GENETIC FACTORS INVOLVED IN THE RESPONSE TO INHALED CORTICOSTEROIDS IN PEDIATRIC ASTHMA

DOCTORAL THESIS

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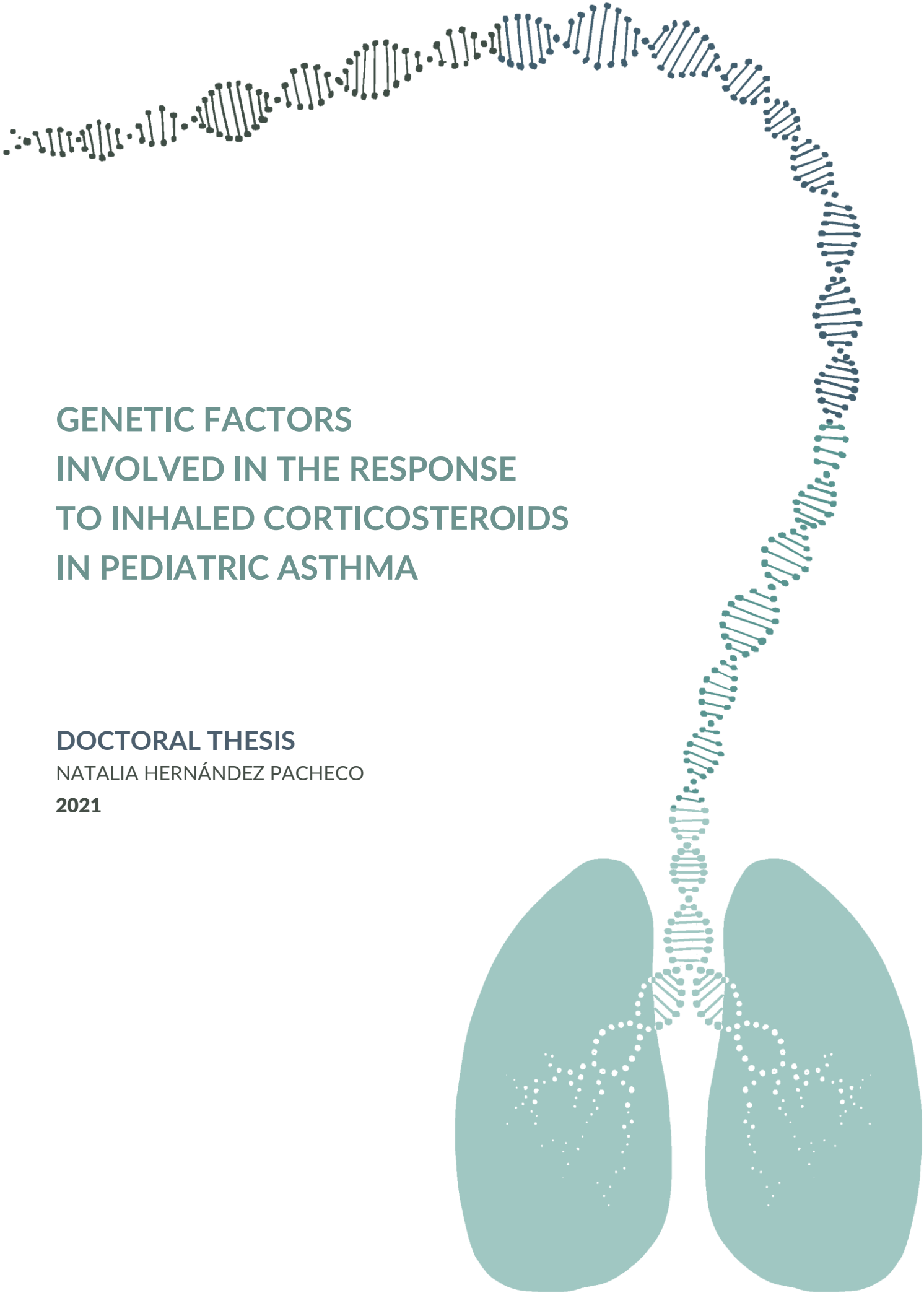

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DOCTORAL THESIS

Genetic factors involved in the response to inhaled corticosteroids in pediatric asthma

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Los doctores Dña. María del Mar del Pino Yanes y D. Carlos A. Flores Infante, directores de la tesis doctoral presentada por Dña. Natalia Hernández Pacheco

Certifican que:

La memoria presentada por la graduada Natalia Hernández Pacheco titulada "*Genetic factors involved in the response to inhaled corticosteroids in pediatric asthma*" ha sido realizada bajo su dirección en la Unidad de Investigación del Hospital Universitario Nuestra Señora de Candelaria y en el Departamento de Bioquímica, Microbiología, Biología Celular y Genética de la Universidad de La Laguna, y considerando que reúne las condiciones de calidad y rigor científico, se autoriza para que pueda ser presentada y defendida ante la comisión nombrada al efecto para optar al grado de Doctor por la Universidad de La Laguna.

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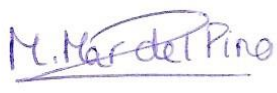

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ABSTRACT

Inhaled corticosteroids (ICS) are the most commonly prescribed and effective medication to control asthma symptoms in children and young adults. High variability in the response to this treatment has been described among individuals and populations. These differences have been suggested to be the result of the interaction of several factors, including an important contribution of the individual's genetic composition. However, the genetic markers of ICS response identified to date are not able to predict the responsiveness to this medication in clinical practice. This doctoral thesis aimed to identify genetic variants involved in the response to asthma treatment with ICS through genomic approaches. A systematic review of the main findings of the genomic studies of asthma susceptibility and treatment response published between 2016 and 2018 was performed, identifying the genetic markers to be followed up for replication. Two genome-wide association studies of asthma exacerbations despite ICS use in admixed and European populations were also completed, revealing two suggestive novel associations. Additionally, a gene-set enrichment analysis in asthma patients of European descent revealed a potential novel drug for asthma. Genetic associations with the change in lung function after a short period of ICS therapy were assessed, suggesting a novel association of a locus that could be involved in the response to this medication. Finally, the combination of transcriptomic data from different cell types with genomic information from asthma patients treated with ICS led to the identification of an additional potential novel locus for ICS response. The findings of this doctoral thesis suggest the existence of genetic markers of asthma treatment response specific to certain ancestry groups and shared among different populations. Moreover, the information about asthma exacerbations was evidenced as a good predictor of the response to this medication through the validation of previous associations described for different measures of ICS response.

ABBREVIATIONS

1KGP	1,000 Genomes Project
APOBEC3B	Apolipoprotein B mRNA editing enzyme catalytic subunit 3B
APOBEC3C	Apolipoprotein B mRNA editing enzyme catalytic subunit 3C
ASM	Airway smooth muscle
ATS	American Thoracic Society
BAL	Bronchoalveolar lavage
BDR	Bronchodilator response
BTS/SIGN	British Thoracic Society/Scottish Intercollegiate Guidelines Network
Ca^{2+}	Calcium ions
CAAPA	Consortium on Asthma among African ancestry Populations in the Americas
CACNA2D3	Calcium voltage-gated channel auxiliary subunit alpha-2/delta-3
COPD	Chronic obstructive pulmonary disease
CpG	Sites enriched in cytosine and guanine
eQTL	Expression quantitative trait loci
ER	Emergency room
ERS	European Respiratory Society
FBLN1	Fibulin 1
FeNO	Fractional concentration of nitric oxide
FEV_1	Forced expiratory volume in one second
FVC	Forced vital capacity
GCs	Glucocorticosteroids
GR	Glucocorticosteroid receptor
GRM4	Glutamate metabotropic receptor 4
GSDMB	Gasdermin B
GWAS	Genome-wide association study
HRC	Haplotype Reference Consortium
ICS	Inhaled corticosteroids
IgE	Immunoglobulin E
IL	Interleukin
IPF	Idiopathic pulmonary fibrosis
LABA	Long-acting beta-2 agonists
LD	Linkage disequilibrium
LTBP1	Latent transforming growth factor β binding protein 1

LTRA	Leukotriene receptor antagonists
NGS	Next-generation sequencing
NK	Natural killer
NSAIDs	Non-steroidal anti-inflammatory drugs
OCS	Systemic corticosteroids
ORMDL3	ORMDL sphingolipid biosynthesis regulator 3
PBMCs	Peripheral blood mononuclear cells
PiCA	Pharmacogenomics in Childhood of Asthma
RNA-seq	Next-generation transcriptome sequencing
ROBO2	Roundabout guidance receptor 2
SABA	Short-acting beta-2 agonists
SLIT	Slit guidance ligand
SNP	Single nucleotide polymorphism
TGF-β1	Transforming growth factor β1
Th2	T helper 2
TOPMed	Trans-Omics for Precision Medicine
TSA	Trichostatin A
WGS	Whole-genome sequencing
WNT	Wingless/integrase 1
WNT5A	Wingless/integrase 1 family member 5A
ZPBP2	Zona pellucida binding protein 2

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1. INTRODUCTION

1. INTRODUCTION

1.1. Description of asthma

Asthma is a complex disease of the respiratory system characterized by chronic inflammation and variable obstruction of the airways that can be partially or completely reversible (Global Initiative for Asthma 2020). This disease can lead to diverse and non-specific symptoms, including wheezing, cough, chest tightness, breathlessness, and airflow limitation, among others. Additionally, asthma has been related to allergic sensitization (atopy), increased levels of blood eosinophils, and bronchial hyperresponsiveness (Global Initiative for Asthma 2020).

1.2. Epidemiology of asthma

Asthma affects approximately 350 million people worldwide and causes around 350 thousand deaths per year (Masoli et al. 2004; Lozano et al. 2013), and it has been estimated that a further 100 million people will be affected by 2025 (Global Asthma Network 2018). A substantial social and economic burden has been attributed to asthma globally (Ferkol and Schraufnagel 2014), representing 1.8% of the total disease morbidity worldwide (Vos et al. 2012). Specifically, this condition causes an important direct economic impact on health care systems due to the numerous hospital admissions, therapies, specialist visits, and emergency care required by patients with uncontrolled asthma (Ferreira de Magalhaes et al. 2017; Nunes et al. 2017). Additionally, asthma causes important indirect financial effects driven by the loss of working days and/or absenteeism from school together with a substantial detriment of the quality of life of patients with severe asthma (Williams et al. 2009; Global Initiative for Asthma 2020), including limitation of physical activities and psychological consequences (Gibson et al. 2013). In this respect, asthma is considered one of the most important pulmonary diseases (Ferrante and La Grutta 2018).

Wide differences in asthma prevalence have been found among countries and populations, ranging from approximately 1.5% to 20.0% (Van Wonderen et al. 2010; Baiz and Annesi-Maesano 2012; Sears 2014), and also among different ethnic groups (Akinbami et al. 2014; Enilari and Sinha 2019). Interestingly, recent studies have evidenced that genetically admixed populations with African ancestry such as Latinos/Hispanics and African Americans are more affected by asthma than Europeans, showing from two to three-fold higher rates of asthma-related complications and deaths (Akinbami et al. 2012; Akinbami et al. 2014; Hernandez-Pacheco et al. 2016; Centers for Disease Control and Prevention 2018).

Different patterns of incidence and prevalence have also been described among age groups (Dharmage et al. 2019). Although asthma symptoms onset can occur at any lifetime, they usually begin during childhood (Dharmage et al. 2019). Nonetheless, remission of childhood asthma sometimes occurs during adolescence (Trivedi and Denton 2019). Asthma is considered the most common chronic disease in children and young adults (Global Initiative for Asthma 2020), where the most severe form of this disorder is usually presented (Akdis and Agache 2013). Interestingly, adult-onset asthma often results to be difficult to differentiate from other respiratory conditions, such as chronic obstructive pulmonary disease (COPD), since the substantial overlap between both diseases (Global Initiative for Asthma 2020). Thus, incidence and prevalence rates are increased in children even though the utilization of healthcare resources and mortality

rates are considerably higher among adults (Centers for Disease Control and Prevention 2018; Dharmage et al. 2019). Additionally, differences driven by gender have also been described, which have been evidenced to vary across the lifespan (Centers for Disease Control and Prevention 2018; Dharmage et al. 2019).

Variability in asthma incidence and prevalence evidence the complex etiology of this disease, being the result of the interaction of several factors such as environmental exposures, individual's genetic composition, sex hormones, immune response, obesity, and socioeconomic status, among others (Nishimura et al. 2013; Oh et al. 2016; Shah and Newcomb 2018; Dharmage et al. 2019). Nonetheless, the important contribution of genetic factors in asthma has been evidenced (Fagnani et al. 2008; Yang et al. 2010; Wang et al. 2017a). Specifically, asthma heritability estimates demonstrate that 55-74% of asthma susceptibility could be explained by the genetic composition in adults (Thomsen et al. 2006; Ober and Yao 2011). Genetic twin studies have estimated asthma heritability in 50-90% (Fagnani et al. 2008; Ullemar et al. 2016), whereas lower estimates (30%) have been revealed by analyzing non-related individuals (Pividori et al. 2019).

1.3. Pathogeny and pathophysiology of asthma

Asthma is a heterogeneous disease driven by diverse events leading to a common clinical expression (King et al. 2019). The main pathogenic effects of asthma take place in the bronchial tree, involved in the distribution of air through the lungs until reaching the alveoli. Bronchi walls are composed of smooth muscle and elastic fibers, which are the components involved in the contraction and relaxation induced by endogenous or exogenous mediators (Grinnan and Truwit 2005; Amador and Varacallo 2020).

In patients with asthma, complex interactions between several cell types and molecules acting as mediators are responsible for several physiological processes that lead to the bronchial obstruction, characterized by the reduction of the diameter of the airways and its ability to distend (Grinnan and Truwit 2005; Amador and Varacallo 2020). Although inflammation plays a central role in the pathophysiology of asthma (Global Initiative for Asthma 2020), important structural changes through the airway and lung parenchyma remodeling also take place (Al-Muhsen et al. 2011; Gelb et al. 2018), which can occur as a consequence of the repair response to chronic inflammation or independently of this process (Grainge et al. 2011).

Inflammation of the airways is mainly caused by bronchoconstriction or contraction of the bronchial smooth muscle in response to several stimuli, resulting in airway narrowing, which can be spontaneously relieved or may need the action of medication (National Heart, Lung, and Blood Institute 2007). Exposure to different stimuli can trigger the immune response driving inflammation patterns similar to the induced in allergic reactions, though the most severe airflow limitation episodes are triggered by viral infections of the respiratory system (Jackson and Johnston 2010). This process causes the activation and production of higher levels of several inflammatory cell types in the airways, including lymphocytes T, predominantly T helper 2 (Th2) cells, which produce specific cytokines (interleukin (IL)-4, IL-5, IL-9, IL-13) (Barnes 2002; Cohn et al. 2004). These inflammatory mediators induce the overproduction of immunoglobulin E (IgE) (Rosenberg et al. 2013), the main antibody involved in allergic reactions, and also the activation of eosinophils in some patients (Williams 2004). Interestingly, reduced regulatory T cells together with

increased activation of natural killer (NK) and dendritic cells are characteristic of patients with asthma, which promote the production of proinflammatory Th2 cytokines (Lambrecht and Hammad 2010; Lloyd and Hessel 2010). High levels of these inflammatory mediators are correlated with asthma severity (Barnes 2011). Indeed, increased activation of neutrophils and macrophages has been related to an enhanced inflammatory response (Macdowell and Peters 2007; Yang et al. 2012).

Osmotic changes induced by physical exercise, allergens, or neuronal stimuli can cause acute bronchoconstriction, driven by high levels of mast cells in bronchial epithelium and airway smooth muscle (ASM) after infiltration (Galli and Tsai 2012). Mast cells release pro-inflammatory and bronchoconstrictor mediators (histamine, tryptase, leukotrienes, prostaglandins, and cytokines), which directly act on ASM (Galli and Tsai 2012). This has been demonstrated to be a process triggered by the production of IgE, although acute airway obstruction can also be caused by means of IgE-independent processes in response to non-steroidal anti-inflammatory drugs (NSAIDs) (Stevenson and Szczeklik 2006). In patients with progressive inflammation underlying persistent asthma symptoms, other events including airway edema and mucus hypersecretion lead to the formation of mucus plugs, which also contribute to airflow limitation (National Heart, Lung, and Blood Institute 2007).

Airway hyperresponsiveness or exaggerated bronchoconstrictor response to endogenous and exogenous stimuli that seem to be innocuous in healthy individuals is one of the main characteristics of asthma pathophysiology (National Heart, Lung, and Blood Institute 2007). This reaction is mainly triggered by inflammation, which causes airflow limitation and intermittent symptoms. For this reason, airway hyperresponsiveness is considered a good indicator of asthma severity (National Heart, Lung, and Blood Institute 2007).

Remodeling of the airways through structural changes is also one of the main processes underlying asthma symptoms (National Heart, Lung, and Blood Institute 2007). Several types of structural cells are activated causing permanent changes that increase the airflow limitation and airway hyperresponsiveness, hampering a proper response to asthma therapies (Holgate and Polosa 2006). Nonetheless, both inflammatory and structural processes have been demonstrated to be highly correlated. The migration of inflammatory cells from blood vessels to the airways is mediated by endothelial cells through the expression of adhesion molecules (National Heart, Lung, and Blood Institute 2007). Additionally, bronchial epithelial cells are highly sensitive to physical modifications, viral infections, and pollutants, promoting inflammation and epithelium injury through chemokines and nitric oxide production (National Heart, Lung, and Blood Institute 2007). These cells are also involved in epithelium reparation after damage, a process that occurs very frequently in asthma patients (Lambrecht and Hammad 2012). Bronchial smooth muscle cells show increased proliferation and growth rates, processes known as hyperplasia and hypertrophy, respectively, leading to the production of high levels of proinflammatory mediators (Koziol-White and Panettieri 2011). Fibroblasts and myofibroblasts are also involved in the structural changes that take place in asthma through the production of components of the connective tissue. Furthermore, airway cholinergic nerves are implicated in the stimulation of the airflow obstruction and mucus production in response to neural reflexes (National Heart, Lung, and Blood Institute 2007).

Airway remodeling can also include tissue modifications, such as thickening of the reticular basement membrane, subepithelial fibrosis, ASM hypertrophy and hyperplasia, dilation and proliferation of airway blood vessels, and hypersecretion and hyperplasia of mucous glands (Cookson 2004). These changes have been associated with a progressive loss of lung function, which involves a reduced capacity to take oxygen into the lungs and distribute it across the body and a decreased ability to breathe out the carbon dioxide. Indeed, it has been demonstrated that the detriment of pulmonary capacity cannot be completely reversible by the therapies available to date (Cookson 2004; National Heart, Lung, and Blood Institute 2007).

Lastly, one of the main characteristics of asthma is the high variability and progression of symptoms and lung function over time, even during the day, independently of the physiologic changes driven by the circadian rhythm (Global Initiative for Asthma 2020).

1.4. Diagnosis of asthma and subtype classification

1.4.1. Clinical diagnosis

Most asthma symptoms might be confused with other chronic respiratory disorders so that precise clinical examination is crucial to avoid misdiagnosis and ensure appropriate treatment (Levy et al. 2009). In clinical practice, the diagnosis of asthma is mainly established based on the presence of respiratory symptoms and airflow limitation. This is commonly detected using standard questionnaires, physical examination, measurement of nitric oxide levels, evaluation of blood eosinophils count, and the assessment of the lung function, among others. The latter is considered the most easily accessible approach used to support the diagnosis of asthma (Saglani and Menzie-Gow 2019). Lung function is evaluated throughout different measurements, mainly forced expiratory volume in one second (FEV_1) and forced vital capacity (FVC) using standard spirometry approaches, which are compared to reference values established for different age, gender, and ethnic groups (Quanjer et al. 2012; Chhabra 2015; Global Initiative for Asthma 2020). FEV_1 measures how the air flows through the lung, whereas FVC represents the total volume of air held in the lungs (Bailey 2012).

The airflow limitation is quantified as the ratio between FEV_1 and FVC (FEV_1/FVC). According to international guidelines, asthma patients with airway obstruction should show FEV_1/FVC values lower than 0.90 in children and 0.70-0.80 in adults (National Institute for Health and Care Excellence 2017; Global Initiative for Asthma 2020). Additionally, obstruction reversibility is often measured through the improvement in FEV_1 a few minutes after the administration of bronchodilators (bronchodilator response or BDR). Most of these parameters are not only used for the diagnosis but also for monitoring disease progression (Gallucci et al. 2019). Indeed, FEV_1<60% has been related to an increased risk to experience future episodes of symptoms worsening in children (Gallucci et al. 2019).

The practical application of spirometry to diagnose asthma in children can be challenging due to the difficulty to measure it (Brand et al. 2008; Saglani and Menzie-Gow 2019) and also the fact that some patients can show normal lung function measurement despite suffering severe symptoms (Bush et al. 2017; Global Initiative for Asthma 2020). Therefore, standard guidelines for the clinical diagnosis of childhood asthma are mostly based on the presence of wheezing (Brand et al. 2008), although allergic rhinitis or eczema symptoms, and family history of asthma or atopy, are also considered (Burke et al. 2003; Global

Initiative for Asthma 2020). Furthermore, the diagnosis of asthma in children younger than six years old can be even more difficult (Chu and Bajaj 2020), where a history of recurrent wheezing episodes, cough, and breathlessness are important criteria considered for the initial diagnosis of asthma (van Aalderen 2012). Nonetheless, these can be sometimes due to acute respiratory infections or congenital airway anomalies instead of asthma (Ng and How 2014; Trivedi and Denton 2019). Thus, the measurement of the fractional concentration of nitric oxide (FeNO) in exhaled air is often carried out, which is a non-invasive and inexpensive technique especially recommended for this age group (van der Valk et al. 2014; Kaplan et al. 2019; Pijnenburg et al. 2019). Nitric oxide is a reactive gas produced in the airway epithelium in response to pro-inflammatory mediators (Pijnenburg and De Jongste 2008), leading to hyperresponsiveness (Ricciardolo 2003). High FeNO levels have been detected in asthma patients even in mild stages of the disease (Lane et al. 2004) and they have been correlated with a higher risk of lung function decline, future exacerbations, and worsening of asthma symptoms (Stern et al. 2011; van der Valk et al. 2012; Pijnenburg et al. 2019). Therefore, the evaluation of FeNO levels can provide a picture of the inflammatory patterns in the airways, supporting asthma diagnosis, mostly in children (Kaplan et al. 2019; Pijnenburg et al. 2019). However, the added value of this technique over other clinical examinations is still under debate (Pijnenburg et al. 2019).

1.4.2. Asthma phenotypes

Asthma is a heterogeneous disease that consists of different recognizable clinical, demographic, and pathological characteristics, known as phenotypes or endotypes (Pembrey et al. 2018; Global Initiative for Asthma 2020). These have been classically categorized based on clinical and physiological characteristics (age at onset, disease severity, presence of severe exacerbations, obesity, treatment response, and temporal symptoms patterns), symptoms triggers (allergy, atopy, physical exercise, viral infections, menstruation, NSAIDs, air pollution, and cigarette smoking), and inflammatory biomarkers (eosinophilic, neutrophilic, and paucigranulocytic) (Fuchs et al. 2017). The importance of the definition of endotypes or asthma subtypes based on different pathological and inflammatory mechanisms has been demonstrated (Bush 2019), although the clinical utility of this phenotypic classification is still limited (Global Initiative for Asthma 2020).

In this sense, the increasing development of novel techniques for sample collection and multi-dimensional analytic methods have opened new research opportunities for accurate classification of different asthma phenotypes. This could provide additional insights into the causes and underlying mechanisms of asthma and improve prevention and management approaches, including therapeutic strategies (Pembrey et al. 2018).

1.4.3. Exacerbations: an important asthma-related trait

Different asthma-related traits have been described, although severe exacerbations, commonly known as asthma attacks, are considered the most important outcome in childhood asthma (Jorgensen et al. 2003). Severe asthma exacerbations are clinically identified as acute episodes outside the patient's usual range of day-to-day disease that occur with a rapid onset (less than three hours), even though they can gradually develop in adults (several days or weeks) (Reddel et al. 2009). The European Respiratory Society (ERS) and the American Thoracic Society (ATS) have defined them as events that require unscheduled and urgent

medical interventions to prevent severe or fatal outcomes. Hospital admissions or emergency room (ER) visits because of asthma that could imply the systemic administration of corticosteroids are often required (Reddel et al. 2009).

These events are considered the major determinant of the global burden in childhood asthma (Bateman et al. 2004) with a substantial economic impact on healthcare systems (Bai et al. 2007). Additionally, severe exacerbations have an important negative impact on the quality of life and education of uncontrolled asthma patients, enhancing the progressive loss of pulmonary capacity in some individuals (Bai et al. 2007). Interestingly, severe exacerbations can trigger future events independently of clinical and demographic characteristics of the patient and increase the risk of asthma-derived death (Miller et al. 2007; Levy and Winter 2015). Therefore, prevention of asthma exacerbations should be a crucial consideration in pediatric asthma management and treatment guidelines (Puranik et al. 2017; Global Initiative for Asthma 2020). For that, protocols of personalized medicine to prevent severe asthma exacerbations should be designed based on the identification of patients at higher risk (Puranik et al. 2017).

Substantial inter and intraindividual variability in asthma exacerbations incidence and intensity has been described so that, these events can occur from mild to highly severe symptoms that may be fatal for the patient. Furthermore, differences among countries and populations have also been found (Park and Tantisira 2017), with African Americans and Latinos/Hispanics showing the highest exacerbation rates (Akinbami et al. 2012; Akinbami et al. 2014; Centers for Disease Control and Prevention 2018). These differences are associated with genetic and non-genetic components such as clinical and demographic characteristics, besides the exposure to asthma risk factors, including viral infections of the respiratory tract (Reddel et al. 2009). The important implication of the individual's genetic composition has been proposed (Ortega and Meyers 2014b; Park and Tantisira 2017). However, the genetic factors involved in asthma exacerbations have not been completely disentangled (Ortega et al. 2013; Herrera-Luis et al. 2019).

1.5. Asthma treatment

Given that there is no cure for asthma yet, the main goals of standard strategies for the management of childhood asthma are to ensure the correct lung development and function, control asthma symptoms, maintain normal activity levels, and to reduce the risk of future exacerbations, asthma-related mortality, and treatment side effects. This is usually carried out employing a multi-layer approach including self-management education, monitoring of clinical parameters, and control of environmental exposures. Nonetheless, pharmacological therapies play a central role in the management of this disease (Chu and Bajaj 2020; Global Initiative for Asthma 2020).

1.5.1. Types of asthma medications

Asthma treatments can be classified into two main groups based on the duration of time taken to make any effects upon administration: controllers and relievers. Controller treatment consists of regular-use maintenance medications to decrease airway inflammation, control asthma symptoms, prevent future exacerbations, and reduce the risk of lung function decline through long-term mechanisms of action (Global Initiative for Asthma 2020). These are prescribed in the presence of frequent symptoms and any risk factors to suffer exacerbations (Tesse et al. 2018). The main medications currently used for asthma control in the

clinical practice are inhaled corticosteroids (ICS), leukotriene receptor antagonists (LTRA), and long-acting beta-2 agonists (LABA), although the latter are only recommended for 6-year-old children and older (Sharma and Chakraborty 2018; Tesse et al. 2018; Global Initiative for Asthma 2020) (**Table 1**).

Severe asthma might also require the addition of further medications, such as monoclonal antibodies (Tesse et al. 2018; Global Initiative for Asthma 2020). These act towards components of the Th2 inflammatory response (Busse 2018), such as IgE, inhibiting its binding to specific receptors on basophils, mast, and dendritic cells (Chang et al. 2007). Anti-IgE antibodies have been recommended for persistent severe allergic asthma patients (>6 years old) with elevated serum IgE levels (Delimpoura et al. 2018). Another main type of monoclonal antibodies is designed to specifically inhibit the activity of IL-5 (Delimpoura et al. 2018), which is the major cytokine involved in increasing eosinophil levels in asthma patients (Sanderson 1992). These are indicated for severe asthma patients with a history of exacerbations and blood eosinophilia (Delimpoura et al. 2018). Long-term anticholinergic agents can be also added to conventional controller medications to treat severe asthma in children, adolescents, and adults (Gosens and Gross 2018). Specifically, these antagonist muscarinic receptors, inhibiting the effects on ASM contraction and mucus secretion triggered by acetylcholine (Gosens and Gross 2018). Additionally, low daily doses of systemic corticosteroids (OCS) might be effective for patients aged >6 years old with severe asthma. Nonetheless, these should be exceptionally considered when symptoms are poorly controlled, and exacerbations occur frequently despite the treatment with standard and additional controller medications given their several potential side effects (Haktanir Abul and Phipatanakul 2019; Global Initiative for Asthma 2020) (**Table 1**).

Reliever or rescue medications are used to ameliorate asthma symptoms a few minutes upon administration during episodes of symptoms worsening or exacerbations (Tesse et al. 2018). They are also used to prevent bronchoconstriction induced by physical exercise (Tesse et al. 2018). Short-acting beta-2 agonists (SABA) are the most effective short-term rescue therapy, which are recommended to be used as needed (van Aalderen 2012; Chu and Bajaj 2020). Nonetheless, the last updates of the international guidelines for the management of asthma include low doses of the combination of ICS and LABA, specifically budesonide and formoterol, as the preferred reliever option for adults and adolescents, given the long-lasting but short time needed for formoterol to act. This is also recommended when needed in patients with mild asthma to relieve symptoms or to prevent them before the exposure to risk factors, such as physical exercise (Global Initiative for Asthma 2020). Additionally, OCS are frequently used as a rescue treatment, which must be promptly administered to patients suffering severe episodes of asthma exacerbations (Global Initiative for Asthma 2020). Short-term anticholinergics have also been suggested to be alternative asthma relievers for patients shown not to properly respond to SABA (British Thoracic Society 2016) (**Table 1**).

Most asthma medications are commonly used with inhaler devices, except for OCS, which are administered via oral tablets, enteric-coated tablets, oral solution, intramuscular or intravenous injections. Although SABA are commonly inhaled, injections and different forms for oral administration are also available. Moreover, anticholinergic agents can be systematically administered, and LTRA are also available in intravenous injections or different forms of oral tablets or solutions, which are specially indicated for young children (Sharma and Chakraborty 2018).

Table 1. Main asthma medications currently used in clinical practice for asthma management in children.

Type of asthma treatment	Medication	Administration form	Main mechanisms of action	Main effects on asthma pathophysiology	Drugs commonly used
Controller	ICS	Inhaler device	▪ Decrease of extravasation of immune cells into airways ▪ Decrease of inflammatory cells survival	Reduction of airway hyperresponsiveness and mucus production	Budesonide Fluticasone Ciclesonide
	LABA [a]	Inhaler device	▪ Binding to β-adrenergic receptor in bronchioles ▪ Relaxation of airway smooth muscle	Reduction of airflow obstruction (long-term action)	Salmeterol Formoterol
	LTRA	Oral film-coated and chewable tablets, oral granules, intravenous injection	▪ Reduction of leukotrienes effects ▪ Decrease of proinflammatory mediators	Reduction of airway inflammation and hyperresponsiveness	Montelukast Zafirlukast
	OCS [a, b]	Oral tablets and enteric-coated tablets, oral solution, intramuscular or intravenous injection	▪ Decrease of extravasation of immune cells into airways ▪ Decrease of inflammatory cells survival	Reduction of airway hyperresponsiveness and mucus production	Prednisone Prednisolone
	Monoclonal antibodies [a]	Subcutaneous or intravenous injection	▪ Decrease of activity of Th2 inflammatory pathway ▪ Decrease eosinophils levels	Reduction of airway inflammation	Omalizumab Mepolizumab Reslizumab Benralizumab Dupilumab
	Anticholinergic agents [a]	Inhaler device, oral extended-release tablets, subcutaneous injection	▪ Block neural signal from muscarinic receptors	Reduction of bronchoconstriction, mucus secretion and airway edema (long-term action)	Tiotropium bromide
Reliever	SABA	Inhaler device, oral film-coated and extended-release tablets, syrup, intramuscular, intravenous or subcutaneous injection	▪ Binding to β-adrenergic receptor in bronchioles ▪ Relaxation of airway smooth muscle	Reduction of airflow obstruction (short-term action)	Albuterol Salbutamol
	Anticholinergic agents [a]	Inhaler device, oral extended-release tablets, subcutaneous injection	▪ Block neural signal from muscarinic receptors	Reduction of bronchoconstriction, mucus secretion and airway edema (short-term action)	Ipratropium bromide
	OCS [a]	Oral tablets and enteric-coated tablets, oral solution, intramuscular or intravenous injection	▪ Decrease of extravasation of immune cells into airways ▪ Decrease of inflammatory cells survival	Reduction of airway hyperresponsiveness and mucus production	Prednisone Prednisolone

[a] Only recommended for ≥6 years old children; [b] Daily low doses.
ICS: inhaled corticosteroids; LABA: long-acting beta-2 agonists; LTRA: leukotriene receptor antagonists; OCS: systemic corticosteroids; SABA: short-acting beta-2 agonists; Th2: Lymphocyte T helper 2.

1.5.2. Stepwise pharmacological management of asthma

International guidelines for asthma management recommend that pharmacological therapies should be applied following specific stepwise strategies for each age group, whose main goal is to efficiently alleviate asthma symptoms and optimize pulmonary capacity (British Thoracic Society and the Scottish Intercollegiate Guidelines Network 2014; Sharma and Chakraborty 2018; Tesse et al. 2018; Global Initiative for Asthma 2020). Therefore, therapies should start at the most appropriate step according to the initial level of symptoms control and disease severity so that, if disease control is not achieved after three months, it should be stepped up. Moreover, regular assessment of risk factors, medication side effects, treatment adherence, inhaler technique, comorbidities, and patient/parents' satisfaction are key in the management of asthma and should be considered before adding further medication. Nonetheless, treatment should be stepped down once disease control is achieved (British Thoracic Society and the Scottish Intercollegiate Guidelines Network 2014).

Although there are updated guidelines from the pharmacological management of asthma at the time of writing (Global Initiative for Asthma 2020), patients participating in each of the asthma studies included in this doctoral thesis had been treated following the recommendations established by the British Thoracic Society and the Scottish Intercollegiate Guidelines Network (BTS/SIGN) in 2014 (British Thoracic Society and the Scottish Intercollegiate Guidelines Network 2014) (**Figure 1**). Based on these guidelines, the first level of standard asthma therapeutic strategies (*Step 1*) mainly consists of administering only SABA as needed, recommended for mild asthma patients with occasional symptoms (British Thoracic Society and the Scottish Intercollegiate Guidelines Network 2014). Other alternatives have also been proposed as short-term relievers, although SABA is the first preferred option for patients with intermittent asthma symptoms from all age groups (British Thoracic Society and the Scottish Intercollegiate Guidelines Network 2014) (**Figure 1**). The presence of frequent symptoms three times a week or more, night awakenings because of asthma once a week, and/or a history of asthma exacerbations in the last two years indicate the need to step up the treatment and addition of regular preventer therapy. Daily low doses of ICS according to disease severity are the most common *Step 2* approach indicated for all age groups. Alternatively, the daily use of low doses of LTRA can be also considered for young children when ICS are not appropriate (**Figure 1**). Patients with persistent symptoms not adequately controlled at *Step 2* may require the addition of further controller medications as part of *Step 3*, whose composition depends on the age of the patients. While the addition of LABA is the most commonly accepted approach in children older than 5 years and adults, daily low doses of ICS and LTRA are the preferred option in young children (<5 years old) since these are not prevented from severe exacerbations by LABA treatment (Chung and Paton 2019; Global Initiative for Asthma 2020). However, asthma control must be evaluated in response to LABA, considering increasing the ICS dose and/or interruption of LABA, and the addition of LTRA in case of inadequate response to LABA therapy. Assessment by a pediatric respiratory specialist is also recommended for children under 2 years that are poorly controlled (British Thoracic Society and the Scottish Intercollegiate Guidelines Network 2014) (**Figure 1**). Non-achievement of disease control and decline of lung function requires stepping up, increasing controller medications, or referring young children patients for specialist assessment to avoid possible medication side effects (*Step 4*). Nonetheless, if symptoms persist or exacerbations are recurrent, evaluation

of adherence to treatment, reassessment of asthma phenotypes, identification, and management of risk factors is needed. Further maintenance treatment options may be required in patients older than 5 years old, which may include the administration of daily low doses of OCS in patients with severe uncontrolled asthma (*Step 5*) (**Figure 1**) (British Thoracic Society and the Scottish Intercollegiate Guidelines Network 2014).

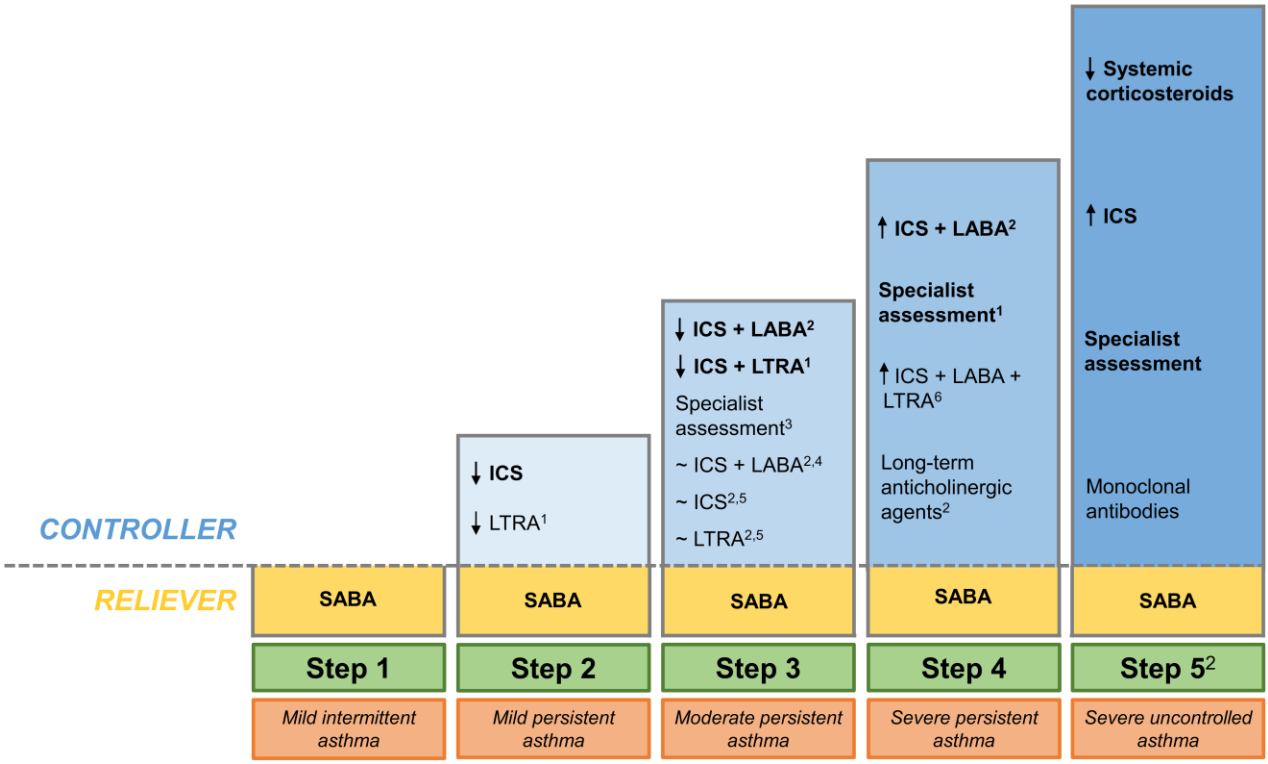

Figure 1. Stepwise pharmacological approach to control asthma symptoms and prevent exacerbations. *Steps 1-5* indicate the most common pharmacological strategies used to treat asthma patients. Medications are indicated for all asthma patients or particular age groups, if specified. Low, medium or high doses are represented by down arrows, tilde symbols or up arrows, respectively. Preferred controller strategies are in boldface. Reliever or rescue medications to be used as needed are indicated in yellow, whereas controllers of daily use are in blue.
[1]Children <5 years old; [2]Children ≥5 years old and adults;[3]Children <2 years old; [4]Patients who benefit from LABA but disease control is still inadequate; [5]Non-responders to LABA therapy; [6]Children >12 years old and adults.
Based on the British Thoracic Society and the Scottish Intercollegiate Guidelines Network (BTS/SIGN) guidelines (2014).
ICS: inhaled corticosteroids; LABA: long-acting β_2 agonists; LTRA: leukotriene receptor antagonists; SABA: short-acting β_2 agonists.

1.5.3. Inhaled corticosteroids: the most prescribed and effective asthma treatment

Despite the increasing amount of novel asthma therapies developed in the last years, ICS are still the most commonly prescribed drugs to control asthma symptoms and decrease the risk of future severe exacerbations in patients with persistent and chronic symptoms, as recommended by international guidelines for asthma management (Scelfo et al. 2018; Global Initiative for Asthma 2020). Interestingly, ICS are used as the first-line asthma controller medication in children and adults (Global Initiative for Asthma 2020).

Several benefits have been attributed to the use of ICS as a maintenance medication, including improvement of symptoms and lung function measurements, and a substantial reduction of the exacerbations and asthma-related mortality rates (Sharma and Chakraborty 2018; Ramadan et al. 2019). Corticosteroids are preferably inhaled administered, minimizing side effects related to systemic administration (Derendorf et al. 2006). Briefly, 80-90% of the ICS inhaled are deposited in the oropharyngeal cavity and lower airways, whereas 10-20% of the total ICS dose is delivered in the lungs, where it exerts the desired pharmacological effects after their activation (Derendorf et al. 2006), including the reduction of airway inflammation, hyperresponsiveness, and bronchoconstriction through numerous molecular and cellular mechanisms of action (Sharma and Chakraborty 2018; Ramadan et al. 2019) (**Figure 2**). However, a fraction of the ICS deposited in the lungs can also reach the bloodstream, triggering potential side effects (Derendorf, Nave et al. 2006). Moreover, part of the medication is retained in the mouth and pharynx after inhalation, which is gastrointestinally absorbed, and then, enters into the systemic circulation after metabolism in the liver. After this, ICS can also reach the lungs through the pulmonary vasculature, although this route can cause potential side effects. Nonetheless, the use of auxiliary devices, such as spacers or holding chambers, or mouth rinsing after administration, can reduce the fraction deposited in the oropharynx or swallowed, increasing the proportion of ICS delivered in the lungs (**Figure 2**).

Therefore, the efficacy and adverse effects of this medication depend on the balance between the dose absorbed pulmonary and orally, receptor-binding affinity, drug activation, and retention time in the lungs. The latter is correlated with lipophilicity, lipid conjugation, and protein-binding capacities (Barnes et al. 1998; Derendorf et al. 2006). Additionally, ICS side effects depend on their metabolism and elimination from the body, which is primarily carried out through hepatic oxidation and urinary excretion, although multiple organs can be also involved (Barnes et al. 1998; Derendorf et al. 2006; Barnes 2010) (**Figure 2**).

ICS's effects are triggered by their binding to the glucocorticosteroid receptor (GR), which is widely expressed in almost all cell types, although asthma beneficial effects of ICS occur mainly in the airways (Derendorf et al. 2006) (**Figure 3**). ICS bind GR located in the cytoplasm after passing through the cell membrane and then, the drug-receptor complex migrates to the nucleus where they bind to specific DNA regions or interact with transcription factors, directly or indirectly regulating gene transcription at inflammatory and airway structural cells and elements, including mucous glands, fibroblasts, epithelial, endothelial, and ASM cells (Barnes and Adcock 2003; Ramadan et al. 2019). Nonetheless, there is some evidence suggesting that ASM cells are the major targets of ICS (Reddy et al. 2009). Although ICS can broadly regulate the transcriptional activity, this medication mostly regulates the expression of genes involved in the transcriptional suppression of pro-inflammatory genes (Barnes 2010) and the enhancement of anti-inflammatory protein synthesis (Barnes and Adcock 2003) (**Figure 3**).

ICS are considered the most effective controllers in the management of asthma (Barnes 2010). The response to asthma medications is commonly measured in clinical practice using different methods based on the control of asthma symptoms (Al Moamary et al. 2012), information about the presence of exacerbations despite treatment (Fuhlbrigge et al. 2012), measurement of lung function (Global Initiative for Asthma 2020), sputum levels of inflammatory cells or skin allergen sensitization tests (Global Initiative for Asthma 2020), and FeNO levels (Smith et al. 2005; Petsky et al. 2018), among others.

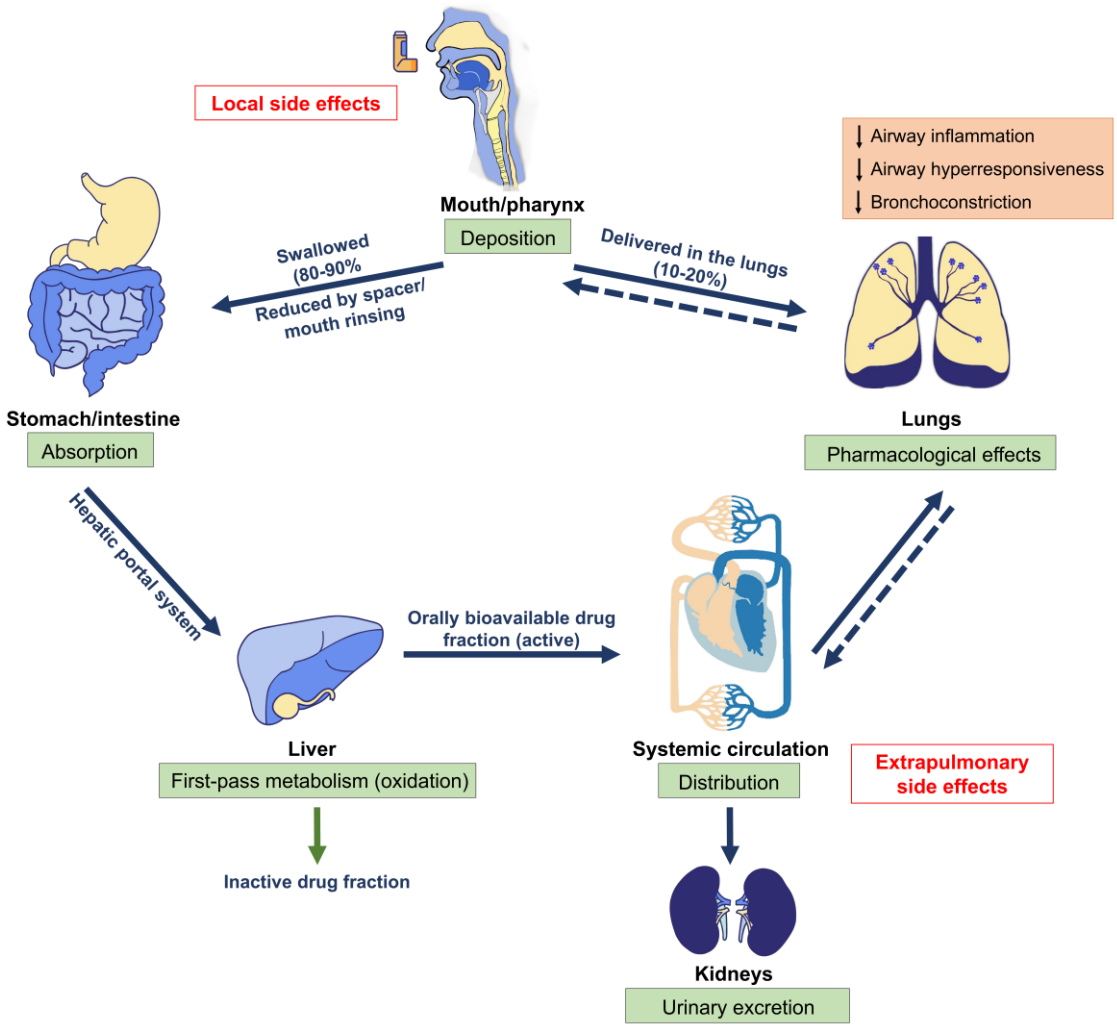

Figure 2. Schematic representation of the pharmacokinetics of ICS. Solid blue arrows show the preferred route, while dashed blue arrows represent alternative routes of ICS. The process taking place in each organ is indicated in green boxes. Potential side effects are shown in red bold face. ICS pharmacological effects in the lungs are labeled into the orange box. Based on Barnes 2010 and Derendorf et al. 2006.

Nonetheless, strong interindividual differences in the response to this treatment have been reported, where 30-40% of the asthmatic children treated with ICS do not show any improvement of their symptoms and 10-15% of them might experience disease worsening despite the regular use of this therapy or can even suffer adverse effects (Szefler et al. 2005). For this reason, the evaluation of ICS response is quite important to identify groups of responders or non-responders to ICS therapy (Ramadan et al. 2019). Childhood-onset asthma patients tend to show a better response to ICS therapy compared to adulthood-onset asthma patients (Kaditis et al. 2007). Moreover, high variability in ICS response has also been described among different ethnic groups and populations (Szefler et al. 2005; Mersha 2015). According to asthma mortality, morbidity, and exacerbation rates, admixed populations have been shown to have decreased ICS response. Indeed, African American patients with asthma experience substantially poorer effectiveness and increased adverse effects of ICS treatment compared to other populations (Ortega and Meyers 2014b). These strong differences in response to ICS therapy have been suggested to be the result of complex interactions among clinical asthma characteristics, comorbidities, environmental exposures, and the genetic composition of each

individual (Ramadan et al. 2019). Nonetheless, the important contribution of genetic factors has been evidenced by means of heritability estimates, suggesting that approximately 60-80% of the total variation in the response to asthma treatment might be explained by genetic components (Drazen et al. 2000; Park et al. 2015; Duong-Thi-Ly et al. 2017).

