EUROPEAN RESPIRATORY *journal*

FLAGSHIP SCIENTIFIC JOURNAL OF ERS

Early View

Original article

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

Natalia Hernandez-Pacheco, Susanne J Vijverberg, Esther Herrera-Luis, Jiang Li, Yang Yie Sio, Raquel Granell, Almudena Corrales, Cyrielle Maroteau, Ryan Lethem, Javier Perez-Garcia, Niloufar Farzan, Katja Repnik, Mario Gorenjak, Patricia Soares, Leila Karimi, Maximilian Schieck, Lina Pérez-Méndez, Vojko Berce, Roger Tavendale, Celeste Eng, Olaia Sardon, Inger Kull, Somnath Mukhopadhyay, Munir Pirmohamed, Katia MC Verhamme, Esteban G Burchard, Michael Kabesch, Daniel B Hawcutt, Erik Melén, Uroš Potočnik, Fook Tim Chew, Kelan G Tantisira, Steve Turner, Colin N Palmer, Carlos Flores, Maria Pino-Yanes, Anke H Maitland-van der Zee

Please cite this article as: Hernandez-Pacheco N, Vijverberg SJ, Herrera-Luis E, *et al*. Genome-wide association study of asthma exacerbations despite inhaled corticosteroids use. *Eur Respir J* 2020; in press (https://doi.org/10.1183/13993003.03388-2020).

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

Natalia Hernandez-Pacheco[1,2], Susanne J Vijverberg[3,4,5], Esther Herrera-Luis[2], Jiang Li[6], Yang Yie SIO[7], Raquel Granell[8], Almudena Corrales[1,9], Cyrielle Maroteau[10], Ryan Lethem[8], Javier Perez-Garcia[2], Niloufar Farzan[3,4,11], Katja Repnik[12,13], Mario Gorenjak[12], Patricia Soares[14,15], Leila Karimi[16], Maximilian Schieck[17,18], Lina Pérez-Méndez[1,9,19], Vojko Berce[12,20], Roger Tavendale[21], Celeste Eng[22], Olaia Sardon[23,24], Inger Kull[25], Somnath Mukhopadhyay[14,21], Munir Pirmohamed[26], Katia MC Verhamme[16], Esteban G Burchard[22,27], Michael Kabesch[17], Daniel B Hawcutt[28,29], Erik Melén[25,30], Uroš Potočnik[12,13], Fook Tim CHEW[7], Kelan G Tantisira[6,31], Steve Turner[32], Colin N Palmer[21], Carlos Flores[1,9,33,34], Maria Pino-Yanes[1,2,9,34*], Anke H Maitland-van der Zee[3,4,5*], on behalf of the PiCA and SysPharmPedia consortia

[1]Research Unit, Hospital Universitario N.S. de Candelaria, Universidad de La Laguna, Spain.

[2]Genomics and Health Group, Department of Biochemistry, Microbiology, Cell Biology and Genetics, Universidad de La Laguna, San Cristóbal de La Laguna, Santa Cruz de Tenerife, Spain.

[3]Department of Respiratory Medicine, Amsterdam UMC, University of Amsterdam, Amsterdam, The Netherlands.

[4]Division of Pharmacoepidemiology and Clinical Pharmacology, Faculty of Science, Utrecht University, Utrecht, The Netherlands.

[5]Department of Paediatric Respiratory Medicine and Allergy, Emma's Children Hospital, Amsterdam UMC, University of Amsterdam, Amsterdam, The Netherlands.

[6]The Channing Division of Network Medicine, Department of Medicine, Brigham & Women's Hospital and Harvard Medical School, Boston, Massachusetts, United States.

[7]Department of Biological Sciences, National University of Singapore, Singapore.

[8]MRC Integrative Epidemiology Unit, Population Health Sciences, Bristol Medical School, University of Bristol, Bristol, United Kingdom.

