as a paranymph. Thank you for being such an important part of my life and for supporting me throughout this *to-be-continued-together* journey. I hope you achieve more love and passion in your life.

My gratitude would be incomplete without acknowledging the greatest source of my strength, my parents. My dear Maman, how I wish you were still on this planet to share this moment with me. Thank you, Baba, for your love and support all along. Without you both, I could not have made this far.

Lastly, to my caring husband Nima Moin, I want to whisper my boundless love and gratitude. Thank you for all your patience and cooperation during disturbances, late meals, early wake up alarms, and missed events. Nima, you are not just my partner in life but also my closest friend. I could never have completed this work without you on my side.

Erasmus University Medical Centre, 2 October 2024
Leila Karimi

ABOUT THE AUTHOR

Leila was born in Rasht, Iran. She studied medicine, earning her Doctor of Medicine, and subsequently worked as a medical doctor in Iran. She took up her role as the head of a public healthcare clinic, while she also collaborated with the research center at Guilan University. Later because of her husband's studies, she moved to Rotterdam Netherlands with her family. By 2014, Leila had graduated with a Master's degree in Clinical Epidemiology from NIHES Erasmus University. The following year, she joined the Medical Informatics Department as a Scientific Researcher and Coordinator for the Online European Master in Pharmacoepidemiology and Pharmacovigilance, while working full-time. Along with this commitment, she earned her Doctor of Science in Genetic Epidemiology in 2017 from NIHES Erasmus University. By September 2017, Leila began her PhD studies while working part-time in the department. She left EMC in December 2019 to take on a new role as Senior Epidemiologist at IQVIA, a leading global provider of advanced analytics, technology solutions, and clinical research services to the life sciences industry. Currently, she is an Associate Director of Pharmacoepidemiology in Real World Evidence (RWE). Her expertise extends to serving as a Senior Oversight Epidemiologist on several influential studies. Leila has a proven track record of fostering extensive collaborations with academic institutions, pharmaceutical companies, and regulatory bodies such as the EMA. Her leadership is further demonstrated by her successful contributions to numerous multinational RWE projects and publications in high-impact scientific journals. Despite working full-time at IQVIA, she continued to pursue her dream and successfully completed her PhD.

ISBN: 978-94-6510-202-3