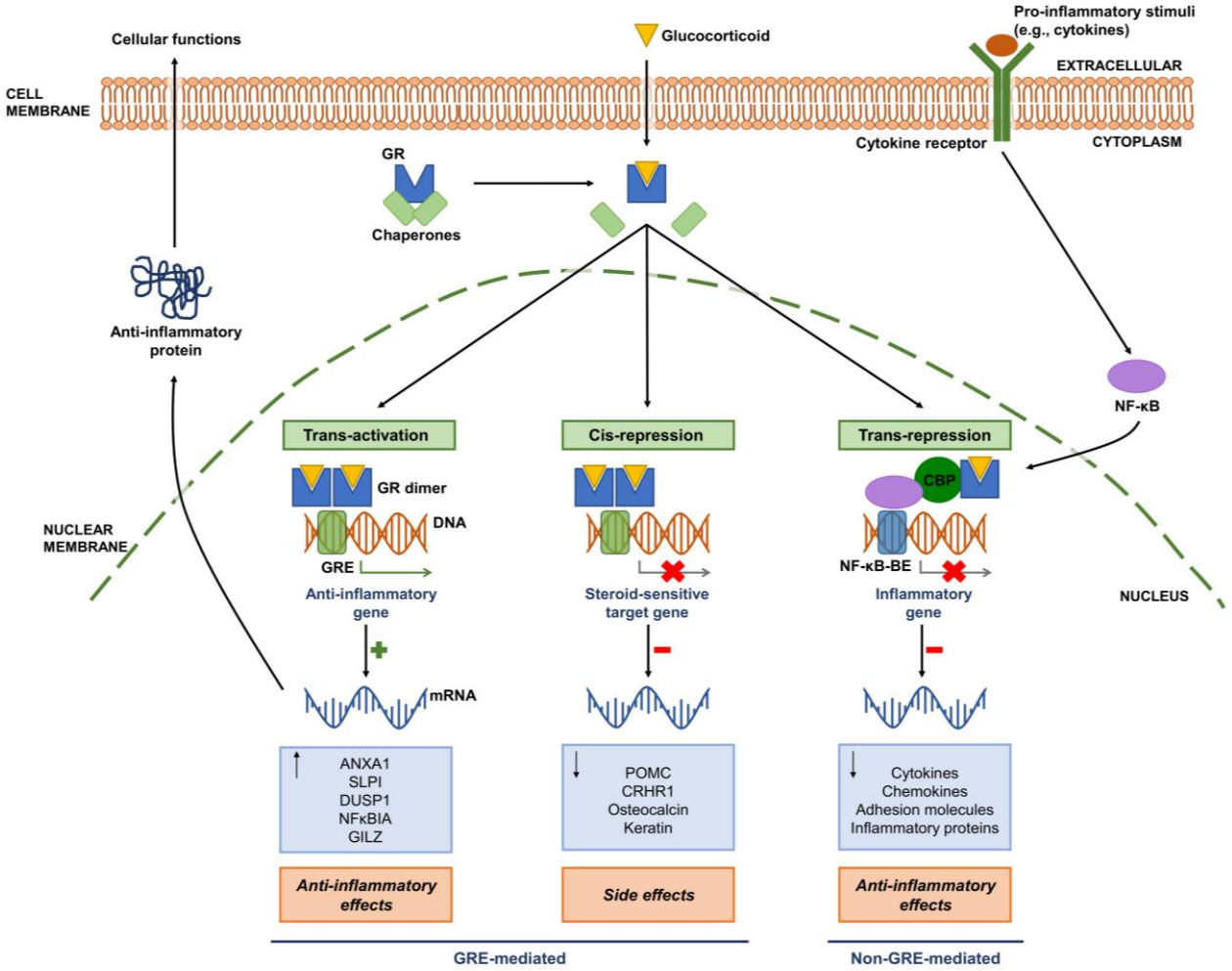

Figure 3. Molecular mechanisms of action of glucocorticosteroids. Glucocorticoid (GC) molecules diffuse across the plasmatic membrane, reaching and binding to the glucocorticosteroid receptor (GR) in the cytoplasm, which causes the dissociation of GR from inhibitory proteins, such as chaperones. This allows the GC-GR complex to translocate into the nucleus through nuclear pores, where it regulates the transcription by means of trans-activation, cis-repression, or trans-repression mechanisms. Once into the nucleus, GC-GR dimerizes and specifically binds to glucocorticosteroid-response elements (GRE) in the promoter region of genes encoding proteins with anti-inflammatory functions, increasing their expression levels. GC-GR homodimers can also repress gene expression through interaction with GRE, but to a lesser extent, which can be related to the side effects of GCs. Moreover, GCs can repress the expression of activated genes encoding pro-inflammatory proteins through non-GRE mediated mechanisms. Inflammatory stimuli activate transcription factors such as, nuclear factor-κB (NF-κB), which translocates to the nucleus, binding to NF-κB-binding elements (NF-κB-BE) and coactivators (e.g., CREB-binding protein (CBP)), promoting the transcription of proteins involved in inflammatory processes. Nonetheless, GC-GR monomers can interact with NF-κB through coactivators, suppressing the expression of pro-inflammatory genes. Green plus or red minus symbols represent the transcription activation or repression, respectively. Decreased or increased levels of proteins encoded are shown by down or up arrows within the light blue boxes, respectively.
ANXA1: annexin-1; CRHR1: corticotropin-releasing hormone receptor 1; DUSP1: dual specificity phosphatase 1; GILZ: glucocorticoid-induced leucine zipper protein; NFκBIA: NF-κB inhibitor alpha; POMC: proopiomelanocortin; SLPI: secretory leukocyte peptidase inhibitor.
Based on Barnes and Adcock 2003 and, Barnes 2010.

1.6. Pharmacogenomics of ICS response

Pharmacogenomics is the discipline focused on the study of the genetic variability across the entire genome that affects the individual's response to drugs, modulating the way the medications are absorbed, distributed, metabolized, and excreted (pharmacokinetics) (Turfus et al. 2017), but also the beneficial and adverse effects according to the drug exposure (pharmacodynamics) (Marian and Seghezzi 2013; Park et al. 2015; Alfirevic and Pirmohamed 2016). However, the former definition of pharmacogenetics consists of the search for the variation in nucleotide sequences of individual genes influencing the drug response (Bardal et al. 2011; Park et al. 2015). Although pharmacogenetics is considered a subset of pharmacogenomics (Park et al. 2015), both can be used interchangeably (Pirmohamed 2001). *Pharmacogenomics* is the term most commonly used referring to this field (Bardal et al. 2011), which will be used from this point forward.

Specifically, pharmacogenomics of ICS response aims to identify multiple genetic markers involved in the response to ICS treatment that could help in the future to determine the responsiveness to ICS therapy and identify those patients who are not able to respond properly to such medication (Pirmohamed 2001). Additionally, the usage of pharmacogenomic information has the potential to contribute to personalizing drug selection and dosage, maximizing their efficacy, and reducing adverse effects (Pirmohamed 2001; Ortega et al. 2015).

Pharmacogenomic research of ICS response has been mainly carried out through association studies, which attempt to correlate the genetic variation and information about ICS-responsiveness to identify genetic variants involved in the response to this asthma treatment. Single nucleotide polymorphisms (SNPs), which represent the vast majority of human genetic variability, are the most widely assessed variation in genetic association studies (Lewis and Knight 2012; Chaudhary et al. 2015). These are sequence positions where the substitution of one nucleotide has occurred so that, two or more possible alleles can be found at a single site for a certain population, although biallelic SNPs with two possible alleles are the most common (Arias et al. 1991). Therefore, the main basis of these studies consists of comparing the frequency of SNP allele or genotype between ICS responders and non-responders (Lewis and Knight 2012), evaluating the association or correlation between the genotypes at single SNPs and the measure of ICS response using statistical models (Sul et al. 2018).

1.6.1. Candidate-gene association studies

For the last decades, most pharmacogenomic studies of ICS response have been performed through approaches based on the evaluation of small sets of SNPs located within or near a single candidate gene with prior knowledge of their implication in asthma pathogenesis or the metabolism or mechanisms of action of this drug (Vijverberg et al. 2018; Keskin et al. 2019). Candidate-gene association studies have been performed analyzing different definitions of ICS response and have allowed the identification of the association of genes encoding proteins involved in processes such as regulation of the immune response (i.e., *CRHR1* and *TBX21*), including the lymphocyte B growth and IgE production (*FCER2*), blood eosinophils levels (*IL1RL1*) and, apoptosis of inflammatory cells induced by glucocorticosteroids (GCs) (*GLCCI1*), in addition to the regulation of the GC signaling pathway (*NR3C1*) (Farzan et al. 2017b; Dijk et al. 2019; Karimi et al. 2019; Keskin et al. 2019). Moreover, some authors have proposed the role of several

genes located at the 17q21 locus in the response to ICS treatment (Keskin et al. 2019). Nonetheless, these studies have been lately regarded as inaccurate and outdated (Vijverberg et al. 2013; Vijverberg et al. 2015) given the numerous limitations attributed to them (Vijverberg et al. 2018). These include a reduced statistical power to detect significant association signals due to limited sample sizes and the number of genetic variants tested in association, in addition to the scarce evidence of replication in independent populations.

1.6.2. Genome-wide association studies

The limitations of the candidate-gene association studies together with the rapid development of genotyping platforms designed for the capture of large amounts of genetic markers across the genome have dramatically decreased the use of this approach in the last years. This has opened the doors for the emerging of genome-wide association studies (GWAS) (Foulkes 2009; Vijverberg et al. 2018). This approach simultaneously assesses the association for millions of SNPs across the genome with no prior knowledge about the functional implication of the genes near their location, which is the main advantage of these studies (Vijverberg et al. 2018; Willis-Owen et al. 2018).

The first GWAS of ICS response was published in 2011 (Tantisira, Lasky-Su et al. 2011) and since then, a total of nine additional studies that are not part of this thesis have been performed until December 2020 (Tantisira et al. 2012; Park et al. 2014a; Park et al. 2014b; Wu et al. 2014; Dahlin et al. 2015; Wang et al. 2015; Leusink et al. 2016; Mosteller et al. 2017; Levin et al. 2019). Given the variability in the asthma pathophysiological processes and phenotypes described between childhood and adulthood asthma (Wang et al. 2011), most pharmacogenomic studies published to date have focused only on either children or adults. Additionally, a few GWAS of ICS response have explored the existence of shared genetic variants across different age groups (Tantisira et al. 2012; Dahlin et al. 2015).

These studies have identified a total of 26 variants at 15 loci associated with different definitions of ICS response, although the improvement in lung function after ICS treatment has been widely assessed. This has been proposed to be an objective measure of treatment responsiveness, although it strongly depends on the patient's characteristics (Ortega and Kumar 2015) and factors inherent to the measurement approach (Cooper 2005; Tepper et al. 2012). A total of six studies explored the association with the difference in the percentage of FEV_1 between the beginning of the ICS therapy and a short-time period later (Tantisira et al. 2011; Tantisira et al. 2012; Park et al. 2014b; Wang et al. 2015; Leusink et al. 2016; Mosteller et al. 2017). These identified a total of 15 variants associated with ICS response in children and/or adults of Asian (Park et al. 2014b) or European descent (Tantisira et al. 2011; Tantisira et al. 2012; Wang et al. 2015) within the *ALLC* gene (Park et al. 2014b), and the intergenic regions of *UMAD1-GLCCI1* (Tantisira et al. 2011), *PDE10A-T*, *HRH4-ZNF521* (Tantisira et al. 2012), *MMS22L-FBXL4*, and *NAV2-HTATIP2* (Wang et al. 2015).

The role of *GLCCI1* in ICS response in European children was revealed by Tantisira *et al.* (2011), which has been attempted for replication in several candidate-gene association studies (Hosking et al. 2014; Izuhara et al. 2014; Vijverberg et al. 2014; Salhi et al. 2019). Although the function of the protein encoded by this gene is still unknown, this has been demonstrated to be targeted by GCs, inducing the apoptosis of cells involved in inflammatory response underlying asthma (Chapman et al. 1995). In fact, *in vitro* experiments

have demonstrated that GCs increase the expression levels of *GLCCI1*, which correlates with improved lung function measures in asthma patients treated with ICS (Tantisira et al. 2011).

The same authors explored in another study genetic associations with the change in lung function after ICS treatment both in children and adults with asthma (Tantisira et al. 2012), revealing the association of variants near genes with different functions related to inflammation and allergic responses (*HRH4*) (Nakamura et al. 2000), regulation of gene expression (*ZNF521*) (Matsubara et al. 2009; Hesse et al. 2010), signal transduction (*PDE10A*) (Fujishige et al. 1999), and developmental processes (*T*) (Herrmann et al. 1990). Among these, the *T* gene seems to be a promising locus for ICS response, which had not been previously associated with any asthma-related traits or mechanisms of action of GCs. The protein encoded by this gene plays a well-known central role in the development of vertebrates, although the *T* gene is also expressed in adult pulmonary tissues. This evidence, together with the fact that it inhibits the development of cartilage mediated by a receptor implicated in GCs resistance, supports the potential role of the *T* gene in the response to asthma treatment with ICS (Tantisira et al. 2012). *ALLC* was associated with the response to ICS treatment in Asian adults with asthma (Park et al. 2014b). This gene encodes an enzyme whose function in humans is unclear since its capacity to participate in the degradation of uric acid seems to have been lost through evolution (Keskin et al. 2019). Although little is known about its potential role in asthma treatment response, some authors have suggested its implication in respiratory processes (van der Plaat et al. 2018). MMS22L and FBXL4 activities are involved in DNA repair and control of the cell cycle (Duro et al. 2010; Gai et al. 2013), and from these, high levels of MMS22L have been detected in individuals with pulmonary diseases (Nguyen et al. 2012), suggesting its plausible implication in asthma. *NAV2* and *HTATIP2* encode proteins implicated in cellular growth, migration, apoptosis, and autophagy (Tong et al. 2009; Wang et al. 2017b), which have been associated with changes in lung function dependent on ICS doses (Wang et al. 2015).

Leusink *et al*. (2016) assessed the association of genetic variants across the genome with the change in FEV_1 and also airway hyperresponsiveness after methacholine administration as measures of ICS response in childhood asthma patients. In that study, variants at the 17q12-21 locus were nominally ($p \leq 0.05$) associated with ICS response, but none of these remained statistically significant after adjusting by multiple testing. Interestingly, this locus is the most widely associated with childhood-onset asthma and severity (Moffatt et al. 2010; Torgerson et al. 2011; Demenais et al. 2018; Stein et al. 2018; Pividori et al. 2019). The 17q12-21 locus was linked to asthma for the first time in 2007 by Moffatt *et al*. (2007) and, since then, it has been highly replicated in independent studies through candidate-gene or GWAS approaches focused on different asthma-related phenotypes (Stein et al. 2018), including the ICS response (Farzan et al. 2018).

One additional study also explored the genomic variation involved in the change in lung function in response to ICS therapy in adults with asthma of diverse populations, although no evidence of significant associations was found. Nonetheless, this study had several advantages, such as the fact that this was the first GWAS of ICS response analyzing multiple populations and it had the largest sample size at the time it was published (Mosteller et al. 2017).

On the other hand, several associations of other spirometric measurements, such as BDR as an indicator of ICS responsiveness, have been reported in European children with asthma (Wu et al. 2014).

Specifically, variants at the *ZNF432* and *ZNF841* genes were associated with BDR after the administration of SABA while on ICS therapy (Wu et al. 2014). These findings suggest the implication of these loci in the reversibility of airflow limitation in response to ICS (Wu et al. 2014).

Nonetheless, definitions of ICS response independent of spirometry measurements have also been tested as a proxy of ICS response. On one hand, Park *et al*. evaluated the responsiveness to ICS treatment through asthma scores based on self-reported symptoms, identifying the association of the G allele of rs10044254, located at *FBXL7*, with worsening of symptoms despite ICS therapy (Park et al. 2014a). This gene encodes one of the members of the F-box protein family involved in the ubiquitination of proteins, promoting cell apoptosis and tissue injury (Coon et al. 2012; Liu et al. 2015). However, further studies are needed to elucidate the role of *FBXL7* in the response to ICS in patients with asthma. On the other hand, before the completion of this thesis, only one GWAS had evaluated the association with the presence of asthma exacerbations despite ICS use (Dahlin et al. 2015), although its significant value as an indicator of treatment response in asthma patients had been evidenced (Fuhlbrigge et al. 2012; Park et al. 2017). This was performed in children and adults from European populations by Dahlin *et al*. (2015), revealing the association of several genetic variants with the risk for asthma exacerbations despite ICS treatment. These were located at genes involved in different functions, such as protection against viral infections (*CMTR1*) (Kato et al. 2003), regulation of cell proliferation and apoptosis (*TRIM24*) (Thenot et al. 1997), regulation of transcription (*L3MBTL4*, *ZNF334*) (Kimura et al. 2006; Liu et al. 2016b), and cytoskeletal rearrangements (*ELMO2*) (Gumienny et al. 2001). Furthermore, evidence of association with the protection against asthma exacerbations under ICS therapy was found for genetic variants located at *MAGI2* and *SHB-ALDH1B1* (Dahlin et al. 2015). From these, *CMTR1* was found to be the strongest association signal. Interestingly, this gene showed increased expression levels in bronchoalveolar lavage (BAL) samples from patients suffering from episodes of asthma exacerbations. Upregulation of this gene could trigger enhanced protective activity against viral infections (Dahlin et al. 2015), which have been demonstrated to be one of the main risk factors to suffer asthma exacerbations (Duenas Meza et al. 2016).

The first GWAS of ICS response focused on admixed populations was recently published by Levin *et al*. (2019). The *EDDM3B* was found to be associated with the change in asthma symptoms control in African American and European American adults, and Hispanic/Latino and African American children and young adults treated with ICS. Specifically, the C allele of the SNP rs3827907 was associated with the improvement in asthma control after ICS treatment, which was found to regulate the expression of genes encoding biomarkers of inflammation in African Americans. These findings suggest that this variant could be a biomarker of the ICS responsiveness in admixed populations (Levin et al. 2019).

1.6.3. Challenges of pharmacogenomic studies of ICS response

Despite the large advantages of GWAS compared to candidate-gene association studies, the associations identified do not explain the response to ICS treatment. As a consequence, these have not provided yet real improvements in the clinical management of asthma (Garcia-Menaya et al. 2019) and additional genetic variants are expected to be involved in the response to this asthma medication (Park et al. 2015).

Some potential explanations of the difficulty in identifying genetic markers involved in the response to asthma treatment have been proposed. The main reason could be the reduced statistical power to detect genetic associations of the GWAS of ICS response performed until December 2020. Most of them have included relatively reduced sample sizes (N<1,000) (Park et al. 2015; Vijverberg et al. 2018), given the difficulty to gather samples fulfilling adequate phenotypic and genotypic criteria. Additionally, Europeans have been the most represented populations in the pharmacogenomic research of ICS response followed by Asians, as in the GWAS of other traits (Ortega and Meyers 2014b; Sirugo et al. 2019). Nonetheless, the strong power of combining genetically and ethnically diverse populations on these studies compared to analyzing populations of European descent alone has been widely postulated (Bien et al. 2019; Sirugo et al. 2019). Indeed, single populations only contain a subset of the total human genetic variation so, they do not seem to be sufficient to disentangle the genetic variants underlying a specific outcome (Bien et al. 2019; Sirugo et al. 2019). Moreover, including diverse populations in GWAS of complex traits such as ICS response can be enriching due to potential discrepancies in disease prevalence and substantial differences in terms of allele frequencies, association effect sizes, and patterns correlation among genetic variants across the genome, known as linkage disequilibrium (LD) (Hernandez-Pacheco et al. 2016; Sirugo et al. 2019).

Specifically, the numerous advantages of recently-admixed populations in the genetic research of asthma-related traits, including the response to medications, have been evidenced (Ortega and Meyers 2014a; Ortega and Meyers 2014b; Hernandez-Pacheco et al. 2016; Levin et al. 2019). There are different populations around the world where the admixture process has occurred at a relatively recent point of their evolutionary history, resulting in the transmission of genes between ancestral populations that were previously genetically isolated (Soares-Souza et al. 2018). Among them, African Americans and Latinos/Hispanics show higher prevalence and complications of asthma (Akinbami et al. 2012; Akinbami et al. 2014; Centers for Disease Control and Prevention 2018; Enilari and Sinha 2019) and poorer response to asthma medications (Ortega and Meyers 2014b; Hernandez-Pacheco et al. 2016) in addition to a particular genetic composition (Hernandez-Pacheco et al. 2016). These are the major admixed populations with African ancestry in the United States, which are the result of the admixture among different continental populations such as Europeans and Africans, though current Latinos/Hispanics also show an important Native American component (Bryc at al. 2015, Soares-Souza et al. 2018). As a result of this process, individuals from current admixed populations show variable proportions of the genetic ancestry of each of the parental populations (Mersha 2015). At chromosome level, they are characterized by large regions where genetic variants are in high LD and mosaics of segments of the genetic ancestry of each of the parental populations (Rosenberg et al. 2010; Hernandez-Pacheco et al. 2016). Additionally, African ancestry in some of these populations has been associated with higher asthma susceptibility and risk to suffer exacerbations (Choudhry et al. 2006; Vergara et al. 2009; Kumar et al. 2010; Flores et al. 2012; Vergara et al. 2013; Pino-Yanes et al. 2015; Rosas-Salazar et al. 2016; da Silva et al. 2019; Grossman et al. 2019b). These characteristics make them an excellent scenario to explore the genetic variation involved in asthma-related traits, although these have been historically poorly represented in GWAS (Mersha 2015; Hernandez-Pacheco et al. 2016; Levin et al. 2019).

In addition to the reduced sample sizes and narrow diversity of the GWAS of ICS response performed until December 2020, a limited number of genetic variants has been tested in association, contributing to the aforementioned reduced statistical power of these studies. This could be mainly explained by the fact that most studies have only analyzed genetic variants captured by genotyping platforms. Although commercial high-throughput genotyping platforms have highly increased the number of genetic markers compared to alternative genotyping methods, these are designed to simultaneously capture between 100 thousand and 2.5 million genetic variants across the genome, which still represent a small fraction of the human genetic variation (Schurz et al. 2019). Nonetheless, this problem can be partially solved through a method known as genotype imputation. Briefly, this consists of the prediction of the genotype for many of those positions not captured in patient samples by genotyping platforms through a statistical inference approach based on LD patterns with nearby variants that are directly genotyped (Marchini and Howie 2010). For that, comparisons between the genotypes available for the sample individuals and a publicly available reference panel are carried out, which contains information about variants from the whole genome obtained by next-generation sequencing (NGS) of representative individuals from populations around the world (Marchini and Howie 2010; Bai et al. 2019).

Imputation allows to notably increase the number of genetic variants that can be tested to around 3-16 million variants, incrementing the statistical power to detect significant associations (Bai et al. 2019). However, the number of imputed variants highly depends on the coverage capacity of the genotyping platform, genetic ancestry correspondence between the sample population and the reference panel, and the haplotype sizes and sequencing quality of the population taken as reference, among others (Bai et al. 2019). It is important to note that current genotyping platforms and reference panels are mostly designed for capturing common variation with a minor allele frequency higher or equal than 1-5%, whereas low frequency or rare variants are unlikely to be captured by GWAS approaches (Willis-Owen et al. 2018).

Additionally, imputation facilitates the combination of the association effects obtained from GWAS performed in different studies in a meta-analysis. Although this is a powerful technique to detect significant associations with a certain trait, it can be challenging since discrepancies in coverage from different platforms used for genotyping of samples from each study. Therefore, imputation can help to attenuate this limitation, allowing to increase the overlap between variants assessed in each study, which results in a significant increase of the statistical power (Marchini and Howie 2010; Bai et al. 2019).

Apart from the studies included in this thesis, only two of the GWAS of ICS response performed until December 2020 have used imputation approaches (Mosteller et al. 2017; Levin et al. 2019) with data from the 1,000 Genomes Project (1KGP) as reference panel (Abecasis et al. 2012). This was the first public large catalog of human genetic variants from different populations around the world based on whole-genome sequencing (WGS) data, which has been widely used in many association studies (Birney and Soranzo 2015). The third phase of 1KGP includes data for 88 million variants across the genomes obtained from 2,504 individuals from 26 different worldwide populations (Auton et al. 2015). Nonetheless, several additional reference panels have been recently released, worth highlighting the resource created by the Haplotype Reference Consortium (HRC). At the time of initiating this thesis, this was the largest catalog of human variation publicly available, and it had not been used in any GWAS of asthma treatment response with ICS.

This reference panel contains haplotype information at 39 million sites obtained from WGS of 32,488 individuals from different populations, although with an important representation of Europeans (McCarthy et al. 2016).

All the facts commented above evidence the need for studies simultaneously exploring a high number of variants across the genome from a larger number of individuals from diverse populations. This would enable increasing the knowledge about the genetic factors underlying responsiveness to asthma treatment with ICS. For this reason, there are emerging collaborative and international initiatives gathering efforts from different research groups to perform large GWAS meta-analyses (Vijverberg et al. 2015), such as the Pharmacogenomics in Childhood of Asthma (PiCA) consortium (Farzan et al. 2017a). This was initiated in 2014 with the aim of collecting genetic and clinical data from more than 14,000 children and young adults with asthma participating in 21 independent asthma studies from different countries worldwide, including European, Hispanic/Latino, African American, and Asian populations (Farzan et al. 2017a). The PiCA consortium includes patients from newborns to young adults aged 25 years old with a diagnosis of mild to severe asthma and available information about the use of different controller and reliever asthma medications, environmental exposures, clinical outcomes measuring treatment response, and genotype data. The main aim of this consortium is to identify novel genetic markers involved in uncontrolled asthma despite treatment use through candidate-gene and GWAS approaches. The ultimate goal of the PiCA consortium is to contribute to guiding asthma therapeutic strategies that ensure the adequate control of asthma symptoms and prevent the occurrence of severe exacerbations (Farzan et al. 2017a).

1.7. Other omics studies of ICS response

Although there is still a long way to completely understand the molecular mechanisms underlying the responsiveness to asthma medications, the main findings of the research of ICS response over the last decades can be attributed to genetic association studies. Only a few studies focused on data obtained from other omics approaches have been performed until now, despite they have been evidenced to be promising strategies for the identification of novel markers of asthma treatment response (Galeone et al. 2018; Tyler and Bunyavanich 2019). To the best of our knowledge, a total of 18 studies of ICS response focused on different single omics sources based on approaches different from association studies have been published until December 2020.

Transcriptomics or the study of the structure and function of the complete set of RNA molecules product of genomic DNA transcription in a specific cell type or tissue (Abdel-Aziz et al. 2020; Golebski et al. 2020) has been the second most used approach to investigate biomarkers underlying asthma treatment response to ICS. A total of eight studies have explored the transcriptome of inflammatory and ASM cells in response to ICS (Misior et al. 2009; Masuno et al. 2011; Himes et al. 2014; Sasse et al. 2017; Qiu et al. 2018; Su et al. 2018; Yeh et al. 2018; Kan et al. 2019), which have been suggested to be the main targets of GCs (Hakonarson et al. 2001; Misior et al. 2009), using high-throughput technologies, such as gene expression platforms or NGS. Most of them have analyzed cell cultures obtained from asthmatic or non-asthmatic individuals exposed to GCs or control solutions *in vitro*. As a result, GCs were found to play a central role in the regulation of the activation of inflammatory cells and the production of pro-inflammatory mediators such as cytokines (Misior et al. 2009; Himes et al. 2014). Yeh *et al.* proposed that specific gene

expression profiles in peripheral blood mononuclear cells (PBMCs) are correlated with the regulation of the GC signaling and inflammatory response in patients with poor asthma control despite ICS therapy (Yeh et al. 2018). Additionally, evidence of differential expression levels in response to GCs was found for several genes (e.g., *BACH1*, *SOCS3*, *CRISPLD2*, and *KLF15*) (Masuno et al. 2011; Himes et al. 2014; Sasse et al. 2017; Qiu et al. 2018; Kan et al. 2019), although it has been proposed that the variability in response to asthma treatment with ICS could be explained by different connectivity patterns between transcription factors and genes (Qiu et al. 2018).

Some authors have assessed the potential changes in the microbiome or genetic composition of commensal and pathogenic microorganisms in different human biological samples (metagenomics) (Abdel-Aziz et al. 2020) that could be due to the ICS use (Durack et al. 2017; Turturice et al. 2017; Zhou et al. 2019). They have explored the variation in microbial communities in samples from the respiratory system from asthma patients treated with ICS, such as BAL (Turturice et al. 2017), bronchial brushing (Durack et al. 2017), or nasal swabs (Zhou et al. 2019). Interestingly, these have been predominantly performed in youth or adults, except for the work by Zhou *et al*. (2019), which could be explained by the fact that invasive techniques are needed for sampling in the lower respiratory system (Sinha et al. 2017). The main findings of these studies suggest the enrichment of bacteria involved in the degradation of xenobiotic and synthetical chemical compounds in those patients who do not respond properly to the treatment with ICS (Durack et al. 2017). Additionally, it has been suggested that ICS use alters the correlation between specific pro-inflammatory mediators and several bacterial species, highlighting the decrease of inflammatory cytokines and *Streptococcus pneumoniae* abundancy, which has been proposed to be a potential biomarker of lower response to ICS therapy (Turturice et al. 2017). Nonetheless, the evaluation of the nasal microbiome revealed that asthma patients with profiles dominated by bacteria from the *Corynebacterium* and *Dolosigranulum* genera could be linked to a lower frequency of asthma exacerbation events while on ICS treatment (Zhou et al. 2019).

Proteomic studies have used large-scale quantification methods to evaluate protein levels and their chemical modifications (Golebski et al. 2020) in response to GCs in lung tissue from mouse models of asthma (Roh et al. 2004; Liu et al. 2013). These have suggested that therapies with GCs in asthma patients reduce cytokine levels and therefore, IgE production and airway inflammation (Roh et al. 2004). Moreover, this medication might decrease proteolysis and alter expression patterns of proteins involved in main cellular processes such as cytoskeleton restructuration (Roh et al. 2004; Liu et al. 2013).

Detection methods such as nuclear magnetic resonance and mass spectrometry have been used for the detection of levels and interactions of low molecular weight endogenous and exogenous metabolites in metabolomic studies of ICS response (Abdel-Aziz et al. 2020; Golebski et al. 2020). The evaluation of urine and serum samples from asthmatic children under ICS treatment has revealed changes in products from the metabolism of glutathione, proposed as the main pathway implicated in ICS resistance (Park et al. 2017). Additionally, combined therapy of ICS and SABA was found to interact with arginine metabolism, reducing asthma symptoms (Quan-Jun et al. 2017).

Three studies published until December 2020 have assessed genomic modifications that regulate the transcription (epigenomics) (Abdel-Aziz et al. 2020; Golebski et al. 2020) driven by the ICS treatment in

asthma patients (Kho et al. 2018; Wang et al. 2019a; Wang et al. 2019b). Kho *et al*. evaluated the association of circulating miRNAs with the presence or absence of asthma exacerbations in children treated with ICS (Kho et al. 2018). A total of 12 miRNAs were associated with the future risk to suffer asthma exacerbations in patients under ICS therapy and two of them (Kho et al. 2018), previously linked to asthma-related traits (Bentley et al. 2009; Collison et al. 2013; Comer et al. 2014; Kho et al. 2016), showed a predictive capacity of the occurrence of asthma exacerbations despite ICS use (Kho et al. 2018). On the other hand, two works published by Wang *et al*. compared the methylation status at sites enriched in in peripheral blood cells from children with asthma showing different patterns of ICS responsiveness (Wang et al. 2019a; Wang et al. 2019b). In the first study, hypermethylation of CpG sites was associated with the improvement of lung function and increased *BOLA2* expression levels (Wang et al. 2019b). The second study found evidence of correlation of the hypomethylation or hypermethylation of two different CpG sites with the absence of severe asthma exacerbations in childhood asthma patients treated with ICS from different populations. These were also associated with the downregulation of *IL12B* and upregulation of *CORT*, respectively (Wang et al. 2019a). These findings suggest that the evaluation of DNA methylation could help to detect ICS sensitivity or resistance in patients with asthma (Wang et al. 2019a).

Integration of information from different omics layers, clinical features, and environmental exposures has been proposed as a powerful strategy to disentangle the molecular and cellular mechanisms underlying asthma (Pecak et al. 2018; Abdel-Aziz et al. 2020), although they have not been extensively applied to the research of ICS response (McGeachie et al. 2018; Levin et al. 2019). Therefore, there is a need for large studies combining data from different omics sources to better understand the processes involved in sensitivity and resistance to ICS treatment to optimize strategies of asthma management and reduce the burden of this disease.

2. HYPOTHESIS AND OBJECTIVES

The **general hypothesis** of this work is that the response to ICS treatment in asthma patients is partly affected by the individual's genetic composition. The identification of genetic variants involved in the response to this medication requires the validation of associations described by previous studies, exhaustive genome-wide explorations, the analysis of a larger number of individuals from diverse populations, the evaluation of different definitions of ICS response, and the combination of data from different omics layers. Therefore, the **main objective** of this doctoral thesis is to attempt to identify novel associations of ICS response and to assess the replication of the association of variants identified by previous studies.

The **specific objectives** of this doctoral thesis are:

1. To perform a systematic review of the genomic studies of asthma susceptibility and treatment response to validate the proposed genetic variants.
2. To identify genetic variants associated with asthma exacerbations despite ICS use in genetically admixed populations with African ancestry.
3. To detect genetic polymorphisms associated with asthma exacerbations in European patients treated with ICS.
4. To evaluate the association of genetic variants with the change in FEV_1 after ICS treatment as an additional measure of the response to this medication.
5. To integrate genomic and transcriptomic data from different cell types in response to GCs in order to identify novel genes involved in ICS response.

To achieve these aims, this dissertation is structured into five chapters that address each of the objectives.

3. CHAPTERS

3.1. A systematic review of genomics of asthma-related traits

This *Chapter* includes a systematic review of the main findings of the published genomic studies of different asthma-related traits to identify associations to be followed up for replication in independent studies. We also aimed to give a perspective about the directions of the asthma research towards integrative approaches including data from different omics sources.

We attempted to complete the review of the main findings of the GWAS of asthma susceptibility published by Vicente *et al*. (2017), also including studies focused on associations with the response to the main asthma medications, interactions between genetic and environmental factors, and the genetic overlap of asthma and other allergic diseases. For that, the GWAS published between May 2016 and September 2018 in NHGRI-EBI GWAS Catalog (Buniello et al. 2019) were searched for using the words 'asthma risk' and 'asthma'. PubMed was used to search for Original Research articles focused on different omics approaches and admixture mapping analyses. All references were manually revised and those reporting results from non-omics approaches were excluded. This literature review revealed the validation of genes previously identified and novel association with different asthma-related traits. Despite the large efforts of the genomic studies of asthma, genes identified to date only represent a small proportion of the total heritability. There is a trend towards the use of integrative approaches that combine data from different biological sources. These are promising and powerful strategies to increase the knowledge about the mechanisms underlying asthma that will allow predicting clinical outcomes in the future. Nonetheless, its application has still been limited in the research of response to asthma medications.

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REVIEW
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Genomic Predictors of Asthma Phenotypes and Treatment Response

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Asthma is a complex respiratory disease considered as the most common chronic condition in children. A large genetic contribution to asthma susceptibility is predicted by the clustering of asthma and allergy symptoms among relatives and the large disease heritability estimated from twin studies, ranging from 55 to 90%. Genetic basis of asthma has been extensively investigated in the past 40 years using linkage analysis and candidate-gene association studies. However, the development of dense arrays for polymorphism genotyping has enabled the transition toward genome-wide association studies (GWAS), which have led the discovery of several unanticipated asthma genes in the last 11 years. Despite this, currently known risk variants identified using many thousand samples from distinct ethnicities only explain a small proportion of asthma heritability. This review examines the main findings of the last 2 years in genomic studies of asthma using GWAS and admixture mapping studies, as well as the direction of studies fostering integrative perspectives involving omics data. Additionally, we discuss the need for assessing the whole spectrum of genetic variation in association studies of asthma susceptibility, severity, and treatment response in order to further improve our knowledge of asthma genes and predictive biomarkers. Leveraging the individual's genetic information will allow a better understanding of asthma pathogenesis and will facilitate the transition toward a more precise diagnosis and treatment.

Keywords: admixture mapping, asthma, genomics, genome-wide association study, multiomics, personalized medicine

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INTRODUCTION

Asthma is a complex respiratory disease characterized by inflammation and reversible obstruction of the airways (1) that can lead to diverse symptoms such as wheeze, breathlessness, chest tightness, and cough (2). Asthma affects approximately 350 million people from all age groups worldwide (3) and causes around 350,000 deaths per year (4). Although asthma is a lifelong disease, it is considered the most common chronic condition in children and young adults (5, 6), where symptoms are usually more severe (7, 8).

A significant global burden has been attributed to asthma, which is mostly driven by direct economic costs on health care systems (9) and indirect social and economic consequences due to substantial productivity loss (10). In this regard, asthma represents one of the most important pulmonary diseases (11). However, wide differences in asthma prevalence have been estimated among countries and populations, ranging from 1.5 to 15.6% (12, 13), and also among ethnic groups within countries (14). These differences could be a result of complex interactions among environmental and genetic factors (15, 16).

Several studies support a large genetic contribution to asthma predisposition, known as heritability (17, 18), with estimates of as much as 55–74% in adults (19, 20), and almost reaching 90% in children (21). In order to elucidate the genes underlying asthma pathogenesis, several genetic approximations have been performed (22). The initial studies were linkage analyses, which are based on small panels of informative markers across the genome that were determined in multigenerational families with multiple affected individuals to allow the identification of the markers that were more frequently co-inherited with the disease (23). After a genomic region is linked to a disease, this could be followed-up using positional cloning, or the genes contained therein might serve as candidate regions for association studies in outbred population samples (23). Although the use of this approach for over 20 years allowed the identification of as few as eight asthma genes [reviewed in (24–26)], it was recognized a lack of power of this approach for detecting small effect sizes of the risk variants (27).

The use of linkage analysis decreased as it was a progressive development and use of candidate-gene association studies (23, 27). The latter were extensively used during the last two decades, mostly to refute or confirm the implication of a single biological candidate gene at a time (28), mainly by comparing the allele frequencies of a small set of single nucleotide polymorphisms (SNPs) near or within the gene of interest among asthma cases and control subjects without the disease (29).

Although candidate-gene association studies largely increased the resolution of genetic studies of asthma compared to linkage analyses, they also complicated the interpretation of overall results. The main reasons for that were that most studies have included small sample sizes and have tested a reduced number of genetic variants, which greatly decreases the power to detect significant associations. Most importantly, replication of findings in, at least, an independent study was not a standard practice. As a consequence, failure in the attempt to consistently replicate the findings in independent populations was common (23, 29). Given these criticisms, its use has progressively decreased while advances in high-throughput polymorphism genotyping platforms were occurring, leading to continuous reductions in costs and the development of key analysis methods to allow much denser genomic scans (29). These advances opened the way for genome-wide association studies (GWAS), which now allow a simultaneous exploration of hundreds of thousands of SNPs across the genome, most commonly determined in samples from unrelated cases and controls (23, 29). The main advantage of this hypothesis-free approach is the ability to detect mild effects of disease genes without any previous knowledge of the condition (23, 29). On the other side, performing GWAS could be challenging as they usually require large sample sizes and the coverage of the largest number of variants as possible to reach enough statistical power to detect significant associations with asthma (29).

Vicente et al. recently discussed the GWAS that were published from the first one in 2007 (30) until the end of 2016 (31), revealing a total of 39 common SNPs independently associated with asthma risk (22). In this review, we aimed to update the main findings of the genomic studies of asthma, treatment response and the overlap of this disease with other allergic conditions performed between 2016 and 2018. Additionally, we discuss the direction of the new generation of genetic studies of asthma to cover the unexplored variation and the forthcoming integrative omics approaches to continue disentangling the genetic predictors of asthma.

GENOME-WIDE ASSOCIATION STUDIES

A search on the NHGRI-EBI GWAS Catalog (32) and on PubMed records revealed that 15 GWAS of asthma and related traits had been published after the period reviewed by Vicente et al. (22), between 1st May 2016 and 19th September 2018 (**Supplementary Table 1**).

Asthma was defined by a physician diagnosis in most of the studies. However, some GWAS also considered other asthma definitions, such as the presence of symptoms or the prescription of any asthma medication, among others. Four GWAS focused on children (33–36) and five on adults (37–41), whereas another five attempted to identify common genetic factors between childhood and adulthood asthma (42–46). Across the 15 GWAS of asthma and related traits reviewed, the largest sample sizes were attained by those focusing on the genetic overlap of asthma and allergic diseases. The largest one included 360,838 individuals (180,129 cases and 180,709 controls) and aimed to disentangle the common genetic basis of asthma, hay fever and eczema in asthmatic children from European populations (45). The smallest comprised 949 individuals and it was focused on a highly specific phenotype, the response to asthma treatment with short-acting β_2 agonists (SABA) (36) (**Supplementary Table 1**).

Although there is an increasing trend to include multiethnic populations in genomic studies of asthma, an underrepresentation of non-European populations is still pervasive (47, 48). In fact, the vast majority of GWAS performed between 2016 and 2018 focused on patients of European ancestry (33, 34, 39, 41–43, 45, 49), presenting a particularly poor representation of Asians and Africans-admixed populations.

In total, 451 genetic variants, including short insertions/deletions and SNPs, were reported as risk factors for asthma and related traits by the GWAS of the last 2 years. From these, 319 SNPs clustered at 167 loci that reached genome-wide significance at a threshold of $p < 5 \times 10^{-8}$ or $p < 3 \times 10^{-8}$ in the discovery or replication phases and/or after performing a meta-analysis with the results from both stages. Among these, 68 were revealed as novel asthma loci, whereas 99 had been previously associated with asthma or any allergic diseases.

In the sections below, we summarize the main findings of these GWAS, distinguishing among those that focused on asthma susceptibility; treatment response; gene-environment

Abbreviations: BDR, bronchodilator response; CNVs, copy number variations; GWAS, genome-wide association study; GWIS, genome-wide interaction study; ICS, inhaled corticosteroids; IL-5, interleukin 5; miRNA, microRNA; NGS, next-generation sequencing; OR, odds ratio; pLoF, predicted loss-of-function; PRS, polygenic risk score; SABA, short-acting β2 agonists; SNP, single nucleotide polymorphism.

interactions and the overlap among asthma and allergic disorders.

Asthma Susceptibility

Eight GWAS evaluated the association with asthma (33, 35, 37, 38, 42–44, 46) (**Supplementary Table 1**), although only four studies revealed genome-wide significant associations (38, 43, 44, 46). These validated the association of 14 loci previously associated with asthma susceptibility (**Table 1**).

The well-known 17q21 asthma locus (50) has been the most replicated signal, although the main driver of this association has not been disentangled to date (43). The gene encoding the zona pellucida-binding protein 2 (*ZPBP2*) has been revealed as a common locus of both childhood and adulthood asthma by several studies, supported by the association of several intronic SNPs as well as variants located within the intergenic region of *ZPBP2* and *GSDMB* (43, 44). A SNP located at the promoter region of *ZPBP2* (rs11557467) showed the most significant association after performing a meta-analysis in 13,556 children and adults from several European populations (43). The risk allele was associated with asthma susceptibility (OR for the T allele = 1.32, $p = 3.29 \times 10^{-15}$) (43) and was also replicated in Latinos/Hispanics (44). This variant was previously evidenced to be a putative site with allele-specific nucleosome occupancy in patients with asthma (51). Similar results were found for *GSDMB*, with a shared signal between both European (min $p = 2.55 \times 10^{-20}$) (43) and Latino/Hispanic populations (min $p = 8.21 \times 10^{-14}$) (44). Furthermore, the association of *ORMDL3* with asthma was validated in Latinos/Hispanics (min $p = 1.90 \times 10^{-15}$) (44), which have been also extensively associated with asthma across different populations (30, 52, 53) (**Table 1**). Interestingly, differences in the expression level of *ZPBP2* and *GSDMB* have been found between European and African populations (54). In fact, early studies had revealed that SNPs associated with asthma co-regulate the expression of *ORMDL3*, *GSDMB,* and *ZPBP2* in Latinos (54).

A large multiethnic GWAS performed in 23,948 asthma cases and 118,538 controls validated the association of several genes already known to be involved in asthma with functions related to immune response and other activities, such as organogenesis, cellular differentiation and transcriptional modulation, among others (46). The most significant association signal was driven by the SNP rs2952156 located at the Erb-B2 Receptor Tyrosine Kinase 2 (*ERBB2*) gene, whose G allele was associated with protection for asthma (OR = 0.87, $p = 2.20 \times 10^{-30}$) in ethnically diverse populations (46) (**Table 1**).

Additionally, 6 loci not previously linked to asthma were identified in European (38), Latino/Hispanic (44) and multiethnic populations (38). In these studies, the *GRM4* gene was the most frequent signal, where a higher number of variants with evidence of association with asthma susceptibility were located (min $p = 5.29 \times 10^{-9}$) (38). *GRM4* encodes the glutamate metabotropic receptor 4, involved in synaptic neurotransmission and maintenance on normal functions of the central nervous system throughout the regulation of the adenylate cyclase cascade (55), although it has been recently linked to tumorigenesis (56). The *GRM4* gene has been associated with several neurological disorders (57–59) and different types of cancer (56, 60) but, it has not been associated with any asthma-related traits and it has not been implicated in any immune-related function. However, early studies had suggested the potential implication of glutamate receptors on asthma worsening by means of triggering airways inflammation (61).

Asthma Treatment Response

The most commonly prescribed medication to treat asthma are SABA and inhaled corticosteroids (ICS) (2). Although most asthma patients treated with these medications experience a decrease in their symptoms (62), wide differences in asthma treatment response have been described among individuals and populations (63, 64). These observations suggest that genetics may play a key role in the response to asthma treatment (64, 65). Therefore, the characterization of multiple genetic markers determining therapeutic responsiveness to asthma medications could contribute in the future to identify specific pharmacogenetic profiles. This would enable clinical identification of those asthma patients that respond unsatisfactorily to these treatments or that experience adverse effects (66). Consequently, the burden of asthma could be reduced by implementing personalized asthma management and therapeutic strategies (67).

SABA are the most commonly prescribed relief asthma medication that quickly reduces bronchoconstriction throughout smooth muscle relaxation of the airways (2). Clinical response to this treatment is frequently assessed as bronchodilator response (BDR), which quantitatively measures the change in airway constriction by means of the change in forced expiratory volume in 1 s after SABA administration (68). However, high variability in BDR among individuals and populations has been described, which has been evidenced to be influenced by environmental and genetic factors (69, 70). In fact, it has been estimated that 47–92% of the total variation in BDR could be attributed to the genetic component (71, 72). Recently, a GWAS of BDR was performed in 949 children with asthma from two African American populations (36) (**Supplementary Table 1**). This revealed the intergenic region of *SPATA31D1* and *RASEF* as population-specific novel loci of BDR in African American children with asthma (rs73650726, β for the A allele = 0.02, $p = 7.69 \times 10^{-9}$). Moreover, they found the *PRKG1* to be implicated in BDR shared between African Americans and Latinos/Hispanics (min $p = 3.94 \times 10^{-8}$) (**Table 2**). This gene encodes a cyclic guanosine monophosphate-dependent protein kinase involved in several biological processes, such as the nitric-oxide signaling pathway (73, 74), which modulates vasodilation in response to β_2 agonists (75). This fact together with evidence of expression of *PRKG1* in pulmonary tissues suggest this could be a plausible gene of BDR in African-admixed asthmatic children (76).

Despite the large improvements in asthma therapeutic strategies in the last decades, ICS are still the most effective and commonly prescribed medication to control symptoms and prevent severe exacerbations in asthma patients (2), which consist of the most important outcome in childhood asthma (77). However, a small proportion of the genetic basis of the ICS response has been disentangled (78–80). In the period reviewed,

TABLE 1 | Summary of the most significant variants identified by the genome-wide association studies of asthma susceptibility.

SNP[a]	Chr. region[b]	Position[c]	Nearest gene (s)	Effect allele	OR[d,e]	*p*-value[e]	References
rs1420101	2q12	102957716	*IL1RL1*	T	1.12	3.90×10^{-21}	(46)
rs10455025	5q22	110404999	*TSLP*	C	1.15	9.40×10^{-26}	
rs20541	5q31	131995964	*IL13*	G	0.89	5.00×10^{-16}	
rs7705042	5q31	141492419	*NDFIP1*	A	1.09	7.90×10^{-9}	
rs9272346	6p21	32604372	*HLA-DQA1*	A	1.16	5.70×10^{-24}	
rs2325291	6q15	90986686	*BACH2*	A	0.91	2.20×10^{-12}	
rs992969	9p24	6209697	*IL33*	G	0.86	7.20×10^{-20}	
rs7927894	11q13	76301316	*LRRC32*	T	1.10	2.20×10^{-14}	
rs167769	12q13	57503775	*STAT6*[f]	T	1.08	3.90×10^{-9}	
rs2033784	15q22	67449660	*SMAD3*	G	1.10	7.40×10^{-15}	
rs2952156	17q12	37876835	*ERBB2*	G	0.87	2.20×10^{-30}	
rs17637472	17q21	47461433	*ZNF652-PHB*	A	1.08	6.60×10^{-9}	
rs200567451	17q21	37902883	*GRB7*[f]	G	NA	5.10×10^{-9}	(44)
rs12946510	17q21	37912377	*GRB7-IKZF3*	T	NA	5.54×10^{-13}	
rs907092	17q21	37922259	*IKZF3*	A	NA	8.70×10^{-14}	
rs36095411	17q21	38031865	*ZPBP2*	G	NA	5.32×10^{-14}	
rs35569035	17q21	38035624	*ZPBP2-GSDMB*	T	NA	6.36×10^{-14}	
rs9303279	17q21	38073968	*GSDMB*	C	NA	8.21×10^{-14}	
rs8076131	17q21	38080912	*ORMDL3*	A	NA	5.19×10^{-13}	
rs7221814	17q21	38089717	*ORMDL3-LRRC3C*	G	NA	9.37×10^{-11}	
rs3095318	6p21	31088145	*PSORS1C1*	NA	1.42	1.61×10^{-11}	(38)
rs1776883	6p21	34156444	*GRM4*[f]	NA	0.80	5.29×10^{-9}	
rs72721166	9p22	27304548	*EQTN*[f]	NA	1.82	3.83×10^{-9}	
rs75446656	10q21	65100016	*JMJD1C*[f]	NA	2.64	3.60×10^{-8}	
rs36080042	10q21	65426785	*REEP3*[f]	NA	2.62	4.70×10^{-8}	
rs62067034	17q21	38063738	*GSDMB*	T	NA	3.55×10^{-12}	
rs9303277	17q21	37976469	*IKZF3*	T	1.31	1.43×10^{-14}	(43)
rs11557467	17q21	38028634	*ZPBP2*	T	1.32	3.29×10^{-15}	
rs2290400	17q21	38066240	*GSDMB*	C	1.31	2.55×10^{-20}	
rs4795405	17q21	38088417	*ORMDL3*	T	1.26	1.90×10^{-15}	

[a] *Only the most significant variants per loci and study are included.*
[b] *Chromosomal region.*
[c] *Positions based on GRCh37/hg19 build.*
[d] *Odds ratio for the effect alleles.*
[e] *Association results of the meta-analysis.*
[f] *Novel locus (no previous evidence of association with asthma). NA: not available.*

Mosteller et al. performed the unique additional GWAS that has explored the association of genetic variants with ICS response (**Supplementary Table 1**). This constitutes the first GWAS of ICS response to include non-European patients. Unfortunately, they did not find any significant finding (40).

In addition to the most common types of medications used to treat asthma, there is an increasing number of emerging therapies, including biological treatments. These have been designed to act directly toward specific components of the T-lymphocyte inflammatory response involved in asthma such as, interleukin 5 (IL-5). This mediator is centrally involved in increasing immunoglobulin E levels and blood and bronchoalveolar eosinophilia in severe asthma. Therefore, the inhibition of IL-5 by using monoclonal antibodies could reduce the high levels of eosinophils (81). A few pharmacogenetic studies have recently evaluated the response to asthma therapies with anti-IL-5 monoclonal antibodies, such as mepolizumab (39) (**Supplementary Table 1**), which has been evidenced to reduce asthma exacerbations rates and enables asthma control (82, 83). Condreay et al. investigated the association of genetic variants with the response to asthma treatment with mepolizumab measured as number of asthma exacerbations, eosinophil count and immunoglobulin E levels in 1,192 asthma patients. Although no variants reached genome-wide significance level ($p \leq 5 \times 10^{-8}$), six SNPs at 6p24 and 9p21 showed suggestive associations with mepolizumab response (**Table 2**) (39).

Unfortunately, despite the large efforts during the last decades, pharmacogenetic findings are still not able to predict clinical outcomes that are directly applied to asthma patients (84). As happened in the past for the asthma field, and although

TABLE 2 | Summary results of the genome-wide association studies of asthma treatment response.