[9]CIBER de Enfermedades Respiratorias, Instituto de Salud Carlos III, Madrid, Spain.

[10]Division of Population Health and Genomics, School of Medicine, University of Dundee, Dundee, United Kingdom.

[11]Breathomix B.V., El Reeuwijk, The Netherlands.

[12]Center for Human Molecular Genetics and Pharmacogenomics, Faculty of Medicine, University of Maribor, Maribor, Slovenia.

[13]Laboratory for Biochemistry, Molecular Biology and Genomics, Faculty for Chemistry and Chemical Engineering, University of Maribor, Maribor, Slovenia.

[14]Academic Department of Paediatrics, Brighton and Sussex Medical School, Royal Alexandra Children's Hospital, Brighton, United Kingdom.

[15]Escola Nacional de Saúde Pública, Lisboa, Portugal

[16]Department of Medical Informatics, Erasmus University Medical Center, Rotterdam, The Netherlands.

[17]Department of Paediatric Pneumology and Allergy, University Children's Hospital Regensburg (KUNO), Regensburg, Germany.

[18]Department of Human Genetics, Hannover Medical School, Hannover, Germany.

[19]Department of Clinic Epidemiology and Biostatistics, Research Unit, Hospital Universitario N.S. de Candelaria, Gerencia de Atención Primaria, Santa Cruz de Tenerife, Spain.

[20]Department of Paediatrics, University Medical Centre Maribor, Maribor, Slovenia.

[21]Population Pharmacogenetics Group, Biomedical Research Institute, Ninewells Hospital and Medical School, University of Dundee, Dundee, United Kingdom.

[22]Department of Medicine, University of California, San Francisco, San Francisco, California, United States.

[23]Division of Paediatric Respiratory Medicine, Hospital Universitario Donostia, San Sebastián, Guipúzcoa, Spain.

[24]Department of Paediatrics, University of the Basque Country (UPV/EHU), San Sebastián, Guipúzcoa, Spain.

[25]Department of Clinical Sciences and Education Södersjukhuset, Karolinska Institutet and Sachs' Children's Hospital, Stockholm, Sweden.

[26]Department of Molecular and Clinical Pharmacology, Institute of Translational Medicine, University of Liverpool, Liverpool, United Kingdom.

[27]Department of Bioengineering and Therapeutic Sciences, University of California, San Francisco, San Francisco, California, United States.

[28]Department of Women's and Children's Health, University of Liverpool, Liverpool, United Kingdom.

[29]Alder Hey Children's Hospital, Liverpool, United Kingdom.

[30]Institute of Environmental Medicine, Karolinska Institutet, Solna, Sweden.

[31]Division of Pulmonary and Critical Care Medicine, Department of Medicine, Brigham and Women’s Hospital, and Harvard Medical School, Boston, Massachusetts, United States.

[32]Child Health, University of Aberdeen, Aberdeen, United Kingdom.

[33]Genomics Division, Instituto Tecnológico y de Energías Renovables (ITER), Santa Cruz de Tenerife, Spain.

[34]Instituto de Tecnologías Biomédicas (ITB), Universidad de La Laguna, San Cristóbal de La Laguna, Santa Cruz de Tenerife, Spain.

** These authors contributed equally to this work*

Corresponding author:

Dr. Maria Pino-Yanes

Genomics and Health Group. Department of Biochemistry, Microbiology, Cell Biology and Genetics. Universidad de La Laguna

Apartado 456, 38200, San Cristóbal de La Laguna, Santa Cruz de Tenerife, Spain

Phone: (+34) 922 316502 6343 Fax: (+34) 922 318 490 e-mail: mdelpino@ull.edu.es

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.