Treatment	SNP	Chr. region[a]	Position[b]	Nearest gene(s)	Effect allele	Beta[c,d]	*p*-value[d]	References
SABA	rs73650726	9q21	85152666	*SPATA31D1-RASEF*[e]	A	−3.80	7.69×10^{-9}	(36)
	rs7903366	10q21	53689774	*PRKG1*	T	1.23	3.94×10^{-8}	
	rs7081864	10q21	53690331	*PRKG1*	A	1.23	4.94×10^{-8}	
	rs7070958	10q21	53691116	*PRKG1*	A	−1.24	4.09×10^{-8}	
Mepolizumab	rs114633080	6q24	145404255	*IFNA14-IFNA22P*[e]	NA	NA	NA	(39)
	rs137893217	6q24	145427162	*IFNA14-IFNA22P*[e]	NA	NA	NA	
	rs78517277	6q24	145465077	*IFNA14-IFNA22P*[e]	NA	NA	NA	
	rs117220641	6q24	145496192	*IFNA14-IFNA22P*[e]	NA	NA	NA	
	rs10811516	9p21	21255049	*IFNA14-IFNA22P*[e]	NA	NA	NA	
	rs10811517	9p21	21256306	*IFNA14-IFNA22P*[e]	NA	NA	NA	

[a] *Chromosomal region.*
[b] *Positions based on GRCh37/hg19 build.*
[c] *Beta values for the effect alleles.*
[d] *Association results of the meta-analysis.*
[e] *Novel locus (no previous evidence of association with asthma). NA: not available.*

asthma pharmacogenetic studies have started to evolve toward GWAS approaches (64, 85), the main reason could be that most published pharmacogenomic studies continue to be performed using the candidate-gene approach (64, 80, 85).

Gene-Environment Interactions

Despite the significant contribution of genetic factors on asthma and related traits, a key role of the gene interactions with exposures to a wide variety of environmental factors has been described (15, 16, 86). Among these, early-life exposures demonstrate a high relevance in the prediction of childhood asthma development, including respiratory infections (87), gut and airway microbiome (87, 88), and tobacco smoke exposure (89). Several strategies have been used to identify gene-environment interactions during the last decades (90, 91), but their application has recently emerged in the form of genome-wide interaction studies (GWIS) (91), which are considered a powerful approach to identify novel disease loci that interact with environmental factors (41).

Two GWIS have attempted to identify gene-environment interactions involved in asthma susceptibility since 2016 (34, 41) (**Supplementary Table 1**). One of them explored for the first time the interaction of genetic variants with traffic-related air pollution, although previous studies used candidate gene approaches (34). Traffic air pollution measured as nitrogen dioxide levels cause deterioration of asthma symptoms by triggering exacerbations and decreasing the lung function (92, 93). This GWIS in European children revealed five loci that were suggestively associated and three of the SNPs were located at *ADCY2*, a known asthma locus (94). The risk alleles at *ADCY2* were also associated with decreased expression of the gene in peripheral blood. Moreover, differential *ADCY2* expression depending on nitrogen dioxide levels was found, suggesting that this gene could have functional implications on asthma under exposure to traffic-related air pollution (34). Similar results were found for a SNP located within the intergenic region of *B4GALT5* and *SLC9A8* (**Table 3**), which were revealed as novel plausible genes with functional implications on childhood asthma in interaction with nitrogen dioxide exposure (34).

Additionally, a GWIS of active tobacco smoking was conducted in 4,057 patients with adulthood-onset asthma of European ancestry (41) (**Supplementary Table 1**). It is well-known that second-hand smoke exposure to tobacco smoke increases childhood asthma risk during prenatal and postnatal stages (95–99). Although active tobacco smoke has been associated with asthma onset during adulthood (100), it is still unclear how the genetic variation could affect asthma susceptibility in interaction with tobacco smoke exposure in adults (41). The intergenic SNPs rs9969775 (OR for the A allele $= 0.50$, $p = 7.63 \times 10^{-5}$) and rs5011804 (OR for the C allele $= 1.50$, $p = 1.21 \times 10^{-4}$), which are located at the *MPDZ-NFIB* and *KRAS-IFLTD1* loci, respectively, showed significant interactions with active tobacco smoking for late-onset asthma. These findings were validated at nominal level in an independent study (41) (**Table 3**). Although none of these loci showed any functions specifically related to asthma and none were previously associated with asthma or related traits, the SNP rs9969775 was postulated to be involved in the regulation of gene expression in the lung (41).

Overlap Among Asthma and Allergic Diseases

Given the firm links in the pathogenesis of asthma and other allergic diseases (20, 101), a few studies used this rationale to explore the overlapping genetic architecture among these diseases (45, 49, 102, 103), including two large-scale GWAS published between 2016 and 2018 (45, 49) (**Supplementary Table 1**).

Ferreira et al. carried out the largest GWAS of asthma and allergic diseases to date (45). They combined data from 360,838 children and adults from 13 different European studies, including 180,129 patients with self-reported or physician-diagnosed asthma, eczema or hay fever, and 180,709 controls (**Supplementary Table 1**). They reported 136 independent SNPs

TABLE 3 | Summary results of the genome-wide association studies of gene-environment interactions.

Environmental exposure	SNP[a]	Chr. region[b]	Position[c]	Nearest gene(s)	Effect allele	Discovery phase		Replication phase		References
						OR[d]	*p*-value	OR[d]	*p*-value	
Nitrogen dioxide	rs727432	5p15	7716078	*ADCY2*	G	1.61	6.67×10^{-5}	1.13	0.016	(34)
	rs4143882	5p15	7717364	*ADCY2*	A	1.61	4.75×10^{-5}	0.88	0.015	
	rs6886921	5p15	7718539	*ADCY2*	C	1.71	7.03×10^{-6}	1.12	0.016	
	rs963146	11q14	83423444	*DLG2*[e]	A	0.67	8.61×10^{-5}	1.12	0.034	
	rs12455842	18q12	32096284	*MOCOS*[e]	C	0.48	6.10×10^{-5}	1.30	0.010	
	rs1057251	18q12	32102579	*MOCOS*[e]	C	0.50	6.18×10^{-5}	1.30	9.40×10^{-3}	
	rs12457919	18q12	32108100	*MOCOS-FHOD3*[e]	A	0.39	5.52×10^{-5}	1.30	0.012	
	rs12457919	18q12	33854102	*MOCOS-FHOD3*[e]	A	0.39	5.52×10^{-5}	NA	0.017	
	rs686237	20q13	47804141	*B4GALT5-SLC9A8*[e]	A	1.69	5.43×10^{-5}	0.89	1.60×10^{-3}	
Tobacco smoke	rs9969775	9p23	13561933	*MPDZ-NFIB*[e]	A	0.50	7.63×10^{-5}	0.65	0.020	(41)
	rs5011804	12p12	25441894	*KRAS-LMNTD1*[e]	C	1.50	1.21×10^{-4}	1.40	0.030	

[a] *Only the variants with evidence of replication are included.*
[b] *Chromosomal region.*
[c] *Positions based on GRCh37/hg19 build.*
[d] *Odds ratio for the effect alleles.*
[e] *Novel locus (no previous evidence of association with asthma). NA: not available.*

at 99 loci as genome-wide significant associations ($p \leq 3 \times 10^{-8}$) with susceptibility to asthma or any allergic disease (**Supplementary Table 2**). A total of 86 variants were located at loci that were already associated with at least one of the diseases under study, whereas 50 other SNPs revealed novel loci that were shared by asthma and allergy (**Table 4**). A high proportion (96%) of these variants showed similar effects between asthma, eczema and hay fever. The most significant variants were located within or near loci with previous evidence of implication on asthma and/or allergic diseases such as, *WNT11-LRRC32*, *IL18R1*, *TLR1,* and *HLA-DQA1*, among others. In fact, the SNP rs7936323 of the intergenic region of *WNT11* and *LRRC32* genes showed the strongest evidence of association (OR for the A allele = 1.09, $p = 2.20 \times 10^{-63}$) (**Supplementary Table 2**). Altogether, the 136 SNPs identified accounted for 3.2, 3.8, and 1.2% of the total variation of asthma, hay fever and eczema, respectively. These findings partially explain the co-existence of these diseases in many patients (45) (**Supplementary Table 2**). Interestingly, evidence of potential functional implication on blood and pulmonary tissues was found for many of the SNPs identified. Specifically, they demonstrated that risk variants shared among asthma, hay fever and eczema are involved in the regulation of gene expression in immune response-related signaling pathways, such as in the B and T cell activation (45). These findings confirmed previous evidence (104) suggesting that these biological processes could be among the ones shared between asthma and allergic diseases (45).

Besides these findings, 29 of the genes identified by Ferreira et al. encode for proteins that are drug targets for several diseases, including allergic and auto-immune diseases. Interestingly, the protective effect of these genes was found to be correlated with the effect of drugs targeting them, attenuating allergy symptoms. These findings suggest that these could be effective targets to treat allergic diseases or asthma and thus, the proteins encoded by these should be prioritized for pre-clinical evaluation (45).

That GWAS was further complemented in a separate study with a gene-based association analysis using an algorithm that was specifically designed to identify shared risk variants among multiple phenotypes. With this approximation, which helps to increase the statistical power to detect novel risk loci, multiple variants near or within each gene are tested rather than focusing on individual SNP tests at a time (103). By relying on the information of SNPs that modify gene expression levels in different tissues and cell types, also known as expression quantitative trait loci (103, 105), they additionally revealed 19 novel risk genes for allergic diseases, which were not revealed by the previous stages of the study (45). Among these genes, nine showed functions that were closely related to well-known mechanisms involved in allergic diseases and asthma. Although further functional validation is needed, these could also represent novel drug targets (103).

Recently, Zhu et al. performed another GWAS in 110,361 Europeans in an attempt to identify genetic variants shared among asthma, hay fever, eczema and rhinitis (**Supplementary Table 1**). After performing a cross-trait meta-analysis, 38 loci were associated with both asthma and allergic diseases at genome-wide significance level (**Supplementary Table 2**). These loci were enriched in essential pathways for several tissues, such as skin, lung and whole blood, among others (49). Among these results, seven hits were novel loci that might contribute to the common genetic architecture of asthma and allergic diseases (49) (**Table 4**). These findings were consistent with the results reported by Ferreira et al. (45). In fact, a high proportion of the loci revealed by Zhu et al. (49) had been

TABLE 4 | Novel loci of asthma and allergic diseases revealed by meta-analyses published between 2016 and 2018.

Phenotypes	SNP	Chr. region[a]	Position[b]	Nearest gene (s)	Effect allele	OR[c,d]	*p*-value[d]	References
Asthma/hay fever/eczema/rhinitis	rs12743520	1p22	93037112	*PIGX*	C	0.93[e]	3.83×10^{-8e}	(49)
	rs61815704	1q21	152893891	*IVL-SPRR2E*	C	1.14[e]	5.16×10^{-9e}	
	rs1214598	1q24	167426424	*RP11-104L21.2*	G	0.95[e]	5.14×10^{-11e}	
	rs4916533	3q29	196373582	*LRRC33*	C	0.93[e]	1.66×10^{-8e}	
	rs56267605	4q27	123363109	*ADAD1-IL2*	A	1.05[e]	2.56×10^{-12e}	
	rs6461503	7p21	20560996	*ABCB5*	C	1.05[e]	3.19×10^{-10e}	
	rs2169282	9p24	6350235	*TPD52L3*	G	1.09[e]	1.80×10^{-10e}	
	rs12413578	10p14	9049253	*SLC7A10*	C	0.91[e]	1.09×10^{-14e}	
	rs10876864	12q13	56401085	*SUOX*	A	1.05[e]	1.41×10^{-13e}	
	rs9911533	17q21	38775476	*SMARCE1*	T	0.92[e]	9.70×10^{-16e}	
	rs10414065	19q13	33721455	*SLC7A10-CEBPA*	C	0.91[e]	2.63×10^{-10e}	
	rs2766664	20q13	52171241	*RP4-724E16.2*	G	1.08[e]	8.07×10^{-11e}	
	rs10033073	1p36	4775401	*EVI5*	G	1.04	1.20×10^{-10}	
Asthma/hay fever/eczema	rs1057258	1p36	234115629	*INPP5D*	C	1.05	1.40×10^{-10}	(45)
	rs10414065	1q21	33721455	*SLC7A10-CEBPA*	C	1.10	6.10×10^{-18}	
	rs10663129	1q21	141321836	*RASA2*	ACT	1.04	1.10×10^{-13}	
	rs10760123	1q42	123650534	*PHF19-TRAF1*	T	1.03	5.20×10^{-9}	
	rs10910095	2p25	2510755	*TNFRSF14-FAM213B*	G	1.04	2.70×10^{-8}	
	rs11169225	2q12	50345671	*AQP2*	A	1.05	1.20×10^{-11}	
	rs12440045	3q28	41782684	*RTF1-ITPKA*	C	1.03	4.90×10^{-10}	
	rs13088318	3q29	101242751	*FAM172BP-TRMT10C*	A	1.03	8.60×10^{-9}	
	rs13153019	4p16	176782218	*LMAN2-RGS14*	C	1.04	1.30×10^{-8}	
	rs13403656	4q24	112269127	*BCL2L11-ANAPC1*	A	1.05	2.20×10^{-8}	
	rs13384448	4q27	228707862	*CCL20-DAW1*	T	1.04	2.80×10^{-12}	
	rs227275	5p13	103593898	*MANBA*	C	1.03	3.70×10^{-11}	
	rs17664743	5q22	50253897	*C7orf72-IKZF1*	A	1.04	6.20×10^{-11}	
	rs250308	5q31	118684297	*TNFAIP8*	T	1.03	4.00×10^{-9}	
	rs2910162	6p21	159909345	*MIR3142-MIR146A*	G	1.03	2.50×10^{-9}	
	rs35469349	7p12	128294709	*PTPRK*	A	1.04	2.30×10^{-10}	
	rs3540	7p21	91045408	*IQGAP1*	G	1.04	3.30×10^{-11}	
	rs4296977	8q21	77018542	*GSAP*	C	1.06	2.10×10^{-13}	
	rs4801001	9p24	52336175	*DYNAP-RAB27B*	T	1.03	5.90×10^{-9}	
	rs4574025	9q33	60009814	*TNFRSF11A*	T	1.03	6.80×10^{-9}	
	rs4671601	9q34	64836267	*LOC339807*	C	1.04	8.80×10^{-9}	
	rs4943794	10p14	41173408	*FOXO1*	C	1.04	7.20×10^{-12}	
	rs5758343	10p14	41816652	*TEF-TOB2*	A	1.05	4.80×10^{-14}	
	rs55726902	10p14	48196982	*HDAC7*	G	1.05	2.60×10^{-16}	
	rs4848612	10q24	112388538	*BCL2L11-ANAPC1*	A	1.04	2.30×10^{-10}	
	rs61192126	11q13	72394852	*LINC00870-RYBP*	T	1.04	8.90×10^{-11}	
	rs59593577	11q13	95425526	*SESN3-FAM76B*	C	1.05	1.60×10^{-11}	
	rs6489785	12q13	121363724	*SPPL3-HNF1A-AS1*	T	1.04	1.60×10^{-15}	
	rs6977955	12q24	28156887	*JAZF1*	T	1.05	7.10×10^{-13}	
	rs697852	12q24	226914734	*ITPKB*	A	1.04	1.60×10^{-9}	
	rs7130753	13q22	111470567	*LAYN-SIK2*	C	1.05	7.00×10^{-15}	
	rs71368508	14q13	4521473	*SMTNL2-ALOX15*	C	1.12	2.00×10^{-9}	
	rs7137828	14q21	111932800	*ATXN2*	T	1.03	2.20×10^{-10}	
	rs7207591	14q24	40414862	*STAT5B*	A	1.04	1.40×10^{-9}	
	rs72033857	14q24	167390671	*RNASET2-MIR3939*	C	1.06	1.20×10^{-9}	
	rs7214661	14q32	43430696	*MAP3K14-ARHGAP27*	G	1.03	1.20×10^{-8}	
	rs73205303	15q15	36467830	*RUNX1*	A	1.04	7.90×10^{-10}	

(Continued)

TABLE 4 | Continued

Phenotypes	SNP	Chr. region[a]	Position[b]	Nearest gene (s)	Effect allele	OR[c,d]	*p*-value[d]	References
	rs74847330	15q22	143831599	*KYNU-ARHGAP15*	A	1.05	1.80×10^{-9}	
	rs75557865	16p13	121652141	*SLC15A2*	G	1.03	1.60×10^{-8}	
	rs76167968	17q21	35681738	*SFPQ-ZMYM4*	T	1.06	1.30×10^{-8}	
	rs76081789	17q21	44846426	*SIK1*	T	1.07	1.30×10^{-8}	
	rs9323612	20q13	75968608	*JDP2-BATF*	A	1.03	8.60×10^{-9}	
	rs9372120	20q13	106667535	*ATG5*	G	1.04	4.20×10^{-11}	
	rs9383820	20q13	157419508	*ARID1B*	C	1.04	1.20×10^{-8}	
	rs9573092	21q22	73627275	*PIBF1-KLF5*	A	1.03	2.70×10^{-8}	
	rs9989163	22q13	103235012	*RCOR1-TRAF3*	A	1.03	1.90×10^{-8}	

[a] *Chromosomal region.*
[b] *Positions based on GRCh37/hg19 build.*
[c] *Odds ratio for the effect alleles.*
[d] *Association results of the meta-analysis.*
[e] *Association results of the discovery phase.*

previously identified by Ferreira et al. (45). Interestingly, most of them had functions related to immune response, inflammation and epithelium maintenance, such as *HLA-DQB1-AS1*, *IL1RL1*, *FLG-AS1*, and *STAT6*, among others (45, 49).

ADMIXTURE MAPPING

It has been evidenced that differences in asthma prevalence and severity among populations and ethnic groups could be partly explained by population-specific genetic factors. Alternative genetic scanning methods take advantage of these population specificities that are not frequently explored by means of GWAS approaches (106, 107), which could contribute to further identify asthma genes. One of such approaches is based on the exploration of the variation in genetic ancestry at chromosome-segment level (termed local ancestry) and the correlation with asthma in populations that are the result of a recent historical admixture, an approach most commonly known as admixture mapping (108, 109). Although both GWAS and admixture mapping are based on genome-wide data obtained by means of genotyping platforms, admixture mapping compares local ancestry estimates with the disease or trait (63). With this approach, a much smaller number of comparisons are involved in the scan, which highly increases the statistical power to detect associations compared to a traditional GWAS approach for a given sample size (110). Besides, this approach gives an opportunity for leveraging the specific genetic architecture of admixed populations, which have been largely underrepresented in genetic studies of asthma (111, 112). Admixed populations are characterized by high correlation over large chromosomal regions resulting from the recent admixture process, therefore these populations simplify gene mapping over longer distances (113–116). Moreover, if the trait of interest affects differentially the parental populations of the admixed, large trait differences are expected among admixed individuals, providing increased power to detect novel associations (117). Besides, the particular allelic configurations of the admixed individuals could interact with genetic risks for asthma, mitigating, or enhancing their effects in disease. In fact, causal variants are transmitted in higher proportion from the parental to the admixed populations, which leads to higher prevalence in the latter (112). As a consequence, it is expected that the proportion of the parental ancestry at those loci will vary between asthma cases and control individuals (110, 113), which would be indicative of ancestry-specific genetic risks (63). Combining admixture mapping with the traditional GWAS is a suitable strategy to identify both asthma risks that are ancestry-specific and those that are shared among different ancestry groups (63, 114). Although recently admixed populations are abundant (63), those with African admixture have been the prevalent in the asthma field (69, 109, 115).

There are two major African-admixed populations in the United States: African Americans and Latinos/Hispanics, which show different proportions of ancestry from each parental population (63). However, both show evident genetic footprints of the African admixture (116). Although very simplified, Latinos/Hispanics are usually modeled as descendants of ancient Native American, European and sub-Saharan African populations (63, 117), whereas current African Americans are modeled as descendants of an admixture event between sub-Saharan African and Europeans (63, 110, 118). Interestingly, compared to European Americans, asthma prevalence is higher in these populations, which also show a decreased response to asthma medications (14, 69, 119).

During the last decades, several loci have been associated with asthma and related traits in African Americans and Latinos/Hispanics using admixture mapping analysis as it has been reviewed by Mersha et al. (63) and Hernandez-Pacheco et al. (109). Additionally, two more admixture mapping analysis of asthma susceptibility and treatment response in African-admixed populations had been published by September 2018 (36, 120).

Spear et al. performed a genome-wide exploration in order to identify those genomic regions in which African ancestry is associated with response to asthma treatment with SABA in 949 African Americans. They found that local African ancestry at the 8p11 locus was suggestively associated with BDR in African

American children with asthma, though the result did not reach significance level after considering the multiple comparisons (β = 1.49, $p = 6.34 \times 10^{-4}$) (36).

Additionally, Gignoux et al. revealed that the risk linked to the 18q21 locus in the admixture mapping peak in Latinos/Hispanics was driven by the Native American ancestry (OR = 1.20, $p = 1.63 \times 10^{-3}$), whereas the European ancestry was protective (OR = 0.86, $p = 8.35 \times 10^{-3}$), which was validated in an independent Hispanic/Latino population. Interestingly, this peak is located within the intergenic region of *SMAD2* and *ZBTB7C*, none of which have been previously associated with any asthma-related trait, even in GWAS analyzing the same study populations (120–122), suggesting that admixture mapping is a powerful approach to identify novel asthma loci in admixed populations (120). The *SMAD2* gene encodes a cofactor involved in regulation of the growth factor β signaling that has been extensively evidenced to play a key role in asthma (69, 123, 124).

ASTHMA PREDICTION AND TRANSLATION INTO THE CLINIC

In the last 2 years, many asthma genes have been discovered and validated in independent populations, strongly supporting that these are generally involved in asthma pathogenesis. However, the genetic risk factors identified to date only represent a small proportion of total asthma heritability (22, 23, 125). Therefore, despite the uncountable advantages of the GWAS compared to previous strategies (23, 27, 126), there are a number of challenges ahead in order to better understand the genetic architecture of asthma (23, 126, 127).

One of the potential explanations of the current difficulty in explaining a larger proportion of the disease could be the reduced effect size of most genetic risks. Current reference panels and genotyping platforms are mainly designed to capture common genetic variants with are anticipated to show small effects in the disease (23, 114, 125). Therefore, the unassessed genomic variation could help to explain the missing heritability of asthma (114, 128).

Most GWAS of asthma have been limited in terms of low statistical power (23, 125, 127) due to limitations in the study design, mostly due to reduced sample sizes or the underrepresentation of genetically diverse populations, among others (20, 47, 48, 114, 129). A solution to this problem has been attempted in the last years with the emergence of large consortia gathering many asthma studies from different countries around the world (121, 122, 130, 131), which have contributed to increase the representation of patients from multiethnic populations (126, 127). However, this continuous need to increase sample sizes might have also led to heterogeneity in asthma definition by means of combination of samples with different asthma phenotypes. Consequently, this could have contributed to the reduction of the statistical power driven by the dilution of the effect size of association signals among different phenotypes (23, 125, 132–134). Thus, there is an increasing need to accurately characterize asthma patients through classification into homogenous groups (23, 134).

On the other hand, a limited number of large-scale studies have explored the role of gene-environment interactions in asthma despite robust evidence of the important contribution of environmental exposures in asthma susceptibility and severity (15, 16, 86, 135, 136). In fact, it has been evidenced underscoring the significant environmental contribution while designing a GWAS could result in reduced effect sizes (22, 137).

Last but not least, the functional implications of most asthma loci still remain unknown. Therefore, further studies are needed to increase our understanding of the impact of these on genes and cellular function, and their contribution on the molecular mechanisms underlying asthma pathophysiology (22). These have been proposed to be disentangled by means of approaches combining GWAS data with information related to biological pathways or processes (138, 139). Nonetheless, only one GWAS-based pathway enrichment analysis of asthma has been performed to date (140).

Because of all of this, our current knowledge of asthma genetics hampers our capacity to predict disease progression and treatment response, preventing its use in the clinical practice (125, 127). As a result, there is still a long way to use this knowledge and their integration with lifestyle and environment exposures (127, 141, 142) to develop precision medicine strategies for accurate prevention, diagnosis, or treatment of asthma (23).

Other Omic Studies and Integration of Multiomics

Other omics technologies, apart from genomics, are powerful tools to increase the current knowledge about asthma pathophysiology (143, 144). These are focused on data from a wide variety of biological sources: genomic modifications (epigenomics), gene transcription (transcriptomics), protein levels and chemical modifications (proteomics), endogenous and exogenous metabolites (metabolomics), and the microbiome (metagenomics), among others (23, 126, 127, 145, 146). The application of omics approximations in asthma is still incipient compared to other diseases (23, 147). Still, several studies have been performed in asthma in the last years as it was reviewed elsewhere (23, 127, 148, 149).

To our knowledge, a total of 26 asthma studies using other omics approaches have been published in the last 2 years (**Supplementary Table 3**). Just like recent GWAS of asthma, these studies have been equally focused on childhood and adulthood asthma since 2016. Moreover, most of them have been carried out in patients of European descent. A total of 18 studies focused on asthma susceptibility or severity (150–167); three focused on the ICS response (168–170); two explored the interactions with environmental factors (171, 172); and three inspected the overlap with other pulmonary diseases (173–175). Nonetheless, other experts have discussed the recent omics advances in asthma in this issue except for those of transcriptomics. Therefore, we focused on summarizing the main findings of studies made using this approach.

Transcriptomics provide a quantitative and qualitative characterization or RNA transcripts (176). These are mainly

focused on comparing gene expression levels in cells or tissues under specific controlled conditions in order to identify differentially expressed genes that could have (alone or in combination) functional implications on the disease under study (127, 177). Rapid development of technologies has made possible the near-complete characterization of the transcriptome, first using arrays and later, by means of RNA sequencing, which has greatly promoted the genome-wide exploration of transcriptomic changes in asthma during the last years (23, 127). A number of advantages of transcriptomics studies in asthma have been extensively described (23, 126). In fact, it has been proposed to be an accurate method to characterize pathways contributing to asthma pathophysiology, and the interactions with exogenous and endogenous factors in different sample types such as, blood, sputum or lung tissues, among others (23, 146).

Transcriptomics is a powerful tool to provide or confirm a mechanistic explanation of asthma loci identified by GWAS (23). Eight transcriptomic studies of asthma have been recently performed (**Supplementary Table 3**). However, most of them were carried out using arrays (157, 158, 167, 170–172) and the majority focused on European populations (157, 163, 170–172) and adults (157, 163, 171, 172, 175). Only three of them explored differential gene expression in children with asthma (158, 167, 170).

The largest transcriptomic study of childhood asthma performed in the last 2 years explored array-based gene expression levels of 133 asthma patients and 11 healthy controls of Asian ancestry (167) (**Supplementary Table 3**). RNA was extracted from a mixed population of T cells that were isolated from peripheral blood (167). Yeh et al. classified asthma patients into three groups based on 2,048 genes differentially expressed in immune cells. These groups showed distinct inflammatory profiles, including one that clustered the patients with higher neutrophil count and the poorest treatment control, suggesting that these could correspond to those patients with the most severe asthma status. When this group was compared with asthma patients included in other groups, 163 genes were found to be upregulated. Most of these genes encoded proteins involved in glucocorticoid signaling pathway and the immune response, suggesting that this could be an accurate method to classify asthma patients based on transcriptomic data (167). In transcriptomic studies of adulthood asthma, solid or liquid airway samples are frequently used such as, sputum or lung tissues (178, 179). However, clinical procedures to obtain these samples are quite invasive and are especially impractical in children (167). For this reason, peripheral blood has been regarded as the most suitable sample for the studies in children (23, 167).

Asthma diagnosis has classically relied just on conventional clinical guidelines and biomarkers for over decades (180), which are considered very inaccurate due to the wide variety of molecular mechanisms underlying the different asthma phenotypes (181, 182). Is in this respect where integrative approaches that combine complete clinical data and the omics sources could contribute to better characterize the biological processes underlying asthma pathophysiology (182, 183), ultimately helping to define asthma subtypes and to improve the prediction of severity and treatment response.

Multiomics approaches, which incorporate information from different omics levels, have been suggested as a promising strategy to fulfill that purpose (144) as they show an increased predictive capacity (166, 184). Five multiomics studies of asthma and related traits have been performed since 2016. Most of them have combined only a few omics levels (166, 185–188) (**Supplementary Table 3**).

Forno et al. conducted the largest multiomics study of asthma to date (186). They proposed a novel vertical approach to combine data from different omics levels (genomics, epigenomics, transcriptomics, and proteomics) with clinical information available for 1,127 Latino/Hispanic children, including 618 asthma patients and 509 children without asthma (**Supplementary Table 3**). Expression of 1,645 genes was associated with cytokine levels in blood, revealing the enrichment of the cytokine signaling pathway. From the 269 genes involved in this pathway, 41 were significantly associated with more than two asthma intermediate phenotypes. As a result, this list was reduced to the *IL5RA* gene, which was found to be the most significant association at the following steps (186). In fact, several transcription factors previously associated with pulmonary diseases showed evidence of association with *IL5RA* (189–191), suggesting that these could be involved in its signaling pathway. Furthermore, low plasma levels of IL-5Rα were found in children with asthma exacerbations, whereas children with earlier age of asthma onset showed increased levels of IL-5Rα, providing firm evidence of implication of *IL5RA* on asthma (186).

Studies as this one suggests that vertical approaches could be a suitable strategy to perform integrative multiomics studies of asthma and even other diseases. However, further validation in independent populations and other complex diseases is needed to confirm the applicability of this method (192). In this respect, a few omics studies of asthma treatment been performed to date, opening an opportunity to identify novel markers that could be applied in the design of precision medicine approaches in asthma and novel therapeutic strategies (67, 193). Although omics approaches have promisingly broken new ground in asthma research, translation into the clinic is still very challenging due to the large amount of information that is obtained.

Unexplored Genetic Variation in Asthma

Exploring non-coding variation has been also proposed as a promising strategy to disentangle the genetic basis of complex diseases (114) such as, microRNAs (miRNAs). These are short, non-coding and single-strand RNA molecules that interact with different genomic elements and regulate gene expression at transcriptional level (148, 184, 194). Interestingly, these are involved in the regulation of the stability of immune cells and the intensity of inflammation (194). In fact, miRNAs have been proposed as potential non-invasive asthma biomarkers that could be used for asthma diagnosis (195, 196). However, although some authors have suggested the implication of miRNAs on asthma susceptibility, severity, and exacerbations (195, 197, 198), there is a lack of studies that have extensively evaluated their role in asthma (197) and further studies are needed (199).

Structural variation, including copy number variations (CNVs), has been proposed to account for part of the missing heritability of complex traits (114, 200). These involve large chromosomal segments such as, duplications or deletions with consequences on regulation of gene expression (201). It has been reported that CNVs comprise 2% of the total genetic variation (202) with effects on approximately 12% of the human genome (203). This type of variation is enriched within protein-coding genes with functions related to immune response, suggesting its implication on disorders with a significant immunological component such as, asthma (204, 205). Although structural variation has been implicated on asthma, these is an insufficient number of studies to date (206). Some have found strong evidence of association of CNVs with asthma susceptibility (206, 207). Although this type of variation might contribute to an accumulation of mutations and allergic sensitization, leading to an increase of asthma susceptibility, CNVs do not seem to be the initial trigger of asthma development (208).

A substantial proportion of the genetic risk for common diseases could also be explained by variants that are at low frequency in the population (209, 210). The rarer the variant the more likely is for the variant to be population-specific (209, 210). Besides, the pathogenic potential of variants tends to accumulate in the lower range of allele frequency. Therefore, rare variants are more likely to be more structured in populations and to have larger effects on the disease (128, 209). As a corollary, rare variants will be underrepresented in reference datasets and, therefore, remain undetected by traditional GWAS. With this scenario in mind, many rare variants with large effects may be contributing to asthma and allergic diseases (128, 209, 211). However, their study will be only available for now applying sequencing-based methods instead of genotyping arrays. Irrespective of this, endogamous populations are especially appropriate to study the role of rare and low-frequency variation in asthma (210, 212) because rare pathogenic variants are predicted to increase their frequency in these populations (213, 214). Despite this, recent studies have also demonstrated the role of rare variants on recently admixed populations (209), whose inherent characteristics also increase the possibilities to uncover the contribution of rare and low-frequency variants on asthma (215).

Predicted loss-of-function (pLoF) variants, which are likely involved in disrupting protein-coding genes, show significant scientific and clinical interest due to their utility for clinical interpretation of sequencing data. In fact, pLoF variants have been suggested to allow direct identification of causal genes (216) and provide direct mechanistic implications of association effects (217). Although this type of variation has been extensively unexplored for over decades (218), Emdin et al. have recently revealed the potential role of these variants in asthma (217). They found evidence of association of pLoF variants located at well-known *IL33* and *GSDMB* asthma loci with lower risk of both asthma and allergic rhinitis (217). Interestingly, similar results have been found for protein-truncating variants in *IL33* and *GSDMB* (219). These have been predicted to shorten the coding sequence by inducing loss or gain-of-function effects (220). These findings also suggested that exploration of either pLoFs or protein-truncating variants could be another powerful tool for the identification of novel therapies for asthma (217, 219).

As mentioned, the main reason for the scarce evaluation of these types of genetic variation in asthma could be attributed to the fact that research strategies on asthma genetics have focused on using SNP genotyping platforms, which are suboptimal for inferring CNVs, and do not capture rare or pLoF variants (23, 29, 114, 125), as these would be optimally detected by means of sequencing approaches. Given that simultaneous sequencing of millions of small DNA fragments is currently possible at great speed and relatively low cost thanks to large improvements in next-generation sequencing (NGS) technologies (221, 222), the interest on the impact of these types of genetic variation in asthma will continue to rise.

Estimation of Polygenic Risk Scores

Another example of the large efforts to try to accelerate the progression toward precision medicine in complex traits is an emerging approach that takes GWAS results as the start point. This consists of stratification of the whole population based on estimates of individual's genetic disease susceptibility measured as polygenic risk scores (PRSs) (223). Just like other complex diseases, the genetic architecture of asthma is polygenic, where many genes contribute to disease development (224). Hence, the overall disease risk could be considered as the result of combined effects driven by common low-risk and rare large-risk variants (225). PRSs are the result of summing up risk alleles from thousand variants revealed by the GWAS (226). Even though most common variants show small effects, combining their effects could explain a significant proportion of the disease variability or at least allow classifying patients into discrete subgroups based on different levels of probabilistic disease risk (223, 226, 227).

Although multilocus profiles of genetic risks for asthma have been constructed using small sets of variants (101), large evaluations of PRSs are lacking for asthma. Nevertheless, previous studies that focused on other complex diseases (228–236) suggest that this approach could be fruitful for asthma. For instance, Khera et al. recently estimated PRSs for five common disorders with major public health impact, including coronary artery disease (236). They found 20-fold greater coronary artery disease risk using a PRSs involving many genetic variants than previous studies based on biomarkers or the mutation panels traditionally used in the clinical practice (236).

Uncountable utilities of PRSs have been demonstrated for the study of common diseases, suggesting its plausibility in a healthcare scenario (227). In fact, it has been proposed that PRS estimation could facilitate the development of accurate preventive, diagnostic and therapeutic strategies (223, 227, 236). Moreover, given the previous evidence of genetic overlap among different diseases (35, 45, 49, 103, 227, 237), an evaluation of individual risks could be assessed simultaneously for multiple traits at a time. This would potentiate the implementation of common therapeutic strategies for different diseases (227, 236). For all these reasons, calculation of PRS has been considered as a feasible approach to translate asthma research findings into healthcare practice for early disease detection (227).

There are many technical, economic, and sociopolitical barriers that should be overcome for the use of PRSs into clinical practice. By one hand, physicians would need additional training to correctly interpret and communicate PRSs to the patients (227). On the other hand, most current PRS estimates are based on loci that were mapped using designs with an overwhelming number of European patients. Therefore, their generalizability in populations of non-European ancestries are questionable (223, 236, 238) due to the large differences in terms of effect sizes, allele frequencies and linkage disequilibrium patterns. Besides this, most PRSs have been estimated in adults. Therefore, an evaluation of their usefulness in other age groups will be needed.

For asthma, the major limitation of PRSs is related to the reduced proportion of heritability explained by the loci identified to date. Given that PRS is a quantitative measure of the individual genetic risk, the more genetic variants are incorporated into the predictive disease risk model, the better the individuals are stratified into the risk subgroups (226). In this scenario, some studies suggest that whole-genome prediction models may account for the unknown genetic risks and, therefore, be able to improve the capacity to predict disease susceptibility, outcomes and treatment response, where the contribution of rare and low-frequency variants will be particularly relevant (223, 239).

On these terms, complex diseases could be comparable to rare disorders, where rare variants with large effect sizes provide disease risk in a small proportion of the population (127, 240). Large-scale sequencing studies will be required to further assess this idea (226, 240).

FUTURE PERSPECTIVES

Despite the large insight provided by GWAS and the admixture mapping scans during the last decades, it remains a large proportion of the missing heritability yet to be ascertained for asthma and related traits (22, 23, 125–127). The future of genetic research in asthma will be driven by NGS approaches, which are expected to significantly increase our knowledge of many other complex diseases (114, 126, 241).

The use of NGS technologies in pulmonary diseases is still emerging (242–246). More specifically, only a few asthma genetics studies have used NGS technologies (242, 243, 246) and large consortia studies are underway (247, 248). Because of its prohibitive costs for large population studies, several strategies have been proposed, such as sequencing the subjects from the extremes of the phenotype distribution (245, 246, 249) or the families where multiple individuals affected (250). The combination of NGS with conventional GWAS approaches has been suggested as another promising strategy (251).

Although the limited knowledge of genetic factors involved in asthma available to date hampers our current capacity to predict disease progression and treatment response (22, 23, 125), the use of genetic information to develop novel therapeutic targets is plausible. For instance, DeBoewe et al. recently found the association of protein-truncating variants with asthma located within widely known asthma susceptibility loci, such as IL33 or GSDMB. This reinforced the evidence suggesting the capacity of the genetic research to find potential asthma therapeutic targets. In fact, as a result of GWAS findings, several drugs targeting IL6R, IL-33, and TSLP are in development or are being evaluated in ongoing clinical trials investigating their efficacy to treat asthma and allergic diseases (22, 252, 253).

CONCLUSIONS

Our knowledge of asthma genetics has been greatly improved over the last decade because of GWAS, revealing a number of novel and firm common risk factors with small effects that overall explain a limited proportion of asthma heritability. Nonetheless, the improvements in high-throughput sequencing technologies and their anticipated cost reductions have the promise to accelerate the transition of this knowledge into the clinical practice and to progressively redirect the field toward an integrative multiomics perspective.

AUTHOR CONTRIBUTIONS

All the authors were involved in the conception, hypotheses delineation and structuration of this article, drafted the article, and approved the final version of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fped.2019.00006/full#supplementary-material

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Table S1. Summary of the GWAS of asthma and related traits published between 2016 and 2018.

Type of study	Trait	Definition of asthma	Phenotype tested	Population	Age group	Sample size (cases/controls)	Genome-wide associations [a]	Reference
GWAS	Asthma susceptibility	Physician diagnosis	Asthma status	Asian	Adults	4,063 (720/3,343)	No [b]	[1]
		Self-reported	Asthma status	Caucasian	Children/adults	6,889 (3,041/3,848)	No [b]	[2]
		Physician diagnosis	Asthma status	Caucasian	Children	9,329 (4,061/5,268)	No [b]	[3]
		Physician diagnosis	Asthma status	Caucasian	Children/adults	13,556	Yes	[4]
		Physician diagnosis/symptoms/ use of medication	Asthma status	Caucasian (D)/ African American (R)	Adults	10,995 (1,750/9,245)	Yes	[5]
		Physician diagnosis/symptoms	Asthma symptoms	Latino	Children	1,246	No [b]	[6]
		Physician diagnosis/symptoms	Asthma status	Latino	Children/adults	5,037 (2144/2,893)	Yes	[7]
		Physician diagnosis/symptoms/use of medication	Asthma status	Mixed (African, Caucasian, Asian, Latino)	Children/adults	142,486 (23,948/118538)	Yes	[8]
	Asthma treatment response	Physician diagnosis/symptoms	Change in FEV_1	Mixed (African American, Asian, Caucasian, Latino, Mixed)	Adults	2,672	No [b]	[9]
		Physician diagnosis	Asthma exacerbations, eosinophil counts, IgE levels	Caucasian	Adults	1,192	Yes	[10]
		Physician diagnosis/symptoms/use of medication	BDR	African American	Children	949	Yes	[11]

Table S1 (continuation). Summary of the GWAS of asthma and related traits published between 2016 and 2018.

Type of study	Trait	Definition of asthma	Phenotype tested	Population	Age group	Sample size (cases/controls)	Genome-wide associations [a]	Reference
GWAS	Overlap among asthma and allergic diseases	Physician diagnosis/symptoms/ use of medication	Asthma/allergic diseases status	Caucasian	Children/adults	360,838 (180,129/180,709)	Yes	[12]
		Physician diagnosis	Asthma/allergic diseases status	Caucasian	Children/adults	191,468 (57,245/134,223)	Yes	[13]
GWIS	Gene-environment interactions	Physician diagnosis	Asthma status	Caucasian	Adults	16,532 (1,690/14,842)	No	[14]
		Physician diagnosis	Asthma status	Caucasian	Children	3,136 (1,097/2,039)	No	[15]

[a] Association results with $p \leq 5 \times 10^{-8}$; [b] These results were not included in this review.
BDR: bronchodilator response; D: discovery phase; FEV_1: forced expiratory volume in 1 second; GWAS: genome-wide association study; GWIS: genome-wide interaction study; IgE: immunoglobin E; NA: not available; R: replication phase.

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Table S2. Summary results of the meta-analysis of asthma and allergic diseases published between 2016 and 2018.

Phenotypes	SNP	Chr. region [a]	Position [b]	Nearest gene(s)	Effect allele	OR [c,d]	*p*-value [d]	Reference
Asthma/hay fever/eczema/ rhinitis	rs12743520	1p22	93037112	*PIGX* [f]	C	0.93 [e]	3.83×10^{-8} [e]	[1]
	rs301805	1p36	8481016	*RERE* [g]	G	0.96 [e]	6.43×10^{-9} [e]	
	rs742230	1p36	25251424	*RUNX3* [g]	A	0.96 [e]	1.02×10^{-8} [e]	
	rs61816766	1q21	152319572	*FLG-AS1*	T	1.14 [e]	4.63×10^{-12} [e]	
	rs61815704	1q21	152893891	*IVL-SPRR2E* [f]	C	1.14 [e]	5.16×10^{-9} [e]	
	rs1214598	1q24	167426424	*RP11-104L21.2* [f]	G	0.95 [e]	5.14×10^{-11} [e]	
	rs10174949	2p25	8442248	*LINC00299* [g]	G	0.94 [e]	1.70×10^{-16} [e]	
	rs72823641	2q12	102936159	*IL1RL1*	T	0.89 [e]	1.58×10^{-27} [e]	
	rs34290285	2q37	242698640	*D2HGDH* [g]	G	0.93 [e]	5.17×10^{-17} [e]	
	rs4916533	3q29	196373582	*LRRC33* [f]	C	0.93 [e]	1.66×10^{-8} [e]	
	rs28393318	4p14	38784267	*TLR10*	A	0.92 [e]	2.14×10^{-19} [e]	
	rs56267605	4q27	123363109	*ADAD1-IL2* [f]	A	1.05 [e]	2.56×10^{-12} [e]	
	rs6881270	5p13	35879095	*IL7R*	C	0.91 [e]	1.53×10^{-14} [e]	
	rs7705653	5q22	110142816	*TSLP*	A	1.14 [e]	1.12×10^{-19} [e]	
	rs1837253	5q22	110401872	*TSLP*	C	0.93 [e]	4.38×10^{-21} [e]	
	rs2548992	5q31	131808668	*C5orf56* [g]	G	1.10 [e]	4.54×10^{-10} [e]	
	rs2706362	5q31	131925187	*RAD50*	T	1.06 [e]	3.75×10^{-11} [e]	
	rs10074523	5q31	132060583	*KIF3A*	A	1.10 [e]	2.37×10^{-10} [e]	
	rs9273374	6p21	32626614	*HLA-DQB1-AS1*	G	0.84 [e]	7.87×10^{-35} [e]	
	rs6461503	7p21	20560996	*ABCB5* [f]	C	1.05 [e]	3.19×10^{-10} [e]	
	rs2136016	8q21	81300681	*TPD52-ZBTB10*	A	1.09 [e]	2.11×10^{-9} [e]	
	rs9775039	9p24	6177453	*IL33*	G	1.18 [e]	4.42×10^{-22} [e]	
	rs2169282	9p24	6350235	*TPD52L3* [f]	G	1.09 [e]	1.80×10^{-10} [e]	
	rs10795656	10p14	8595839	*GATA3-CELF2*	G	1.05 [e]	4.07×10^{-10} [e]	
	rs12413578	10p14	9049253	*SLC7A10* [f]	C	0.91 [e]	1.09×10^{-14} [e]	
	rs61839660	10p15	6094697	*IL2RA* [g]	C	1.12 [e]	2.30×10^{-11} [e]	
	rs7936070	11q13	76293527	*LRRC32*	G	1.08 [e]	2.81×10^{-28} [e]	
	rs659529	11q23	111436896	*LAYN*	A	0.95 [e]	6.03×10^{-11} [e]	
	rs10876864	12q13	56401085	*SUOX* [f]	A	1.05 [e]	1.41×10^{-13} [e]	
	rs1059513	12q13	57489709	*STAT6* [g]	T	0.92 [e]	7.65×10^{-13} [e]	
	rs8008961	14q24	68752643	*RAD51B* [g]	C	1.05 [e]	1.24×10^{-8} [e]	
	rs56062135	15q22	67455630	*SMAD3* [g]	C	1.16 [e]	1.56×10^{-22} [e]	
	rs36045143	16p13	11224966	*CLEC16A*	A	0.93 [e]	1.83×10^{-21} [e]	
	rs869402	17q12	38068043	*GSDMB* [g]	T	1.12 [e]	4.15×10^{-17} [e]	
	rs9911533	17q21	38775476	*SMARCE1* [f]	T	0.92 [e]	9.70×10^{-16} [e]	
	rs10414065	19q13	33721455	*SLC7A10-CEBPA* [f]	C	0.91 [e]	2.63×10^{-10} [e]	
	rs2766664	20q13	52171241	*RP4-724E16.2* [f]	G	1.08 [e]	8.07×10^{-11} [e]	
	rs3208007	20q13	62322288	*RTEL1* [g]	C	0.95 [e]	3.22×10^{-10} [e]	

Table S2 (continuation). Summary results of the meta-analysis of asthma and allergic diseases published between 2016 and 2018.

Phenotypes	SNP	Chr. region [a]	Position [b]	Nearest gene(s)	Effect allele	OR [c,d]	*p*-value [d]	Reference
Asthma/hay fever/eczema	rs10883723	1p34	104225832	*C10orf95-ACTR1A*	C	1.03	1.60×10^{-8}	[2]
	rs10033073	1p36	4775401	*EVI5* [f]	G	1.04	1.20×10^{-10}	
	rs10876864	1p36	56401085	*SUOX-IKZF4*	G	1.05	1.40×10^{-19}	
	rs1057258	1p36	234115629	*INPP5D* [f]	C	1.05	1.40×10^{-10}	
	rs10174949	1q21	8442248	*LINC00299* [g]	G	1.07	7.30×10^{-31}	
	rs10414065	1q21	33721455	*SLC7A10-CEBPA* [f]	C	1.10	6.10×10^{-18}	
	rs1048990	1q21	35761675	*PSMA6*	G	1.04	1.00×10^{-8}	
	rs10865050	1q21	102941311	*IL18R1*	G	1.13	7.00×10^{-61}	
	rs10663129	1q21	141321836	*RASA2* [f]	ACT	1.04	1.10×10^{-13}	
	rs10486391	1q23	20376018	*ITGB8*	A	1.03	6.80×10^{-9}	
	rs10519067	1q24	61068347	*RORA*	G	1.06	9.30×10^{-13}	
	rs10068717	1q24	141494934	*NDFIP1*	T	1.04	4.80×10^{-15}	
	rs1064213	1q25	198950240	*PLCL1*	G	1.04	5.40×10^{-12}	
	rs10760123	1q42	123650534	*PHF19-TRAF1* [f]	T	1.03	5.20×10^{-9}	
	rs11255968	2p14	8936162	*GATA3-SFTA1P*	C	1.09	7.70×10^{-9}	
	rs10910095	2p25	2510755	*TNFRSF14-FAM213B* [f]	G	1.04	2.70×10^{-8}	
	rs11169225	2q12	50345671	*AQP2* [f]	A	1.05	1.20×10^{-11}	
	rs11204896	2q12	151796742	*RORC*	C	1.06	2.40×10^{-12}	
	rs111914382	2q13	38097001	*FOXA1-TTC6*	TG	1.04	8.10×10^{-9}	
	rs112401631	2q13	38764524	*CCR7-SMARCE1*	A	1.26	2.20×10^{-26}	
	rs1143633	2q13	113590467	*IL1B*	C	1.03	1.70×10^{-10}	
	rs11464691	2q22	38770641	*CCR7-SMARCE1*	TA	1.05	1.40×10^{-21}	
	rs1102705	2q33	172700868	*FASLG-TNFSF18*	G	1.06	3.10×10^{-10}	
	rs11236814	2q36	76343428	*WNT11-LRRC32*	A	1.07	4.00×10^{-14}	
	rs11255753	2q37	8605553	*GATA3-SFTA1P*	T	1.04	2.00×10^{-12}	
	rs10947428	2q37	33647058	*ITPR3*	C	1.05	3.50×10^{-13}	
	rs12470864	3p13	102926362	*IL1RL2-IL18R1*	A	1.06	4.20×10^{-26}	
	rs12551834	3p22	131613191	*C9orf114-LRRC8A*	G	1.06	3.00×10^{-9}	
	rs11652139	3q12	38149033	*PSMD3*	A	1.05	7.50×10^{-22}	
	rs12596613	3q13	11491007	*RMI2-LITAF*	C	1.03	6.10×10^{-9}	
	rs11644510	3q23	11277358	*CLEC16A-RMI2*	C	1.07	6.10×10^{-38}	
	rs12413578	3q27	9049253	*GATA3-SFTA1P*	C	1.10	1.30×10^{-27}	
	rs12365699	3q27	118743286	*DDX6-CXCR5*	G	1.06	5.10×10^{-18}	
	rs12440045	3q28	41782684	*RTF1-ITPKA* [f]	C	1.03	4.90×10^{-10}	
	rs12123821	3q28	152179152	*RPTN-HRNR*	T	1.11	6.80×10^{-17}	
	rs13088318	3q29	101242751	*FAM172BP-TRMT10C* [f]	A	1.03	8.60×10^{-9}	
	rs144829310	4p14	6208030	*RANBP6-IL33*	T	1.09	1.20×10^{-35}	
	rs13153019	4p16	176782218	*LMAN2-RGS14* [f]	C	1.04	1.30×10^{-8}	
	rs13403656	4q24	112269127	*BCL2L11-ANAPC1* [f]	A	1.05	2.20×10^{-8}	
	rs1444789	4q27	9064361	*GATA3-SFTA1P*	C	1.07	1.50×10^{-22}	

Table S2 (continuation). Summary results of the meta-analysis of asthma and allergic diseases published between 2016 and 2018.