Total manuscript word count: 5,559 words

Number of figures and tables: 3 figures, 3 tables

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 analysed 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 for PACMAN, GoSHARE, PASS, SLOVENIA, and ESTATe (**Table 1**) [16]. Definitions of moderate asthma exacerbations were used in BREATHE-PAGES, BREATHE, and followMAGICS (**Table 1**), since no information was available for any of the previous variables [16]. Therefore, data related to unscheduled general practitioner or respiratory system specialist visits and school absence were also considered in the definition of asthma exacerbations for BREATHE-PAGES, BREATHE, and followMAGICS (**Table 1**), as described elsewhere [15].

Replication phase

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

Assessment of ICS associations in non-European populations

Association signals with evidence of replication ($p \leq 0.05$) 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 analysed 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-analysed. 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 analysed. Association with asthma exacerbations despite the use of ICS treatment was considered at suggestive significance level (p-value $\leq 5 \times 10^{-6}$), which was arbitrarily set based on the criteria commonly adopted in GWAS studies [15].

Independent association signals were detected from these results through conditional and joint multiple-SNP analyses (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-analysed 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 validation 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, 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 analysed 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, analysing 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 $\alpha = 0.05$/number of independent variants analysed, 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 analysed 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 analysed 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 analysed 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-analysed. No major evidence of genomic inflation due to population stratification was found when each study was individually analysed (**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 10 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.

Of the 10 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-analysing 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 analysed 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 12- month period. This could be explained by the fact that a wider timeframe makes exacerbation events likely to occur, but also by the larger sample size analysed compared to the studies with information based on the previous 6 months (n=1,557 vs. n=1,124).

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 did not account for the association with the occurrence of asthma exacerbations (OR for C allele: 1.24, 95% CI: 1.14-1.34, $p = 2.30 \times 10^{-7}$). 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, $p = 2.98 \times 10^{-6}$ in the model not adjusted by ICS dose). Similar results were found adjusting by a categorical variable related to ICS dose based on age groups: OR for C allele: 1.23, 95% CI: 1.14-1.34, $p = 2.02 \times 10^{-7}$) (**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 foetal lung fibroblasts and other foetal 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 ($p \leq 0.01$) 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, $p = 5.97 \times 10^{-5}$), *UMAD1-GLCCI1* (rs13235500, OR for G allele: 0.71, 95%CI: 0.60-0.85, $p = 2.44 \times 10^{-4}$), *SHB-ALDH1B1* (SNP with min *p*-value: rs341488, OR for A allele: 2.24, 95%CI: 1.48-3.40, $p = 1.44 \times 10^{-4}$), *ZNF432-ZNF841* (SNP with min *p*-value: rs67834224, OR for A allele: 0.65, 95%CI: 0.52-0.82, $p = 2.86 \times 10^{-4}$), *ELMO2-ZNF334* (SNP with min *p*-value: rs11087003, OR for C allele: 0.77, 95%CI: 0.66-0.89, $p = 5.84 \times 10^{-4}$) (**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 $p \leq 2.78 \times 10^{-5}$).

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 ($p \leq 1 \times 10^{-4}$) 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 $p \leq 1 \times 10^{-4}$ in our GWAS had been previously proposed as targets of TSA, since changes in expression levels were found to be triggered by the exposure to this drug (**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 to *CACNA2D3* have been recently associated with different lung function measurements, which are important predictors of asthma severity and progression [40,41]. Specifically, these associations include forced expiratory volume in 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 a powerful approach to support asthma diagnosis in children [46] and to monitor the adherence and response to medications [47]. These findings suggest that *CACNA2D3* could be involved in asthma progression, including the risk of asthma exacerbations, even in patients under ICS therapy.

WNT5A encodes for the WNT family member 5A, a lipid-modified glycoprotein that activates diverse signalling pathways [48]. This protein has been evidenced to play a crucial role in development during embryogenesis, oncogenesis, and regulation of inflammatory processes in infectious disorders [49]. Moreover, other genes encoding for ligands involved in the WNT signalling pathway are associated with impaired lung function in asthmatic children [50]. This

suggests that *WNT5A* could be also involved in the pulmonary capacity in asthma. Interestingly, genes associated with asthma susceptibility have been linked to WNT signalling through a gene-set enrichment analysis [30]. Specifically, this biological process seems to play regulatory and suppressive roles through the modulation of inflammation and structural changes in airways. WNT ligands have been proposed to act on the major players implicated on inflammatory processes such as dendritic and T-helper type 2 (Th2) cells and macrophages [51]. Indeed, WNT molecules regulate the homeostasis of these cells, avoiding dysregulated immune responses, which could trigger several diseases, including allergic asthma [51].