Phenotypes	SNP	Chr. region [a]	Position [b]	Nearest gene(s)	Effect allele	OR [c,d]	*p*-value [d]	Reference
	rs13384448	4q27	228707862	*CCL20-DAW1* [f]	T	1.04	2.80×10^{-12}	
	rs227275	5p13	103593898	*MANBA* [f]	C	1.03	3.70×10^{-11}	
	rs2228145	5p13	154426970	*IL6R*	C	1.04	4.30×10^{-13}	
	rs16922576	5p15	5064193	*JAK2*	C	1.04	3.20×10^{-10}	
	rs2507978	5p21	31351664	*HLA-B-MICA*	G	1.04	1.20×10^{-10}	
	rs17664743	5q22	50253897	*C7orf72-IKZF1* [f]	A	1.04	6.20×10^{-11}	
	rs2104047	5q22	68754417	*RAD51B* [g]	T	1.04	1.60×10^{-13}	
	rs2070901	5q22	161185058	*NDUFS2-FCER1G*	T	1.04	1.30×10^{-11}	
	rs17607589	5q22	188402586	*LPP*	C	1.05	1.80×10^{-14}	
	rs1814576	5q23	110159879	*SLC25A46-TSLP*	C	1.12	1.40×10^{-22}	
	rs2025758	5q31	8841669	*GATA3-SFTA1P*	T	1.04	4.70×10^{-15}	
	rs2134814	5q31	90987512	*BACH2*	C	1.05	1.70×10^{-17}	
	rs250308	5q31	118684297	*TNFAIP8* [f]	T	1.03	4.00×10^{-9}	
	rs150254607	5q31	123454110	*IL2-IL21*	ATAT	1.08	4.60×10^{-14}	
	rs2030030	5q31	187793833	*BCL6-LPP-AS2*	T	1.04	1.00×10^{-8}	
	rs1837253	5q34	110401872	*SLC25A46-TSLP*	C	1.07	1.60×10^{-31}	
	rs16903574	5q35	14610309	*FAM105A*	G	1.07	1.40×10^{-12}	
	rs2854001	6p21	31323012	*HLA-B*	A	1.06	1.20×10^{-19}	
	rs28895016	6p21	31574525	*NCR3-AIF1*	C	1.10	9.40×10^{-16}	
Asthma/hay fever/eczema	rs2893907	6p21	64382359	*ZNF365*	C	1.03	1.80×10^{-9}	[2]
	rs2910162	6p21	159909345	*MIR3142-MIR146A* [f]	G	1.03	2.50×10^{-9}	
	rs2988277	6p21	167431352	*CD247*	C	1.04	4.00×10^{-14}	
	rs34004019	6p22	32626403	*HLA-DQA1-HLA-DQB1*	A	1.10	3.80×10^{-52}	
	rs2766678	6q15	52208356	*ZNF217*	G	1.06	5.00×10^{-18}	
	rs34290285	6q21	242698640	*D2HGDH* [g]	G	1.08	4.00×10^{-33}	
	rs301806	6q22	8482078	*RERE* [g]	T	1.05	1.80×10^{-20}	
	rs3091307	6q23	131989136	*RAD50-IL13*	G	1.06	3.60×10^{-21}	
	rs343478	6q25	6051399	*RANBP6-IL33*	G	1.03	2.60×10^{-10}	
	rs3097670	6q27	33046752	*HLA-DPA1*	G	1.06	7.70×10^{-12}	
	rs35469349	7p12	128294709	*PTPRK* [f]	A	1.04	2.30×10^{-10}	
	rs4090390	7p15	173146921	*TNFSF18-TNFSF4*	A	1.05	1.30×10^{-15}	
	rs3787184	7p21	50157837	*NFATC2*	A	1.05	1.10×10^{-12}	
	rs3540	7p21	91045408	*IQGAP1* [f]	G	1.04	3.30×10^{-11}	
	rs3749833	7q11	131799626	*C5orf56* [g]	C	1.04	3.30×10^{-11}	
	rs4296977	8q21	77018542	*GSAP* [f]	C	1.06	2.10×10^{-13}	
	rs4145717	8q24	123316076	*ADAD1*	T	1.06	9.20×10^{-27}	
	rs4747846	9p24	6074451	*IL2RA* [g]	C	1.04	1.00×10^{-11}	
	rs4801001	9p24	52336175	*DYNAP-RAB27B* [f]	T	1.03	5.90×10^{-9}	
	rs479844	9p24	65551957	*AP5B1-OVOL1*	G	1.04	1.60×10^{-13}	
	rs4574025	9q33	60009814	*TNFRSF11A* [f]	T	1.03	6.80×10^{-9}	

Table S2 (continuation). Summary results of the meta-analysis of asthma and allergic diseases published between 2016 and 2018.

Phenotypes	SNP	Chr. region [a]	Position [b]	Nearest gene(s)	Effect allele	OR [c,d]	*p*-value [d]	Reference
Asthma/hay fever/eczema	rs4671601	9q34	64836267	*LOC339807* [f]	C	1.04	8.80×10^{-9}	[2]
	rs4943794	10p14	41173408	*FOXO1* [f]	C	1.04	7.20×10^{-12}	
	rs5758343	10p14	41816652	*TEF-TOB2* [f]	A	1.05	4.80×10^{-14}	
	rs55726902	10p14	48196982	*HDAC7* [f]	G	1.05	2.60×10^{-16}	
	rs55646091	10p14	76299431	*WNT11-LRRC32*	A	1.18	2.30×10^{-40}	
	rs5029937	10p14	138195151	*TNFAIP3*	G	1.08	2.40×10^{-8}	
	rs519973	10p14	187633268	*BCL6-LPP-AS2*	A	1.03	4.50×10^{-10}	
	rs5743618	10p15	38798648	*TLR1*	C	1.10	3.30×10^{-58}	
	rs56375023	10p15	67448363	*SMAD3* [g]	A	1.07	8.20×10^{-32}	
	rs56129466	10q21	128158189	*KIRREL3-AS3-ETS1*	A	1.05	1.90×10^{-13}	
	rs4848612	10q24	112388538	*BCL2L11-ANAPC1* [f]	A	1.04	2.30×10^{-10}	
	rs6461503	11q13	20560996	*ITGB8-ABCB5*	T	1.04	1.70×10^{-14}	
	rs61192126	11q13	72394852	*LINC00870-RYBP* [f]	T	1.04	8.90×10^{-11}	
	rs59593577	11q13	95425526	*SESN3-FAM76B* [f]	C	1.05	1.60×10^{-11}	
	rs60946162	11q13	188133336	*LPP*	T	1.04	8.60×10^{-15}	
	rs61839660	11q21	6094697	*IL2RA* [g]	T	1.08	4.40×10^{-19}	
	rs6011033	11q23	62322699	*RTEL1* [g]	G	1.05	3.50×10^{-14}	
	rs63406760	11q23	123742692	*C12orf65-CDK2AP1*	T	1.05	3.00×10^{-13}	
	rs61816761	11q24	152285861	*FLG*	A	1.22	7.40×10^{-21}	
	rs6776757	12q13	33069091	*GLB1*	G	1.03	3.10×10^{-10}	
	rs1059513	12q13	57489709	*STAT6* [g]	T	1.08	1.00×10^{-22}	
	rs6594499	12q13	110470137	*WDR36-CAMK4*	C	1.08	4.60×10^{-46}	
	rs6489785	12q13	121363724	*SPPL3-HNF1A-AS1* [f]	T	1.04	1.60×10^{-15}	
	rs6977955	12q24	28156887	*JAZF1* [f]	T	1.05	7.10×10^{-13}	
	rs6869502	12q24	110166083	*SLC25A46-TSLP*	T	1.08	6.40×10^{-29}	
	rs697852	12q24	226914734	*ITPKB* [f]	A	1.04	1.60×10^{-9}	
	rs6990534	13q14	128814091	*MYC*	A	1.04	6.40×10^{-14}	
	rs7130753	13q22	111470567	*LAYN-SIK2* [f]	C	1.05	7.00×10^{-15}	
	rs71368508	14q13	4521473	*SMTNL2-ALOX15* [f]	C	1.12	2.00×10^{-9}	
	rs7137828	14q21	111932800	*ATXN2* [f]	T	1.03	2.20×10^{-10}	
	rs7207591	14q24	40414862	*STAT5B* [f]	A	1.04	1.40×10^{-9}	
	rs72033857	14q24	167390671	*RNASET2-MIR3939* [f]	C	1.06	1.20×10^{-9}	
	rs7214661	14q32	43430696	*MAP3K14-ARHGAP27* [f]	G	1.03	1.20×10^{-8}	
	rs73205303	15q15	36467830	*RUNX1* [f]	A	1.04	7.90×10^{-10}	
	rs72782676	15q22	9032555	*GATA3-SFTA1P*	C	1.30	3.20×10^{-11}	
	rs74847330	15q22	143831599	*KYNU-ARHGAP15* [f]	A	1.05	1.80×10^{-9}	
	rs740474	15q26	140925362	*DIAPH1*	C	1.03	5.60×10^{-11}	
	rs75557865	16p13	121652141	*SLC15A2* [f]	G	1.03	1.60×10^{-8}	
	rs7512552	16p13	150265704	*C1orf54-MRPS21*	C	1.03	1.40×10^{-9}	
	rs7714574	17p13	40492655	*DAB2-PTGER4*	T	1.03	5.90×10^{-10}	

Table S2 (continuation). Summary results of the meta-analysis of asthma and allergic diseases published between 2016 and 2018.

Phenotypes	SNP	Chr. region [a]	Position [b]	Nearest gene(s)	Effect allele	OR [c,d]	*p*-value [d]	Reference
Asthma/hay fever/eczema	rs7936323	17q12	76293758	*WNT11-LRRC32*	A	1.09	2.20×10^{-63}	[2]
	rs760805	17q21	25251923	*RUNX3* [g]	T	1.04	6.40×10^{-13}	
	rs76167968	17q21	35681738	*SFPQ-ZMYM4* [f]	T	1.06	1.30×10^{-8}	
	rs7717955	17q21	35862841	*IL7R*	C	1.07	9.10×10^{-36}	
	rs76081789	17q21	44846426	*SIK1* [f]	T	1.07	1.30×10^{-8}	
	rs7824394	17q21	81292599	*MIR5708-ZBTB10*	A	1.05	3.50×10^{-20}	
	rs80064395	17q21	196372546	*FBXO45-CEP19*	C	1.07	1.60×10^{-12}	
	rs921650	18q21	38069076	*GSDMB* [g]	A	1.06	5.70×10^{-30}	
	rs848	18q21	131996500	*IL13*	A	1.07	1.50×10^{-24}	
	rs9259819	19q13	29893575	*HLA-J*	G	1.04	2.40×10^{-9}	
	rs9323612	20q13	75968608	*JDP2-BATF* [f]	A	1.03	8.60×10^{-9}	
	rs9372120	20q13	106667535	*ATG5* [f]	G	1.04	4.20×10^{-11}	
	rs9383820	20q13	157419508	*ARID1B* [f]	C	1.04	1.20×10^{-8}	
	rs9889262	21q22	47398070	*ZNF652*	A	1.04	9.70×10^{-16}	
	rs9573092	21q22	73627275	*PIBF1-KLF5* [f]	A	1.03	2.70×10^{-8}	
	rs9989163	22q13	103235012	*RCOR1-TRAF3* [f]	A	1.03	1.90×10^{-8}	

[a] Chromosomal region; [b] Positions based on GRCh37/hg19 build; [c] Odds ratio for the effect alleles; [d] Association results of the meta-analysis; [e] Association results of the discovery phase; [f] Novel locus (no previous evidence of association with asthma); [g] Loci identified by both studies.

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Table S3. Summary of the omics studies except for genomics published between 2016 and 2018.

Type of study	Trait	Definition of asthma	Phenotype tested	Strategy (sample type)	Population	Age group	Sample size (cases/controls)	Reference
EWAS	Asthma susceptibility	Physician diagnosis/symptoms/ use of medication	Asthma status/symptoms	DNA microarray (blood)	Caucasian	Children	3,751 (842/2,909)	[1]
	Asthma susceptibility	Physician diagnosis	Asthma status	DNA microarray (nasal brushing)	Caucasian	Children	58 (29/29)	[2]
	Asthma susceptibility	Physician diagnosis/symptoms/ use of medication	Asthma status	DNA microarray (blood)	Caucasian	Children	4,904 (655/4,249)	[3]
Metabolomics	Asthma susceptibility	Physician diagnosis/symptoms/ use of medication	Asthma status	LC-MS (blood)	Latino	Children	100 (50/50)	[4]
	Asthma susceptibility	Self-reported/clinical records	Asthma severity	LC-HRMS (blood)	NA	Adults	76 (54/22)	[5]
	Asthma susceptibility	Physician diagnosis/ exacerbations/symptoms	Asthma severity	LC-MS (blood)	Latino	Children	380	[6]
	Overlap with other diseases	Physician diagnosis/symptoms/ use of medication	COPD/asthma severity	NMR spectroscopy (exhaled breath condensate)	NA	Adults	75	[7]
	Asthma susceptibility	Physician diagnosis/ use of medication	Asthma status	MS (blood)	NA	Children	237 (46/191)	[8]
	Asthma susceptibility	Physician diagnosis/symptoms	Age of asthma-onset	UPLC-mass spectrometry (urine)	Caucasian	Children	45 (32/13)	[9]
	Asthma susceptibility	Physician diagnosis	Asthma status	Flow cytometry, fluorescence imaging (BAL fluid)	NA	Adults	20 (11/9)	[10]
	Asthma susceptibility	Physician diagnosis/symptoms/ use of medication	Asthma status	NMR spectroscopy (urine)	Asian	Children	60 (30/30)	[11]

Table S3 (continuation). Summary of the omics studies except for genomics published between 2016 and 2018.

Type of study	Trait	Definition of asthma	Phenotype tested	Strategy (sample type)	Population	Age group	Sample size (cases/controls)	Reference
Metagenomics	Asthma susceptibility	Physician diagnosis	Asthma severity	V3-V5 16S rRNA sequencing (sputum)	Caucasian	Adults	56 (44/12)	[12]
	Asthma treatment response	Lung function	Atopic asthma status	V4 16S rRNA sequencing (oral wash, bronchial brushing)	NA	Adults	84 (63/21)	[13]
	Asthma susceptibility	Physician diagnosis	Asthma severity/ inflammatory type	V3-V5 16S rRNA sequencing (sputum)	Asian	Adults	64 (49/15)	[14]
	Asthma treatment response	Symptoms	Asthma status	V1-V3 16S rRNA sequencing (BAL fluid)	Caucasian	Young adults	19 (13/6)	[15]
	Asthma susceptibility	NA	Asthma status	V4 16S rRNA sequencing (feces)	Caucasian	Children	690	[16]
Multiomics	Asthma susceptibility	Physician diagnosis/ use of medication	Asthma status	DNA microarray (genotyping, methylation), RNA-seq (bronchial brushing)	Mixed (African American, Caucasian, Other)	Adults	85 (58/27)	[17]
	Gene-environment interactions	Physician diagnosis/ symptoms	Asthma status, asthma-intermediate phenotypes, cytokine levels	DNA microarray (genotyping, methylation), immunoassay, RNA microarray (blood)	Latino	Children	1,127 (618/509)	[18]
	Gene-environment interactions	Physician diagnosis/ symptoms	Smoking status	Aptamer-based proteomics assay, RNA microarray (blood, bronchial biopsies, bronchial brushing, sputum)	Caucasian	Adults	88 (70/18)	[19]
	Gene-environment interactions	Physician diagnosis/ use of medication	Asthma status	ChIP-seq, RNA-seq (blood)	Caucasian	Children	48 (32/16)	[20]
	Asthma susceptibility	Physician diagnosis/ exacerbations/symptoms	FEV_1, FEV_1/FVC	DNA microarray, RNA microarray, mass spectrometry (blood)	Latino	Children	325	[21]

Table S3 (continuation). Summary of the omics studies except for genomics published between 2016 and 2018.

Type of study	Trait	Definition of asthma	Phenotype tested	Strategy (sample type)	Population	Age group	Sample size (cases/controls)	Reference
Proteomics	Asthma susceptibility	Physician diagnosis	Asthma subtypes	HPLC/LC-MS (blood, sputum)	Asian	Adults	117 (87/30)	[22]
	Overlap among asthma and allergic diseases	NA	Pulmonary disease status	Immunoblotting, LC-MS (BAL fluid)	Caucasian	Children	12 (6/6)	[23]
Transcriptomics	Gene-environment interactions	Physician diagnosis/symptoms	Asthma severity	RNA microarray (blood)	Caucasian	Adults	498 (411/87)	[24]
	Gene-environment interactions	Physician diagnosis/symptoms	Asthma severity	RNA microarray (sputum)	Caucasian	Adults	418	[25]
	Asthma susceptibility	Several definitions (lung function, symptoms	Asthma severity	RNA microarray (BAL fluid, bronchial brushing)	Caucasian	Adults	65 (46/19)	[26]
	Overlap among asthma and allergic diseases	Physician diagnosis	CARAS status	RNA microarray (blood)	Asian	Adults	6	[27]
	Asthma susceptibility	Physician diagnosis	Asthma status	RNA microarray (blood)	Mixed (African American/ Caucasian/Latino)	Children	99	[28]
	Asthma treatment response	Physician diagnosis	Change in FEV_1	RNA microarray (blood)	Caucasian	Children	95 (48/47)	[29]
	Asthma susceptibility	Uncontrolled asthma [a]	Asthma status	RNA-seq (bronchial brushing)	Caucasian	Adults	NA	[30]
	Asthma susceptibility	Physician diagnosis	Asthma severity	RNA microarray (blood)	Asian	Children	144 (133/11)	[31]

[a] Asthma Control Questionnaire score≥1.5.

BAL: bronchoalveolar lavage; CARAS: combined allergic rhinitis and asthma syndrome; ChIP-seq: chromatin immunoprecipitation sequencing; COPD: chronic obstructive pulmonary disease; FEV_1: forced expiratory volume in 1 second; FVC: forced vital capacity; HPLC: high performance liquid chromatography; LC-HRMS: liquid chromatography-high-resolution mass spectrometry; LC-MS: liquid chromatography-tandem mass spectrometry; MS: multi-platform mass spectrometry; NA: not available; NMR: nuclear magnetic resonance; RNA-seq: RNA sequencing; rRNA: ribosomal RNA; UPLC: ultra-performance liquid chromatography.

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3.2. Genome-wide association study of asthma exacerbations despite ICS use in admixed populations

ICS are the most effective and commonly prescribed medication to control asthma symptoms and prevent exacerbations. Nonetheless, high variability in the response to this medication has been described among individuals and populations, evidencing the important contribution of the individual's genetic composition to the response. To date, only a few genetic markers of ICS response have been identified and mostly in European and Asian populations. In this *Chapter*, a GWAS of asthma exacerbations was performed in 1,347 admixed (Hispanic/Latino and African American) children and youth treated with ICS. Genetic variants shared among populations were explored through the validation of suggestive associations in 1,697 children and youth with asthma of European ancestry. Moreover, loci of ICS response identified by previous GWAS of ICS response were followed up for replication in the admixed populations.

A total of 15 variants were suggestively associated with asthma exacerbations despite ICS use in asthma patients of admixed ancestry ($p \leq 5x10^{-6}$). From these, one variant in the intergenic region of *APOBEC3B-APOBEC3C* showed nominal evidence of replication with asthma exacerbations in European asthma patients treated with ICS. This association was also validated with the improvement in lung function after six weeks of ICS therapy in one of the studies in Europeans analyzed. Additionally, the intergenic region of *L3MBTL4* and *ARHGAP28*, a potential locus of ICS response identified by previous GWAS, was found associated with asthma exacerbations despite ICS treatment in Hispanic/Latino and African American children and young adults. This is one of the few studies evaluating the genetic factors involved in ICS response in asthma patients from admixed populations, whose results suggest the existence of genetic variation shared among different populations.

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ORIGINAL ARTICLE

Asthma and Rhinitis

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

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Summary

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), analysing 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

1 | 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.

2 | METHODS

2.1 | Study populations

A total of eight independent studies participating in the Pharmacogenomics in Childhood of Asthma (PiCA) consortium [17] were analysed 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).

2.1.1 | 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 analysed 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).

2.1.2 | 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 analysed 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.

2.2 | 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.

2.3 | 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) $\geq$ 1% and with imputation quality (Rsq) $\geq$ 0.3 in GALA II and SAGE, and shared among both populations, were meta-analysed 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 $\leq 5 \times 10^{-6}$ 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.

2.4 | 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 $\leq$ 0.05 in a meta-analysis of all the European studies and consistent directions of effects in both discovery and validation populations.

2.5 | 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 $\geq$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.

2.6 | 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]

2.7 | 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 analysed 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 α = 0.05/number of independent variants.

3 | RESULTS

3.1 | Characteristics of the study populations

The characteristics of the 1347 admixed asthmatic patients from GALA II and SAGE analysed 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 exacerbations 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 admixed populations analysed in the discovery phase

	GALA II (n = 854)	SAGE (n = 493)
Gender (% male)	57.3	54.2
Mean age ± SD (y)	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.

3.2 | 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 $\leq 5 \times 10^{-8}$) 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 $\leq 5 \times 10^{-6}$) 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.

3.3 | 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

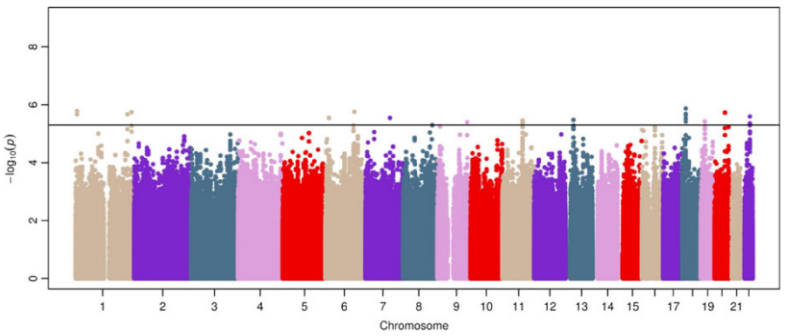

FIGURE 1 Manhattan plot of association results of ICS response in the discovery phase. Association results are represented as -$\log_{10}$ *P*-value on the *y*-axis along the chromosomes (*x*-axis). The suggestive significance threshold for replication is indicated by the black line ($P \leq 5 \times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

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).

3.4 | 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.

3.5 | *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 monomethylation (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]

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.
The significant replication result is shown in bold.
[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-effects model was applied since heterogeneity was found between admixed/European populations.

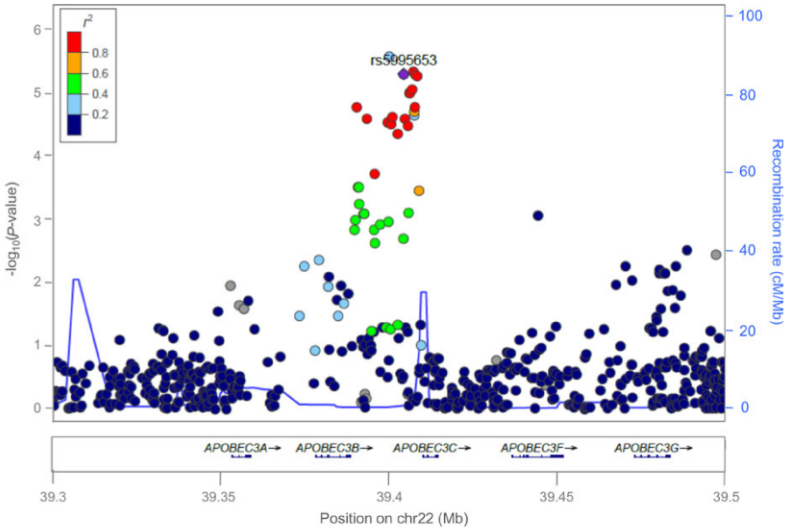

FIGURE 2 Regional plot of association results in the discovery phase for the *APOBEC3B-APOBEC3C* intergenic region, which represents a novel association with ICS response. Statistical significance of association results ($-\log_{10}$ *P*-value) (*y*-axis) is represented by chromosome position (*x*-axis) 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 colour-coded based on their LD with this SNP, indicated by pairwise r^2 values for American populations from the 1KGP

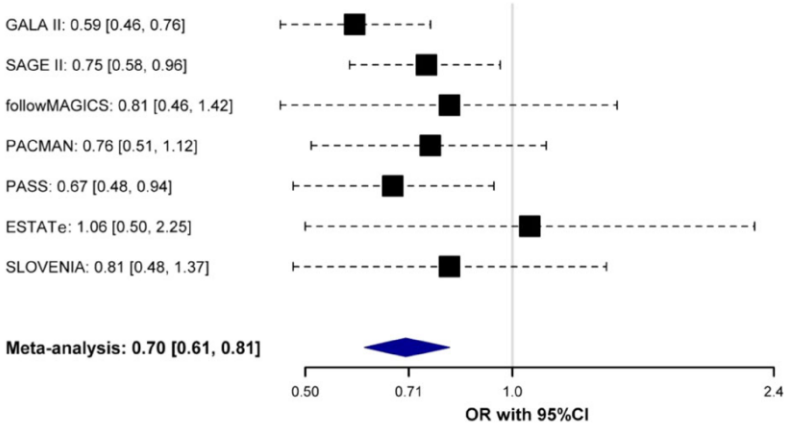

FIGURE 3 Forest plot of association effect of rs5995653 across studies. Odds ratio (OR) for the effect allele (A) is shown for each study and after combining them by black boxes and a blue diamond. Black dash lines indicate the corresponding 95% confidence intervals (95% CI) for each individual study

3.6 | 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 analysed 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, $P = 1.57 \times 10^{-5}$).

4 | 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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CONFLICT OF INTEREST

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The rest of authors have no conflict of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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SUPPORTING INFORMATION

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

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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 a physician's diagnosis of asthma. During the follow-up phase of the same children and young adults (followMAGICS), now aged 7 to 25 years, the 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 adrenal suppression. Children and young adults aged 5 to 18 years old with a clinical diagnosis of asthma, inhaled corticosteroids (ICS) therapy under pediatric supervision, and clinical concern about adrenal suppression were recruited from the United Kingdom. A detailed description of 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 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 the 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 a physician diagnosis of asthma recruited at primary and secondary care units from the United Kingdom. A detailed clinical history and, demographic and anthropometric information were 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 the 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 individuals 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 100,000 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 the 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 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 the availability of information related to the presence/absence of severe asthma exacerbations and medication use. After QC, a total of 487,050 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 526,121 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 the availability of clinical information. Furthermore, a total of 176,412 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 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 560,996.

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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 African-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}
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}

[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 S2 (continuation). Summary of the SNPs suggestively associated with asthma exacerbations in African-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
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. Summary of the conditional regression models for each gene region with suggestive associations in African-admixed populations.

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}		NA
	rs3789494	1	6370476	0.55 (0.43 – 0.70)	2.14×10^{-6}	rs11121611	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]		0.315
	rs2125362	11	86167136	1.31 (0.68 – 2.56)	3.53×10^{-6} [d]	rs2125362	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}		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}	rs540731596, rs12959468	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

[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 S3 (continuation). Summary of the conditional regression models for each gene region with suggestive associations in African-admixed populations.

				Meta-analysis		Conditional regression model	
Nearest gene(s)	SNP	Chr. [a]	Position [b]	OR (95% CI) [c]	*p*-value	Conditioned on	*p*-value
KCNN1	**rs2278992**	19	18095769	0.59 (0.47 – 0.74)	3.76×10^{-6}		NA
	rs2278993	19	18096073	0.59 (0.47 – 0.74)	3.76×10^{-6}	rs2278992	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}		NA
	rs5995653	22	39404249	0.66 (0.56 – 0.79)	4.80×10^{-6}	rs6001366, rs5995653	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 = 1,347)
		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 African-admixed individuals.

					GALA II (n=854)			SAGE II (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	1
	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	
	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	

[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.
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Table S5 (continuation). Results for SNPs previously associated with ICS response. Evidence found in African-admixed individuals.

Nearest gene(s)	SNP	Chr. [a]	Position [b]	A1/A2	GALA II (n=854)			SAGE II (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	
PDE10A-T	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	5
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. Genome-wide association between *GLCCI1* and response to glucocorticoid therapy in asthma. *N Engl J Med* 2011; 365:1173-1183.

Table S6. Gene-level association results in the discovery phase within 100 kb of previously reported genes.

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.

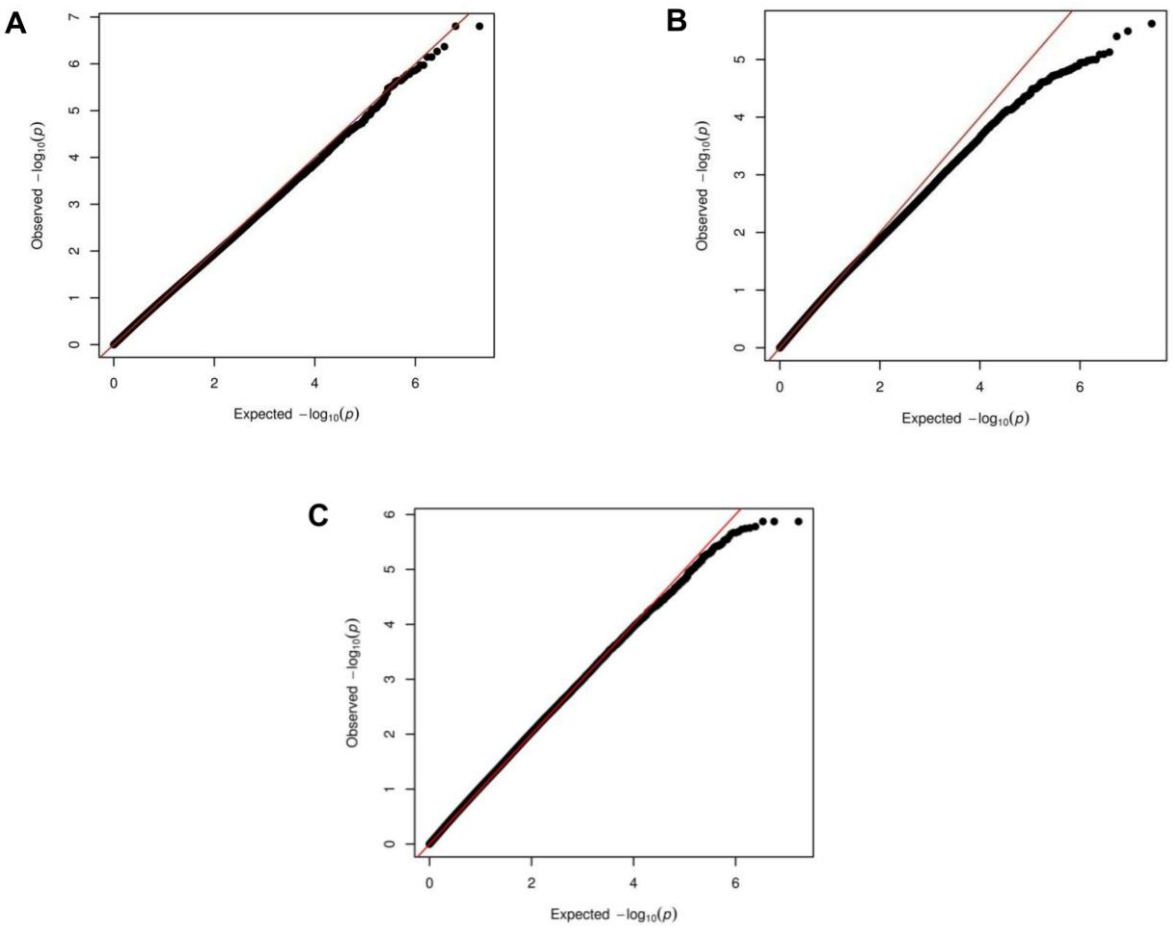

Figure S1. Quantile-quantile plots of association results of ICS response in the discovery phase. Observed and expected association results are represented as -log10 *p*-value on the *y*-axis and *x*-axis, respectively. A) Q-Q plot of association results in Latinos/Hispanics (GALA II) (λ_{GC}=1.03);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

3.3. Genome-wide association study of asthma exacerbations despite ICS use in European populations

Only a few genes involved in the response to asthma treatment with ICS have been identified to date through studies including reduced sample sizes and a scarce exploration of the variability of the genome. Thus, in this *Chapter*, a GWAS of asthma exacerbations despite ICS use was carried out in children and young adults of European descent. Association signals were also evaluated in non-European populations, including African Americans, Asians, and Hispanics/Latinos. Variants previously revealed to be involved in the ICS response by means of GWAS approaches were followed up for replication. This was complemented with gene-enrichment analyses focused on pharmacological therapeutic targets.

A total of ten variants were associated with asthma exacerbations despite ICS use in Europeans at the suggestive significance level ($p \leq 5x10^{-6}$). The association of one variant located in the intergenic region of the genes *CACNA2D3* and *WNT5A* was validated in independent studies, suggesting this could be a novel locus of asthma exacerbations despite ICS treatment. Nonetheless, this was not found associated in non-European populations. Additionally, evidence of replication was detected for five other loci previously associated with the response to ICS treatment. The gene-set enrichment analyses revealed that trichostatin A could be implicated in the molecular mechanisms underlying the response to asthma treatment, allowing to hypothesize that it could be a potential asthma therapy.

This *Chapter* is presented as a manuscript draft entitled ‘Genome-wide association study of asthma exacerbations despite inhaled corticosteroids use' recently accepted for publication in the *European Respiratory Journal* (doi.org/10.1183/13993003.03388-2020).

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

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TAKE-HOME MESSAGE

A genome-wide association study of asthma exacerbations despite inhaled corticosteroids treatment in childhood asthma revealed a novel association at the *CACNA2D3-WNT5A* locus and suggested trichostatin A as a potential asthma therapy.

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 1,773 non-Europeans. Variants revealed by published GWAS were assessed for replication. Additionally, gene-set enrichment analysis focused on drugs was performed.

Results. Ten independent variants were associated with asthma exacerbations despite ICS treatment in the discovery phase ($p \leq 5 \times 10^{-6}$). 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.

Keywords: childhood asthma, Europeans, exacerbations, pharmacogenomics, treatment, trichostatin A.

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 interindividual variability in ICS response has been described[3]. Studies have shown that 30 to 40% of the asthmatic children treated with ICS do not show an improvement of their symptoms and that 10 to 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 the 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. We also explored whether a gene-set enrichment analysis of the GWAS results could suggest alternative 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 fourteen 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 collecting 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-Europeans 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 Paediatric Asthma Gene-Environment Study (PAGES); BREATHE; the Genetics of the Scottish Health Research Register (GoSHARE); the Pharmacogenetics of Adrenal Suppression study (PASS); SLOVENIA; the follow-up stage of the Multicenter Asthma Genetics in Childhood Study (followMAGICS); Effectiveness and Safety of Treatment with Asthma Therapy in Children (ESTATe). All these studies included children and young adults aged 2 to 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 last 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[16] for PACMAN, GoSHARE, PASS, SLOVENIA, and ESTATe (**Table 1**). 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 followMAGICS (**Table 1**), as described elsewhere[15].

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	654	540	472	402	182	182	147	102
Gender (% male)	61.6	60.4	24.8	55.0	57.1	59.3	59.9	58.8
Mean age ± SD (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[a]	13.8	51.7[a]	34.1	52.7[a]	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 (%) [b]	6.1	NA	NA	NA	28.0	NA	7.5	NA
OCS use (%) [c]	6.7	35.0	13.8	51.7	12.6	48.4	NA	35.3
Hospitalizations (%) [d]	NA	13.5	0.21	NA	9.9	46.7	3.4	12.7 [h]
GP visits (%) [e]	NA	NA	NA	NA	NA	NA	49.0	NA
Specialist visits (%) [f]	NA	NA	NA	NA	NA	NA	21.8	NA
School absences (%) [g]	NA	43.1	NA	NA	NA	47.2	NA	NA

[a] Asthma exacerbations-related data was available for the 6 precedent months of the study enrolment; [b] Proportion of patients with any exacerbations who sought emergency care due to asthma; [c] Proportion of patients with any exacerbations who needed the use oral corticosteroids because of asthma; [d] Proportion of patients with any exacerbations who needed to be hospitalized because of asthma; [e] Proportion of patients with any exacerbations who needed any unscheduled general practitioner visits because of asthma; [f] Proportion of patients with any exacerbations who needed any respiratory system specialist visits because of asthma; [g] Proportion of patients with any exacerbations who were absent from school because of asthma; [h] 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 1 (continuation). Clinical and demographic characteristics of the European populations included in the discovery phase.

	PACMAN	BREATHE-PAGES	GoSHARE	PASS	SLOVENIA	BREATHE	followMAGICS	ESTATe
Treatment steps [i]								
Step 2 (%) [j]	70.6	37.6	97.3	7.5	NA	61.0	29.3	63.7
Step 3 (%) [k]	20.8	32.6 [m,n]	2.5 [m,n]	32.1 [n]	NA	29.1 [m,n]	59.8 [n]	33.3 [n]
Step 4 (%) [l]	5.4	29.8 [ñ]	0.2 [ñ]	57.2	NA	9.9 [ñ]	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)

[a] Asthma exacerbations-related data was available for the 6 precedent months of the study enrolment; [b] Proportion of patients with any exacerbations who sought emergency care due to asthma; [c] Proportion of patients with any exacerbations who needed the use oral corticosteroids because of asthma; [d] Proportion of patients with any exacerbations who needed to be hospitalized because of asthma; [e] Proportion of patients with any exacerbations who needed any unscheduled general practitioner visits because of asthma; [f] Proportion of patients with any exacerbations who needed any respiratory system specialist visits because of asthma; [g] Proportion of patients with any exacerbations who were absent from school because of asthma; [h] ER visits and hospitalizations were considered as a single variable; [i] Adapted from British Thoracic Society/Scottish Intercollegiate Guidelines Network guidelines; [j] As-needed SABA plus regular ICS; [k] As-needed SABA plus regular ICS and LABA; [l] As-needed SABA plus regular ICS, LABA and LTRA; [m] As-needed SABA plus combinations of ICS and LABA; as-needed SABA plus ICS and combinations of ICS and LABA; [n] As-needed SABA plus ICS and LTRA was also considered; [ñ] 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.

LABA: long-acting β2 agonists; LTRA: leukotriene receptor antagonists; SABA: short-acting β2 agonists; SD: standard deviation; ER: emergency room; OCS: systemic corticosteroids; GP: general practitioner; NA: not available.

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 ($p \leq 0.05$) among Europeans were evaluated in Latinos/Hispanics from the Genes-Environment and Admixture in Latino Americans (GALA II) study, African Americans included from the Study of African Americans, Asthma, Genes and Environments (SAGE), and Asians from The Singapore Cross-Sectional Genetic Epidemiology Study (SCSGES). Information about the presence or absence of asthma exacerbations despite ICS use in the 12 previous months to study enrolment was considered. The details on these studies are described in 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 with the Axiom™ Precision Medicine Research Array (Affymetrix Inc.) by Centro Nacional de Genotipado (CeGen; www.cegen.org). The same QC procedures described in Hernandez-Pacheco *et al*. were applied to all the studies[15]. 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 **Table S1.** Similarly, the genotyping methods used for the non-European studies (**Table S2**) are described in the Supplementary Material.

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 3 of the 1000 Genomes Project (1KGP) 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, gender, and the PCs needed to control for population stratification within each study.

Results for single nucleotide polymorphisms (SNPs) with a minor allele frequency (MAF) ≥1% and imputation quality (Rsq) ≥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*-value ≤$5x10^{-6}$), 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 (COJO), as implemented in GCTA 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 (LD) correlation matrix obtained with the data from PACMAN, the largest study included in the discovery phase. Independent SNPs associated (*p*-value≤$5x10^{-6}$) 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 **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*-value≤0.05), and with the same direction of the effects as in the discovery phase were meta-analyzed following the same methodology as described above.

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 (**Table S2**), association with asthma exacerbations was evaluated using logistic regressions adjusted by age and gender using PLINK 1.9[22].

Evidence of replication was considered if the variant assessed showed a *p*-value≤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 only study with available information for this variable based on medical prescriptions, 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 and the Scottish Intercollegiate Guidelines Network (BTS/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 (GTEx) 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 (**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 ($p \leq 0.05$). Additionally, replication was also assessed as genomic regions, analyzing variants located within 100 kilobases (kb) upstream and downstream from the gene limits. A Bonferroni-corrected significance threshold was estimated for each genomic region as $\alpha = 0.05/\text{number of independent variants analyzed}$, 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 SNPs associated ($p \leq 5 \times 10^{-6}$) with asthma exacerbations despite ICS treatment in the discovery phase were included, but also those significant at $p \leq 1 \times 10^{-4}$ were 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, 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) ≤0.05).

RESULTS

Characteristics of the study populations

A total of 2,681 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 (**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, Latinos/Hispanics from GALA II had the highest proportion of asthma exacerbations occurrence despite the treatment with ICS (66.4%) (**Table S2**).

Association results in European populations

Association results for a total of 8.1 million common SNPs (MAF≥1%) with Rsq≥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 (**Figure S1A-S1H**), neither after combining them in a meta-analysis (λ_{GC}=1.04, **Figure S1I**). Although no associations were detected at the genome-wide significance level (p-value≤5x10^{-8}), a total of 19 variants near or within ten loci showed p-value≤5x10^{-6} in European children and young adults (**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 ten independent signals were detected (**Table 2**), which were followed up for replication.

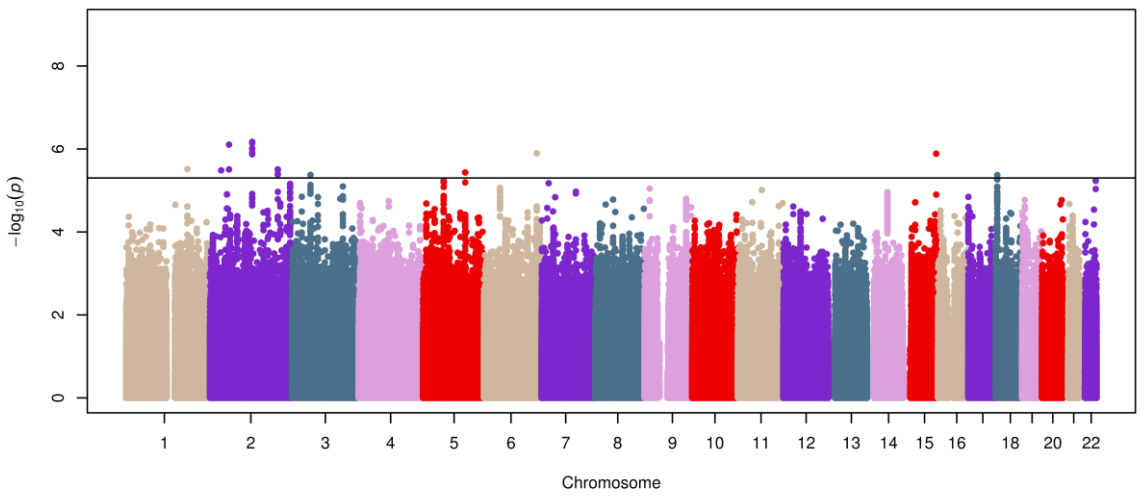

Figure 1. Manhattan plot of association results of asthma exacerbations in ICS users included in the discovery phase. Association results are represented as -log$_{10}$ p-value on the y-axis along the chromosomes (x-axis). The horizontal black line shows the suggestive significance threshold for replication (p≤5x10^{-6}).

Table 2. Summary of the conditional regression models for each locus suggestively associated with ICS response in the discovery phase.

						Meta-analysis (n=2,681)		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}		NA
	rs72805125	2	56684554	T/C	0.063	2.09 (1.53 – 2.85)	3.11×10^{-6}	rs113364932	0.888
CNTNAP5	rs76496334	2	125427606	T/C	0.022	2.29 (1.64 – 3.19)	9.69×10^{-7}		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}	rs144289311	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} [e]		0.847
	rs7587871	2	201505269	A/C	0.318	1.09 (0.75 – 1.58)	3.10×10^{-6} [e]		NA
	rs7420798	2	201506713	G/A	0.318	1.09 (0.75 – 1.58)	3.24×10^{-6} [e]	rs7587871	NA
	rs12988162	2	201507154	A/T	0.318	1.08 (0.75 – 1.57)	4.14×10^{-6} [e]		NA
CACNA2D3-WNT5A	**rs67026078**	3	55162698	C/T	0.085	1.50 (0.93 – 2.43)	4.22×10^{-6} [e]	NA	NA
ZNF608-GRAMD3	**rs444610**	5	125315286	A/T	0.398	1.36 (1.09 – 1.69)	3.68×10^{-6} [e]	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

[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); [e] Random-effect model was applied since heterogeneity was found between European studies.
CI: Confidence Interval; E: Effect allele; NA: not available; NE: Non-effect allele; SNP: single-nucleotide polymorphism.
Independent SNPs of each gene region are in boldface.

Of the ten variants associated with asthma exacerbations despite ICS treatment in the discovery phase (*p*-value≤5x10^{-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 (odds ratio (OR) for C allele:1.83, 95% Confidence Interval (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*=4.22x10^{-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*=4.34x10^{-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 twelve-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=1,557 vs. n=1,124).

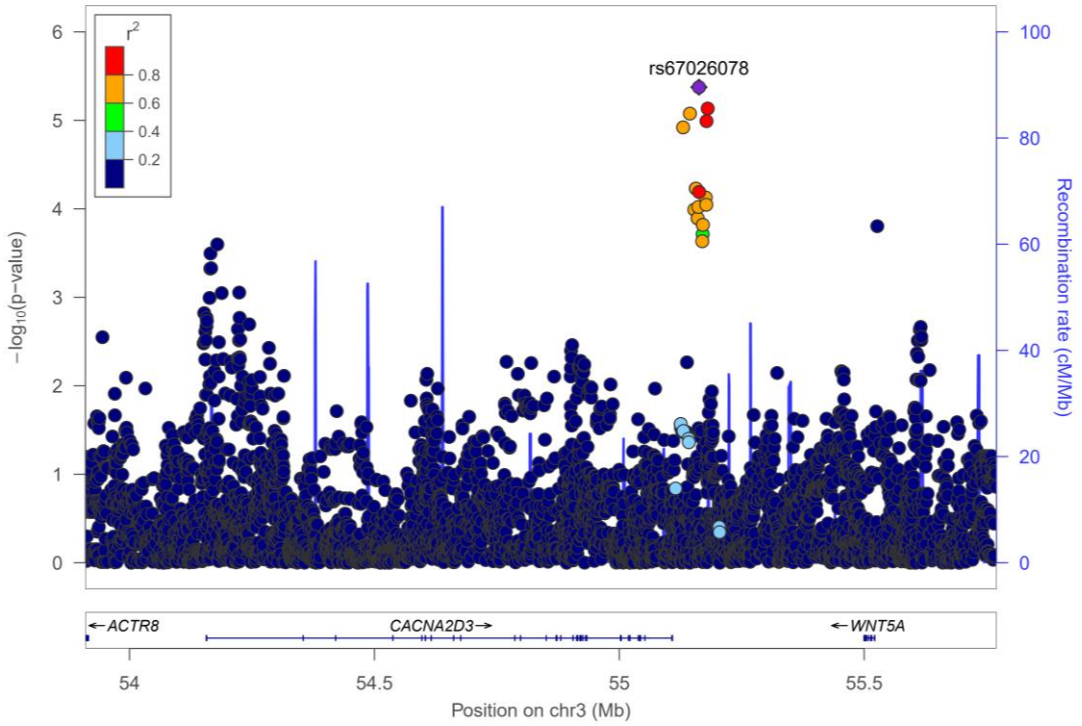

Figure 2. Regional plot of association results for the *CACNA2D3-WNT5A* locus for the European populations included in the discovery phase. Logarithmic transformation of the association results (-log$_{10}$ *p*-value) is represented in the *y*-axis by chromosome position (*x*-axis) for each SNP as a 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 grey color-coded based on pairwise r^2 values with that SNP for European populations from 1KGP.

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=2,681)		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

[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);
[e] Random-effect model was applied since heterogeneity was found between European studies.
CI: Confidence Interval; E: Effect allele; NA: not available; NE: Non-effect allele; SNP: single-nucleotide polymorphism.
SNPs with evidence of replication in independent European populations are in boldface.

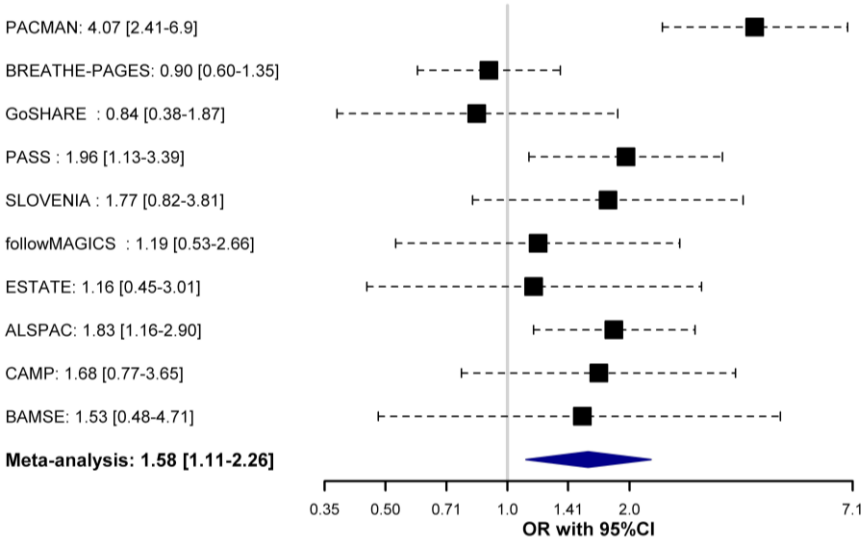

Figure 3. Forest plot of association effect of rs67026078 across European studies included in the GWAS of asthma exacerbations despite ICS treatment. Association effects are shown in terms of odds ratio (OR) for the effect allele (C) for each study and after meta-analyzing the results from both phases by black boxes and a blue diamond, respectively. Black dash lines indicate the corresponding 95% Confidence Intervals (95% CI) for each study. The effect of association results is not given for BREATHE since rs67026078 did not pass quality control checks.

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 (**Table S5**). In Asians, this variant was not consistently associated with asthma exacerbations in SCSGES neither (**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 phases (5.7-9.4%), compared to the non-European populations. Specifically, this variant had a frequency of 4.7%, 4.9%, and 1.4% in Hispanics/Latinos, African Americans, and Asians, 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 dose 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=2.30x10^{-7}). These results are equivalent in terms of significance to those obtained applying the original association model for the same individuals with complete data, but the effect sizes are smaller (OR for C allele:4.30, 95% CI:2.33 – 7.92, p=2.98x10^{-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=2.02x10^{-7}) (**Table S6**).

Association analyses adjusted by asthma severity based on treatment steps classification were performed in 2,282 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=1.05x10^{-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=1.03x10^{-5}).

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, the SNP rs67026078 had been previously significantly identified (p≤0.01) as pQTL and meQTL. Specifically, Sun *et al.* found this variant to be associated with protein expression levels for 16 different proteins in plasma[27,28,34] (**Table S7**). 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 Bonder *et al*.[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=6.58x10^{-3}) (**Table S8**). Considering the genomic regions where these genes reside, 33,096 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 x 10^{-5}), *UMAD1-GLCCI1* (rs13235500, OR for G allele:0.71, 95%CI:0.60 – 0.85, p=2.44 x 10^{-4}), *SHB-ALDH1B1* (SNP with min p-value: rs341488, OR for A allele:2.24, 95%CI:1.48 – 3.40, p=1.44 x 10^{-4}), *ZNF432-ZNF841* (SNP with min p-value:rs67834224, OR for A allele:0.65, 95%CI:0.52 – 0.82, p=2.86 x 10^{-4}), *ELMO2-ZNF334* (SNP with min p-value: rs11087003, OR for C allele:0.77, 95%CI:0.66 – 0.89, p=5.84 x 10^{-4}) (**Table S9**). However, none of these associations were significant after correction for the total number of SNPs tested across all genomic regions (1,799 independent SNPs: Bonferroni-like correction significance threshold of p≤2.78x10^{-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≤1x10^{-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 (**Table S10**). Of those, trichostatin A (TSA) remained statistically significant after adjusting for multiple comparisons (FDR=0.035) (**Table S10**). Specifically, a total of 30 of the 83 genes associated at p≤1x10^{-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 (**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*) (**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, ten independent variants were found to be suggestively associated with asthma exacerbations despite ICS treatment in children and young adults with asthma. 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 Latinos/Hispanics, African Americans, or Asians, 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, which could regulate molecular mechanisms involved in asthma pathogenesis.

CACNA2D3 encodes a member of the alpha-2/delta subunit family, which are voltage-dependent calcium channels consisting of a complex of alpha-1, alpha-2/delta, beta, and gamma subunits. Specifically, CACNA2D3 modulates the calcium current density through the regulation of the influx of calcium ions into the cell upon membrane polarization[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 *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 one second (FEV_1), forced vital capacity (FVC), and the ratio FEV_1/FVC, in chronic obstructive pulmonary disease (COPD) patients from the large cohort of European descent UKBiobank[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 also been associated with the fractional concentration of nitric oxide (FeNO) in exhaled air[45], which is a good indicator of inflammatory patterns in the airways and potentially an alternative approach to support asthma diagnosis in children[46,47] and to monitor the adherence and response to medications[48]. 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[49]. This protein has been evidenced to play a crucial role in development during embryogenesis, oncogenesis, and regulation of inflammatory processes in infectious disorders[50]. Moreover, other genes encoding for ligands involved in the WNT signaling pathway are associated with impaired lung function in asthmatic children[51]. 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[52]. Indeed, WNT molecules regulate the homeostasis of these cells, avoiding dysregulated immune responses, which could trigger several diseases, including allergic asthma[52].

Specifically, expression of *WNT5A* has been positively associated with Th2-mediated airway inflammation in asthmatic patients[53]. 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[54]. It is well known that eosinophilia at blood and tissue levels is one of the most important phenotypes in asthma patients[55], 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 β_1 (TGF-β_1) molecules[56]. 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[54]. 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[57], throughout inhibition of WNT ligands biogenesis, secretion and blocking their ligand-receptor interactions through small pharmacological molecules[50]. 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[58].

The C allele of the SNP rs67026078, which is located 54.1 kb from the 3' UTR 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 the biologic drivers of asthma exacerbations. Nonetheless, this did not show 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[59,60].

TSA is a hydroxamic acid extracted from the bacterial genus *Streptomyces* with a wide range of histone deacetylase (HDAC) inhibitor activities in mammalian cells[61]. 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[62]. Nonetheless, HDAC inhibitors have been also demonstrated to act on diverse non-histone substrates involved in several functions such as cell signaling, chromatin structure, and DNA repair, among others[63].

Interestingly, the potential clinical utility of HDAC inhibitors in asthma has been investigated[63]. Several studies in animal models[63-65] 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[66]. These findings, together with evidence that HDACs regulate sensitivity to glucocorticosteroids[63], suggest that histone acetylation may play a key role in asthma development[67], 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[67]. 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[64]. Additionally, Banerjee *et al.* also found that TSA could have important functions in the inhibition of bronchoconstriction by inducing remodelling changes[64]. 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[64].

Although the effects of TSA on chromatin structure and regulation of gene expression in pulmonary tissues are still unclear[64], 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 non-response might be more likely to occur in 12 months. Third, although the standard definition of severe asthma exacerbations established by the European Respiratory Society (ERS) and the American Thoracic Society (ATS) 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 ICS non-responders. 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 MATERIAL

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

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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 Paediatric 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 multicentre 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 an 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 14,541 (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 14,676 fetuses, resulting in 14,062 live births and 13,988 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 14,541 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 15,454 pregnancies, resulting in 15,589 fetuses. Of these 14,901 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, using support from 23andMe.

The UK Medical Research Council and Wellcome (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 centers 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, 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 analyzed, 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 1,233 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, 398,634 autosomal variants and 1,012 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 ICS prescription data available, 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 β2 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 β2 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}

[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; NA: not available; NE: Non-effect allele; SNP: single-nucleotide polymorphism.

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

					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

[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; NA: not available; NE: Non-effect allele; SNP: single-nucleotide polymorphism.

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

					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**	**Citation**	**OR (95% CI)** [c]	***p*-value**
ALLC	rs17445240	2	3703041	G/A	% ΔFEV_1	1.43 (1.25 – 1.65)	5.01×10^{-7}	1	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}	2	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}	3	1.09 (0.92 – 1.27)	0.320
MMS22L-FBXL4	rs6924808	6	98358575	A/G	% ΔFEV_1	NA	5.31×10^{-7}	4	1.09 (0.96 – 1.25)	0.194
PDE10A-T	rs6456042	6	166534742	C/A	% ΔFEV_1	NA	6.67×10^{-6}	5	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

[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.

Citations:

1. Park TJ, *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, *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, *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 S8 (continuation). 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**	**Citation**	**OR (95% CI)** [c]	***p*-value**
UMAD1-GLCCI1	rs37972	7	8007509	C/T	% ΔFEV_1	NA	0.010	6	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	3	1.11 (0.95 – 1.29)	0.207
TRIM24	rs6467778	7	138178222	G/A	Asthma exacerbations	1.01 (1.00 – 1.03)	0.021	3	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}	3	0.97 (0.82 – 1.15)	0.702
NAV2-HTATIP2	rs1353649	11	20253599	G/A	% ΔFEV_1	NA	3.92×10^{-9}	4	0.93 (0.79 – 1.09)	0.353
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}		1.10 (0.93 – 1.29)	0.283
	rs3450	19	52552999	C/T	BDR	1.03 (1.02 – 1.04)	1.93×10^{-6}	8	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.

Citations:

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2. Park HW, *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, *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}**

[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 S9 (continuation). 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
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	rs112008128	G/T	1.46 (1.16 – 1.86)	1.62×10^{-3}
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}**

[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 S9 (continuation). 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
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, photodamaged 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, postherpetic 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.

[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 boldface.

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

Drug	*p*-value	Adjusted *p*-value (FDR)	# Enriched genes	Use [a]
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.
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 tachycardias, 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.
Omeprazole	0.039	0.787	4	Gastric acid-related disorders.

[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 boldface.

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

Drug	*p*-value	Adjusted *p*-value (FDR)	# Enriched genes	Use [a]
Clarithromycin 476 mg	0.040	0.787	4	Bacterial infections.
Phentolamine	0.041	0.787	4	Hypertension, pheochromocytoma, vasospasm of Raynaud disease and frostbite, clonidine withdrawal syndrome, impotence, peripheral vascular disease.
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 boldface.

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

				Meta-GWAS of ICS response in Europeans (n=2,681)		
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)	1.24×10^{-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

TGF-β1: transforming growth factor β1.

Table S12 (continuation). 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
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 Lung function measurements	Almoguera *et al. Am J Respir Crit Care Med* 2017; 195:456-463 Wyss *et al. Nat Commun* 2018; 9:2976
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.

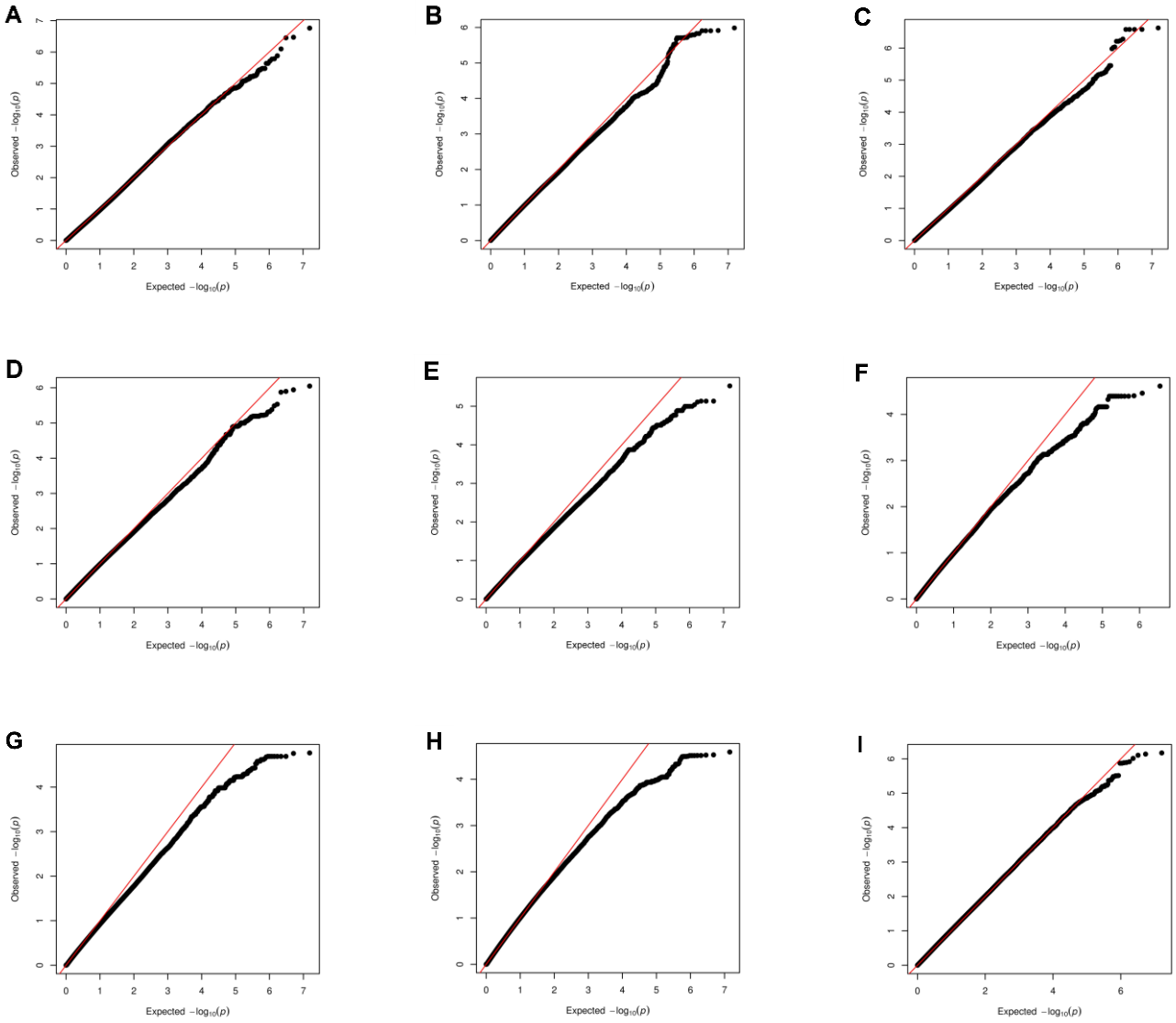

Figure S1. Quantile-quantile plots of association results of asthma exacerbations in patients treated with ICS from the European studies analyzed in the discovery phase. Observed and expected association results are represented as -log10 *p*-value on the *y*-axis and *x*-axis, respectively. Figures S1A-H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

3.4. Genome-wide association study of the change in lung function after ICS use

The difference in lung function measured at the beginning of asthma treatment and a short period later has been evidenced to be a good clinical predictor of the response to asthma therapy. This fourth *Chapter* presents the results of a GWAS of the change in FEV_1 after 6 weeks of ICS use in 166 childhood asthma patients of European ancestry. The genetic variants found were assessed for association with asthma exacerbations despite ICS use in 4,028 children and young adults from ten independent studies in Europeans and non-Europeans. Additionally, genes previously associated with different measures of ICS response, mostly in Europeans and Asians, were attempted for validation with the change in FEV_1 after ICS use.

A variant at the *ROBO2* gene was identified to be suggestively associated with the change in FEV_1 after a short period of ICS use. The association of this gene was validated with asthma exacerbations despite ICS at the level of the genomic region in Europeans, but not in African Americans and Hispanics/Latinos. These findings suggest that *ROBO2* could play a role in ICS response in patients of European descent. Additionally, the association of the intergenic region of the *PDE10A* and *T* genes was validated with the change in FEV_1 after ICS treatment, demonstrating the validity of this measure as a marker of the response to asthma therapy.

This *Chapter* is presented as a manuscript draft entitled ‘Genome-wide association study of change in lung function after inhaled corticosteroids treatment in children and young adults with asthma’ that will be submitted to an international scientific journal for its revision.

Genome-wide association study of change in lung function after inhaled corticosteroids treatment in children and young adults with asthma

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ABSTRACT

Background. Asthma is the most common chronic disease in children, and it is usually treated with inhaled corticosteroids (ICS) as controller medication. High variability in ICS response has been described, evidencing an important contribution of the individual's genetic composition. Here, we aimed to identify novel genetic markers involved in asthma treatment response with ICS.

Methods. A genome-wide association study (GWAS) of ICS response defined as the change in lung function after 6 weeks of ICS treatment was performed in 166 asthma patients from the SLOVENIA study. Prioritized variants were assessed for association with asthma exacerbations despite ICS use in European (n=2,681) and admixed (n=1,347) children and youth from the Pharmacogenomics in Childhood Asthma (PiCA) Consortium. Variants associated with ICS response by previous GWAS were also assessed for replication.

Results. The SNP rs1166980 from the *ROBO2* gene was suggestively associated with ICS response measured with a binary outcome (OR for G allele: 7.01, 95% CI: 3.29 – 14.93, p=4.61x10^{-7}) and with the quantitative measurement of lung function change (β for G allele: -6.54, 95% CI: -9.74 – -3.34, p=9.41x10^{-5}). *ROBO2* showed gene-level evidence of replication with asthma exacerbations despite ICS use in Europeans (p=1.44x10^{-5}), but not in admixed individuals. The previously described association of *PDE10A*-*T* with ICS response was validated.

Conclusions. We identified *ROBO2* as a novel locus of ICS response measured as the change in lung function and asthma exacerbations despite ICS use in European populations, which could be a potential novel locus of the response to this medication.

Keywords: childhood asthma, exacerbations, forced expiratory volume in one second, inhaled corticosteroids.

INTRODUCTION

Asthma is the most common chronic disease in childhood and causes a high impact on the quality of life of the patients and their families, as well as in economic terms on the healthcare system, school and/or work absenteeism[1,2]. This is a complex respiratory disorder characterized by inflammation and reversible obstruction of airways[3], and symptoms such as wheeze, breathlessness, chest tightness and, cough, among others[1].

Inhaled corticosteroids (ICS) are the most effective and widely prescribed asthma preventive medication[1,2,4,5]. Patients with asthma benefit from ICS therapy through decreased airway inflammation, improved lung function, and reduced asthma-related symptoms and exacerbations[6]. Although ICS has demonstrated efficacy in improving symptoms in most children with asthma, between 30 and 40% do not respond to ICS treatment. Furthermore, 10-15% of the children treated with ICS may experience worsening of asthma exacerbations or even suffer severe adverse effects[4,7]. Not only interindividual differences in ICS response have been described, but also among different populations and ethnic groups[4,8-9].

Different clinical markers that have been commonly used to evaluate ICS response include the asthma control test[10], asthma symptoms scores[11,12], information about exacerbations[13-15], and change in lung function after therapy[1,16]. Among these, performing serial measurements of lung function after the start of the therapy is the most commonly used marker for the assessment of treatment response[6,14]. The difference between forced expiratory volume in one second (FEV_1) values measured at the beginning of treatment and a few weeks[17,18] or months[19] later provides substantial information about ICS response[19]. Importantly, the change in FEV_1 after 6 weeks of treatment with ICS has been proposed to be a good predictor of long-term asthma control[17,18]. Although some limitations have been attributed to the evaluation of lung function (e.g., measurement variability during the day, experience and potential errors driven by the operator, type and calibration of the equipment, and the interpretative algorithm), this approach provides a quantitative and objective measure of the response to asthma treatment[20,21].

Some authors have suggested that variability in ICS response may be explained by the interaction of several factors including the individual's genetic composition[22,23]. It has been estimated that between 40 and 60% of the total variation in ICS response may be explained by genetic factors[24]. Pharmacogenetic studies of ICS have been recently carried out mostly using the genome-wide association study (GWAS) approach[25]. To date, a total of ten published GWAS have explored the association with ICS response mostly in European populations[26-35]. These have identified the association of 26 genetic variants located within or near 15 genes with different definitions of ICS response, being the change in FEV_1 after a short period of treatment with ICS the most common definition. Nonetheless, the validation of some of these associations has suggested that the assessment of the presence or absence of asthma exacerbations despite ICS treatment can also be used as a proxy of asthma treatment response in different populations[36]. Despite the effort of these studies, the number of genes associated with ICS response to date is scarce and the effect sizes of the associations detected are small[25].

In the current study, we aimed to identify novel genetic markers associated with ICS treatment response by means of a GWAS of the change in FEV_1 after initiating ICS therapy in asthma patients of

European descent. Association with asthma exacerbations of the markers identified was attempted in children and young adults treated with ICS from different populations.

METHODS

Ethics statement

All studies included were approved by their local institutional review boards and all participants or their parents/caregivers gave their informed consent for inclusion before they participated in the study. All methods were applied in accordance with guidelines and regulations for human subject research under the principles of the Declaration of Helsinki. Further details of the ethical approval of each study are available in the Supplementary Material.

Study population analyzed in the discovery phase

Asthma patients from the SLOVENIA study were included in the GWAS of change in lung function after ICS treatment. Children and young adults (5-18 years old) of Slovenian origin with mild or moderate persistent asthma were included in this study. Patients were excluded if they had other chronic inflammatory disorders, except for asthma and atopic diseases[36-38].

A subset of patients with reported use of any type of ICS and/or combination with long-acting beta-2 agonists (LABA) at least once in the 12 months preceding the study enrolment were analyzed. Availability of genome-wide genotypes, data on the change in FEV_1 after 6 weeks of ICS therapy and information regarding asthma exacerbations were considered as inclusion criteria in the GWAS analyses. FEV_1 expressed as the percentage of the predicted value based on sex, age, and height of the patients was measured before the beginning of ICS treatment (when the patients were ICS-naïve) and 6 weeks after the start of the treatment using a Vitalograph 2150 spirometer (Compact, Buckingham, UK), according to the standard guidelines established by the European Respiratory Society (ERS) and the American Thoracic Society (ATS)[38,39]. Based on these measurements, the percent change in FEV_1 (ΔFEV_1) was calculated as (post-FEV_1 – pre-FEV_1)/(pre-FEV_1) x 100. Based on a threshold of 8% FEV_1 improvement, which has been shown to be a good predictor of asthma treatment response in children[36,40], participants were classified as ICS responders ($\Delta FEV_1 \geq 8\%$) or non-responders ($\Delta FEV_1 < 8\%$).

Genotyping and imputation of genetic variants in SLOVENIA

The SLOVENIA samples were genotyped using the Illumina Global Screening Array-24 v1.0 BeadChip (Illumina Inc.). Quality control (QC) analyses were carried out with PLINK 1.9[41,42] following the procedures described elsewhere[36]. Genetic variants across the whole genome were imputed with the Michigan Imputation Server[43] using Minimac3[44], performing the haplotype reconstruction with SHAPEIT v2.r790[45]. Data from the second release of the Haplotype Reference Consortium (r1.1 2016) was used as a reference panel[36,46].

Association testing with the change in FEV_1 defined as a binary variable

The association of genetic variants with the binary variable of ICS response was tested using logistic regression models with the binary Wald test implemented in EPACTS 3.2.6[47], including age and gender as covariates. Association analyses were also adjusted by the first two principal components (PCs) of genetic ancestry estimated by means of EIGENSOFT[48]. This model was selected since it showed the best fit with the expected values in terms of significance assuming no association as null hypothesis attending to λ_{GC} values, estimated through the R package *gap*[49], and quantile-quantile plots.

Results were filtered to retain single nucleotide polymorphisms (SNPs) with a minor allele frequency (MAF) ≥1% and imputation quality (Rsq) ≥0.3 and variants that reached a significance threshold of *p*-value≤$5x10^{-6}$ were deemed suggestively associated and followed up for replication in additional studies. This threshold was set arbitrarily, following what has been commonly adopted by previous GWAS[36,37].

Association with the quantitative change in FEV_1 after ICS treatment

SNPs suggestively associated with the binary outcome related to ΔFEV_1 after 6 weeks of ICS treatment were assessed in the same group of asthma patients from the SLOVENIA study, but evaluating the association with the quantitative form of this outcome. Linear regression models were performed through linear Wald tests in EPACTS 3.2.6[47] adjusted by the same aforementioned covariates.

Replication of results analyzing the association with asthma exacerbations despite ICS use in additional studies

The genetic markers found to be associated with ΔFEV_1 after ICS treatment were attempted for validation with the absence or presence of asthma exacerbations despite the use of ICS. This was done in ten independent studies included in the PiCA consortium[50]. Association analyses were carried out in asthma patients (2-25 years old) treated with ICS in the previous year, separately performed in two groups of studies based on their ancestry.

On one hand, eight independent European studies were analyzed: the Pharmacogenetics of Asthma Medication in Children: Medication with Anti-inflammatory effects (PACMAN); the Paediatric Asthma Gene-Environment Study (PAGES); BREATHE; Genetics of the Scottish Health Research Register (GoSHARE); the Pharmacogenetics of Adrenal Suppression study (PASS); SLOVENIA; the follow-up stage of the Multicenter Asthma Genetics in Childhood Study (followMAGICS); Effectiveness and Safety of Treatment with Asthma Therapy in Children (ESTATe) (**Table S1**). Additionally, two recently admixed populations with African ancestry were also included in association analyses: Latinos/Hispanics and African Americans from the Admixture in Latino Americans (GALA II) study, and African Americans included in the Study of African Americans, Asthma, Genes, and Environments (SAGE), respectively (**Table S2**).

Asthma exacerbations were defined as the need for emergency care, hospitalizations, or systemically administered corticosteroids because of asthma in the previous 6 or 12 months depending on the study. Information regarding any of these events was not available for BREATHE-PAGES, BREATHE, and followMAGICS so that alternative definitions including unscheduled general practitioner or respiratory system specialist visits and school absences were used instead (**Table S1** and **Table S2**). ICS use was defined

using the same criteria described for participants in the SLOVENIA study. Further description of the characteristics of the study populations, genotyping, imputation, and association analyses are available in the Supplementary Material and elsewhere[36,37].

Association with the presence/absence of asthma exacerbations despite ICS use was tested for each study using logistic Wald tests implemented in EPACTS 3.2.6[47]. Regression models also included age, gender, and PCs as covariates. A meta-analysis of the association results was carried out within each ancestry group using METASOFT[51]. The absence or presence of genetic heterogeneity was accounted for applying fixed-effects or random-effects models, respectively, through Cochran Q-test[51].

The association of the SNPs identified in the discovery phase was separately evaluated in each ancestry group of studies. Replication was carried out at the SNP-level, but also genomic regions were considered, including variants located within a 100 kilobases (kb) window upstream and downstream from the limits of the genes where the variants were located. Only common SNPs with MAF≥1% and Rsq≥0.3 shared among the populations included in each group were included. Replication results were considered significant for those SNPs that reached the Bonferroni-corrected significance threshold, estimated as α=0.05/number of independent signals within each genomic region. For this, independent variants were separately estimated for Europeans and non-Europeans through empirical autocorrelations based on the -$\log_{10}$ *p*-value of each SNP analyzed using the R package *coda*[36,52].

Sensitivity analyses of asthma severity

Sensitivity analyses were carried out for the variants identified to ascertain whether the association effect detected was driven by disease severity rather than the response to asthma medications. On one hand, association analyses were adjusted by the baseline asthma severity of SLOVENIA patients measured as the pulmonary capacity before the beginning of ICS treatment. The original regression models evaluating the association with the binary and quantitative outcomes related to ΔFEV_1 after 6 weeks under ICS therapy were applied, but also including basal FEV_1 as a covariate.

On the other hand, logistic regressions were performed evaluating the association with the presence/absence of asthma exacerbations under ICS therapy accounting for asthma severity based on a modified classification into treatment steps established by the British Thoracic Society and the Scottish Intercollegiate Guidelines Network (BTS/SIGN)[53]. These were performed in asthma patients with reported use of ICS in the previous 6 or 12 months from the studies included in the replication phase with available information about the use of the medications included in the classification into treatment steps. Therefore, individuals were classified as follows: *Step 1*, as-needed use of short-acting beta-2 agonists (SABA); *Step 2*, as-needed use of SABA plus regular ICS; *Step 3*, as-needed use of SABA plus regular ICS and LABA; *Step 4*, as-needed use of SABA plus regular ICS, LABA and leukotriene receptor antagonists (LTRA). Alternatively, *Step 3* also included 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. Moreover, *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. It is important to note that all patients were classified

into *Step 2* or above since being under ICS therapy was 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[53]. Association results were combined in a meta-analysis using METASOFT[51]. Nonetheless, sensitivity analyses accounting for asthma severity measured as treatment steps could not be performed in SLOVENIA since incomplete information about the different medications included in the definition of treatment steps.

Validation of previous associations with ICS response

Previous GWAS have identified a total of 26 SNPs near or within 15 genes associated with ICS response in several populations not overlapping with the ones analyzed in the current study (**Table S3**). Validation of these associations was attempted at SNP-level using the results of the GWAS of the binary variable of ΔFEV_1 after ICS treatment performed in the SLOVENIA study. Evidence of replication was considered for significant variants at the nominal level ($p \leq 0.05$) with the same direction of the association effect as in the discovery phase. Replication was also evaluated at the genomic-region level, including variants located within 100 kilobases (kb) upstream and downstream from the limits of the genes of ICS response previously identified. A Bonferroni-like correction was applied accounting for the number of independent variants analyzed within each genomic region. Evidence of replication was considered for those association signals reaching the Bonferroni-corrected significance threshold estimated as $\alpha = 0.05/\text{number of independent variants}$[36,37].

RESULTS

Characteristics of the study populations

A total of 166 children and young adults with asthma from the SLOVENIA study with reported use of ICS in the last 12 months were included in the discovery phase (**Table 1**). Of these, 94 were ICS non-responders (cases) and 72 were responders (controls). Individuals included were 10.9 ± 3.4 years old on average, showing similar mean age in both groups (cases: 10.7 ± 3.2 years, controls: 11.2 ± 3.5 years). ICS responders showed a substantial improvement in lung function after 6 weeks of treatment with ICS (16.9% ± 8.7%). While 37.2% of the patients defined as non-responders to ICS showed asthma exacerbations in the previous year, 29.2% of the controls had suffered these events in the same period (**Table 1**).

Patients from the European and non-European studies included in the replication showed a similar mean age to those in the SLOVENIA study, except for followMAGICS, which included older participants (17.2 ± 3.0 years) (**Table S1**, **Table S2**). Since asthma exacerbations were differentially defined among studies, there was variation in the exacerbation rates, ranging from 11.0% in PACMAN to 66.4% in GALA II (**Table S1**, **Table S2**).

Table 1. Clinical and demographic characteristics of the asthma patients from the SLOVENIA study included in the GWAS of change in FEV_1 after ICS treatment.

	Total	ICS non-responders [a]	ICS responders [b]	*p*-value
Sample size	166	94	72	-
Gender (% male)	59.0	62.8	54.2	0.264[h]
Mean age ± SD (years)	10.9 ± 3.4	10.7 ± 3.2	11.2 ± 3.5	0.461[i]
Lung function				
Mean basal FEV_1 ± SD (%) [c]	87.1 ± 14.8	91.3 ± 12.7	81.6 ± 15.5	< 0.001[i]
Mean post-treatment FEV_1 ± SD (%) [d]	93.7 ± 14.4	90.1 ± 13.6	98.5 ± 14.2	< 0.001[i]
Mean ΔFEV_1 ± SD (%)	6.7 ± 12.1	-1.2 ± 7.8	16.9 ± 8.7	< 0.001[i]
Asthma exacerbations in the last 12 months (%)	33.7	37.2	29.2	0.276[h]
ER visits (%) [e]	27.7	29.8	25.0	0.495[h]
OCS use (%) [f]	12.0	12.8	11.1	0.746[h]
Hospitalizations (%) [g]	9.0	8.5	9.7	0.787[h]

[a] Asthma patients with ΔFEV_1<8% after 6 weeks of ICS treatment; [b] Asthma patients with ΔFEV_1≥8% after 6 weeks of ICS treatment; [c]FEV_1 measured at the beginning of ICS treatment; [d]FEV_1 measured after 6 weeks of ICS treatment; [e] Proportion of patients with any exacerbations who sought emergency care due to asthma; [f] Proportion of patients with any exacerbations who needed the use oral corticosteroids because of asthma; [g] Proportion of patients with any exacerbations who needed to be hospitalized because of asthma; [h] Pearson $\chi 2$ test (df=1; α=0.05); [i] Mann-Whitney U test.
ER: emergency room; FEV_1: forced expiratory volume in one second; ΔFEV_1: change in FEV_1 after 6 weeks of ICS treatment; OCS: systemic corticosteroids; SD: standard deviation; NA: not available.

Association results of the change in FEV_1 after ICS treatment

A total of 7.5 million common SNPs (MAF≥1%) with Rsq≥0.3 were tested for association with the binary outcome related to ΔFEV_1 after ICS treatment in asthma patients from the SLOVENIA study. No evidence of genomic inflation due to population stratification effects was revealed by the value of λ_{GC}=1.00 (**Figure S1**). No associations were found at genome-wide significance level (*p*-value≤$5x10^{-8}$), but the SNP rs1166980 located in the *ROBO2* gene was found to be suggestively associated with ICS responsiveness in asthma patients (odds ratio (OR) for G allele: 7.01, 95% confidence interval (CI): 3.29 – 14.93, *p*=$4.61x10^{-7}$) (**Figure 1**, **Figure 2**). The association of rs1166980 was also found for the quantitative measurement of ΔFEV_1 in SLOVENIA, since the risk allele for non-response to ICS, was also associated with lower lung function improvement (β for G allele: -6.54, 95% CI: -9.74 – -3.34, *p*=$9.41x10^{-5}$).

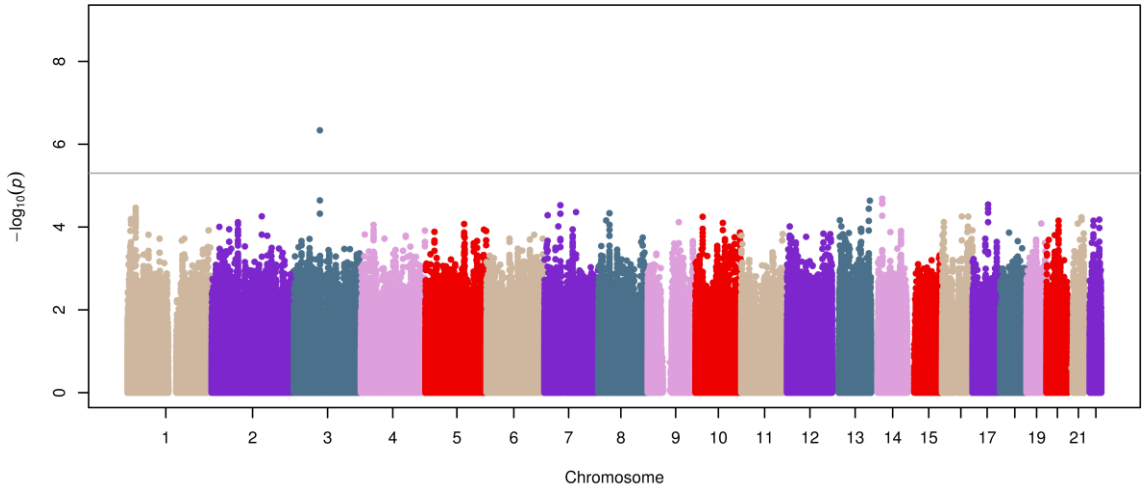

Figure 1. Manhattan plot of association results of the binary variable of the change in FEV_1 after ICS treatment in the discovery phase. The logarithmic transformation of the association results (-$\log_{10}$ *p*-value) is represented on the *y*-axis along with the chromosome position (*x*-axis). The suggestive significance threshold ($p \leq 5 \times 10^{-6}$) considered for evidence of association with ICS response is indicated by the gray line.

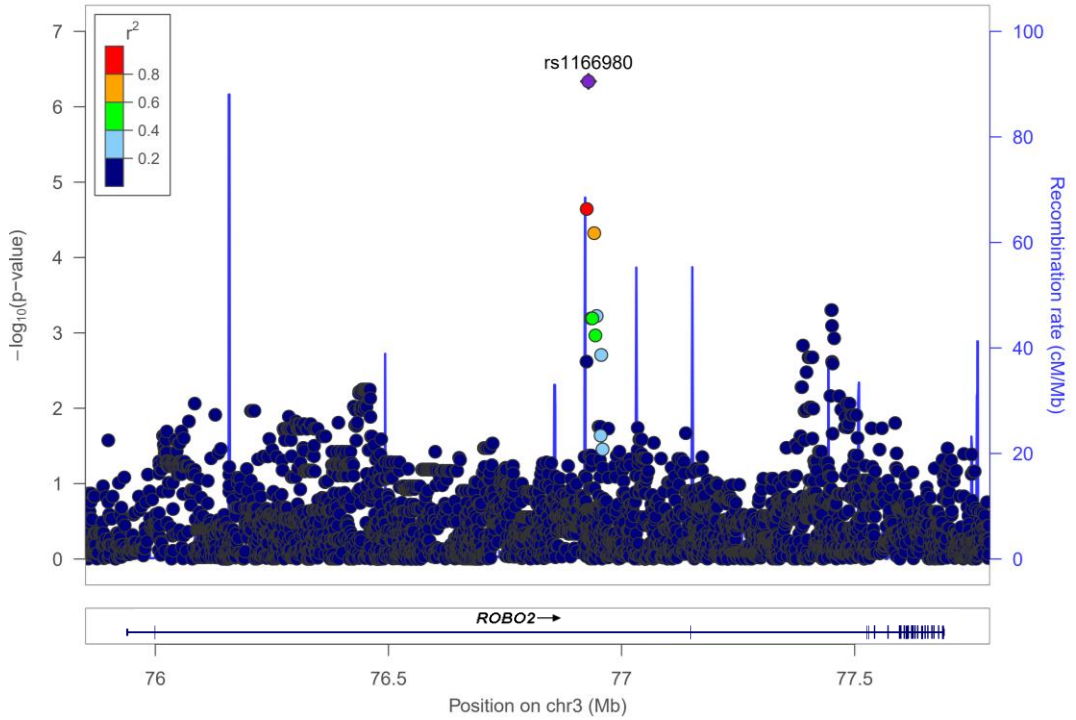

Figure 2. Regional plot of association results with the binary variable related to the change in FEV_1 after ICS treatment in the SLOVENIA study. Association results are represented on the *y*-axis (-$\log_{10}$ *p*-value) by chromosome position (*x*-axis) for each SNP as a dot. The diamond corresponds to the variant suggestively associated with ICS response (rs1166980). The remaining SNPs are color-coded based on pairwise linkage disequilibrium (r^2 values) with that SNP for European populations from 1KGP (GRCh37/hg19 build).

Validation of the association with asthma exacerbations despite ICS use

The association of the SNP rs1166980 was not replicated in Europeans, Latinos/Hispanics, and African Americans when assessing asthma exacerbations despite ICS use as an outcome. At the genomic-region level, a total of 5,919 variants within 100 kb upstream and downstream from *ROBO2* were assessed in Europeans. From these, eleven SNPs were significantly associated with asthma exacerbations despite ICS use after accounting for the 164 independent variants located within this region (Bonferroni-like correction significance threshold of $p \leq 3.04 \times 10^{-4}$). The SNP rs72891545 was the most significant association with ICS response using asthma exacerbations as the outcome (OR for A allele: 4.79, 95% CI: 2.36 – 9.73, $p=1.44 \times 10^{-5}$) (**Table S4**, **Figure S2**). A total of 6,453 variants within a +/-100 kb window from *ROBO2* were evaluated in admixed populations. However, no significant associations with asthma exacerbations despite ICS were found after applying a Bonferroni-like correction ($p \leq 1.22 \times 10^{-4}$ for 411 independent variants).

Sensitivity analyses accounting for asthma severity

Association analyses adjusted by basal FEV_1 as a proxy of asthma severity revealed no major differences from the original results obtained for rs1166980 in SLOVENIA neither when the binary (OR for G allele: 7.21, 95% CI: 3.15 – 16.50, $p=2.95 \times 10^{-6}$) nor quantitative ΔFEV_1 variables were evaluated (β for G allele: -5.58, 95% CI: -8.72 – -2.44, $p=6.42 \times 10^{-4}$). Additionally, sensitivity analyses accounting for asthma severity based on treatment steps classification were performed in 2,282 individuals from six of the eight studies from populations of European descent included in the replication phase with available information about the medications included in the definition of treatment steps. Specifically, these were carried out for the variant within the genomic region of *ROBO2* most significantly associated with asthma exacerbations despite ICS in European populations (rs72891545). As a result, no major differences were found (OR for A allele: 2.66, 95% CI: 1.44 – 4.89, $p=1.71 \times 10^{-3}$) compared to the original association models performed for the patients with available information about treatment steps (OR for A allele: 3.66, 95% CI: 1.88 – 7.12, $p=1.32 \times 10^{-4}$).

Validation of previous associations with ICS response

Among the 26 SNPs previously associated with ICS response through GWAS approaches, two variants were found to be nominally associated with the binary outcome related to ΔFEV_1 after 6 weeks of ICS treatment in the SLOVENIA study: rs2395672 at *CMTR1* (OR for G allele: 1.78, 95% CI: 1.03 – 3.05, $p=0.037$) and rs3827907 at *EDDM3B* (OR for C allele: 0.52, 95% CI: 0.32 – 0.84, $p=7.40 \times 10^{-3}$) (**Table S5**). However, these did not remain significant after adjusting for the total number of variants assessed ($p \leq 1.92 \times 10^{-3}$). At the genomic-region level, a total of 33,617 variants located within a 100 kb window from genes previously associated with ICS response were assessed. This resulted in evidence of suggestive replication for two variants located in the intergenic region of *PDE10A* and *T* after Bonferroni-like correction of the significance threshold within each genomic region: rs9365939 (OR for G allele: 0.41, 95% CI: 0.26 – 0.65, $p=1.92 \times 10^{-4}$) and rs2118353 (OR for T allele: 0.41, 95% CI: 0.26 – 0.65, $p=1.92 \times 10^{-4}$) (**Table S6**). However, no significant association was found after correcting for the total number of independent SNPs tested across all the genomic regions ($p \leq 2.89 \times 10^{-5}$ for 1728 independent variants).

DISCUSSION

The current study describes the results of one of the few GWAS of the response to inhaled corticosteroids in asthma carried out in ICS-naïve patients to date. A variant located in the *ROBO2* gene was the most significantly associated with a binary variable of ICS responsiveness based on FEV_1 change after 6 weeks of ICS treatment. Consistent with this finding, the same result was also found with the quantitative variable of FEV_1 change. This association was validated at genomic-region level by analyzing asthma exacerbations despite ICS use in Europeans, but not in Latinos/Hispanics, and African Americans. Moreover, this association effect seems to be driven by the response to asthma treatment rather than disease severity.

ROBO2 encodes one of the members of the roundabout guidance receptor's family, which are immunoglobulins highly conserved across species. Four ROBO proteins have been identified in humans[55]. These are transmembrane receptors binding Slit guidance ligands [SLIT][56,57] with well-known functions in the nervous system, including the modulation of axon guidance and cell migration during neuronal development[56-58]. However, they have also been demonstrated to be involved in different processes, such as regulation of the immune response[59] and tissue morphogenesis[60]. Furthermore, the ROBO signaling pathway has been suggested to be involved in lung development[58,61]. Previous studies have found that the inactivation of ROBO induces the attraction of immune cells, remodeling the extracellular matrix and reducing the number of alveoli[62]. Specifically, ROBO2 is involved in the signal transduction of SLIT2[58], which has been shown to have an important function in pulmonary diseases[58,63,64]. SLIT2 is implicated in the migration of different cell types, including eosinophils[65], which are known to be elevated in certain types of asthma. In addition, SLIT2 has been found to inhibit the differentiation of fibrocytes from monocytes, preventing fibrotic processes in several diseases, including pulmonary fibrosis[66]. Moreover, Lin *et al.* detected decreased levels of ROBO2 and SLIT2 in chronic obstructive pulmonary disease (COPD) patients[64].

ROBO2 has been suggestively associated with post-bronchodilator spirometric measures in African Americans[67]. More interestingly, it has been suggested that this gene may play an important role in asthma through the induction of airway constriction[68]. This gene was identified to be a shared genetic factor for asthma susceptibility among European, African American, and Latino/Hispanic populations[69]. Ding *et al*. also suggested that *ROBO2* may be part of biological networks related to inflammatory diseases and disorders of the immune system[69]. This evidence suggests that *ROBO2* could play an important role in asthma phenotypes, including response to ICS in asthma.

As part of this study, we also assessed the replication of SNPs and genes that have previously been implicated in ICS response, providing evidence of an association of the intergenic region of the *PDE10A* and *T* genes with FEV_1 change after ICS treatment. However, this association was found for different SNPs from those described in the study reporting the association of this genomic region with ICS response[27].

This study has several limitations that need to be acknowledged. First, the sample size of the discovery phase was limited, which could cause that only one variant was suggestively associated with ICS responsiveness, with a lack of genome-wide significant associations. Nonetheless, the fact that FEV_1 was measured in ICS-naïve patients with asthma, an approach that is rare among studies, explains part of the

difficulty in achieving a larger sample size. Second, information related to spirometry recordings before and after a short period of ICS treatment was not available in independent populations to assess replication using the same measurement. However, the association of *ROBO2* with ICS response among asthma patients was also found evaluating the association with asthma exacerbations despite ICS treatment. Third, different definitions of asthma exacerbations were used based on retrospective information from European and admixed asthmatic children treated with ICS, which could not be fully informative about the response to asthma treatment. Fourth, information about the specific ICS used and the doses administered, or indices of adherence to treatment were not available for any of the studies evaluated, not allowing to include these factors as covariates in the regression models.

In conclusion, this study identified an association with a variant in *ROBO2* and ΔFEV_1 after ICS treatment in European children and young adults with asthma. This association was validated using asthma exacerbations despite ICS use as an alternative outcome in independent European populations. Taken together with the biological plausibility regarding the role of *ROBO2* in pulmonary and immune functions, *ROBO2* potentially represents a novel locus influencing the response to ICS in patients with asthma. Larger studies coupled with functional evaluation are required to fully understand the role of *ROBO2* in responsiveness to ICS in patients with asthma.

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SUPPLEMENTARY MATERIAL

Genome-wide association study of change in lung function after inhaled corticosteroids treatment in children and young adults with asthma

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SUPPLEMENTARY METHODS

Design and characteristics of the studies included in association analyses with asthma exacerbations despite ICS use

European populations

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) with self-reported use of any asthma medications. Recruitment was carried out through records of community pharmacies in the Netherlands[S1].

PAGES (n = 437)

The Paediatric Asthma Gene-Environment Study (PAGES) is a cross-sectional study that includes asthma patients (2-16 years old) with a pediatrician's diagnosis, recruited at secondary care clinics at different centers across the United Kingdom: Aberdeen, Edinburg, Glasgow, Kilmarnock, and Brighton. Clinical assessment through questionnaires about dietary and quality of life was complimented, and saliva samples were collected. The coexistence of any respiratory diseases or significant health problems were considered as exclusion criteria[S2].

BREATHE (n = 288)

The BREATHE study recruited participants aged 3 to 22 years old with a physician's diagnosis of asthma. Participants were recruited at primary and secondary care centers from the United Kingdom[S3-S5]. From the BREATHE samples included, genotypes from 182 patients had been obtained using the Illumina Infinium CoreExome-24 BeadChip (Illumina) array, whereas 103 samples were genotyped using the Axiom™ Precision Medicine Research Array (Affymetrix Inc.). Quality control procedures were applied as described in Hernandez-Pacheco *et al*.[S6]. Association analyses were performed for the latter together with PAGES samples due to similarities of study design and sample characteristics, denoted as BREATHE-PAGES.

GoSHARE (n = 472)

The Genetic of Scottish Health Research Register (GoSHARE) recruited children and young adults (3-18 years old) in Tayside (Scotland) through complete electronic medical records (EMR) available at databases from the National Health Service[S7].

PASS (n = 402)

The Pharmacogenetics of Adrenal Suppression study (PASS) includes children and young adults (5-18 years old) from the United Kingdom. Participants had a physician's diagnosis of asthma and were treated with inhaled corticosteroids (ICS) under medical supervision. Clinical concern about adrenal suppression was also considered as an inclusion criterion since this study was initially conceived to explore the effects of corticosteroids on adrenal suppression[S8-S9].

SLOVENIA (n = 182)

The SLOVENIA study recruited patients (5-18 years old) with a physician diagnosis of mild to moderate persistent asthma and hospital records at tertiary health centers in Slovenia. Part of the participants was regularly treated with ICS under medical supervision. The coexistence of other chronic inflammatory diseases was considered as an exclusion criterion[S10].

followMAGICS (n = 147)

Participants with persistent asthma symptoms from the follow-up phase of the Multicenter Asthma Genetics in Childhood Study (followMAGICS) were aged from 7 to 25 years old. Children with a physician's diagnosis of asthma were recruited at secondary and tertiary centers from Germany and Austria[S11-S14].

ESTATe (n = 102)

Children and young adults (4-19 years old) with a physician's diagnosis of asthma were included in the case-control Effectiveness and Safety of Treatment with Asthma Therapy in children (ESTATe) study. Patients using any asthma controller medication were recruited at primary care units from the Netherlands based on electronic medical records[S6].

Admixed populations

GALA II (n = 854)

Genes-Environment and Admixture in Latino Americans (GALA II) is a case-control study including asthma patients (8-21 years old) with a physician's diagnosis, active symptoms, and reported use of any asthma medications recruited in the United States and Puerto Rico. Hispanic/Latino origin based on four grandparents belonging to that ancestry group was used as an inclusion criterion[S15].

SAGE (n = 493)

The Study of African Americans, Asthma, Genes, and Environments (SAGE) recruited asthma patients following the same protocols used in GALA II. Individuals with four grandparents of African American ancestry were recruited in several centers across the United States[S15-S16].

Ethical approval of each study included

The Slovenian National Medical Ethics Committee (Ljubljana, Slovenia) approved SLOVENIA (reference number: 0120-569/2017/4). 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. 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. 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).

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Table S1. Clinical and demographic characteristics of the European populations included in the evaluation of the association with asthma exacerbations.

	PACMAN	BREATHE-PAGES	goSHARE	PASS	SLOVENIA	BREATHE	followMAGICS	ESTATe
Sample size	654	540	472	402	182	182	147	102
Gender (% male)	61.6	60.4	24.8	55.0	57.1	59.3	59.9	58.8
Mean age ± SD (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[a]	13.8	51.7[a]	34.1	52.7[a]	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 (%) [b]	6.1	NA	NA	NA	28.0	NA	7.5	NA
OCS use (%) [c]	6.7	35.0	13.8	51.7	12.6	48.4	NA	35.3
Hospitalizations (%) [d]	NA	13.5	0.21	NA	9.9	46.7	3.4	12.7 [h]
GP visits (%) [e]	NA	NA	NA	NA	NA	NA	49.0	NA
Specialist visits (%) [f]	NA	NA	NA	NA	NA	NA	21.8	NA
School absences (%) [g]	NA	43.1	NA	NA	NA	47.2	NA	NA

[a] Asthma exacerbations-related data was available for the 6 precedent months of the study enrollment; [b] Proportion of patients with any exacerbations who sought emergency care due to asthma; [c] Proportion of patients with any exacerbations who needed the use oral corticosteroids because of asthma; [d] Proportion of patients with any exacerbations who needed to be hospitalized because of asthma; [e] Proportion of patients with any exacerbations who needed any unscheduled general practitioner visits because of asthma; [f] Proportion of patients with any exacerbations who needed any respiratory system specialist visits because of asthma; [g] Proportion of patients with any exacerbations who were absent from school because of asthma; [h] 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 S1 (continuation). Clinical and demographic characteristics of the European populations included in the evaluation of the association with asthma exacerbations.

	PACMAN	BREATHE-PAGES	goSHARE	PASS	SLOVENIA	BREATHE	followMAGICS	ESTATe
Treatment steps [i]								
Step 2 (%) [j]	70.6	37.6	97.3	7.5	NA	61.0	29.3	63.7
Step 3 (%) [k]	20.8	32.6 [m,n]	2.5 [m,n]	32.1 [n]	NA	29.1 [m,n]	59.8 [n]	33.3 [n]
Step 4 (%) [l]	5.4	29.8 [ñ]	0.2 [ñ]	57.2	NA	9.9 [ñ]	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)

[a] Asthma exacerbations-related data was available for the 6 precedent months of the study enrollment; [b] Proportion of patients with any exacerbations who sought emergency care due to asthma; [c] Proportion of patients with any exacerbations who needed the use oral corticosteroids because of asthma; [d] Proportion of patients with any exacerbations who needed to be hospitalized because of asthma; [e] Proportion of patients with any exacerbations who needed any unscheduled general practitioner visits because of asthma; [f] Proportion of patients with any exacerbations who needed any respiratory system specialist visits because of asthma; [g] Proportion of patients with any exacerbations who were absent from school because of asthma; [h] ER visits and hospitalizations were considered as a single variable; [i] Adapted from British Thoracic Society/Scottish Intercollegiate Guidelines Network guidelines; [j] As-needed SABA plus regular ICS; [k] As-needed SABA plus regular ICS and LABA; [l] As-needed SABA plus regular ICS, LABA and LTRA; [m] As-needed SABA plus combinations of ICS and LABA/as-needed SABA plus ICS and combinations of ICS and LABA; [n] As-needed SABA plus ICS and LTRA was also considered; [ñ] As-needed SABA plus LABA, combinations of ICS and LABA, and LTRA/as-needed SABA plus ICS, combinations of ICS and LABA, and LTRA/as-needed SABA plus combinations of ICS and LABA, and LTRA was also considered.

LABA: long-acting β2 agonists; LTRA: leukotriene receptor antagonists; SABA: short-acting β2 agonists; SD: standard deviation; ER: emergency room; OCS: systemic corticosteroids; GP: general practitioner; NA: not available.

Table S2. Clinical and demographic characteristics of admixed populations included in the evaluation of the association with asthma exacerbations despite ICS treatment.

	GALA II (n=854)	**SAGE (n=493)**
Gender (% male)	57.3	60.4
Mean age ± SD (years)	12.1 ± 3.2	10.2 ± 3.5
Recruitment country	United States	United States
Ancestry	Latino/Hispanic	African American
Asthma exacerbations in the last 12 months (%)	66.4	51.9
Definition	ER visits/ hospitalizations/ OCS use	ER visits/ hospitalizations/ OCS use
ER visits (%) [a]	56.6	43.2
OCS use (%) [b]	40.2	29.4
Hospitalizations (%) [c]	12.6	5.7

[a] Proportion of patients with any exacerbations who sought emergency care due to asthma; [b] Proportion of patients with any exacerbations who needed the use oral corticosteroids because of asthma; [c] Proportion of patients with any exacerbations who needed to be hospitalized because of asthma.
SD: standard deviation; ER: emergency room; OCS: systemic corticosteroids; NA: not available.

Table S3. Genes associated with ICS response by genome-wide association studies 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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4. 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.

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Table S4. Genomic-region replication of *ROBO2* with asthma exacerbations despite ICS use in European populations. Evidence for significant variants after Bonferroni-like correction.

SNP	Chr. [a]	Position [b]	E/NE	OR (95% CI) [c]	*p*-value
rs72891542	3	77183058	T/C	4.21 (2.12 – 8.37)	3.96×10^{-5}
rs72891545	3	77186033	A/G	4.79 (2.36 – 9.73)	**1.44×10^{-5}**
rs80109563	3	77189324	T/C	6.38 (2.60 – 15.65)	5.26×10^{-5}
rs77698848	3	77191168	A/G	6.06 (2.37 – 15.52)	1.72×10^{-4}
rs75844835	3	77192763	G/T	9.02 (3.04 – 26.79)	7.53×10^{-5}
rs75804244	3	77193590	A/G	6.47 (2.74 – 15.30)	2.11×10^{-5}
rs75336627	3	77197609	A/G	4.95 (2.18 – 11.27)	1.38×10^{-4}
rs77225325	3	77199127	A/C	4.95 (2.18 – 11.27)	1.38×10^{-4}
rs76099377	3	77199443	G/A	4.95 (2.18 – 11.27)	1.38×10^{-4}
rs7623806	3	77201032	C/T	4.95 (2.18 – 11.27)	1.38×10^{-4}
rs72891555	3	77205263	G/T	4.94 (2.09 – 11.71)	2.82×10^{-4}

[a] Chromosome; [b] Positions based on GRCh37/hg19 build; [c] Odds ratio for the effect alleles.
CI: Confidence Interval; E: Effect allele; ICS: inhaled corticosteroids; NA: not available; NE: Non-effect allele; SNP: single-nucleotide polymorphism.
The most significant SNP is in boldface.

Table S5. Results of SNP-level replication of previous associations of ICS response in the results of the GWAS of ΔFEV_1 after ICS treatment performed in the SLOVENIA study.

					Published GWAS of ICS response				GWAS of ΔFEV_1 after ICS use (n = 166)	
Nearest gene(s)	**SNP**	**Chr.** [a]	**Position** [b]	**A1/A2**	**Definition of ICS response**	**OR (95% CI)** [c]	***p*-value**	**Citation**	**OR (95% CI)** [c]	***p*-value**
ALLC	rs17445240	2	3703041	G/A	% ΔFEV_1	1.43 (1.25 – 1.65)	5.01×10^{-7}	1	1.23 (0.62 – 2.43)	0.555
	rs13418767	2	3704830	T/G	% ΔFEV_1	1.40 (1.22 – 1.62)	2.77×10^{-6}		1.11 (0.57 – 2.16)	0.753
	rs6754459	2	3707423	T/C	% ΔFEV_1	1.43 (1.24 – 1.65)	5.73×10^{-7}		1.32 (0.81 – 2.16)	0.268
	rs17017879	2	3713658	C/G	% ΔFEV_1	1.40 (1.22 – 1.61)	2.49×10^{-6}		1.12 (0.53 – 2.36)	0.766
	rs7558370	2	3714261	C/A	% ΔFEV_1	1.39 (1.21 – 1.60)	3.73×10^{-6}		1.16 (0.60 – 2.23)	0.660
	rs11123610	2	3723026	A/G	% ΔFEV_1	0.69 (0.60 – 0.80)	3.57×10^{-7}		0.78 (0.49 – 1.24)	0.287
FBXL7	rs10044254	5	15783596	G/A	Asthma symptoms	3.29 (1.94 – 5.58)	1.02×10^{-5}	2	0.84 (0.50 – 1.42)	0.511
CMTR1	rs2395672	6	37428577	G/A	Asthma exacerbations	1.08 (1.04 – 1.12)	1.86×10^{-5}	3	1.78 (1.03 – 3.05)	**0.037**
MMS22L-FBXL4	rs6924808	6	98358575	A/G	% ΔFEV_1	NA	5.31×10^{-7}	4	0.95 (0.61 – 1.50)	0.841
PDE10A-T	rs6456042	6	166534742	C/A	% ΔFEV_1	NA	6.67×10^{-6}	5	0.78 (0.49 – 1.23)	0.282
	rs3127412	6	166535561	T/C	% ΔFEV_1	NA	9.68×10^{-6}		0.78 (0.49 – 1.23)	0.282
	rs1134481	6	166571164	G/T	% ΔFEV_1	NA	NA		0.87 (0.56 – 1.35)	0.524
	rs2305089	6	166579270	T/C	% ΔFEV_1	NA	NA		0.86 (0.54 – 1.36)	0.520
	rs3099266	6	166581147	C/T	% ΔFEV_1	NA	NA		0.94 (0.60 – 1.48)	0.801

[a] Chromosome; [b] Positions based on GRCh37/hg19 build; [c] Odds ratio for the effect alleles.
A1: Effect allele; A2: Non-effect allele; ACT: asthma control test; BDR: bronchodilator response; CI: Confidence Interval; GWAS: genome-wide association study; ICS: inhaled corticosteroids; NA: not available; 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.
Citations:

1. Park TJ, *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, *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, *et al. CMTR1* is associated with increased asthma exacerbations in patients taking inhaled corticosteroids. *Immun Inflamm Dis* 2015; 3:350-359.
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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 S5 (continuation). Results of SNP-level replication of previous associations of ICS response in the results of the GWAS of ΔFEV_1 after ICS treatment performed in the SLOVENIA study.

					Published GWAS of ICS response				GWAS of ΔFEV_1 after ICS use (n = 166)	
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**
UMAD1-GLCCI1	rs37972	7	8007509	C/T	% ΔFEV_1	NA	0.010	6	0.97 (0.63 – 1.48)	0.876
MAGI2	rs2691529	7	77803275	T/C	Asthma exacerbations	0.97 (0.94 – 1.00)	0.051	3	1.01 (0.57 – 1.81)	0.966
TRIM24	rs6467778	7	138178222	G/A	Asthma exacerbations	1.01 (1.00 – 1.03)	0.021	3	0.67 (0.39 – 1.16)	0.151
SHB-ALDH1B1	rs4271056	9	38232043	C/T	Asthma exacerbations	0.96 (0.93 – 0.99)	6.71×10^{-3}	3	1.16 (0.66 – 2.03)	0.615
NAV2-HTATIP2	rs1353649	11	20253599	G/A	% ΔFEV_1	NA	3.92×10^{-9}	4	0.83 (0.51 – 1.35)	0.444
EDDM3B	rs3827907	14	21238798	C/T	ACT	NA	7.79×10^{-8}	7	0.52 (0.32 – 0.84)	7.40×10^{-3}
L3MBTL4-ARHGAP28	rs9303988	18	6667583	C/T	Asthma exacerbations	1.03 (1.00 – 1.05)	0.012	3	1.30 (0.81 – 2.11)	0.279
HRH4-ZNF521	rs9955411	18	22074720	T/A	% ΔFEV_1	NA	1.28×10^{-4}	5	0.94 (0.56 – 1.58)	0.814
ZNF432-ZNF841	rs3752120	19	52552021	T/C	BDR	1.03 (1.02 – 1.05)	4.58×10^{-6}		0.97 (0.55 – 1.71)	0.917
	rs3450	19	52552999	C/T	BDR	1.03 (1.02 – 1.04)	1.93×10^{-6}	8	0.89 (0.52 – 1.54)	0.681
	rs12460587	19	52586919	G/T	BDR	1.04 (1.02 – 1.05)	5.69×10^{-7}		0.98 (0.56 – 1.73)	0.948
ELMO2-ZNF334	rs279728	20	45080421	T/C	Asthma exacerbations	1.02 (1.01 – 1.03)	6.45×10^{-3}	3	2.59 (0.96 – 6.99)	0.061

[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.

Citations:

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Table S6. Genomic-region replication of previous associations of ICS response. Evidence of association with the ΔFEV_1 after ICS treatment in asthma patients.

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	970	112	4.48×10^{-4}	NA	rs11538545	C/G	1.20 (0.71 – 2.05)	0.496
FBXL7	1251	69	7.19×10^{-4}	NA	rs1019810	G/A	0.46 (0.28 – 0.77)	2.88×10^{-3}
CMTR1	588	31	1.61×10^{-3}	NA	rs56242039	C/G	0.30 (0.14 – 0.67)	3.40×10^{-3}
MMS22L-FBXL4	4218	100	5.01×10^{-4}	NA	rs1206129	A/G	0.50 (0.31 – 0.80)	3.77×10^{-3}
PDE10A-T	3872	138	3.63×10^{-4}	rs9365939	rs9365939	G/A	0.41 (0.26 – 0.65)	**1.92×10^{-4}**
				rs2118353	rs2118353	T/C	0.41 (0.26 – 0.65)	**1.92×10^{-4}**
UMAD1-GLCCI1	2508	196	2.55×10^{-4}	NA	rs200043140	A/G	0.23 (0.10 – 0.52)	3.98×10^{-4}
MAGI2	5798	415	1.21×10^{-4}	NA	rs17417090	C/T	2.51 (1.44 – 4.37)	1.20×10^{-3}
TRIM24	796	29	1.74×10^{-3}	NA	rs17837468	G/A	0.40 (0.19 – 0.86)	0.018
SHB-ALDH1B1	2009	18	2.75×10^{-3}	NA	rs3808862	A/G	1.99 (1.25 – 3.15)	3.42×10^{-3}
				NA	rs7047650	C/G	1.99 (1.25 – 3.15)	3.42×10^{-3}
				NA	rs4878179	T/C	1.99 (1.25 – 3.15)	3.42×10^{-3}
				NA	rs10814650	G/A	1.99 (1.25 – 3.15)	3.42×10^{-3}
				NA	rs10814651	T/C	1.99 (1.25 – 3.15)	3.42×10^{-3}

[a] Odds ratio for the effect alleles.
CI: Confidence Interval; E: Effect allele; ICS: inhaled corticosteroids; NA: not available; NE: Non-effect allele; SNP: single-nucleotide polymorphism; ΔFEV_1: change in forced expiratory volume in one second.
Significant *p*-values after multiple comparison adjustment are in boldface.

Table S6 (continuation). Genomic-region replication of previous associations of ICS response. Evidence of association with the ΔFEV_1 after ICS treatment in asthma patients.

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
NAV2-HTATIP2	2694	114	4.36×10^{-4}	NA	rs73429709	C/T	2.55 (1.42 – 4.57)	1.63×10^{-3}
EDDM3B	912	84	5.97×10^{-4}	NA	rs57736800	G/A	0.49 (0.30 – 0.80)	4.17×10^{-3}
				NA	rs61552195	C/T	0.49 (0.30 – 0.80)	4.17×10^{-3}
L3MBTL4-ARHGAP28	3434	135	3.70×10^{-4}	NA	rs8083583	T/C	0.45 (0.27 – 0.78)	4.11×10^{-3}
HRH4-ZNF521	2983	147	3.39×10^{-4}	NA	rs11877115	C/T	2.34 (1.24 – 4.42)	8.43×10^{-3}
ZNF432- ZNF841	875	57	8.76×10^{-4}	NA	rs111463681	T/C	3.33 (1.36 – 8.13)	8.42×10^{-3}
ELMO2-ZNF334	709	83	6.02×10^{-4}	NA	rs76086573	C/A	0.12 (0.03 – 0.58)	8.03×10^{-3}

[a] Odds ratio for the effect alleles.
CI: Confidence Interval; E: Effect allele; ICS: inhaled corticosteroids; NA: not available; NE: Non-effect allele; SNP: single-nucleotide polymorphism; ΔFEV_1: change in forced expiratory volume in one second.
Significant *p*-values after multiple comparison adjustment are in boldface.

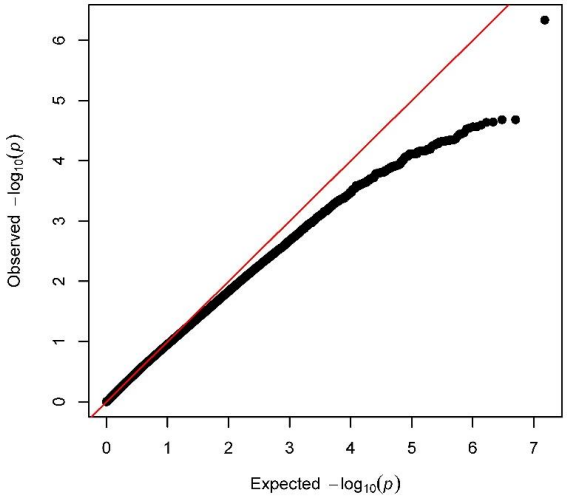

Figure S1. Quantile-quantile plot of association results of ICS response measured as the binary outcome related to the change in FEV_1 after ICS treatment. The logarithmic transformation of the observed and expected association results (-log10 *p*-value) is represented on the *y*-axis and *x*-axis, respectively (λ_{GC}=1.00).

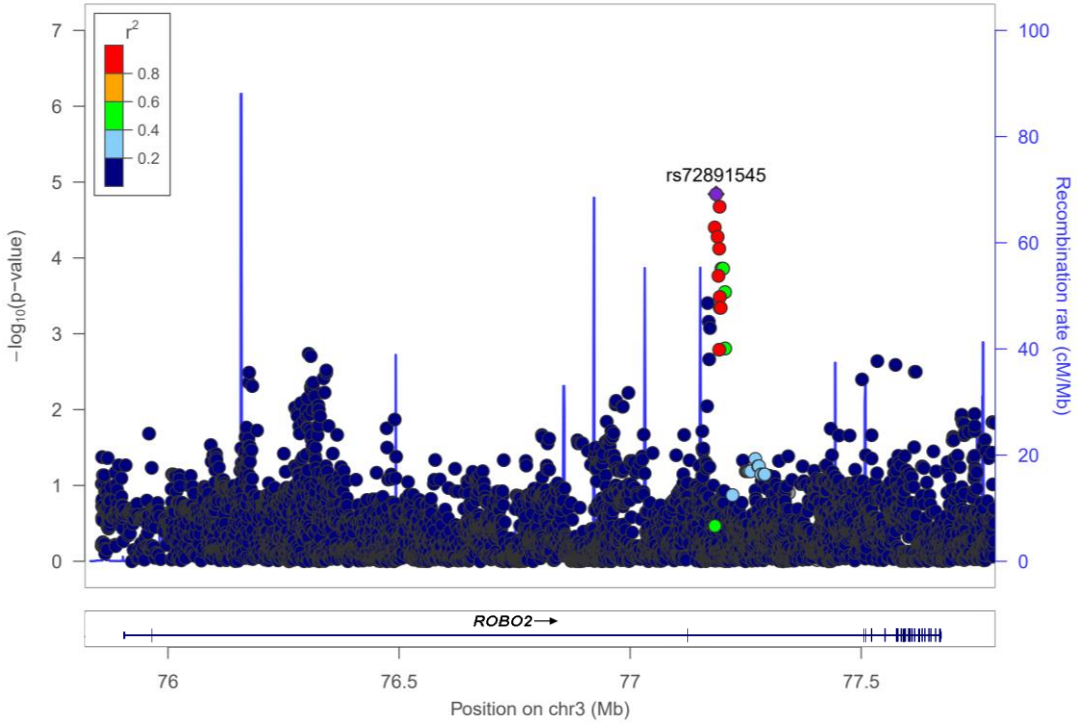

Figure S2. Regional plot of association results with asthma exacerbations despite ICS use in European children and young adults. The *y*-axis represents the logarithmic transformation of the association results (-$\log_{10}$ *p*-value) by chromosome position (*x*-axis) for each SNP as a dot. The most significant variant after Bonferroni-like correction is represented by a diamond (rs72891545). The remaining SNPs are color-coded based on pairwise linkage disequilibrium (r^2 values) with that SNP for European populations from 1KGP (GRCh37/hg19 build).

3.5. Combination of genomics and transcriptomics in response to glucocorticosteroids

A few studies have explored the genes contributing to ICS response using a combination of different omics data, although with a scarce combination of several omics layers. This *Chapter* includes the results of a study combining transcriptomic and genomic data to identify novel markers involved in the response to ICS treatment. This work was partially performed as part of a research internship at the Center for Human Molecular Genetics and Pharmacogenomics at the Faculty of Medicine, University of Maribor (Slovenia) under the supervision of Prof. Dr. Uroš Potočnik between 14th January 2019 and 3rd June 2019 funded by an M-AES Research Scholarship (Instituto de Salud Carlos III, MV18/00038). Differential gene expression was evaluated using publicly available transcriptome data from ASM cells *in vitro* treated with GCs. Genes with significant changes in expression levels were also inspected in PBMCs from responders and non-responders to ICS therapy based on the absence or occurrence of asthma exacerbations, respectively. Further validation was carried out using three publicly available transcriptome datasets from ASM cells experimentally exposed to GCs or control solutions. Genetic variants within the genes with dysregulated expression levels across all the datasets evaluated were tested for association with asthma exacerbations despite ICS using genomic data from children and young adults from European and non-European populations.

A total of 24 genes showed evidence of differential expression in ASM treated with GCs and PBMCs from ICS responder asthma patients. From these, six genes were found to be overexpressed in independent transcriptomic datasets from ASM cells treated with GCs compared to those exposed to control solutions. Genetic variants at *LTBP1* were associated with asthma exacerbations despite ICS use in European and non-European populations, which could be a potential novel locus of ICS response in children and youth with asthma. These results suggest that the combination of data from different omics sources could be a powerful strategy to identify novel markers involved in the response to asthma medications.

This *Chapter* is presented as an extended version of the article entitled “Combined analysis of transcriptomic and genetic data for the identification of loci involved in glucocorticosteroid response in asthma” published as a *Letter to the Editor* in *Allergy* in 2020 (doi.org/10.1111/all.14552). This article is reproduced under the terms of John Wiley and Sons license (number 4913680940940).

Combined analysis of transcriptomic and genetic data for the identification of loci involved in glucocorticosteroid response in asthma

Running title: Combination of omics in asthma treatment response

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ABSTRACT

Background. Inhaled corticosteroids (ICS) are the most commonly prescribed medication to control asthma symptoms. Both environmental and genetic factors are involved in the variability in response to this medication. However, few biomarkers have been associated with response to asthma treatment with ICS. Here, we aimed to analyze transcriptomic and genomic data to identify novel markers of ICS response.

Methods. Differential gene expression analyses were performed using transcriptome data from airway smooth muscle (ASM) cells treated *in vitro* with glucocorticosteroids (GCs). Genes with changes in expression levels after GCs exposure were examined in peripheral blood mononuclear cells (PBMCs) from responders and non-responders to ICS treatment based on asthma exacerbations occurrence. Validation was performed using three publicly available ASM transcriptomic datasets. Genes with evidence of differential expression in response to GCs were tested for association with exacerbations despite ICS use in European (n=2,681) and admixed (n=1,347) children and youth with asthma.

Results. A total of 24 genes showed significant changes in expression levels after GCs treatment in ASM cells and PBMCs from responders to ICS. Evidence of overexpression of six of them was validated in independent transcriptomic datasets of ASM exposed to GCs. Variants within *LTBP1* were associated with asthma exacerbations despite ICS use in Europeans ($p = 3.28 \times 10^{-6}$) and admixed populations (minimum $p = 6.76 \times 10^{-5}$).

Conclusions. *LTBP1* was found to be a potential novel locus of ICS response in childhood asthma. These findings suggest that integrating data from different omic sources could provide insights about molecular mechanisms involved in asthma treatment response.

Keywords: childhood asthma, exacerbations, glucocorticosteroids, pharmacogenomics, transcriptome.

INTRODUCTION

An increasing number of therapies are available to treat asthma, the most common chronic childhood disease. However, inhaled corticosteroids (ICS) are still the most commonly prescribed and effective controller medication for this disease[1,2]. While most children with asthma treated with ICS have been demonstrated to show an improvement of their symptoms, 30-40% of individuals fail or poorly respond to this medication[3]. Moreover, 10-15% of children treated with ICS still experience asthma exacerbations despite regular use of this medication[3]. While adherence is well recognized as a reason for treatment failure, it can occur despite optimal usage. Substantial differences in response to asthma treatment with ICS have also been reported among different populations and ethnic groups[3,4]. However, the molecular mechanisms underlying non-ICS-responsiveness among individuals and populations remain unknown[5].

Beyond adherence, the variability in ICS response has been suggested to be the result of the interaction of multiple factors including clinical phenotypes, ancestry, environmental and genetic factors[6,7]. A high contribution of the individual genetic composition in ICS response has been attributed to heritability estimates of 40-60%[8]. However, to date, the number of genetic markers involved in ICS response is limited and they are not sufficient to predict whether an individual will achieve control of their asthma using ICS[9]. Therefore, the findings from genetic studies have not provided a real improvement in asthma management in clinical practice[9,10].

Pharmacogenomics has been the most predominant approximation to ICS response research. In this respect, only a few studies have attempted to identify markers involved in asthma treatment response with ICS using data from other single omic sources apart from genomics[9]. Nevertheless, the power of these strategies to provide insights about asthma treatment response mechanisms has been demonstrated[9,11], especially when several -omics layers are integrated[12]. To our knowledge, multi-omic studies of ICS response in asthma patients are scarce[13,14].

Diverse mechanisms of action have been attributed to ICS, though many of them involve a reduction of airway inflammation[2] through regulation of gene transcription[15]. Several cell types are suggested to be the source of inflammatory mediators in asthma such as structural airways cells, including fibroblasts, airway smooth muscle (ASM), endothelial, and epithelial cells[15]. Nevertheless, the action of GCs on other important asthma subphenotypes has been described, including airway muscle contractility, hyperresponsiveness, and bronchodilation[16,17]. It has been suggested that epithelial and ASM cells may be the main targets of GCs[18,19], in addition to inflammatory cells (e.g. eosinophils, lymphocytes, mast, and dendritic cells)[15]. ASM cells are involved in the major asthma-related phenotypes, although the specific molecular mechanisms of action of GCs on ASM cells are not well known[20]. A few studies have recently explored the gene expression patterns in ASM cells in response to GCs treatment by applying next-generation sequencing to the analysis of the transcriptome (i.e., RNA-seq)[20,21]. In the present study, we aimed to identify novel markers involved in the response to GCs by combining transcriptome data obtained from ASM and peripheral blood mononuclear cells (PBMCs) with genomic data from patients with different patterns of ICS response.

METHODS

Exploration of changes in the transcriptome of ASM cells in response to GCs

As the discovery phase of this study, differential gene expression analyses were performed on RNA-seq data obtained from ASM cells isolated from four non-asthmatic male lung transplant donors of European ancestry publicly available at the Sequence Read Archive (SRA) (https://www.ncbi.nlm.nih.gov/sra) (accession number SRP033351)[20] (**Figure S1**). Briefly, this study performed *in vitro* experiments on cell lines from each donor treating them with control solution or 1 μM dexamethasone for 18 h[20] and carried out RNAseq to analyze the effects of treatment in gene expression[20].

RNA-seq raw FASTA files were downloaded from SRA, converted to fastq format using a perl script code assuming a quality score of 40. Subsequent analyses were performed with a modified pipeline in R 3.4.4[22], including initial quality control (QC) analysis based on parameters obtained with the FastQC 0.11.8 software[23] to detect overrepresented sequences, GC contents, length distributions, and duplication levels. Alignment to the GRCh37/hg19 reference genome was performed for paired-end reads using the *Rsubread* package[24,25]. Mapped sequence reads were assigned to genomic features at gene-level using the *featureCounts* function implemented in *Rsubread*[26]. Counts per million (CPM) were obtained using *edgeR*[27]. Very low expressed genes were filtered out based on CPM values corresponding to 10 or fewer read counts. Retained genes were normalized according to library sizes using the trimmed mean of M values (TMM) method implemented in *edgeR*[28]. Subsequently, mean-variance modeling at the observational level (VOOM) transformation was applied to filter and normalize counts[29]. Differential gene expression levels in cells treated with GCs compared to those treated with control solution were evaluated considering that cells exposed to both experimental conditions were obtained from the same donors. Linear regression models were applied through moderated t-tests allowing for sample-pair effects using the package LIMMA[30]. Differentially dysregulated genes in response to GCs treatment were identified after multiple comparison adjustment with a false discovery rate (FDR) of 5% (q-value≤0.05)[31].

Analysis of genes dysregulated by GCs treatment in PBMCs from asthma patients treated with ICS

Genes differentially expressed in ASM cells treated with GCs were examined to determine if they also showed changes in PBMCs obtained from asthma patients treated with ICS from the SLOVENIA study (approved by the Slovenian National Medical Ethics Committee (Ljubljana, Slovenia), ethical approval number: 0120-569/2017/4)[32,33]. RNA was extracted from six children and young adults with asthma under regular use of ICS during the previous 12 months of study enrollment and libraries were sequenced using the BGISEQ-500 instrument (BGI Inc.). Based on data on emergency asthma care, hospitalizations, and/or administration of oral corticosteroids because of asthma symptoms in the past 12 months, patients were classified as ICS non-responders (n=3), if they had a history of any of such events or, responders to ICS (n=3) if they did not experience any of those types of events (**Table S1)**. Further details are described in the Supplementary Material.

QC analyses were performed on the RNA-seq data obtained from PBMCs following the same methodology described above for the ASM cells. The relative abundance of each PBMC type was estimated

through the CIBERSORT method using PBMC LM22 signature matrix[34]. Differential gene expression analyses were carried out using linear models implemented on LIMMA[30]. The relative percentage of lymphocytes B and T, natural killer cells, and monocytes among total PBMCs were included as covariates in the analyses. To equate for fold change (FC) direction, differential gene expression was assessed for responders to ICS or GCs treated ASM cells relative to ICS non-responder patients or control (not GCs exposed) ASM cells, respectively. FDR was used for multiple comparison adjustments accounting for the genes analyzed in this dataset (**Figure S1**).

Validation of transcriptome changes in additional datasets from ASM cells

Validation of significant differentially expressed genes found in both ASM cells treated with GCs and PBMCs from asthma patients treated with ICS was sought using additional datasets of gene expression profiles of ASM cells after GCs exposure (GSE13168, GSE34313, and SRP098649) (**Table S2**, **Figure S1**). Differential expression analyses comparing ASM cells exposed to GCs treatment or control solutions were performed using Reproducible Analysis and Validation of Expression Data (RAVED) (https://github.com/HimesGroup/raved) through the online tool Reducing Associations by Linking Genes And omics Results (REALGAR) (http://realgar.org/)[35,36]. Differential expression results obtained from these three independent ASM transcriptomic datasets were combined in a meta-analysis using a random-effects model accounting for the variance among studies through the *metaVolcanoR* package[37]. Evidence of replication was considered for genes with significantly consistent changes in expression levels in cells treated with GCs relative to the controls (q-value≤0.05).

Association of genetic variants within identified genes with asthma exacerbations despite ICS treatment in children and young adults with asthma

The association of genetic variants located within 100 kilobases (kb) upstream or downstream from the genes with evidence of differential expression in all the transcriptomic datasets with asthma exacerbations despite ICS use was explored (**Figure S1**). For that, ten studies participating in the Pharmacogenomics in Childhood of Asthma (PiCA) consortium[38] were analyzed. A total of eight studies in European descent populations were included: the Pharmacogenetics of Asthma Medication in Children: Medication with Anti-inflammatory effects (PACMAN); the Paediatric Asthma Gene-Environment Study (PAGES); BREATHE; Genetics of the Scottish Health Research Register (GoSHARE); the Pharmacogenetics of Adrenal Suppression study (PASS); SLOVENIA; the follow-up stage of the Multicenter Asthma Genetics in Childhood Study (followMAGICS); Effectiveness and Safety of Treatment with Asthma Therapy in Children (ESTATe). Additionally, two studies in admixed populations were also analyzed: Latinos/Hispanics from the Genes-Environment and Admixture in Latino Americans (GALA II) study, and African Americans included in the Study of African Americans, Asthma, Genes and Environments (SAGE)[33]. Details about ethical approval of each study are described in the Supplementary Material and additional information about the studies is described elsewhere[33].

Children and young adults with asthma (2-25 years old) with available genome-wide genotype data, reported use of ICS, and data related to the ICS response based on the occurrence of asthma exacerbations

during the 6 or 12 months preceding the study enrollment were included (Supplementary Material). Asthma exacerbations were defined by the need for emergency care, hospitalizations, or administration of systemic corticosteroids because of asthma (**Table S3** and **Table S4**). Alternative definitions were used for those studies without available information regarding any of these events, such as unscheduled general practitioner or respiratory system specialist visits and school absences (**Table S3**). Individuals with asthma exacerbations despite ICS treatment were considered as ICS non-responders and those without asthma exacerbations as ICS responders.

Association between imputed genetic variants and asthma exacerbations despite ICS treatment was tested for each study through Wald logistic regressions using EPACTS 3.2.6[39]. Principal components (PCs) of genetic ancestry were calculated to account for population stratification using PLINK v1.9[40,41] and EIGENSOFT 6.14[42]. Subsequently, regression models were adjusted by age, gender, and PCs. The number of PCs used as covariates was chosen comparing different regression models including different numbers of PCs and that showing the best lambda fit (λ_{GC}~1), estimated through the *Gap* v1.2.2 R package, was selected. Further details have been described elsewhere[33]. Two separate meta-analyses were performed based on the ancestry of the studies included: European and non-European. Single nucleotide polymorphisms (SNPs) with a minor allele frequency (MAF)≥1% and imputation quality (Rsq)≥0.3 shared among the studies included in each group were meta-analyzed applying fixed-effects or random-effects models depending on the Cochran Q-test implemented on METASOFT[43]. Independent variants within each gene region evaluated were separately estimated for Europeans and non-Europeans through empirical autocorrelations based on the -$\log_{10}$ *p*-value of each SNP analyzed using *coda* R package[44]. A Bonferroni-like correction was applied accounting for the total number of independent variants tested for each population group. Evidence of association with ICS response was considered for those variants reaching the threshold established as α=0.05/total number of independent variants.

Sensitivity analyses were carried out for the variants identified through the same association models but also adjusting for the treatment step as a proxy of asthma severity[45]. Only patients with available data about the medications included in the definition of medication steps were included. Logistic regressions were carried out with R 3.4.4[22] including a covariate classifying individuals as follows: *Step 1*, as-needed use of short-acting β2 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 β2 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.

RESULTS

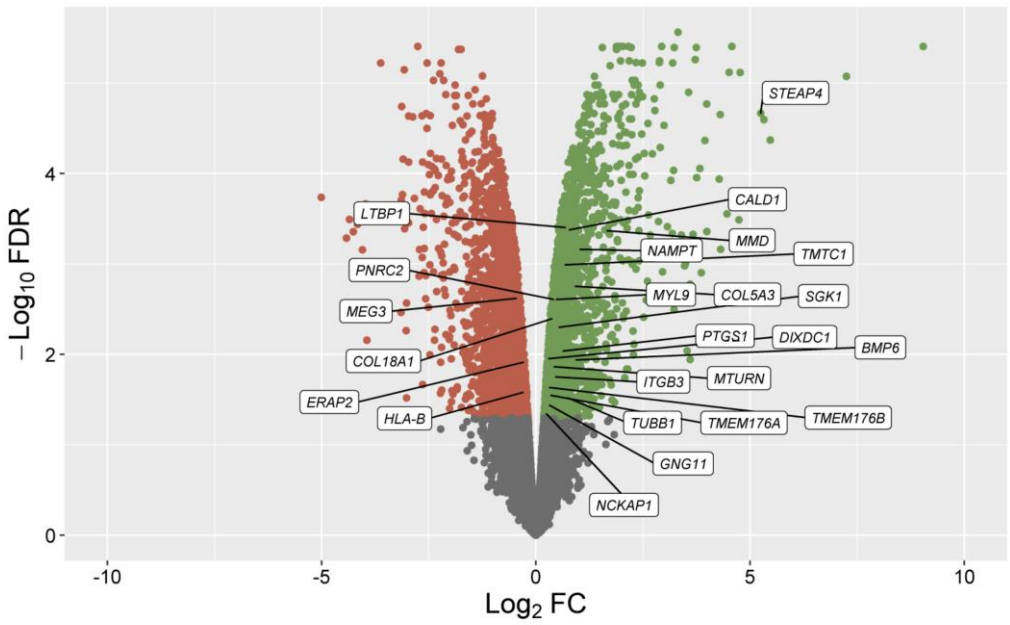
Differential gene expression analyses in airway smooth muscle cells in response to GCs

An average of 48.8 million total reads was obtained per sample and an average of 81.9% of the reads was successfully mapped to the GRCh37/hg19 build of the human reference genome. A total of 14,707 genes were analyzed and 4,718 of them were found to be differentially expressed in cells treated with GCs compared to those exposed to control solution (*q*-value≤0.05) (**Figure 1A**).

Analysis of changes in gene expression driven by GCs exposure in peripheral blood mononuclear cells from asthma patients treated with ICS

The relative abundance of each PBMC type showed no evidence to be statistically different between asthma patients with patterns of responsiveness and non-responsiveness to ICS therapy (*p*-value>0.05) from the SLOVENIA study (**Figure S2**). Genes with altered expression levels in ASM cells after treatment with GCs were followed up for replication using PBMCs transcriptome profiles obtained from six patients treated with ICS from the SLOVENIA study (**Table S1**). From the 4,718 genes found differentially expressed in ASM cells after *in vitro* GCs exposure, 32 genes showed significant changes in expression levels in PBMCs depending on the ICS response status (*q*-value≤0.05). Of those, 24 genes showed consistent alteration in expression levels compared to samples taken as controls in both datasets based on $\log_2$ FC values (**Figure 1B**, **Table S5**).

A



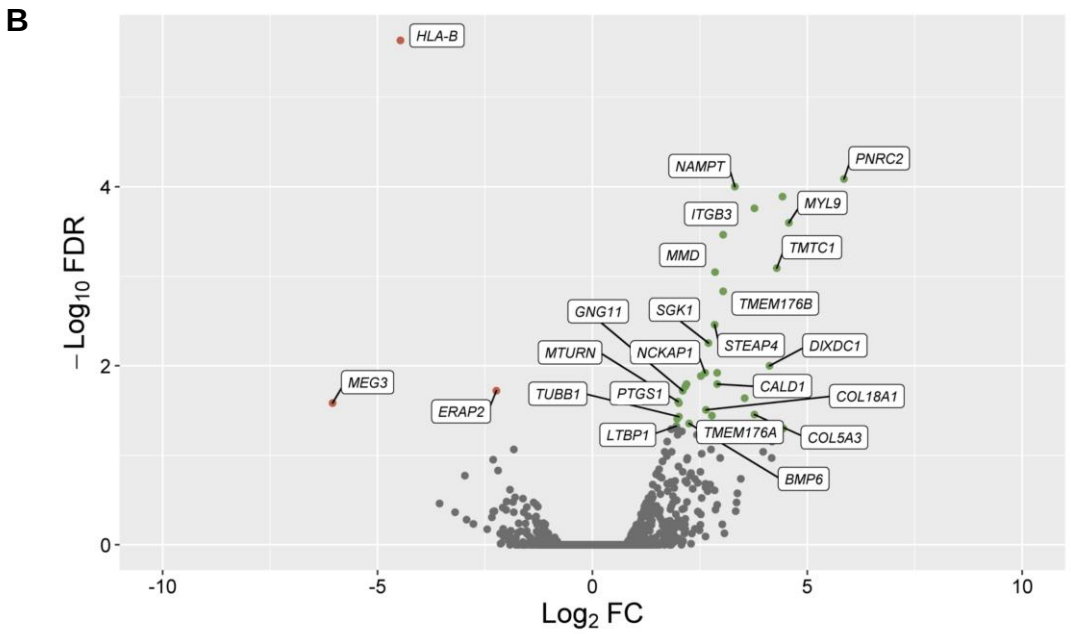


Figure 1. Results of differential expression in response to GCs in ASM cells and PBMCs. Volcano plot of differential expression results in ASM cells after exposure to GCs compared to a control solution (panel A). Results of differential expression in PBMCs from ICS responders are shown for genes with significant changes in expression levels in ASM cells in response to GCs after adjusting with a false discovery rate (FDR) of 5% (q-value≤0.05) (panel B). Results are represented in terms of $\log_2$ fold change ($\log_2$ FC) (x-axis) and the logarithmic transformation of FDR ($-\log_{10}$ FDR) (y-axis). Genes significantly (q-value≤0.05) found to be up-regulated ($\log_2$ FC>0) or downregulated ($\log_2$ FC<0) are represented by means of green or red dots, respectively. Genes with consistent alteration of expression levels in ASM cells and PBMCs are labeled into white boxes.

Validation of transcriptome changes in response to GCs in additional datasets from ASM cells

From the 24 genes with consistent changes in expression levels in response to GCs stimulation and related to asthma exacerbations despite ICS treatment, six genes were up-regulated in cells experimentally treated with different GCs compared to those exposed to control solutions after combining the publicly available ASM transcriptomic datasets: *LTBP1* (q-value=7.46x10^{-4}), *MTURN* (q-value=3.92x10^{-3}), *NAMPT* (q-value=3.77x10^{-7}), *CALD1* (q-value=5.22x10^{-5}), *MMD* (q-value=5.84x10^{-4}), *COL18A1* (q-value=1.93x10^{-3}) (**Table S6**). The potential implication of these genes on asthma severity was assessed by evaluating their expression in PBMCs according to baseline lung function, showing no significant changes (**Table S7**). Additionally, only *LTBP1* remained significantly differentially expressed in response to ICS treatment after including baseline lung function as a covariate (**Table S7**).

Association of genetic variants within identified genes with asthma exacerbations despite ICS use in children and young adults with asthma

Association of 7,042 SNPs within 100 kb upstream and downstream from the genes *LTBP1*, *MTURN*, *NAMPT*, *CALD1*, *MMD*, and *COL18A1* with asthma exacerbations despite ICS use was evaluated in European children and young adults with asthma. After applying a Bonferroni-like correction considering the total number of independent variants tested across all the gene regions (p≤4.96x10^{-5} for 1,007 independent variants tested), the SNP rs11681246 located within *LTBP1* (**Figure 2**) was significantly associated with

asthma exacerbations despite ICS use in Europeans (OR for G allele: 0.72, 95% CI: 0.63 – 0.83, p = 3.28x10^{-6}) (**Figure 3**, **Table S8**). The effect of this association was similar after adjusting by treatment step, as a proxy of disease severity, in a subset of 2,282 patients with complete information about asthma medication (OR for G allele: 0.74, 95% CI: 0.63 – 0.86, p=1.13x10^{-4}). However, this SNP was not replicated in admixed populations, which could be explained by differences in frequency of the effect allele between Europeans (39.4-47.2%), Hispanics/Latinos (25.7%), and African Americans (29.2%). Nonetheless, we assessed the association of alternative polymorphisms in this genomic region in admixed populations, revealing the association of six intronic *LTBP1* variants in high linkage disequilibrium (r^2>0.95) (**Table S8**), all dependent on the association of rs76390075 (OR for C allele: 0.40, 95% CI: 0.26 – 0.63, p=6.76x10^{-5}), after adjustment for 234 independent variants tested (p≤2.14x10^{-4}) (**Figure 4**). Moreover, this association was robust to the adjustment by medication step, OR for C allele: 0.41, 95% CI: 0.26 – 0.65, p=1.12x10^{-4}. However, the association signals detected in European and admixed populations are not in LD (r^2<0.01).

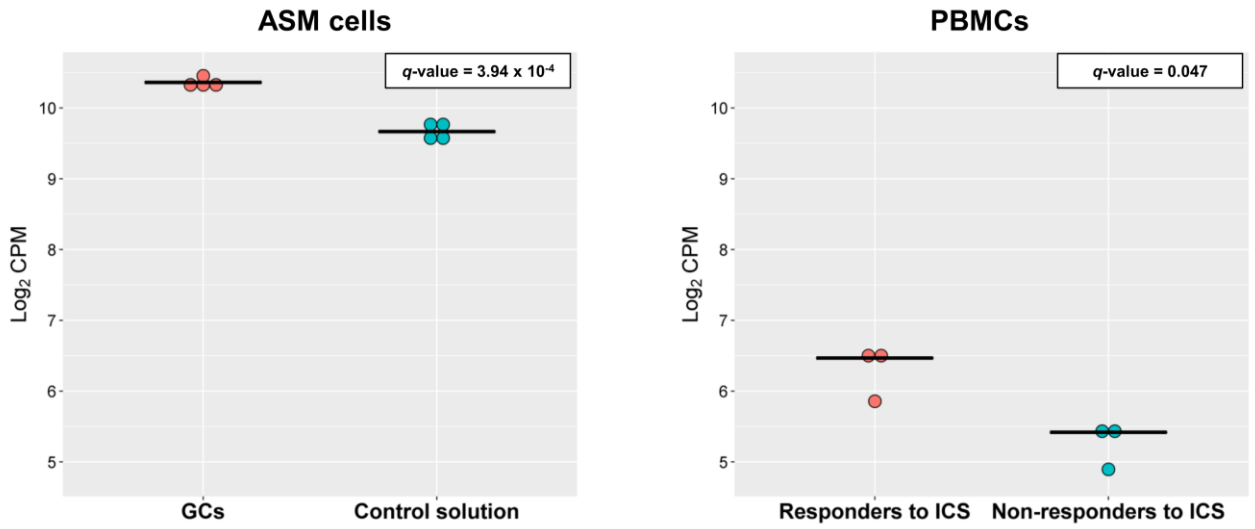


Figure 2. Dot plots of differential expression for *LTBP1* in ASM cells and PBMCs in response to GCs. Gene expression levels are represented in terms of log_2 counts per million (CPM) in the *y*-axis as dots for cases (red) and control (blue) samples. The median expression level is represented for each sample group by a black horizontal line. *P*-values adjusted by false discovery rate are shown (*q*-value).

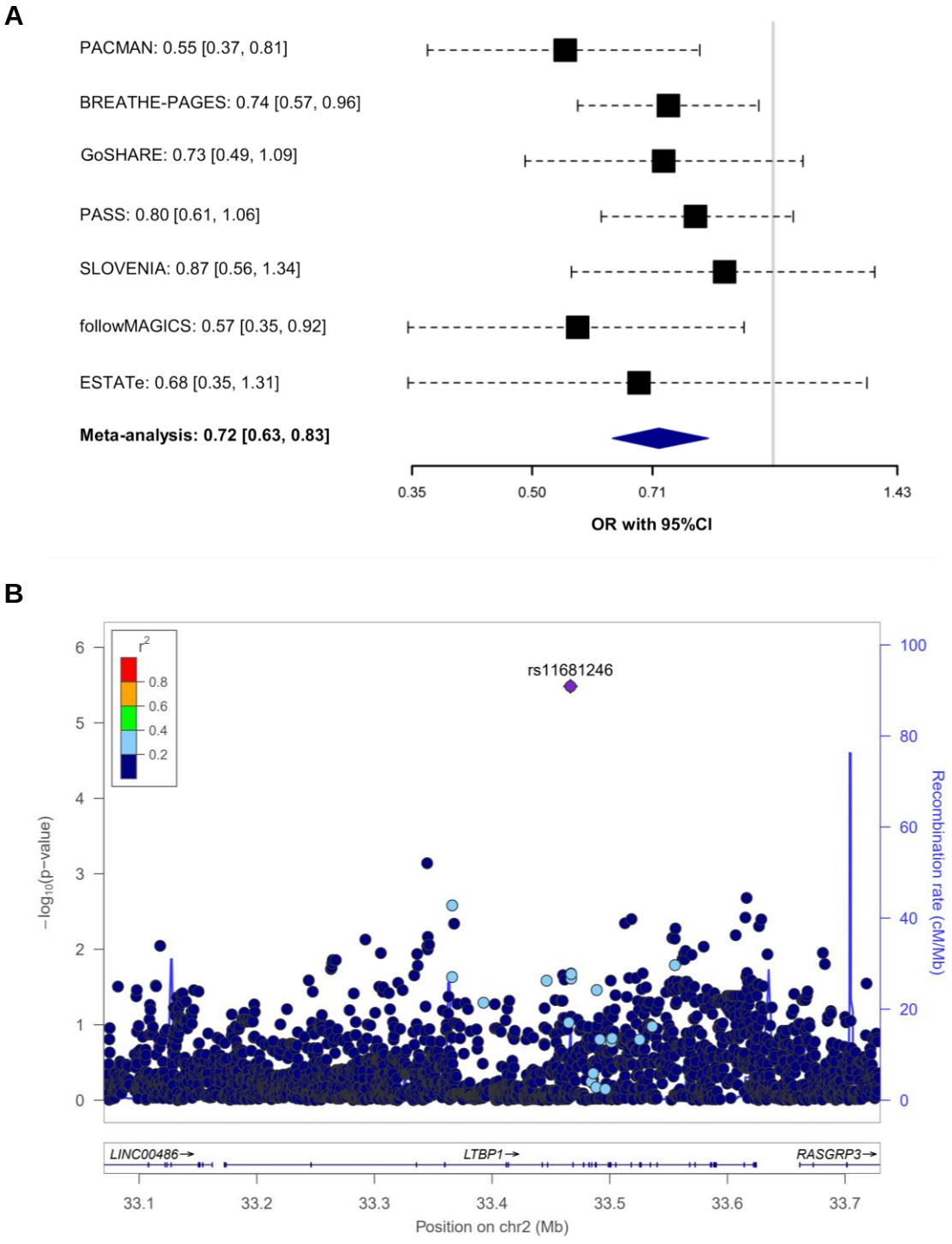


Figure 3. Association results with asthma exacerbations despite ICS treatment for *LTBP1* in European populations. Forest plot of association effects SNP rs11681246 across the European studies included in the association analyses (panel A). Association effects are shown in terms of odds ratio (OR) for the effect allele (G) for each study and after performing a meta-analysis of the results by black boxes and a blue diamond, respectively. The 95% Confidence Intervals (95% CI) are represented by black dash lines. Results are not provided for BREATHE since rs11681246 did not pass quality control checks in this study. Regional plot of association results with asthma exacerbations despite ICS use (panel B). The 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 most significant variant after Bonferroni-like correction is represented by a diamond. The remaining SNPs are color-coded based on pairwise linkage disequilibrium (r^2 values) with that SNP for European populations from 1KGP (GRCh37/hg19 build)[62].

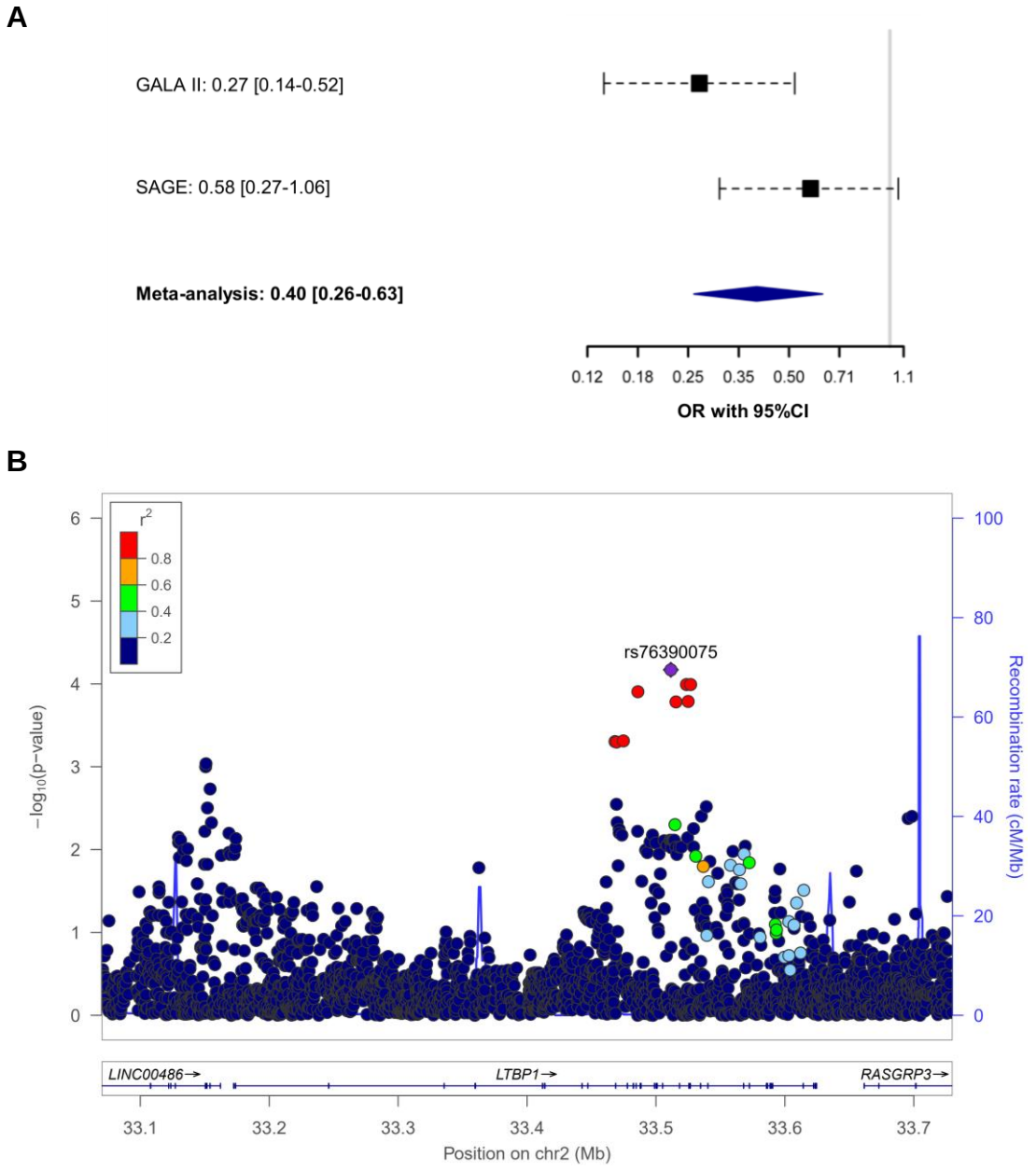


Figure 4. Association results with asthma exacerbations for *LTBP1* in Latinos/Hispanics and African Americans treated with ICS. Forest plot of association effects of the most significant *LTBP1* variant associated with asthma exacerbations despite ICS use in admixed populations (rs76390075) (panel A). Association effects are shown as odds ratio (OR) for the effect allele (G) for each study and after performing a meta-analysis of the results for admixed populations by black boxes and a blue diamond, respectively. The 95% Confidence Intervals (95% CI) are represented by black dash lines. Regional plot of association results of *LTBP1* variants with asthma exacerbations despite ICS use in admixed populations (panel B). The *y*-axis represents the logarithmic transformation of the association results ($-\log_{10}$ *p*-value) by chromosome position (*x*-axis) for each SNP as a dot. The diamond corresponds to the most significant variant after Bonferroni-like correction. The remaining SNPs are color-coded based on pairwise linkage disequilibrium (r^2 values) with that SNP for Admixed American populations from 1KGP (GRCh37/hg19 build)[62].

DISCUSSION

This study describes the results of transcriptomic analyses of several datasets to reveal candidate genes related to ICS response among asthma patients. After combining RNA-seq data from ASM cells treated with GCs with data from PBMCs from asthma patients with different patterns of ICS response based on the occurrence of asthma exacerbations, 24 genes showed consistent changes in expression levels. From these, overexpression of six genes after GCs exposure was validated in three independent ASM transcriptomic datasets. The implication of *LTBP1* in the response to GCs was validated by revealing the association of seven variants within this gene with asthma exacerbations despite ICS use among Europeans, Latinos/Hispanics, and African Americans. GCs were found to increase *LTBP1* expression levels in ASM cells experimentally exposed independently of the type of GCs. Similar effects were detected in PBMCs obtained from ICS responder asthma patients, but not in the ICS non-responders. This suggests that the ICS treatment may not influence *LTBP1* expression in PBMCs from patients who experience asthma exacerbations despite ICS treatment.

LTBP1 encodes a member of the family of latent-transforming growth factor-beta (TGF-β) binding proteins. LTBP1 is involved in the regulation of the TGF-β1 activity[46], including its activation from a precursor form[47], folding, secretion out from the cell[48], and deposition at the extracellular matrix[49] through interactions with fibrillin molecules[50]. Interestingly, TGF-β1 has been proposed to play a key role in cell growth and differentiation, immune response, and airway remodeling[51]. Specifically, increased levels of the active form of TGF-β1 have been detected in asthma patients, which has been suggested to recruit myofibroblasts triggering an increased collagen deposition and the development of subepithelial fibrosis in asthma[52]. *LTBP1* has been also proposed to be involved in allergic diseases and idiopathic pulmonary fibrosis (IPF)[53], where LTBP1 has been found to interact with fibulin 1c (FBLN1), modulating lung remodeling and fibrosis through the regulation of TGF-β1 activation[53]. Additionally, TGF-β1 induces the conversion of fibroblasts to myofibroblasts, which is significantly reduced through inhibition of FBLN1. For this reason, the inhibition of FBLN1 binding to LTBP1 has been proposed as a therapeutic strategy to reduce fibrotic processes[53]. Additionally, genetic variants near or within *LTBP1* have been associated with lung function (forced expiratory volume in 1 second and forced vital capacity) among participants from the UKBiobank (http://www.nealelab.is/uk-biobank/)[54]. Despite the limited sample size of the gene expression datasets evaluated, these findings support that *LTBP1* could play an important role in asthma-related phenotypes. Additionally, our sensitivity analyses suggested that *LTBP1* could be involved in the response to asthma therapy with ICS rather than disease severity. Nonetheless, validation of our findings in independent populations analyzing larger sample sizes and functional studies are needed. These will provide insights about the biological mechanisms implicating *LTBP1* in ICS response and to test whether its clinical relevance predicting the treatment response.

Interestingly, in this study, we found the *LTBP1* variants rs11681246 and rs76390075 were found to be the most significant associations with asthma exacerbations in patients treated with ICS from European and admixed populations, respectively. Previous evidence suggests that both could be involved in the regulation of gene expression in pulmonary cells, according to the Encyclopedia of DNA Elements (ENCODE)[55,56]. The SNP rs11681246 has been related to histone marks such as the monomethylation of

histone H3 at lysine 4 (H3K4me1) and acetylation of histone H3 at lysine 27 (H3K27ac) in several cell lines, including fetal fibroblasts and adult lung fibroblast primary cells. Moreover, this has been proposed to be a DNAse hypersensitivity site in pulmonary fibroblast primary cells[55,56]. On the other hand, the most significantly associated variant with asthma exacerbations despite ICS use (rs76390075) is in LD (r^2=1) in admixed populations with variants that had been evidenced to play a potential functional role in pulmonary tissues, including epigenetic modifications such as the acetylation of histone H3 at lysine 9 and, H3K4me1 and H3K27ac marks (rs75486357 and rs3820912), and the location at DNAse hypersensitivity sites in fetal fibroblasts and pulmonary fibroblast primary cells (rs3820912)[55,56]. Nonetheless, no evidence of implications as expression quantitative trait loci (eQTL) was found for any of these variants[57]. Therefore, this evidence supports the potential role of associated variants in gene expression regulation.

To the best of our knowledge, our study is one of the few combining diverse populations and different omics layers to identify genetic markers of ICS response[12,58,59]. The strengths of our study are related to the assessment of several different transcriptomic datasets and the fact that association analyses were carried out using data from the largest consortium studying the pharmacogenetic factors involved in asthma treatment response in children and youth, including populations with different ancestries. This is in contrast with previous studies, which have explored the association of a low number of variants in reduced sample sizes[9,33,60].

However, we acknowledge some limitations of our study. First, gene expression levels in response to GCs or control exposure were compared between reduced groups of ASM cells in the discovery phase of this study. Moreover, these cells were obtained from non-asthmatic individuals belonging to European-descent males, not representing the two genders and different ethnic groups[20]. Additionally, the sample size of PBMCs transcriptomics set consisted of a minimum of biological replicates needed to identify significantly differentially expressed genes. Second, gene expression profiles obtained using microarrays and RNA-seq assays were compared, even though some discrepancies have been attributed between these approaches[61]. In order to avoid bias, the two sources of data were analyzed in different stages of the analyses. Third, transcriptome data from different cell types were compared, although cell specificity has been attributed to the effects of GCs[58]. Fourth, transcriptomic datasets with different study designs were analyzed, including cells experimentally exposed to GCs and those extracted from patients under ICS therapy. Fifth, the occurrence of exacerbations despite ICS therapy was considered based on retrospective information, which could not reflect the real state at the time of the study enrollment. Additionally, no specifications about ICS dose, the type used, nor adherence were available, which are factors that could also influence treatment response.

In summary, our study revealed *LTBP1* as a novel locus for ICS response in asthma patients. These results indicate that combining publicly available data from different omic sources could be a powerful approach to provide novel insights about the mechanisms involved in the response to ICS treatment and thus, to develop alternative diagnosis and therapeutic strategies that could improve asthma management strategies in clinical practice.

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Author contribution

NH-P and MG equally contributed to this work. UP and MP-Y were involved in the conception and design of the study. NH-P, MG, SJ, AC, SJV, VB, MS, LSB, RT, SM, MP, KMV, MK, DBH, ST, CNAP, EGB, AHM-Z, UP, and MP-Y participated in the acquisition of data. NH-P, MG, AJ, LK, SJV, MA-H, MK, LSB, JV, CF, and MP-Y contributed to the analysis and/or interpretation of data. All authors drafted the article and/or participated in the final approval of the manuscript.

Conflict of Interest Statement

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SUPPLEMENTARY MATERIAL

Combined analysis of transcriptomic and genetic data for the identification of loci involved in glucocorticosteroid response in asthma

Running title: Combination of omics in asthma treatment response

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SUPPLEMENTARY METHODS

Analysis of genes dysregulated by GCs treatment in peripheral blood mononuclear cells from asthma patients treated with ICS

The SLOVENIA study includes children and young adults (5-18 years old) with a physician's diagnosis of mild or moderate persistent asthma of Slovenian origin. Part of the asthmatic patients included in the study were under regular treatment with inhaled corticosteroids (ICS) following the PRACTALL[S1] and NAEPP guidelines[S2]. Therefore, patients younger than 12 years old received a daily dose of 200 mcg of fluticasone, whereas those older than 12 years old were treated with 400 mcg of fluticasone. Adherence to ICS therapy was confirmed in consequent follow-ups based on parents/patient self-reports and, prescription refills. None of the patients were on high dose ICS therapy. Detailed information about the study design has been described elsewhere[S3,S4].

RNA was extracted from peripheral blood mononuclear cells (PBMCs) obtained from patients with asthma treated with ICS in the previous 12 months using the TRI Reagent commercial kit (Sigma-Aldrich, Inc.). The quality and integrity of RNA samples were measured using the Agilent RNA 6000 Nanochip (Agilent Technologies, Inc.) and the Agilent 2100 Bionanalyzer (Agilent Technologies, Inc.). LncRNA and mRNA 100 bp libraries were constructed using MGIEasy rRNA Depletion Kit (MGITech Co., Ltd) and MGIEasy RNA Library Prep Set (MGITech Co., Ltd). Paired-end sequencing was performed using the BGISEQ-500 instrument (BGI Inc.) at BGI-Europe facilities (BGI Inc.).

Design and characteristics of the studies included in the validation using genomic data

European populations

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) with a declared use of any asthma medications. This information is based on records from community pharmacies in the Netherlands[S5].

PAGES (n = 437)

The Paediatric 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 in the United Kingdom. Clinical assessment through questionnaires and saliva sample collection was carried out. Candidates with any respiratory diseases apart from asthma or significant health problems were excluded[S6].

BREATHE (n = 288)

The BREATHE study recruited participants (3-22 years old) with a physician diagnosis of asthma from the United Kingdom[S7-S9]. From the BREATHE samples included, genotypes from 182 patients had been obtained using the Illumina Infinium CoreExome-24 BeadChip (Illumina) array, whereas 103 samples were genotyped using the Axiom™ Precision Medicine Research Array (Affymetrix Inc.). Association analyses

were performed for the latter together with PAGES samples due to similarities of study design and sample characteristics, denoted as BREATHE-PAGES.

GoSHARE (n = 472)

Children and young adults aged 3 to 18 years old participating in the Genetic of Scottish Health Research Register (GoSHARE) study were recruited in Tayside (Scotland) using databases from the National Health Service. Further details have been reported in previous publications[S10].

PASS (n = 402)

Participants (5-18 years old) with a physician diagnosis of asthma and under treatment with inhaled corticosteroids (ICS) from the Pharmacogenetics of Adrenal Suppression study (PASS) were included. This study was initially designed to assess associations between the use of corticosteroids and adrenal suppression so that, clinical concern about adrenal suppression was also considered as an inclusion criterion[S11, S12].

SLOVENIA (n = 182)

Children and young adults (5-18 years old) with mild and moderate persistent asthma recruited from tertiary health centers in Slovenia were included in the SLOVENIA study. Lung function was measured as the forced expiratory volume in 1 second (FEV_1) before and after 6 weeks after treatment with ICS using the Vitalograph 2150 spirometer (Compact, Buckingham, UK) according to ERS/ATS guidelines[S3].

followMAGICS (n = 147)

Participants from the observational Multicenter Asthma Genetics in Childhood Study (MAGICS) were included in the follow-up phase of this study (followMAGICS), including patients with persistent asthma symptoms, now aged from 7 to 25 years. Recruitment was carried out at secondary and tertiary centers from Germany and Austria[S13-S16].

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 (4-19 years old). Patients with a physician diagnosis of asthma were recruited at primary care units at the Netherlands based on electronic medical records. Only those individuals with reported use of asthma controller therapy were included[S4].

Admixed populations

GALA II (n = 854)

Genes-Environment and Admixture in Latino Americans (GALA II) is a case-control study including patients with a physician diagnosis of asthma and controls (8-21 years old) with four Latino grandparents. Participants were recruited from five different centers in the United States and Puerto Rico[S17].

SAGE (n = 493)

The Study of African Americans, Asthma, Genes and Environments (SAGE) is a cross-sectional asthma study, whose participants show similar characteristics as GALA II, although only those with four

grandparents of African American ancestry were included in this study. In this case, subjects were recruited in centers from the United States[S17, S18].

Ethical approval of the studies of childhood asthma from the PiCA consortium

All studies included were approved by their local institutional review boards and written informed consent was provided by participants or their parents/caregivers. 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 (ethical approval 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 Center (Rotterdam, the Netherlands) (ethics approval number: MEC-2011-474) approved ESTATe. 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).

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Table S1. Clinical and demographic characteristics of the asthma patients from the SLOVENIA study included in RNA-seq experiments.

	Total	Responders to ICS [a]	Non-responders to ICS [b]
Sample size	6	3	3
Gender (% male)	50.0	66.7	33.3
Mean age ± SD (years)	10.0 ± 2.0	11.3 ± 0.6	10.7 ± 3.1
Mean ΔFEV_1 ± SD (%)	10.8 ± 13.8	13.0 ± 19.0	8.7 ± 10.2
Asthma exacerbations in the last 12 months (%)	50.0	0.0	100.0
ER visits (%) [c]	50.0	0.0	100.0
OCS use (%) [d]	50.0	0.0	100.0
Hospitalizations (%) [e]	50.0	0.0	100.0

[a] Asthma patients treated with ICS who were prevented from suffering asthma exacerbations in the last 12 months; [b] Asthma patients who suffered any exacerbations in the last 12 months despite ICS treatment; [c] Proportion of patients with any exacerbations who sought emergency care due to asthma; [d] Proportion of patients with any exacerbations who needed the use oral corticosteroids because of asthma; [e] Proportion of patients with any exacerbations who needed to be hospitalized because of asthma.
ER: emergency room; FEV_1: forced expiratory volume in one second; ΔFEV_1: change in FEV_1 after 6 weeks of ICS treatment; OCS: systemic corticosteroids; SD: standard deviation.

Table S2. Publicly available transcriptomic datasets evaluating the effects of GCs in ASM cells.

Accession number	Sample type	Treatment (concentration)	Time exposure	Sample size		Experiment type	Reference
				Controls [a]	Cases [b]		
SRP033351	Non-asthmatic lung transplant donors	Dexamethasone (1 μM)	18 h	4	4	RNA-seq	1
GSE13168	Tracheae (non-asthmatic lung transplant donors)	Fluticasone (0.01 μM)	30 min	3	3	Gene expression microarray	2
GSE34313	Tracheae (asthmatic/healthy lung transplant donors)	Dexamethasone (1 μM)	24 h	6	6	Gene expression microarray	3
SRP098649	Non-asthmatic lung transplant donors	Budesonide (0.1 μM)	24 h	8	8	RNA-seq	4

[a] Cells exposed to control solution; [b] Cells treated with GCs.

ASM: airway smooth muscle; GCs: glucocorticosteroids; RNA-seq: next-generation sequencing of the transcriptome.

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Table S3. Clinical and demographic characteristics of the European studies included in the validation of genes with asthma exacerbations despite ICS use.

	PACMAN (n=654)	BREATHE-PAGES (n=540)	GoSHARE (n=472)	PASS (n=402)	SLOVENIA (n=182)	BREATHE (n=182)	followMAGICS (n=147)	ESTATe (n=102)
Gender (% male)	61.6	60.4	24.8	55.0	57.1	59.3	59.9	58.8
Mean age ± SD (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[a]	13.8	51.7[a]	34.1	52.7[a]	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 (%) [b]	6.1	NA	NA	NA	28.0	NA	7.5	NA
OCS use (%) [c]	6.7	35.0	13.8	51.7	12.6	48.4	NA	35.3
Hospitalizations (%) [d]	NA	13.5	0.21	NA	9.9	46.7	3.4	12.7 [h]
GP visits (%) [e]	NA	NA	NA	NA	NA	NA	49.0	NA
Specialist visits (%) [f]	NA	NA	NA	NA	NA	NA	21.8	NA
School absences (%) [g]	NA	43.1	NA	NA	NA	47.2	NA	NA

[a] Asthma exacerbations-related data was available for the 6 precedent months of the study enrollment; [b] Proportion of patients with any exacerbations who sought emergency care due to asthma; [c] Proportion of patients with any exacerbations who needed the use oral corticosteroids because of asthma; [d] Proportion of patients with any exacerbations who needed to be hospitalized because of asthma; [e] Proportion of patients with any exacerbations who needed any unscheduled general practitioner visits because of asthma; [f] Proportion of patients with any exacerbations who needed any respiratory system specialist visits because of asthma; [g] Proportion of patients with any exacerbations who were absent from school because of asthma; [h] 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 S4. Clinical and demographic characteristics of admixed populations included in the validation of genes with asthma exacerbations despite ICS use.

	GALA II (n=854)	SAGE (n=493)
Gender (% male)	57.3	60.4
Mean age ± SD (years)	12.1 ± 3.2	10.2 ± 3.5
Recruitment country	United States	United States
Ancestry	Latino/Hispanic	African American
Asthma exacerbations in the last 12 months (%)	66.4	51.9
Definition	ER visits/ hospitalizations/OCS use	ER visits/ hospitalizations/OCS use
ER visits (%) [a]	56.6	43.2
OCS use (%) [b]	40.2	29.4
Hospitalizations (%) [c]	12.6	5.7

[a] Proportion of patients with any exacerbations who sought emergency care due to asthma; [b] Proportion of patients with any exacerbations who needed the use of oral corticosteroids because of asthma; [c] Proportion of patients with any exacerbations who needed to be hospitalized because of asthma.
SD: standard deviation; ER: emergency room; OCS: systemic corticosteroids; NA: not available.

Table S5. Genes differentially expressed in ASM cells and PBMCs obtained from asthma patients in response to GCs.

				ASM cells[1]			*PBMCs*		
Gene	**Chr.** [a]	**Position begin 5'** [b]	**Position end 3'** [b]	**log_2 FC (95% CI)** [c]	***p*-value**	***q*-value** [d]	**log_2 FC (95% CI)** [e]	***p*-value**	***q*-value** [f]
PNRC2	1	24285599	24289952	0.41 (0.26 – 0.57)	2.54×10^{-4}	2.47×10^{-3}	5.85 (3.46 – 8.24)	1.45×10^{-5}	8.19×10^{-5}
LTBP1	2	33172039	33624576	0.69 (0.50 – 0.87)	1.82×10^{-5}	3.94×10^{-4}	1.95 (0.52 – 3.38)	8.62×10^{-3}	0.047
NCKAP1	2	183773843	183903586	0.25 (0.06 – 0.43)	0.014	0.045	2.62 (-1.61 – 1.13)	2.21×10^{-3}	0.012
ERAP2	5	96211643	96255420	-0.29 (-0.45 – -0.14)	2.35×10^{-3}	0.012	-2.24 (-3.70 – -0.78)	3.49×10^{-3}	0.019
BMP6	6	7727011	7881961	0.94 (0.45 – 1.44)	2.16×10^{-3}	0.012	2.25 (0.62 – 3.88)	8.00×10^{-3}	0.044
HLA-B	6	31321649	31324989	-0.30 (-0.50 – -0.11)	6.69×10^{-3}	0.026	-4.47 (-5.96 – -2.98)	4.08×10^{-7}	2.31×10^{-6}
SGK1	6	134490384	134639250	0.55 (0.31 – 0.78)	6.96×10^{-4}	5.00×10^{-3}	2.70 (1.17 – 4.24)	9.96×10^{-4}	5.56×10^{-3}
MTURN	7	30174426	30202381	0.43 (0.20 – 0.67)	2.75×10^{-3}	0.014	1.99 (0.65 – 3.33)	4.56×10^{-3}	0.025
STEAP4	7	87905744	87936228	5.25 (4.50 – 5.99)	1.27×10^{-7}	2.16×10^{-5}	2.84 (1.29 – 4.38)	6.21×10^{-4}	3.47×10^{-3}
GNG11	7	93551011	93557922	0.32 (0.10 – 0.55)	0.010	0.037	2.10 (0.74 – 3.47)	3.41×10^{-3}	0.019
NAMPT	7	105888731	105926772	1.04 (0.73 – 1.34)	4.20×10^{-5}	6.90×10^{-4}	3.31 (1.94 – 4.69)	1.76×10^{-5}	9.93×10^{-5}
CALD1	7	134429003	134655480	0.78 (0.57 – 0.99)	1.99×10^{-5}	4.18×10^{-4}	2.90 (1.05 – 4.74)	2.97×10^{-3}	0.016
TMEM176B	7	150488373	150498448	0.32 (0.12 – 0.52)	5.60×10^{-3}	0.023	3.04 (1.51 – 4.57)	2.63×10^{-4}	1.47×10^{-3}
TMEM176A	7	150497491	150502208	0.35 (0.12 – 0.58)	7.39×10^{-3}	0.028	2.78 (0.82 – 4.74)	6.62×10^{-3}	0.036
PTGS1	9	125132824	125157982	0.64 (0.32 – 0.96)	1.59×10^{-3}	9.15×10^{-3}	2.01 (0.65 – 3.37)	4.73×10^{-3}	0.026
DIXDC1	11	111797868	111893308	0.30 (0.14 – 0.45)	2.06×10^{-3}	0.011	4.12 (1.64 – 6.61)	1.77×10^{-3}	0.010

[a] Chromosome; [b] Positions based on GRCh37/hg19 build; [c] Base 2 logarithmic transformation of fold change obtained for ASM cells treated with GCs compared to untreated cells; [d] *p*-value adjusted by false discovery rate; [e] Base 2 logarithmic transformation of fold change obtained for PBMCs from ICS responders compared to ICS non-responder patients; [f] *p*-value adjusted by false discovery rate accounting for the total number of followed up genes.

ASM: airway smooth muscle; CI: confidence interval; GCs: glucocorticosteroids; ICS: inhaled corticosteroids; PBMCs: peripheral blood mononuclear cells.

1. Himes BE, Jiang X, Wagner P, Hu R, Wang Q, Klanderman B et al. RNA-Seq transcriptome profiling identifies *CRISPLD2* as a glucocorticoid responsive gene that modulates cytokine function in airway smooth muscle cells. *PLoS One* 2014;9: e99625 (accession number: SRP033351).

Table S5 (continuation). Genes differentially expressed in ASM cells and PBMCs obtained from asthma patients in response to GCs.

				ASM cells[1]			*PBMCs*		
Gene	**Chr.** [a]	**Position begin 5'** [b]	**Position end 3'** [b]	**log_2 FC (95% CI)** [c]	***p*-value**	***q*-value** [d]	**log_2 FC (95% CI)** [e]	***p*-value**	***q*-value** [f]
TMTC1	12	29653746	29937692	0.68 (0.47 – 0.90)	7.31×10^{-5}	1.02×10^{-3}	4.29 (2.23 – 6.35)	1.44×10^{-4}	8.08×10^{-4}
MEG3	14	101245747	101327368	-0.46 (-0.64 – -0.29)	2.44×10^{-4}	2.40×10^{-3}	-6.05 (-10.14 – -1.96)	4.74×10^{-3}	0.026
ITGB3	17	45331208	45421658	0.46 (0.19 – 0.73)	3.87×10^{-3}	0.018	3.04 (1.67 – 4.42)	6.09×10^{-5}	3.42×10^{-4}
MMD	17	53469974	53499353	1.67 (1.22 – 2.11)	2.08×10^{-5}	4.28×10^{-4}	2.85 (1.47 – 4.23)	1.60×10^{-4}	8.97×10^{-4}
COL5A3	19	10070237	10121147	0.92 (0.59 – 1.25)	1.57×10^{-4}	1.76×10^{-3}	3.77 (1.12 – 6.41)	6.42×10^{-3}	0.035
MYL9	20	35169887	35178228	0.48 (0.30 – 0.67)	2.53×10^{-4}	2.46×10^{-3}	4.57 (2.56 – 6.59)	4.48×10^{-5}	2.52×10^{-4}
TUBB1	20	57594309	57601709	0.75 (0.25 – 1.24)	8.11×10^{-3}	0.030	2.01 (0.59 – 3.43)	6.72×10^{-3}	0.037
COL18A1	21	46825052	46933634	0.37 (0.21 – 0.53)	5.11×10^{-4}	4.03×10^{-3}	2.64 (0.81 – 4.46)	5.71×10^{-3}	0.031

[a] Chromosome; [b] Positions based on GRCh37/hg19 build; [c] Base 2 logarithmic transformation of fold change obtained for ASM cells treated with GCs compared to untreated cells; [d] *p*-value adjusted by false discovery rate; [e] Base 2 logarithmic transformation of fold change obtained for PBMCs from ICS responders compared to ICS non-responder patients; [f] *p*-value adjusted by false discovery rate accounting for the total number of followed up genes.

ASM: airway smooth muscle; CI: confidence interval; GCs: glucocorticosteroids; ICS: inhaled corticosteroids; PBMCs: peripheral blood mononuclear cells.

1. Himes BE, Jiang X, Wagner P, Hu R, Wang Q, Klanderman B et al. RNA-Seq transcriptome profiling identifies *CRISPLD2* as a glucocorticoid responsive gene that modulates cytokine function in airway smooth muscle cells. *PLoS One* 2014;9: e99625 (accession number: SRP033351).

Table S6. Validation of identified differentially expressed genes in publicly available transcriptomic datasets evaluating the effect of GCs.

				GSE13168[1]		GSE34313[2]		SRP098649[3]		Meta-analysis [e]	
Gene	**Chr.** [a]	**Position begin 5'** [b]	**Position end 3'** [b]	**log_2FC (95% CI)** [c]	***q*-value** [d]	**log_2 FC (95% CI)** [c]	***q*-value** [d]	**log_2 FC (95% CI)** [c]	***q*-value** [d]	**log_2 FC (95% CI)** [c]	***q*-value** [d]
PNRC2	1	24285599	24289952	-0.17 (-0.45 – 0.14)	0.737	0.01 (-0.17 – 0.20)	0.902	-0.01 (-0.23 – 0.21)	0.959	-0.03 (-0.16 – 0.10)	0.768
LTBP1	2	33172039	33624576	0.32 (0.12 – 0.52)	0.144	0.70 (0.53 – 0.87)	7.99×10^{-4}	0.97 (0.82 – 1.12)	9.56×10^{-35}	0.67 (0.30 – 1.04)	**7.46×10^{-4}**
NCKAP1	2	183773843	183903586	0.14 (-0.17 – 0.44)	0.804	0.04 (-0.18 – 0.25)	0.837	0.00 (-0.14 – 0.15)	0.969	0.03 (-0.08 – 0.14)	0.743
ERAP2	5	96211643	96255420	-0.30 (-0.56 – -0.06)	0.284	0.36 (0.20 – 0.51)	0.011	-0.45 (-0.64 – -0.27)	1.32×10^{-5}	-0.13 (-0.62 – 0.37)	0.743
BMP6	6	7727011	7881961	-0.23 (-0.43 – -0.03)	0.331	1.20 (0.97 – 1.42)	2.03×10^{-4}	1.21 (0.54 – 1.89)	1.64×10^{-3}	0.70 (-0.26 – 1.66)	0.380
HLA-B	6	31321649	31324989	-0.12 (-0.30 – 0.07)	0.686	0.24 (0.08 – 0.39)	0.050	-0.20 (-0.42 – 0.01)	0.143	-0.02 (-0.29 – 0.25)	0.889
SGK1	6	134490384	134639250	0.39 (0.18 – 0.60)	0.108	-0.42 (-0.60 – -0.22)	0.014	-0.12 (-0.42 – 0.16)	0.600	-0.05 (-0.52 – 0.42)	0.835
MTURN	7	30174426	30202381	NA	NA	0.80 (0.61 – 0.99)	5.81×10^{-4}	0.44 (0.32 – 0.58)	2.64×10^{-11}	0.61 (0.26 – 0.97)	**3.92×10^{-3}**
STEAP4	7	87905744	87936228	-0.10 (-0.30 – 0.10)	0.773	0.16 (-0.06 – 0.38)	0.310	0.82 (-0.14 – 1.78)	0.204	0.09 (-0.20 – 0.38)	0.666
GNG11	7	93551011	93557922	0.34 (0.15 – 0.53)	0.099	0.01 (-0.17 – 0.20)	0.906	0.58 (0.40 – 0.75)	3.15×10^{-10}	0.31 (-0.01 – 0.64)	0.122
NAMPT	7	105888731	105926772	0.77 (0.57 – 0.97)	5.10×10^{-3}	0.48 (0.28 – 0.67)	9.63×10^{-3}	0.94 (0.71 – 1.17)	5.91×10^{-15}	0.72 (0.46 – 0.99)	**3.77×10^{-7}**
CALD1	7	134429003	134655480	0.42 (0.16 – 0.68)	0.144	0.28 (0.07 – 0.46)	0.072	0.19 (0.06 – 0.32)	0.012	0.26 (0.14 – 0.38)	**5.22×10^{-5}**
TMEM176B	7	150488373	150498448	0.04 (-0.15 – 0.25)	0.904	1.05 (0.86 – 1.24)	1.56×10^{-4}	-0.25 (-0.43 – -0.06)	0.029	0.28 (-0.49 – 1.05)	0.666
TMEM176A	7	150497491	150502208	0.04 (-0.14 – 0.23)	0.893	1.04 (0.70 – 1.37)	2.67×10^{-3}	-0.12 (-0.34 – 0.11)	0.523	0.31 (-0.39 – 1.01)	0.530

Table S6 (continuation). Validation of identified differentially expressed genes in publicly available transcriptomic datasets evaluating the effect of GCs.

Gene	Chr. [a]	Position begin 5' [b]	Position end 3' [b]	GSE13168[1]		GSE34313[2]		SRP098649[3]		Meta-analysis [e]	
				log_2FC (95% CI)[c]	*q*-value[d]	log_2 FC (95% CI)[c]	*q*-value[d]	log_2 FC (95% CI)[c]	*q*-value[d]	log_2 FC (95% CI)[c]	*q*-value[d]
PTGS1	9	125132824	125157982	-0.17 (-0.45 – 0.11)	0.708	-0.86 (-1.03 – -0.67)	3.70×10^{-4}	-0.34 (-0.54 – -0.15)	1.83×10^{-3}	-0.47 (-0.87 – -0.06)	0.078
DIXDC1	11	111797868	111893308	-0.10 (-0.29 – 0.08)	0.734	0.43 (0.20 – 0.66)	0.024	-0.17 (-0.38 – 0.04)	0.245	0.05 (-0.32 – 0.42)	0.792
TMTC1	12	29653746	29937692	NA	NA	1.41 (1.23 – 1.58)	2.54×10^{-5}	0.16 (-0.04 – 0.38)	0.262	0.79 (-0.44 – 2.01)	0.625
MEG3	14	101245747	101327368	NA	NA	NA	NA	NA	NA	NA	NA
ITGB3	17	45331208	45421658	-0.14 (-0.36 – 0.10)	0.726	0.23 (0.01 – 0.44)	0.150	-0.42 (-0.62 – -0.23)	1.16×10^{-4}	-0.11 (-0.48 – 0.26)	0.666
MMD	17	53469974	53499353	2.19 (1.95 – 2.42)	6.36×10^{-5}	0.84 (0.59 – 1.08)	1.68×10^{-3}	2.14 (1.88 – 2.41)	1.67×10^{-57}	1.72 (0.86 – 2.59)	**5.84×10^{-4}**
COL5A3	19	10070237	10121147	-0.10 (-0.32 – 0.11)	0.768	-0.22 (-0.51 – 0.08)	0.342	2.29 (1.93 – 2.66)	4.92×10^{-34}	0.65 (-0.95 – 2.25)	0.530
MYL9	20	35169887	35178228	0.00 (-0.20 – 0.19)	0.994	0.80 (0.59 – 1.01)	9.86×10^{-4}	0.28 (-0.09 – 0.63)	0.271	0.36 (-0.12 – 0.84)	0.206
TUBB1	20	57594309	57601709	-0.06 (-0.25 – 0.14)	0.875	0.19 (0.01 – 0.36)	0.151	NA	NA	0.07 (-0.18 – 0.31)	0.582
COL18A1	21	46825052	46933634	0.25 (0.04 – 0.45)	0.305	0.51 (0.33 – 0.68)	3.39×10^{-3}	0.43 (-0.25 – 1.11)	0.381	0.39 (0.18 – 0.61)	**1.93×10^{-3}**

[a] Chromosome; [b] Positions based on GRCh37/hg19 build; [c] Base 2 logarithmic transformation of fold change obtained for ASM cells exposed to GCs vs. control solution; [d] Adjusted *p*-value after adjusting for false discovery rate; [e] Random-effects model was applied taking into account the variance among studies.

ASM: airway smooth muscle; CI: confidence interval; GCs: glucocorticosteroids; ICS: inhaled corticosteroids; NA: not available; PBMCs: peripheral blood mononuclear cells.

Genes with significantly consistent changes in expression levels in ASM cells exposed to GCs vs. control solution after combining the publicly available datasets are in boldface. Results taken from www.realgar.org.

1. Misior AM, Deshpande DA, Loza MJ, Pascual RM, Hipp JD, Penn RB. Glucocorticoid- and protein kinase A-dependent transcriptome regulation in airway smooth muscle. *Am J Respir Cell Mol Biol* 2009; 41:24-39.

2. Masuno K, Haldar SM, Jeyaraj D, Mailloux CM, Huang X, Panettieri RA et al. Expression profiling identifies Klf15 as a glucocorticoid target that regulates airway hyperresponsiveness. *Am J Respir Cell Mol Biol* 2011; 45:642-9.

3. Kan M, Koziol-White C, Shumyatcher M, Johnson M, Jester W, Panettieri RA et al. Airway Smooth Muscle-Specific Transcriptomic Signatures of Glucocorticoid Exposure. *Am J Respir Cell Mol Biol* 2019;61:110-120.

Table S7. Results of differential expression analyses in PBMCs for genes differentially expressed across datasets analyzed using different regression models.

	Asthma exacerbations + age + %FEV$_1$ [a, b]			*Predicted baseline FEV$_1$* [c, d]		
Gene	**log$_2$FC (95% CI)** [e]	***p*-value**	***q*-value** [f]	**log$_2$FC (95% CI)** [e]	***p*-value**	***q*-value** [f]
LTBP1	1.05 (0.45 – 1.65)	4.28 x 10^{-3}	**0.022**	-0.07 (-0.26 – 0.11)	0.369	0.576
MTURN	0.59 (-0.16 – 1.34)	0.106	0.480	0.01 (-0.17 – 0.19)	0.907	0.951
NAMPT	-1.21 (-3.17 – 0.74)	0.186	0.700	0.07 (-0.21 – 0.35)	0.561	0.731
CALD1	1.45 (0.45 – 2.46)	0.011	0.061	-0.07 (-0.40 – 0.26)	0.611	0.769
MMD	0.84 (-0.14 – 1.82)	0.083	0.399	-0.08 (-0.33 – 0.16)	0.439	0.634
COL18A1	0.58 (-0.35 – 1.50)	0.186	0.700	0.00 (-0.21 – 0.21)	0.993	0.996

[a] Absence/presence of asthma exacerbations despite ICS use was evaluated as the dependent variable; [b] The predicted FEV_1 measured at baseline before starting ICS therapy and age were included as covariates; [c] Predicted FEV_1 measured at baseline before starting ICS therapy was evaluated as the dependent variable; [d] Estimated relative percentages of lymphocytes B and T, natural killer cells and monocytes were included as covariates; [e] Base 2 logarithmic transformation of fold change obtained for PBMCs from asthma patients with different patterns of ICS responsiveness based on the occurrence of asthma exacerbations; [f] *p*-value adjusted by false discovery rate accounting for the genes differentially expressed genes in the ASM analyzed in the discovery phase.
Genes with significant changes in expression levels are in boldface.
ASM: airway smooth muscle; CI: confidence interval; ICS: inhaled corticosteroids; FEV_1: forced expiratory volume in one second; PBMCs: peripheral blood mononuclear cells.

Table S8. Association results of genetic variants significantly associated with ICS refractoriness in children and young adults with asthma after Bonferroni-like correction.

Population	SNP	Chr. [a]	Position [b]	Effect/ Non-effect allele	OR (95% CI) [c]	*p*-value
European	rs11681246	2	33466620	G/A	0.72 (0.63 – 0.83)	**3.28×10^{-6}**
Admixed	rs3769534	2	33485965	A/C	0.41 (0.26 – 0.64)	1.24×10^{-4}
	rs76390075	2	33511545	C/T	0.40 (0.26 – 0.63)	**6.76×10^{-5}**
	rs75486357	2	33515538	C/T	0.43 (0.14 – 1.30)	1.65×10^{-4}
	rs3820912	2	33523658	G/A	0.39 (0.15 – 0.99)	1.02×10^{-4}
	rs3754830	2	33524956	C/T	0.43 (0.14 – 1.29)	1.63×10^{-4}
	rs10495788	2	33526895	G/C	0.39 (0.15 – 0.99)	1.02×10^{-4}

[a] Chromosome; [b] Positions based on GRCh37/hg19 build; [c] Odds ratio for the effect alleles.
CI: Confidence Interval; SNP: single-nucleotide polymorphism.
Significant SNPs after accounting for the total number of independent variants tested are in boldface.

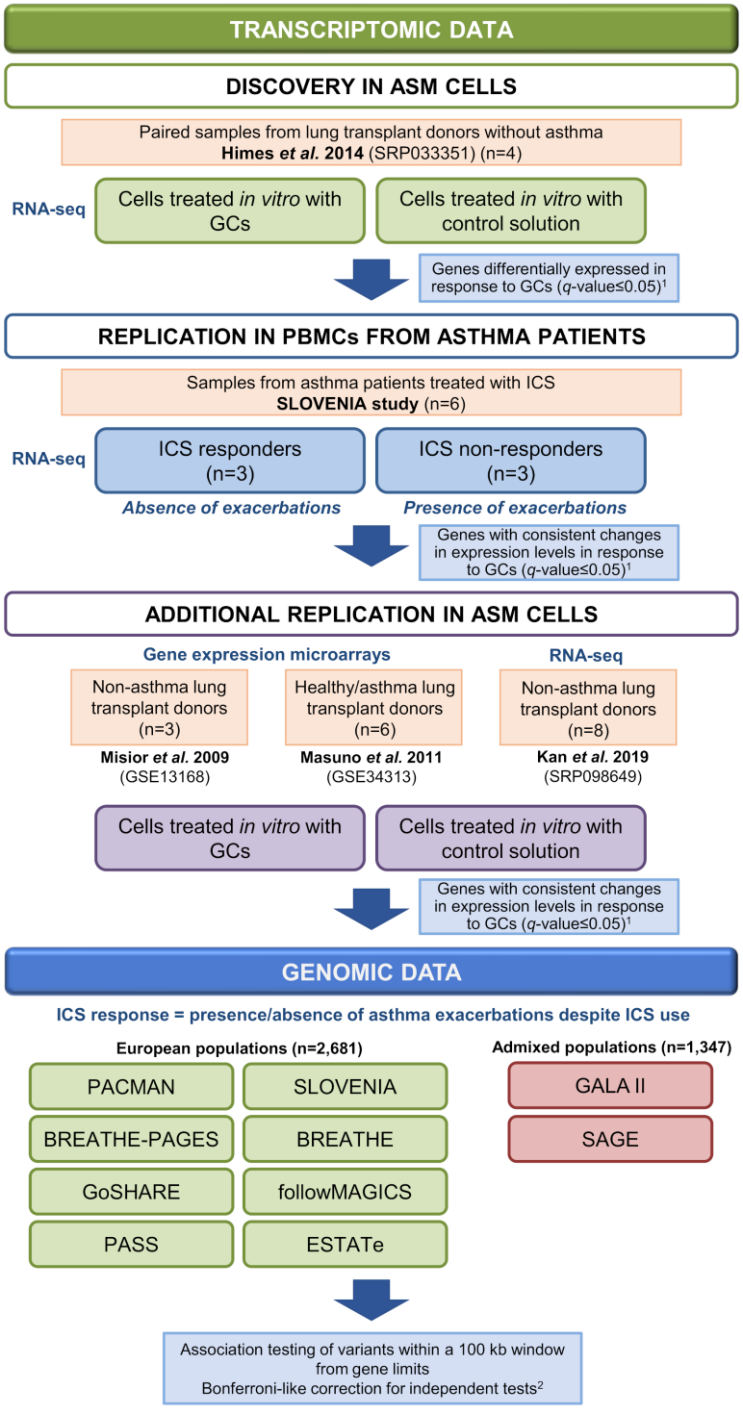

Figure S1. Flow diagram summarizing the methodological approach followed to identify novel loci of ICS response through the combination of transcriptomic and genomic data. Differential gene expression analyses in response to glucocorticosteroids (GCs) were carried out in different groups of airway smooth muscle (ASM) cells treated with GCs *in vitro* or peripheral blood mononuclear cells (PBMCs) extracted from asthma patients with different patterns of inhaled corticosteroids (ICS) responsiveness. The orange boxes show the source of the transcriptomic datasets, whereas the criteria followed to select the genes that were attempted for validation in the replication stage or additional evaluation are indicated within light blue boxes. The names of the studies participating in the Pharmacogenomics in Childhood Asthma Consortium (PiCA) from populations of European or admixed ancestry included in the association analyses of asthma exacerbations despite ICS use are shown within green or red boxes, respectively. [1]*p*-value adjusted by false discovery rate; [2]α= 0.05/number of independent variants. ASM: airway smooth muscle; GCs: glucocorticosteroids; ICS: inhaled corticosteroids; kb: kilobases; PBMCs: peripheral blood mononuclear cells; RNA-seq: RNA sequencing.

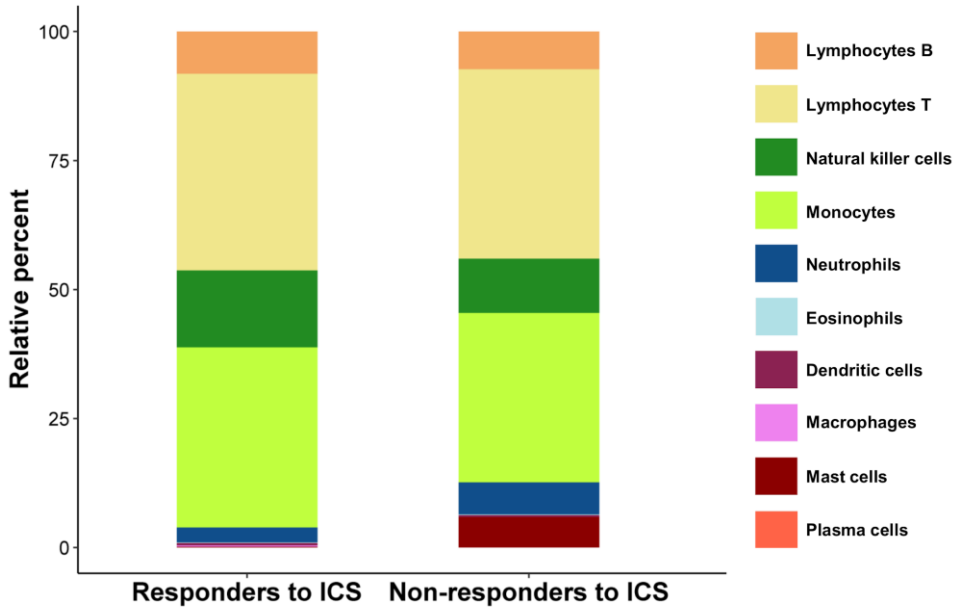

Figure S2. Bar plot of PBMC composition in asthma patients with different patterns of ICS responsiveness. The estimated relative percentage of each cell type among the total population of peripheral blood mononuclear cells (PBMCs) (*y*-axis) in inhaled corticosteroids (ICS) responder and non-responder asthma patients is represented. PBMC types are color-coded as it is shown in the legend on the right.

4. DISCUSSION

4. DISCUSSION

This doctoral thesis includes the results of exploring the genetic variation underlying the response to asthma treatment with ICS through association studies and transcriptomic approaches. To achieve the objectives of this work, we have: i) reviewed the main findings of the genomic studies of different asthma-related phenotypes published between 2016 and 2018; ii) explored the genetic markers involved in asthma exacerbations despite ICS use in children and youth from diverse populations; iii) evaluated the genetic associations with the change in lung function after ICS use; and iv) combined transcriptomic profiles from different cells exposed to GCs with genomic information from asthma patients treated with ICS. The findings of these studies propose four novel suggestive loci that could be involved in the response to asthma therapy with ICS and support the existence of genetic variation involved in the response that is exclusive or shared among different populations. We have also validated several previous associations with different measures of ICS response, suggesting that the information about the occurrence or absence of asthma exacerbations could be a good measurement of the response to asthma treatment with ICS. Additionally, a gene-set enrichment analysis revealed a potential novel medication that could be assessed for asthma treatment.

4.1. Current trends of genomics of asthma-related traits and future perspectives

The main genomic approaches applied to different asthma-related phenotypes between 2016 and 2018 were reviewed, as presented in *Chapter 1*. A brief overview of the evolution of genetic approaches in asthma was provided, from linkage analyses and candidate-gene association studies to GWAS. The results from recent genomic studies and the future perspectives of this field were discussed, which are anticipated to be led by high-throughput sequencing technologies. This supplemented the work published by Vicente *et al*., where the main results of the first decade of GWAS in the asthma field were described (Vicente et al. 2017). Apart from studies focused on candidate genes with previous evidence of implication in any molecular mechanisms underlying asthma, GWAS has been the most common approach in the last decade (Hernandez-Pacheco et al. 2019). This has been mostly applied to the study of the genetic variation involved in asthma susceptibility, with the 17q21 locus being the most replicated signal through the association of genes encoding zona pellucida binding protein 2 (*ZPBP2*), gasdermin B (*GSDMB*), or the ORMDL sphingolipid biosynthesis regulator 3 (*ORMDL3*), among others (Nieuwenhuis et al. 2016; Yan et al. 2017). Although they have well-known functions that seem not to be directly related to asthma pathophysiology, these have been widely evidenced to be associated with asthma (Moffatt et al. 2007; Ono et al. 2014; Das et al. 2017; Yan et al. 2017). Indeed, dysregulated expression of these genes has been related to the protection or risk to develop asthma (Berlivet et al. 2012; Miller et al. 2018). Interestingly, some authors have suggested that these genes could be involved in airway inflammation and structural changes (Kay et al. 2004; Paulenda and Draber 2016). These findings suggest that the genetic variation harbored at the 17q21 locus could play an important role in asthma phenotypes.

The GWAS published to date have also revealed novel association signals of asthma susceptibility in different populations including *GRM4*, encoding glutamate metabotropic receptor 4. Although this is involved in well-established functions in the central nervous system (Hovelso et al. 2012), some authors have linked

glutamate metabotropic receptors to airflow obstruction mediated by several processes (Kc and Martin 2010), including an increased phospholipase A2 activity, which triggers the liberation of pro-inflammatory mediators (Pniewska and Pawliczak 2013).

Genetic variation involved in treatment response has also been explored in asthma but to a lesser extent. Most of the pharmacogenomic studies of the response to asthma therapy with ICS published within the period reviewed have been carried out through candidate-gene association approaches in addition to a scarce exploration of the genomic variability (Keskin et al. 2019). This might be the explanation for the reduced number of biomarkers identified and their limited predictive capacity for treatment response (Hernandez-Pacheco et al. 2019). Moreover, their potential implications in the molecular mechanisms of asthma have not been completely inspected and there is no strong evidence supporting their clinical use (Garcia-Menaya et al. 2019). Thus, these have not provided real improvements in the pharmacological management of this disease (Park et al. 2015; Keskin et al. 2019). Although GWAS strategies have been used in the field of asthma genetics for more than a decade, their application in pharmacogenomics of asthma is relatively recent (Vijverberg et al. 2018; Perez-Garcia et al. 2020). Specifically, only a few GWAS of asthma treatment response had been published in the period reviewed, which were focused on the most prescribed medications to treat asthma (SABA and ICS) (Mosteller et al. 2017; Spear et al. 2018). Additionally, some recent large-scale GWAS have demonstrated the existence of shared genetic factors among asthma and allergic diseases (Ferreira et al. 2017; Zhu et al. 2018), confirming previous evidence of clinical and molecular similarities among them (Ober and Yao 2011; Farh et al. 2015). These findings suggest the power of genomic strategies to identify potential novel targets for alternative treatments in asthma (Hernandez-Pacheco et al. 2019).

Despite the numerous advantages that high-throughput genotyping technologies have provided to the research of genetics of asthma through GWAS compared to previous approaches, the genetic polymorphisms associated with different asthma-related phenotypes until now are not enough to completely understand the genetic architecture of this disease (Willis-Owen et al. 2018). This could be explained by the inherent limitations of genotyping platforms and those derived from inappropriate study designs, leading to the reduction of the statistical power to detect significant association signals (Willis-Owen et al. 2018). The reduced sample size is one of the most recurrent limitations of these studies. Genetic variants involved in complex diseases such as asthma have been evidenced to provide small effects, explaining a minor proportion of the total heritability (Bien et al. 2019). Thus, larger sample sizes are required to achieve the necessary statistical power (Manolio et al. 2009). This has been attempted to be solved by gathering many asthma studies from diverse populations around the world by large emerging consortia (Moffatt et al. 2010; Bousquet et al. 2011; Torgerson et al. 2011; Farzan et al. 2017a). Some examples of these efforts have been shown in *Chapters 2-5* with the results of several studies exploring the genetic variation underlying the response to the most prescribed controller asthma medication combining several populations participating in the PiCA consortium. These attempted to examine not only population-specific genetic factors but also those shared among different ancestry groups, including asthma patients from European, Asian, and admixed ancestry. Nonetheless, the scarce representation of genetically diverse populations has been one of the major limitations of most GWAS of asthma until December 2020 (Wells et al. 2016; Gautam et al. 2017; Bien

et al. 2019; Sirugo et al. 2019), despite the uncountable benefits attributed to including recently admixed populations for the genetic research of complex traits (Hernandez-Pacheco et al. 2016; Bien et al. 2019).

The genomic research of asthma has also been characterized by the limited exploration of the interaction between genes and the environment despite the large evidence of the important contribution of environmental exposures in asthma development, progression, and treatment response (Bonnelykke and Ober 2016; Hernandez-Pacheco et al. 2019). Additionally, the functional implications at the molecular and cellular levels of the asthma loci identified to date have been scarcely investigated (Hernandez-Pacheco et al. 2019). Interestingly, gene-set enrichment analyses have been proposed to be a promising strategy to provide insights about the biological processes underlying asthma and proxies for the discovery of potential alternative pharmacological therapies (Kao et al. 2017), as it has been demonstrated by the findings described in *Chapter 3*. *In silico* evaluations also provide valuable approximations about the functional contribution in asthma pathophysiology using publicly available information (The ENCODE Project Consortium 2012), but there is a need for more experimental functional studies of the genetic variation identified by genomic studies.

Nonetheless, traditional genomic strategies have also hampered the evaluation of the role of genetic variants apart from common polymorphisms captured by genotyping platforms, such as non-coding, low frequency, and structural variation, which have been proposed to be part of the missing heritability of asthma and related phenotypes (Du et al. 2012; Willis-Owen et al. 2018; Bien et al. 2019). This evidence draws the future directions of the asthma genetic research driven by NGS, which could optimally capture the undetected variation by genotyping-related approaches (Marx 2013; Wang and Chen 2018; Hernandez-Pacheco et al. 2019). Despite the large technical improvements in NGS technologies and the progressive reduction of its costs (Rizzo and Buck 2012), the implementation of this approach in large sample size studies is still challenging (Kulkarni and Frommolt 2017; Petersen et al. 2017). This could be one of the explanations for the scarce usage of NGS in respiratory diseases, including asthma (Campbell et al. 2014; Mak et al. 2018). Therefore, the application of GWAS approaches still seems promising in the search of genetic markers involved in asthma and related traits that have been scarcely explored, such as the treatment response (Hernandez-Pacheco et al. 2019).

Apart from association studies, other omics approaches have demonstrated to be powerful to provide insights that could help to better understand the molecular mechanisms taking place in asthma, although they are still emerging in this field (Pirih and Kunej 2018; Ivanova et al. 2019), especially in the research of asthma treatment response (Galeone et al. 2018; Tyler and Bunyavanich 2019). Therefore, in the literature review presented in *Chapter 1*, the need for studies integrating data from different biological sources to increase our knowledge about the mechanisms involved in asthma development, progression, and different response patterns to pharmacological therapies was identified (Pecak et al. 2018; Ivanova et al. 2019; Abdel-Aziz et al. 2020).

4.2. *APOBEC3B-APOBEC3C:* a suggestive novel locus for asthma exacerbations in patients treated with ICS from different populations

One of the few GWAS of response to asthma treatment with ICS in admixed populations published to date, presented in *Chapter 2*, revealed for the first time the association of one variant located in the intergenic region of the *APOBEC3B* and *APOBEC3C* genes with asthma exacerbations despite ICS use. Specifically, the suggestive association of the effect allele of the SNP rs5995653 with the protection against asthma exacerbations under ICS therapy found in Hispanic/Latino and African American children and youth was validated at nominal level in six independent European studies of childhood asthma.

APOBEC3B and *APOBEC3C* encode two cytidine deaminases belonging to the APOBEC3 (apolipoprotein B mRNA editing enzyme catalytic subunit 3) family (Desimmie et al. 2014), arranged in tandem in chromosome 22 (Jarmuz et al. 2002), which had not been previously associated with any asthma-related traits. These are involved in several functions through RNA editing activities, including the innate immune response against mobile genetic elements, such as a wide variety of exogenous viruses (e.g., retrovirus, hepatitis B virus, human immunodeficiency virus 1, and adenovirus) (Janahi and McGarvey 2013). APOBEC3 proteins have been demonstrated to target mRNA or nascent single-strand DNA (Cullen 2006) after the reverse transcription of genomic RNA, causing viral hypermutations and subsequent replication inhibition, although some authors have suggested that they could also inhibit the completion of the capsid assembly after cell infection (Janahi and McGarvey 2013). Interestingly, viral infections of the respiratory system have been evidenced to be important risk factors to develop childhood asthma (Mikhail and Grayson 2019) and the main triggering of exacerbations in children and adults with asthma (Edwards et al. 2013). A broad spectrum of viruses has been associated with the occurrence of asthma exacerbations, highlighting the contribution of rhinovirus (Mikhail and Grayson 2019). Some studies have proposed several molecular mechanisms that could be involved, including low levels of type I interferons found in some asthma patients (Wark et al. 2005; Djukanovic et al. 2014). This could explain a diminished response against viral infections (Wark et al. 2005; Djukanovic et al. 2014) through uncontrolled virus replication (Mikhail and Grayson 2019) and decreased induction of apoptosis of infected epithelial cells (Wark et al. 2005). Therefore, asthma patients with respiratory viral infections could experience a detriment in lung function and increased inflammation in response to reduced levels of type I interferon, developing acute exacerbation episodes (Wark et al. 2005). Additionally, it has been suggested that increased levels of neutrophils in the airway epithelium in children with respiratory viral infections could enhance the expression of IgE receptors in dendritic cells and IgE production. As a consequence, increased levels of Th2 cells and cytokines result in airway hyperresponsiveness and increased proliferation of mucous secretory cells (Mikhail and Grayson 2019). Taking together these pieces of evidence, it could be hypothesized that asthma patients carrying copies of the minor allele of rs5995653 (A allele) could have a better response to respiratory infections, decreasing the risk of asthma exacerbations under ICS therapy.

Previous studies have detected high levels of *APOBEC3B* and *APOBEC3C* mRNA in pulmonary fibroblasts (Kapushesky et al. 2010; GTEx Consortium 2013). The *in silico* functional evaluation carried out as part of this thesis also suggested that rs5995653 could be implicated in the regulation of the transcription of nearby genes in blood cells (The ENCODE Project Consortium 2012; Westra et al. 2013; Ward and Kellis

2016; Fishilevich et al. 2017). Specifically, this variant has been evidenced to be located within a histone mark and a DNase hypersensitivity site in different blood cell types, including primary lymphocytes B and T, monocytes, hematopoietic stem, and NK cells (The ENCODE Project Consortium 2012). Moreover, this polymorphism is in high LD (r^2>0.9) with expression quantitative trait loci (eQTL) associated with the expression levels of several genes in whole blood cells (rs9607601 and rs5995654) (The ENCODE Project Consortium 2012; Westra et al. 2013; Ward and Kellis 2016). Interestingly, both variants have been evidenced to be eQTLs for nearby genes (*APOBEC3A*, *CBX6*, and *APOBEC3G*) (The ENCODE Project Consortium 2012; Westra et al. 2013; Ward and Kellis 2016). These have also been nominally associated with asthma, allergic diseases, and the use of asthma medications in different studies (Ferreira et al. 2017; Demenais et al. 2018; Kichaev et al. 2019) according to the Open Targets Genetics portal (Carvalho-Silva et al. 2019). Additionally, *APOBEC3G* and *CBX6* had been previously linked to lung function measurements in COPD patients of European ancestry (Carvalho-Silva et al. 2019; Kichaev et al. 2019).

Altogether, these findings indicate that *APOBEC3* genes could be implicated in asthma pathophysiology and the development of exacerbations. The study presented in *Chapter 2* suggests the implication of the *APOBEC3B-APOBEC3C* locus in ICS response in asthma patients, which was associated with two different clinical measures of asthma treatment response. Although further validation in independent populations from different ancestry groups is needed, these results indicate that the association of this locus with ICS response is shared among Latinos/Hispanics, African Americans, and Europeans. Evidence of association with the change in FEV_1 after a short period of ICS use was also provided. Additionally, evidence of replication of variants in *L3MBTL4-ARHGAP28* was found in admixed populations, which had been previously associated with asthma exacerbations in European children and adults treated with ICS (Dahlin et al. 2015), suggesting its contribution to ICS response across different age groups.

A gene-set enrichment analysis performed as part of the study described in *Chapter 2*, using the summary association results of the GWAS, revealed evidence of enrichment for processes related to well-known functions of *APOBEC3* genes (e.g., deaminase activity, innate immune response, regulation of viral genome replication, metabolic processes involving pyrimidines, DNA modifications) (results not shown in *Chapters'* section). This analysis reinforced the evidence about the possible implication of other members of the *APOBEC3* gene family (e.g., *APOBEC3D*, *APOBEC3F*, and *APOBEC3G*) in asthma exacerbations in patients under ICS therapy. Additionally, several genes with variants that did not reach the suggestive significance threshold in the GWAS performed in admixed populations but included in the enrichment analysis had been previously implicated in the regulation of the transcription of *RORA* (McLean et al. 2011) (*CNTN6*, *INPP5A*, *RYR1*, *SYNE1*, *ZDHHC21*, and *ZFP36L1*), which represents one of the most replicated associations for asthma susceptibility and asthma-related traits (Acevedo et al. 2013; Gaertner et al. 2019; Lima et al. 2019).

Recently admixed populations have been widely underrepresented in genomic studies of complex traits such as asthma (Mersha 2015; Hernandez-Pacheco et al. 2016; Sirugo et al. 2019) and even more in the pharmacogenomic research of ICS response (Hernandez-Pacheco et al. 2016; Levin et al. 2019). Specifically, the study presented in *Chapter 2* together with the work by Levin *et al*. (2019) represent the few GWAS of ICS response on admixed populations, which were published almost simultaneously. Interestingly,

both studies suggested the existence of genetic determinants of ICS response shared among children and adults. However, different loci were revealed, which could be due to differences in study design. Therefore, the *EDDM3B* gene, found to be associated by Levin *et al*., did not reach the suggestive significance level set to consider significant associations with response to asthma treatment with ICS in the study performed as part of this doctoral thesis, which could be also the explanation of the fact that the association of *APOBEC3B-APOBEC3C* was not detected in that second GWAS.

Moreover, the loci identified were associated with different definitions of response to asthma treatment with ICS, suggesting the validity of the information about the occurrence of asthma exacerbations as a good predictor of response to asthma medications. Nonetheless, although the study described in *Chapter 2* proposed the existence of genetic variation underlying ICS response shared among different populations, Levin *et al.* did not find any evidence of replication in asthma patients of European descent (Levin et al. 2019).

Ancestry groups with diverse backgrounds differ from homogeneous populations in terms of allele frequencies, LD patterns with disease causal markers, genetic architecture, gene-gene, and gene-environment interactions (Sirugo et al. 2019). Populations that have experienced recent admixture events are characterized by wide ancestry-specific LD blocks that facilitate the identification of genomic regions likely to harbor causal variants with potential functional implications on the disease (Bien et al. 2019). Despite numerous studies advising the inclusion of genetically and ethnically diverse populations in genetic investigations of complex traits, Europeans, and to a lesser extent, Asians are still overrepresented, hampering the application of their findings to different populations (Bien et al. 2019; Sirugo et al. 2019). Consequently, the genetic variants identified have limited applicability and accuracy in the development of predictive models of complex disease risk for underrepresented populations (Bien et al. 2019; Sirugo et al. 2019). Therefore, disentangling the genetic variation specific of ancestrally diverse populations seems to be crucial to help accurately predict the disease development, complications, and drug response in the future. This would improve the clinical management of the disease on a global scale and increase the benefits of medicine from a personalized perspective (Bien et al. 2019; Sirugo et al. 2019). More inclusive genetic investigations have been proposed to be carried out through large studies recruiting individuals from multiple ancestry groups or combining several modest sample-sized cohorts from different populations in transethnic meta-analyses (Bien et al. 2019).

Nonetheless, the inclusion of samples from admixed individuals with African and Native American ancestries in the exploration of genetic factors involved in complex traits shows inherent disadvantages that need to be discussed (Shriner et al. 2011; Ortega and Meyers 2014b; Mersha 2015). Specifically, one of the major limitations of GWAS in admixed populations is related to the fact that these are poorly represented in most of the reference panels for genotype imputation available to date (Ortega and Meyers 2014b; Vergara et al. 2018; Schurz et al. 2019), hampering the capacity to increase the number of genetic variants beyond those captured by commercial genotyping platforms (Vergara et al. 2018). Therefore, the total number of genetic variants tested in association and the statistical power to detect significant association signals are significantly diminished.

At the time the analyses of the study presented in *Chapter 2* were performed, HRC was the largest publicly available catalog of genetic variants (McCarthy et al. 2016). The release of this reference panel dramatically improved the capacity of imputation of genetic variants together with the development of publicly available computational servers, such as The Michigan Imputation Server (Das et al. 2016; Bien et al. 2019). Nonetheless, HRC was built mostly with samples of European descent (McCarthy et al. 2016). Large sample size reference panels with information about a high proportion of sites across the genome, such as HRC, have been reported to substantially increase the number of good quality and accurately imputed variants, even at low frequency (Deelen et al. 2014; McCarthy et al. 2016). For this reason, higher coverage of imputed variants using HRC compared to previous imputation panels was expected. Nonetheless, the similarity in terms of genetic ancestry between the reference panel and the population under study also needs to be considered in the selection of the most appropriate panel for imputation (Vergara et al. 2018).

There are just a few available reference panels with modest sample sizes focused on populations with African ancestry, such as the Consortium on Asthma among African ancestry Populations in the Americas (CAAPA) (Mathias et al. 2016). Nonetheless, several studies comparing different reference datasets have suggested that large and multiethnic panels (e.g., the third phase of 1KGP) provide more accurate haplotype information about the parental populations for the imputation of admixed samples (Auton et al. 2015; Vergara et al. 2018) compared to population-specific reference panels with limited sample sizes. Thus, 1KGP phase 3 has been shown to increase the number of imputed variants in recently admixed populations with African ancestry despite its smaller size compared to HRC (Vergara et al. 2018). This could be one of the potential explanations of the fact that no genome-wide associations (p-value≤$5x10^{-8}$) were detected in the GWAS of asthma exacerbations despite ICS use focused on Latinos/Hispanics and African Americans described here.

Interestingly, the SNP located at *APOBEC3B-APOBEC3C* associated with asthma exacerbations despite ICS use in admixed and European populations was slightly more significant after combining Latinos/Hispanics and African Americans when 1KGP phase 3 (Auton et al. 2015; Sudmant et al. 2015) was used as a reference panel (results not shown in *Chapters*' section) compared to the original GWAS results obtained with variants imputed with HRC r1.1 (McCarthy et al. 2016) presented in *Chapter 2*: p=9.64 x 10^{-7} *vs*. p=4.80 x 10^{-6}. Moreover, it approached the genome-wide significance level after performing a meta-analysis of this variant across the admixed and European populations included in the discovery and replication phases using 1KGP phase 3 and HRC r1.1 datasets, respectively: p=6.09 x 10^{-8} *vs*. p=2.66 x 10^{-7} (results not shown in *Chapter*'s section). These results suggest that the imputation of genetic variants in admixed populations with 1KGP phase 3 as a reference panel could have increased the statistical power to detect novel loci of ICS response in the study described in *Chapter 2*. Moreover, combining different panels for the genotype imputation of each study could also have improved the imputation accuracy, as it has been proposed elsewhere (Huang et al. 2009; Vergara et al. 2018).

4.3. Identification of a novel European-specific locus for exacerbations despite ICS use and a promising asthma therapy

Some studies have previously suggested the existence of genetic variation involved in the response to asthma treatment with ICS shared among different populations, whereas a proportion of markers might be

exclusive of certain ancestry groups (Wells et al. 2016; Hernandez-Pacheco et al. 2019; Levin et al. 2019). However, further investigation is needed to confirm this preliminary evidence. Even though most of the studies performed to date have been focused on populations of European descent, these have included reduced sample sizes, hampering the capacity to contribute to the knowledge about the heritability of response to asthma therapy with ICS. In *Chapter 3*, the results of the largest GWAS of asthma exacerbations despite ICS use in children and young adults performed until December 2020 are described, combining eleven independent studies of childhood asthma from European populations. As a result, one variant located within the intergenic region of *CACNA2D3* and *WNT5A* (rs67026078) was suggestively associated with exacerbations in asthma patients under ICS therapy. Nonetheless, this did not show evidence of implication in asthma exacerbations in Latinos/Hispanics, African Americans nor Asians, suggesting that it could have an ancestry-specific effect.

CACNA2D3 encodes a member of the auxiliary alpha-2/delta subunit family of voltage-gated calcium channels, which consist of a pore-forming and three regulatory subunits (Qin et al. 2002; Davies et al. 2007). Calcium channels activated by voltage mediate the flux of calcium ions (Ca^{2+}) into the cell from the extracellular space through polarization of the plasma membrane, which is one of the main sources of intracellular Ca^{2+} (Qin et al. 2002; Davies et al. 2007). Specifically, CACNA2D3 is involved in the acceleration of the activation of calcium channels through the modulation of the Ca^{2+} current density (Gurnett et al. 1996). Ca^{2+} is a secondary messenger involved in a broad range of key biological processes, including muscle contraction, neurotransmission, cell differentiation, and regulation of the transcription, among others (Parkash and Asotra 2010).

Interestingly, *CACNA2D3* has been related to different asthma-related clinical biomarkers (van der Valk et al. 2014; Wain et al. 2017). Specifically, it has been associated with measurements of lung function in adults of European descent with COPD from UKBiobank (Wain et al. 2017; Carvalho-Silva et al. 2019). Although both COPD and asthma are pulmonary diseases characterized by airflow obstruction and inflammation of the airways sharing several molecular mechanisms, clinical and functional features, they also show many differences (Cukic et al. 2012; Gaspar Marques et al. 2020). Nonetheless, the evaluation of the pulmonary capacity through spirometric measures is considered a gold standard for the diagnosis, assessment of disease control, and progression in both diseases (Quezada et al. 2016; Khan et al. 2018; Grossman et al. 2019a; Grossman et al. 2019b). Specifically, the quantification of the improvement in lung function after the administration of bronchodilators is a valuable tool in the management of COPD and asthma (Rabe et al. 2007; Pellegrino et al. 2010; Coverstone et al. 2019). Interestingly, *CACNA2D3* has been associated with BDR in individuals of European and African American descent with COPD (Lutz et al. 2015). Measures of lung function have been evidenced to be reliable markers of the risk to experience future episodes of asthma exacerbations. Therefore, trajectories of pulmonary capacity during childhood might be a predictor of asthma severity (Quezada et al. 2016; Khan et al. 2018; Grossman et al. 2019a; Grossman et al. 2019b). BDR has also been demonstrated to be a powerful marker of the response to asthma medications (Chhabra 2015). Indeed, this has been reported to be a good predictor of treatment responsiveness in asthma patients under therapy with ICS (Tantisira et al. 2006; Galant et al. 2014; Wu et al. 2014).

On the other hand, *WNT5A* flanks the downstream limit of the intergenic region where the association signal detected in the study described in *Chapter 3* is located. This gene encodes for a lipid-modified glycoprotein, which is the member 5A of the large family of proteins involved in the Wingless/integrase 1 (WNT) signal transduction pathway (van Amerongen et al. 2008). The regulation of a wide range of key cellular processes has been attributed to WNT signaling (Komiya and Habas 2008), which can occur through several transduction cascades dependent or independent of β-catenin, also known as canonical or non-canonical, respectively (Komiya and Habas 2008; Kumawat and Gosens 2016). Specifically, WNT5A participates in the non-canonical branch of this biological process through Ca^{2+} signaling, inducing the transcription nuclear factor of activated lymphocytes T (Pashirzad et al. 2017). WNT5A is also involved in the regulation of embryonic development, tissue homeostasis, cell adhesion, and migration, and has been related to several human diseases, including various types of cancer (McDonald and Silver 2009; Kumawat and Gosens 2016).

Importantly, WNT5A has been associated with the maintenance of innate immunity in homeostatic and pathological states. This protein participates in several inflammatory disorders (Kumawat and Gosens 2016; Pashirzad et al. 2017), not only promoting the production of pro-inflammatory chemokines and cytokines but also regulating the recruitment of immune effectors (Kumawat and Gosens 2016). It has been demonstrated that the WNT biological process could modulate the activity of macrophages and neutrophils, two cell types with central roles in the inflammatory immune response (Yang et al. 2012; Kumawat and Gosens 2016; Reuter et al. 2016). Indeed, WNT5A prolongs the survival of macrophages in homeostatic conditions, but also induces anti-inflammatory activities mediated by macrophages during acute inflammation (Reuter et al. 2016). Nonetheless, the expression of WNT5A receptors in neutrophils has been associated with increased migration of these cells from the lymph nodes to the airways (Kumawat and Gosens 2016). Neutrophils are the major source of TGF-β1 (transforming growth factor β1), activating epithelial and mesenchymal cells that drive structural changes in the airways (Januskevicius et al. 2016). Moreover, WNT proteins could regulate the function of dendritic cells (Reuter et al. 2016), which are activated in asthma patients (Lambrecht and Hammad 2010; Lloyd and Hessel 2010). In fact, *in vitro* exposure of dendritic cells to WNT ligands has been demonstrated to produce anti-inflammatory mediators (Oderup et al. 2013) and induce regulatory lymphocytes T (Holtzhausen et al. 2015), suppressing the adaptive immune response (Reuter et al. 2016). However, it is still unclear how this evidence could be applied to inflammatory processes in the lung (Reuter et al. 2016). Nonetheless, some studies have suggested the participation of this signaling pathway in pulmonary diseases, such as asthma. The upregulation of *WNT5A* has been found in mild-to-moderate asthma patients with high Th2-driven inflammation patterns compared to patients with low Th2 inflammation and healthy controls (Choy et al. 2011).

Apart from inflammation, WNT signaling is also implicated in structural modifications of the airways taking place in asthma. Specifically, the WNT signaling independent of β-catenin, where WNT5A is involved, seems to participate in the regulation of the transition from epithelial to mesenchymal cells together with the action of TGF-β1 (Hackett et al. 2009; Reuter et al. 2016). This suggests the central role of WNT ligands and TGF-β1 in structural processes in the airways. Indeed, expression of TGF-β1 and WNT5A in ASM cells at gene and protein levels have been positively correlated (Kumawat and Gosens 2016), and they have also

been related to the Th2 inflammatory signature, which is characteristic of patients with mild-to-moderate asthma (Choy et al. 2011; Halwani et al. 2011). Low doses of TGF-β1 have been associated with increased ASM mass, through the proliferation, migration, and inhibition of the apoptosis of ASM cells (Halwani et al. 2011). TGF-β1 also induces the apoptosis of epithelial cells in response to pro-inflammatory mediators and promotes the differentiation and proliferation of myofibroblasts, increasing the deposition of elements of the extracellular matrix and causing subepithelial fibrosis (Halwani et al. 2011). Interestingly, high expression levels of *WNT5A* have been detected in fibroblasts from patients with idiopathic pulmonary fibrosis (IPF) (Vuga et al. 2009), a chronic disease characterized by interstitial lung fibrosis (Liu et al. 2019). This suggests that *WNT5A* could be also involved in fibrotic processes, including the ones observed in asthma pathogenesis.

Additionally, a gene-set enrichment analysis revealed the enrichment of genes associated with asthma susceptibility in Europeans involved in the WNT signaling pathway, including *WNT5A* (Barreto-Luis et al. 2017). Many authors have widely proposed that members of the WNT signaling, and specifically WNT5A, could have a central role in molecular and cellular mechanisms underlying asthma. Therefore, this could be a promising pharmacological target to treat asthma patients, although further investigation is needed (Koopmans et al. 2016; Reuter et al. 2016; Koopmans and Gosens 2018). These pieces of evidence suggest that the association signal described in *Chapter 3* could reveal *CACNA2D3* and *WNT5A* as potential novel loci for asthma exacerbations despite ICS treatment.

The *in silico* functional evaluation carried out as part of this study revealed that rs67026078 at *CACNA2D3*-*WNT5A* could be involved in the regulation of gene expression in fetal and adult lung fibroblasts (The ENCODE Project Consortium 2012). Moreover, this variant had been previously associated with the expression levels of different proteins involved in the Wnt signaling pathway and asthma-related mechanisms (Staley et al. 2016; Stelzer et al. 2016; Sun et al. 2018; Kamat et al. 2019), and with the regulation of the methylation patterns or a CpG site in blood (Staley et al. 2016; Bonder et al. 2017; Kamat et al. 2019). These findings suggest the role of this association signal in the response to asthma medications, even though evidence of replication was found in Europeans, but not in Latinos/Hispanics, African Americans, or Asians, suggesting that this variant could have ancestry-specific effects.

Additionally, five loci previously associated with different definitions of ICS response in Europeans through GWAS approaches were validated. Evidence of replication was found for a variant at *UMAD1*-*GLCCI1*, previously associated with the improvement in lung function after ICS treatment (Tantisira et al. 2011). Additionally, validation of the association of *UMAD1*-*GLCCI1* and *PDE10A*-*T* was found, assessing alternative genetic variants to the reported in the original work describing their association (Tantisira et al. 2011; Tantisira et al. 2012). Moreover, an association signal detected analyzing BDR as a measure of ICS response was validated with asthma exacerbations despite ICS use in European populations (*ZNF432*-*ZNF841*) (Wu et al. 2014). Loci previously evidenced to be associated with asthma exacerbations despite ICS treatment (*SHB*-*ALDH1B1* and *ELMO2*-*ZNF334*) (Dahlin et al. 2015) were also validated. Nonetheless, none of the loci previously identified to be associated with ICS response in Asian (Park et al. 2014b) or admixed populations (Levin et al. 2019) were validated in Europeans.

The GWAS of asthma exacerbations despite ICS use of European populations was complemented with a gene-set enrichment analysis of variants reaching $p \leq 1x10^{-4}$ focusing on drugs or small molecules with previous evidence of implication in the regulation of gene expression. As a result, an enrichment in association signals within genes whose expression levels are affected by trichostatin A (TSA) treatment was found, suggesting that this could be involved in molecular mechanisms underlying ICS response (Chen et al. 2013; Kuleshov et al. 2016). TSA has been evidenced to be a potent inhibitor of the histone deacetylase, whose effects have been widely related to anticancer activities (Mogal and Abdulkadir 2006; Royce and Karagiannis 2012; Ma et al. 2017). Additionally, findings of *in vivo* experiments in animal models suggest that this drug could play a central role in the regulation of the main asthma pathophysiological processes, such as airway inflammation, bronchoconstriction, and hyperresponsiveness (Adcock et al. 2007; Banerjee et al. 2012) through different molecular mechanisms of standard asthma medications (Choi et al. 2005; Adcock et al. 2007; Banerjee et al. 2012; Toki et al. 2016). Nonetheless, further investigation is needed to understand the potential benefits and side effects of this drug in asthma patients.

These results together with those obtained from the gene-set enrichment analysis performed in admixed populations evidence the potential of this approach to identify novel genetic markers of asthma treatment response, alleviating the stringent requirements of significance level conventionally applied in GWAS approaches (Kao et al. 2017; Sun et al. 2019). Thus, this method allows detecting enrichment for associations with a specific trait even when the significance threshold is not reached by individual variants (Zhu et al. 2018).

4.4. Detection of the association of *ROBO2* with different measures of ICS response in European patients with asthma

The results described in *Chapters 2* and *3* of this doctoral thesis suggest the potential of the information about the history of asthma exacerbations to assess the clinical response to ICS therapy. *Chapter 4* presents a study aimed to evaluate the association with an additional definition of treatment response to identify further genetic loci involved in ICS response. Specifically, a GWAS of the change in FEV_1 after 6 weeks of ICS treatment was carried out. ICS-naïve asthma patients from the only PiCA study with available information about this variable at the time of performing the analyses (SLOVENIA) were included in the analyses. This study revealed the suggestive association of the intronic *ROBO2* variant rs1166980 with the change in lung function in Slovenian children and young adults with asthma. Specifically, the effect allele of this SNP was found to be associated with a lower improvement in FEV_1 after a short period of ICS use. Even though *ROBO2* had not been previously associated with the response to asthma pharmacological therapies, it is not the first time this gene is linked to asthma-related traits (Parameswaran et al. 2007; Ding et al. 2013; Lutz et al. 2015).

ROBO2, located at 3p12.3, encodes an immunoglobulin of the family of transmembrane roundabout guidance receptors (Barak et al. 2019), which specifically bind Slit guidance ligands [SLIT] (Kidd et al. 1998; Li et al. 1999), secreted proteins associated with the extracellular matrix (Dickinson and Duncan 2010). The role of ROBO proteins was initially linked to the development of the nervous system (Li et al. 1999), although their broad range of functional implications is currently well-known, being many of them involved in some of

the main pathophysiological processes taken place in asthma. The ROBO/SLIT signal transduction has been related to cell adhesion, migration, growth, and survival (Dickinson and Duncan 2010). Indeed, it has been related to the morphogenesis of several tissues during fetal development (Xian et al. 2001; Dickinson and Duncan 2010), including normal or aberrant pulmonary tissues (Xian et al. 2001). Several studies have also demonstrated the implication of ROBO/SLIT in the regulation of the innate immune response (Tole, Mukovozov et al. 2009; Lin, Zhong et al. 2019). Moreover, variants near *ROBO1* have been associated with virus diversity as a proxy of infection susceptibility, suggesting the implication of this pathway in the response to viral infections (Fumagalli et al. 2010), which are one of the main risk factors to develop asthma exacerbations (Mikhail and Grayson 2019). Specifically, the SLIT2 ligand binding to ROBO2 (Xian et al. 2001) has been linked to the regulation of chemotaxis and inhibition of the migration of several types of immune cells from the blood circulatory system to the target tissue where they exert their functions, preventing inflammatory responses (e.g., leukocytes, lymphocytes T, dendritic cells, macrophages, and neutrophils) (Wu et al. 2001; Tole et al. 2009; Pilling et al. 2019). The inhibition of ROBO expression has been related to the increased production of chemoattractants, promoting the migration of immune cells and airway remodeling and, decreasing the number of alveoli (Branchfield et al. 2016). Additionally, ROBO2-SLIT2 has been linked to the prevention of fibrotic processes in the lung (Pilling et al. 2014). Interestingly, it has been suggested that SLIT2 is involved in the inhibition of the migration of monocytes from the blood circulation to pulmonary tissues, where they differentiate into fibrocytes, promoting fibroblasts proliferation and collagen production through TGF-β secretion. Experiments in mice models have demonstrated that fibroblasts and epithelial cells secrete SLIT2 in a healthy state, preventing the development of lung fibrosis. Moreover, patients with pulmonary fibrosis have shown reduced levels of SLIT2 (Pilling et al. 2014).

These findings suggest that SLIT ligands could exert anti-inflammatory and anti-fibrotic effects (Tole et al. 2009; Pilling et al. 2014). Thus, ROBO proteins could also play a key role in processes occurring in the lung later in life with potential implications in pulmonary diseases. Additionally, a gene encoding another member of the family of ROBO proteins, *ROBO3*, had been previously associated with FVC in COPD patients (Kichaev et al. 2019). Moreover, the expression levels of the *ROBO2* and *SLIT2* have been negatively correlated with disease progression in patients with COPD (Lin et al. 2019), a disease with underlying mechanisms shared with asthma, as was discussed above. Specifically, it has been hypothesized that the downregulation of *ROBO2* and *SLIT2* expression activates the Cdc42 and Rac2 GTPases, promoting the migration of neutrophils and lymphocytes T into the lung and, causing the characteristic inflammatory COPD patterns (Lin et al. 2019). Therefore, the *ROBO2/SLIT2* system could also play a similar key role in the inflammatory processes in asthma.

Interestingly, *ROBO2* has been proposed to be an important factor triggering the constriction of the airways in pulmonary obstructive diseases, such as asthma and COPD (Parameswaran et al. 2007). Indeed, this gene has been associated with clinical markers of the reversibility of airflow limitation in adults of African American descent. Specifically, evidence of suggestive association with FEV_1 and FEV_1/FVC measured a few minutes after the administration of SABA was found for three *ROBO2* intronic variants (Lutz et al. 2015). Additionally, *ROBO2* has been suggested as a potential locus of susceptibility to develop childhood asthma (Ding et al. 2013). Nevertheless, the findings presented in *Chapter 4* suggest that *ROBO2* could exert

ancestry-specific effects in the response to asthma therapy with ICS. Indeed, the association of *ROBO2* was validated with the information about the recent history of asthma exacerbations despite ICS treatment in European populations, but not in Latinos/Hispanics, and African Americans.

Although the *in silico* functional evaluation carried out did not reveal major implications for any of the variants at *ROBO2* associated with ICS response, the gene-set enrichment analysis performed as part of the study presented in *Chapter 3* revealed this gene as a potential target of TSA. All these pieces of evidence described here suggest the potential implications of *ROBO2* in asthma-related traits, including the response to asthma therapy with ICS, although further investigation is needed to confirm these findings.

Additionally, the association of *PDE10A-T,* which had been previously identified in children and adults of European ancestry (Tantisira et al. 2012), was validated in *Chapter 4* with FEV_1 change after ICS use in Slovenian asthma patients. Interestingly, evidence of replication had also been found for this locus with ICS response measured as the occurrence/absence of asthma exacerbations in Europeans, as presented in *Chapter 3*. These findings consistently suggest that *PDE10A-T* could play an important role in the response to asthma treatment with ICS at least in populations of European descent.

4.5. Lessons from the application of GWAS approaches to the evaluation of genetic variation of ICS response in different populations

Chapters 2-4 describe the results of the application of GWAS approaches in several ancestry groups evaluating different clinical markers of ICS responsiveness. The findings obtained reinforce previous evidence suggesting the existence of genetic variation specific and common to different populations. The nominal replication in European populations of *APOBEC3B-APOBEC3C*, identified in Latinos/Hispanics and African Americans, together with the validation in these admixed populations of the association signal located at *L3MBTL4-ARHGAP28*, previously identified in Europeans (Dahlin et al. 2015), suggest the existence of genetic markers shared among different populations (*Chapter 2*). However, some findings of the work described in *Chapters 3* and *4* also suggest the contribution of ancestry-specific genetic markers to the response to asthma treatment with ICS. First, no evidence of replication of the *CACNA2D3-WNT5A* locus was found in non-Europeans and, the association of *CACNA2D3-WNT5A* and *ROBO2* was shared among independent European populations. Moreover, the association of different loci previously identified in Europeans (Tantisira et al. 2011; Tantisira et al. 2012; Wu et al. 2014; Dahlin et al. 2015) was validated in the same population group analyzed as part of this thesis, but none of the genes revealed by the few GWAS of ICS response in Asian or admixed populations published until December 2020 (Park et al. 2014b; Levin et al. 2019) were validated in the European populations analyzed.

Differences between childhood and adulthood asthma have been described for their clinical manifestations (Chung and Paton 2019; Pividori et al. 2019; Withers and Green 2019), prevalence rates (Shah and Newcomb 2018; Dharmage et al. 2019), and heritability estimates (Moffatt et al. 2010; Ullemar et al. 2016; Pividori et al. 2019). Furthermore, a study has recently identified the association of variants near the *THSD4* and *HIVEP2* genes with differential risk or protection against asthma exacerbations while on ICS therapy depending on the patient's age (Dahlin et al. 2020). Nonetheless, the findings of this thesis suggest the contribution of common genetic variants to ICS response across different age groups through the

validation in patients with childhood asthma of the association of two loci previously identified in children and adults (*Chapters 2-4*) (Tantisira et al. 2012; Dahlin et al. 2015). Therefore, the differences and similarities between adulthood and childhood asthma in terms of the genetic contribution in the response to ICS therapy is still unclear, being evident the need for further investigation.

Several limitations have been attributed to the spirometric assessment of the pulmonary capacity driven by the fact that symptoms worsening and disease progression are not always reflected by a decline in lung function (Global Initiative for Asthma 2020), together with a high dependence on patient and measure operation-related factors (Cooper 2005; Global Initiative for Asthma 2020). Lung function measurements are widely considered as objective and quantitative markers commonly used in clinical practice for the asthma diagnosis (Global Initiative for Asthma 2020), evaluation of the progression and control of the symptoms (Szefler et al. 2002; Gorelick et al. 2004; Martin et al. 2007), and to measure the response to pharmacological therapies, such as ICS (Global Initiative for Asthma 2020). Nevertheless, the results of the GWAS presented in *Chapters 2-4* reinforce the validity of the information about the recent history of asthma exacerbations as a good measurement of treatment response. Specifically, several associations described by previous publications analyzing lung function (Tantisira et al. 2011; Tantisira et al. 2012; Wu et al. 2014; Dahlin et al. 2015) (*Chapters 3* and *4*) or by the GWAS of the change in FEV_1 (*Chapter 4*) were validated considering the presence/absence of asthma exacerbations despite the use of ICS as outcome. Additionally, the association of the *APOBEC3B-APOBEC3C* locus with ICS response measured as asthma exacerbations in children and young adults of admixed ancestry was validated with the change in FEV_1 after ICS treatment (*Chapter 2*).

4.6. Potential role of *LTBP1* in the response to asthma treatment with ICS revealed by the combination of transcriptomic and genomic data

The results described in *Chapters 2-4* demonstrate that the genome-wide evaluation of the association of genetic variants is still a powerful strategy to identify novel loci that could potentially play an important role in the response to ICS in asthma patients. Nonetheless, an alternative approach was applied in *Chapter 5*, combining transcriptomic and genomic data to further contribute to disentangling the molecular mechanisms underlying the responsiveness to this medication in asthma patients. Changes in expression levels in response to GCs were assessed using the only transcriptome datasets obtained through NGS (RNA-seq) that were available at the time of performing the analyses. Specifically, gene expression profiles from ASM cells *in vitro* exposed to GCs (Himes et al. 2014) and PBMCs from asthma patients with different responsiveness patterns to ICS therapy based on a recent history of asthma exacerbations were assessed. Further validation was performed using three additional publicly available ASM transcriptome datasets experimentally treated with different types of GCs or control solutions (Misior et al. 2009; Masuno et al. 2011; Kan et al. 2019). Six genes showed evidence of significant overexpression in ASM treated with GCs or PBMCs from ICS responders. None of these had been previously linked to the response to asthma medications, although some had been previously associated with asthma-related traits, including measurements of lung function (*LTBP1*, *NAMPT*, *CALD1, COL18A1*) (Kichaev et al. 2019) or asthma susceptibility (*NAMPT*) (Bonnelykke et al. 2014; Pickrell et al. 2016). Additionally, the evaluation of genetic associations within genes with consistent differential expression in ASM cells and PBMCs revealed that

LTBP1 could be also involved in asthma exacerbations despite ICS use in populations of European or admixed ancestry.

LTBP1 is located at 2p22.3 and belongs to a gene family encoding extracellular binding proteins to the latent form of TGF-β (Torrego et al. 2007). LTBPs are involved in the organization of microfibrils in the extracellular matrix and assembly of elastic fibers, although they play a key role in the regulation of TGF-β signaling (Torrego et al. 2007; Robertson and Rifkin 2013; Robertson et al. 2015). The activity of TGF-β cytokines has been linked to cell growth, apoptosis, and inflammatory processes (Taipale et al. 1998), among other functions. Indeed, TGF-β molecules are synthesized in a latent state and then, these are folded, secreted and, deposited in the extracellular matrix through the interaction with fibrillin and fibronectin in a process mediated by their binding to LTBPs (Miyazono et al. 1991; Isogai et al. 2003; Annes et al. 2004; Hyytiainen et al. 2004; Massam-Wu et al. 2010; Robertson and Rifkin 2013).

TGF-β proteins are expressed in inflammatory (neutrophils and eosinophils) and structural cells in the lung (fibroblasts, epithelial, and ASM cells) (Wong et al. 1993; Duvernelle et al. 2003). Cytokines of the TGF-β family and LTBPs participate in multiple pulmonary processes from the morphogenesis during fetal development to mechanisms involved in many respiratory diseases (Aschner and Downey 2016; Saito et al. 2018), including structural changes in the airways (Torrego et al. 2007; Peng et al. 2011). Specifically, TGF-β cytokines increase the production of extracellular matrix proteins from pulmonary fibroblasts and growth factors of the connective tissue from ASM cells (Torrego et al. 2007).

Some studies have demonstrated that LTBP1 predominantly binds to TGF-β1, which is the most abundant TGF-β isoform (Saharinen and Keski-Oja 2000; Chen et al. 2005). Although *LTBP1* is expressed in a broad range of cell types and tissues, it is especially abundant in vital organs, such as the lung (Robertson et al. 2015). TGF-β1 is a potent inductor of fibrotic processes in the lung through the differentiation of fibroblasts into myofibroblasts and the inhibition of cell growth and repair of the alveolar epithelium (Saito et al. 2018). Indeed, the TGF-β signaling and LTBP1 have been recently proposed as important elements in IPF. Indeed, a mice model of pulmonary fibrosis showed increased levels of fibulin 1 (FBLN1) in fibrotic lungs (Liu et al. 2019), which has been suggested to regulate the activation of TGF-β1 and, therefore, the airways remodeling and fibrotic processes (Liu et al. 2019). Interestingly, the participation of FBLN1 through LTBP1 has also been proposed in COPD and asthma (Liu et al. 2016a; Liu et al. 2017). Liu *et al.* (2019) hypothesized that the binding of FBLN1C to LTBP1 in the extracellular space is required to trigger the activation of the TGF-β signaling, promoting the differentiation of fibroblasts into myofibroblasts and collagen deposition.

All the findings described above suggest that TGF-β signaling might play a key role in asthma, not only inducing fibrotic processes but also other structural changes in the airways and regulating the immune response (Tirado-Rodriguez et al. 2014). Indeed, TGF-β proteins are important immunomodulators in asthma and allergic diseases (Tirado-Rodriguez et al. 2014), which share both molecular mechanisms and genetic factors (Ober and Yao 2011; Belsky et al. 2013; Hinds et al. 2013; Ferreira et al. 2017; Ferreira et al. 2018; Zhu et al. 2018). TGF-β signaling mediates the migration of leukocytes to the airways, maintaining the inflammatory response (Tran 2012), but it can also exert anti-inflammatory functions (Tirado-Rodriguez et al. 2014). Furthermore, eosinophils are the major source of TGF-β in patients with asthma or allergy (Al-Alawi et

al. 2014) and, interestingly, increased levels of TGF-β1 have been detected in BAL samples from asthma patients (Ohno et al. 1996; Redington et al. 1997; Lau et al. 2010; Al-Alawi et al. 2014). Therefore, a strong correlation between TGF-β1 expression, eosinophil levels, and severity of asthma has been proposed (Al-Alawi et al. 2014). Additionally, it has been postulated that TGF-β1 might underlie the proliferation of ASM cells, inducing bronchoconstriction, and airway hyperresponsiveness (Worthington et al. 2012; Ojiaku et al. 2018; Saito et al. 2018).

Interestingly, several published GWAS have revealed the association of the genes encoding TGF-β1 and its receptor with asthma susceptibility (Chiang et al. 2013; Frischmeyer-Guerrerio et al. 2013; Yao et al. 2016). On the other hand, *LTBP1* has been associated with different spirometric measures (Carvalho-Silva et al. 2019; Kichaev et al. 2019), markers commonly used in the clinical management of asthma (Grossman et al. 2019; Global Initiative for Asthma 2020). Based on these pieces of evidence together with the results described in *Chapter 5* suggesting its implication in ICS response in asthma patients, it seems feasible to speculate that *LTBP1* could be involved in molecular and cellular mechanisms underlying asthma-related traits. Indeed, the *in silico* functional evaluation revealed potential implications of the *LTBP1* variants associated with asthma exacerbations despite ICS use in the regulation of the gene expression in pulmonary cells (The ENCODE Project Consortium 2012). Moreover, the gene-set enrichment analyses performed as part of the study described in *Chapter 3* showed that *LTBP1* was one of the genes whose expression was affected by TSA exposure, suggesting that it could be a target of this potential novel alternative medication in asthma patients.

Nonetheless, the findings of the work presented in *Chapter 5* not only propose the potential implication of *LTBP1* in the response to asthma treatment with ICS but also reinforce previous evidence of the important role of TGF-β1 in asthma pathophysiology (Halwani et al. 2011; Al-Alawi et al. 2014). Interestingly, proteins encoded by some of the novel loci of ICS response suggested by the studies included in *Chapters 3-5* of this doctoral thesis (*WNT5A*, *ROBO2* and, *LTBP1*) are directly or indirectly involved in processes related to TGF-β1. As described above, LTBP1 is the main regulator of the activity of TGF-β1, whereas WNT5A and ROBO2 are indirectly implicated in structural changes in the airways mediated by the interaction with TGF-β1. Therefore, TGF-β1 could be hypothesized as a central player in the mechanisms underlying the response to asthma treatment with ICS, which has been evidenced to ineffectively inhibit TGF-β1 (Chakir et al. 2003). This opens the door to the development of alternative therapeutic asthma strategies personally designed for those patients without a proper response to ICS therapy.

4.7. Study limitations

The studies performed as part of this doctoral thesis have contributed to the knowledge about the genetic factors involved in the response to asthma treatment with ICS, suggesting four novel loci and potential alternative asthma medication. Nevertheless, some limitations need to be acknowledged. First, no genome-wide significant associations were found with none of the different definitions of ICS response in admixed nor European populations, not even after combining the association results obtained in the discovery and replication phases of each study. Although the genome-wide significance level of *p*-value$\leq 5x10^{-8}$ is the most widely accepted threshold to consider significant associations in studies using

GWAS approaches (Willer et al. 2006; Pe'er et al. 2008) to differentiate true associations from false positives (Kaler and Purcell 2019), it is not always reached mainly due to limitations in terms of statistical power. In the GWAS presented in *Chapters 2-4*, the lack of genome-wide significant signals could be due to differences in the design and clinical definitions of episodes of asthma exacerbations despite the regular use of ICS among the different studies included. Nonetheless, it has been proposed that this threshold is not absolute, and it is dependent on the characteristics of the data, such as the origin of the genetic information, minor allele frequencies, and LD patterns due to differences among ancestry groups (Panagiotou and Ioannidis 2012). Moreover, association signals at a less stringent significance level have also been revealed to account for part of the heritability of many traits (Bjorkegren et al. 2015). Even though there is no standard threshold to consider suggestive associations, several arbitrarily defined less stringent cutoffs have been used by GWAS of different traits to select association signals to be followed up for replication in independent populations (Duggal et al. 2008). However, the suggestive significance threshold of p-value$\leq 5x10^{-6}$ has been the most commonly used by GWAS approaches (Reed et al. 2015; Sanders et al. 2017; Griebeler and Werner 2018; Roosenboom et al. 2018). This was reached in the discovery phase of the studies presented in this doctoral thesis and evidence of replication was found at nominal level in independent samples.

Second, the studies presented in this thesis included modest sample sizes, which could limit the significance of the findings. Nonetheless, it is important to notice the difficulty to include homogeneous groups of asthma patients fulfilling several demographical, clinical, ancestry-related, and genetic criteria in GWAS of asthma and related traits, which restricts the total number of individuals included. Of notice, the GWAS of asthma exacerbations despite ICS use in admixed (*Chapter 2*) and European populations (*Chapter 3*) included the largest sample sizes analyzed in any GWAS of ICS response in children and young adults with asthma published until December 2020. However, genetic associations with the change in FEV_1 after ICS use were evaluated in *Chapter 4* in a reduced sample size study, which was the only one participating in the PiCA consortium with available information about this measure at the time of performing these analyses. Moreover, this represents one of the few GWAS carried out in ICS-naïve asthma patients. Specifically, these are advantaged by the capacity to provide an approximation of the real response to the asthma treatment in patients that had not been previously exposed to this medication, making it a unique phenotype that has been explored before.

Third, episodes of asthma exacerbations despite ICS use as a measure of the response to this medication were not homogeneously defined across the studies included in *Chapters 2-5*. Although the standard definition of severe asthma exacerbations established by ATS and ERS was used (Reddel et al. 2009), information about any of the unscheduled medical care events considered was incomplete for some of the studies from European descent populations so that moderate asthma attacks were assessed instead. Additionally, this measure was based on partly self-reported retrospective information from two different timeframes (6 or 12 months before study enrolment) also due to the non-availability of data in some studies. Therefore, the information about asthma exacerbations could have been biased by the patient's subjectivity and the time-dependent probability of episodes occurrence. All of this could have not reflected the real effectiveness of asthma therapy with ICS at the time of study enrolment. The association of the loci identified in *Chapters 2-5* could lead to questioning whether these could exert a direct effect on the disease severity

regardless of the medication use. However, sensitivity analyses correcting for the groups of medications prescribed or baseline lung function in *Chapters 3-5*, as proxies of disease severity, revealed that these could be involved in the response to asthma treatment with ICS rather than asthma severity. Nonetheless, treatment steps were defined based on the guidelines established by the BTS/SIGN in 2014 (British Thoracic Society and the Scottish Intercollegiate Guidelines Network 2014). Although at the moment of performing these analyses, there were available updated guidelines, which had slightly modified the medication composition of each treatment step, the BTS/SIGN were used given that patients had been treated based on this approach at the time of study enrolment.

Fourth, other factors that could affect ICS response could not be included as covariates in the association analyses. Although the exposure to environmental factors in asthma-related traits (Ramadan et al. 2019; Global Initiative for Asthma 2020) could differentially affect the probability and severity of asthma exacerbations (Wenzel 2012; Fuchs et al. 2017; Murrison et al. 2019), this information was not considered in the association analyses, given the lack of such information. ICS responsiveness was defined as the occurrence or absence of asthma exacerbations despite ICS treatment based on retrospective information about asthma medication use, but no specifications about the start time of this therapy were available. Moreover, no details about the pharmacological asthma therapy were available, such as the specific ICS type or adherence to medical treatment prescription. Information about the specific daily dose of ICS based on medical prescriptions was not available in most of the studies, so that sensitivity analyses adjusted by this variable could be carried out only in one study, although these did not reveal major effects on the association of the variant identified in European populations with the occurrence of asthma exacerbations (*Chapter 3*). Despite all these factors driving a heterogeneous definition of the occurrence of asthma exacerbations in patients treated with ICS and potential bias in the interpretation of asthma control due to the regular use of ICS, the loci identified in the discovery phase of the studies presented in *Chapters 2-4* were validated in independent studies, suggesting the robustness of the association signals detected.

Fifth, scarce validation of the association signals of asthma exacerbations despite ICS treatment could be attempted with the change in FEV_1 after ICS use. Even though the variant at *APOBEC3B-APOBEC3C* showed evidence of replication with this additional measure of ICS response, this was not evaluated for variants at *CACNA2D3-WNT5A* nor *LTPB1*. Moreover, assessment of the association of *ROBO2* with the difference in spirometry measures after a short period of ICS treatment could not be carried out in independent populations. Therefore, further validation of the findings with the change in lung function included several independent studies is needed.

Sixth, the effective number of independent genetic markers tested within each genomic region attempted for validation was estimated based on empirical autocorrelations of summary significance results using the R package *coda* (Plummer et al. 2006). Although it is a feasible method to account for multiple comparisons tested that has been applied in genetic association studies (Sobota et al. 2015), this is only based on the logarithmic transformation of *p*-values. Thus, more accurate methods based on LD patterns among the variants tested should have been applied.

Seventh, gene expression levels were compared in reduced groups of cell lines experimentally exposed to GCs or control solutions, and patients with different patterns of ICS responsiveness in *Chapter 5*.

This could be partly explained by the fact that this study was aimed to evaluate the differential gene expression in response to GCs using RNA-seq data. Despite the substantial dramatic improvements in NGS technologies in the last years (Rizzo and Buck 2012; Kulkarni and Frommolt 2017; Petersenet al. 2017), including large sample sizes is still cost limiting. This needs to be added to the fact that invasive techniques are required to obtain ASM samples, extracted from deceased lung transplant donors, which strongly hampers the capacity to include large numbers of samples. Additionally, ASM cells included in the discovery phase were obtained from a group of non-asthmatic individuals biased by age gender, and ancestry, even though this was the only ASM transcriptome dataset obtained through NGS at the time of performing the analyses. Changes in expression levels in response to GCs were validated in independent transcriptome datasets from PBMCs and ASM cells despite differences among RNA-seq and gene expression microarrays (Li et al. 2016), and the well-known GCs cell-specific effects (Pratt et al. 2006).

Finally, the functional implications of the loci identified with different definitions of ICS response could be only assessed through *in silico* approaches using experimental evidence available at public databases. Therefore, further investigation including *in vitro* experiments is needed to better understand the molecular mechanisms implicating these loci in the response to asthma treatment with ICS.

4.8. Future directions of pharmacogenomics of ICS response

Despite the advances that this doctoral thesis has provided to the field of pharmacogenomics of response to asthma treatment with ICS, further studies are needed to continue identifying the genetic markers underlying the differential ICS responsiveness to contribute in the future to improve asthma management and design alternative pharmacological strategies that could reduce the substantial burden of this disease on the society. Nonetheless, future investigations should consider including large sample sizes and individuals from diverse populations. Homogeneous phenotypic definitions of asthma and clinical measures of the response to ICS therapy and, the inclusion of ICS-naïve patients should be also attempted. Furthermore, associations of the genetic ancestry at the chromosome level with ICS response should be further assessed through admixture mapping scans, taking advantage of the numerous benefits of genetically admixed populations.

Large reference panels for imputation with high coverage of genetic variants across the genome and good representation of the ancestry groups under study should be also taken into account. Remarkably, the recent release of the reference panel from the Trans-Omics for Precision Medicine (TOPMed) program (March 2020) (Taliun et al. 2019) is expected to make a substantial change in human genomics. This is the largest catalog of human genetic variation available to date with information about 410 million genetic variants, including 240 million SNPs, short insertions/deletions, and high coverage of rare variants. Unlike previous reference panels, TOPMed has been constructed uniquely through deep coverage WGS of 53,831 individuals, providing an exceptional source for high resolution, accuracy, and quality imputation of genetic variants. Additionally, it contains a wide representation of different ancestry groups, thus it is expected to benefit numerous studies for disentangling the genetic architecture of a broad range of human diseases in diverse populations (Taliun et al. 2019). Therefore, future genomic studies of ICS response in asthma

patients should consider this valuable resource as an opportunity to substantially increase the statistical power to detect novel genetic markers of asthma treatment response.

Nevertheless, it has been suggested that the future of the genetic research in asthma and related-traits will be predominantly led by NGS strategies (Marx 2013; Wang and Chen 2018; Hernandez-Pacheco et al. 2019), even though its application to pulmonary diseases has been scarce to date (Pouladi et al. 2016; Wang and Chen 2018). This will enable the exploration of the genetic component beyond the common variation. Specifically, the role of non-coding, structural, and low-frequency variants in asthma treatment response across the whole genome could be assessed. These approaches in combination with information from different omics sources are expected to increase our knowledge about the genetic factors underlying the response to asthma medications.

5. CONCLUSIONS

1. Genetic factors associated with asthma and treatment response through genomic studies to date only explain a proportion of the total estimated heritability of these traits and, many of these genetic factors have not been validated in independent studies.

2. The *APOBEC3B-APOBEC3C* locus was suggested to be associated with the occurrence of asthma exacerbations in children and young adults treated with ICS from admixed and European populations.

3. The *CACNA2D3-WNT5A* region was identified as a novel locus potentially associated with asthma exacerbations despite ICS treatment in patients of European ancestry, but not in other populations.

4. An enrichment of nominally significant associations at genes with differential expression in response to trichostatin A treatment was found in the genomic analysis of asthma exacerbations despite ICS use in Europeans.

5. The association of several polymorphisms at the *ROBO2* gene with the change in lung function after ICS therapy and asthma exacerbations in patients treated with ICS was suggested in European children and young adults with asthma.

6. The existence of genetic factors of ICS response shared among different populations was demonstrated. The validity of the information about asthma exacerbations as a good marker of the response to asthma treatment was also confirmed.

7. *LTBP1* showed overexpression in different cell types in response to treatment with GCs. Several variants at this gene were nominally associated with asthma exacerbations despite ICS use in admixed and European populations.

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