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

The C allele of the SNP rs67026078, which is located 54.1 kb from the 3' UTR of *CACNA2D3*, was found to be associated with an increased risk of asthma exacerbations despite the ICS treatment across the European studies analysed in the discovery and replication phases. Sensitivity analyses accounting for baseline asthma severity suggested that the effect of this association is related to the response to asthma medications or to the 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 [58,59].

TSA is a hydroxamic acid extracted from the bacterial genus *Streptomyces* with a wide range of histone deacetylase (HDAC) inhibitor activities in mammalian cells [60]. Specifically, TSA belongs to a family of compounds acting on metal-dependent HDACs, inhibiting histone deacetylation, and causing hyperacetylation of core histones, which is one of the major regulators of the chromatin structure [61]. Nonetheless, HDAC inhibitors have been also

demonstrated to act on diverse non-histone substrates involved in several functions such as cell signalling, chromatin structure, and DNA repair, among others [62].

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

Although the effects of TSA on chromatin structure and regulation of gene expression in pulmonary tissues are still unclear [63], these findings suggest that TSA could potentially play an important role in asthma through epigenetic modifications and regulate the molecular mechanisms involved in response to ICS. Nonetheless, to the best of our knowledge, the effect of TSA on asthma patients has not been tested in clinical trials yet and little is known about the

potential side effects of this drug. For this reason, there is still a long way for the potential introduction of TSA as controller therapy in clinical practice.

The current study has some limitations that need to be acknowledged. First, the genome-wide significance level was reached neither in the discovery phase nor after combining the results with independent European studies. Although to our knowledge our study includes the largest sample size analysed 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 aetiology 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.

ACKNOWLEDGEMENTS

The authors acknowledge the patients, families, recruiters, health care providers and community clinics for their participation in all the studies included in the PiCA consortium (http://pica-consortium.org). The authors thank the contribution of Teide High-Performance Computing facilities (http://teidehpc.iter.es) provided by the Instituto Tecnológico y de Energías Renovables (ITER, S.A.) to the results of this research and also the Centro Nacional de Genotipado-Plataforma de Recursos Biomoleculares-Instituto de Salud Carlos III (CeGen-PRB3-ISCIII; www.cegen.org) for the genotyping services provided.

We acknowledge all the families who took part in this ALSPAC, the midwives for their help in recruiting them, and the whole ALSPAC team, which includes interviewers, computer and laboratory technicians, clerical workers, research scientists, volunteers, managers, receptionists, and nurses.

The GALA II study collaborators include Shannon Thyne, UCSF; Harold J. Farber, Texas Children's Hospital; Denise Serebrisky, Jacobi Medical Center; Rajesh Kumar, Lurie Children's Hospital of Chicago; Emerita Brigino-Buenaventura, Kaiser Permanente; Michael A. LeNoir, Bay Area Pediatrics; Kelley Meade, UCSF Benioff Children's Hospital, Oakland; William Rodriguez-Cintron, VA Hospital, Puerto Rico; Pedro C. Avila, Northwestern University; Jose R. Rodriguez-Santana, Centro de Neumologia Pediatrica; Luisa N. Borrell, City University of New York; Adam Davis, UCSF Benioff Children's Hospital, Oakland; Saunak Sen, University of Tennessee and Fred Lurmann, Sonoma Technologies, Inc.

The authors acknowledge the families and patients for their participation and thank the numerous health care providers and community clinics for their support and participation in GALA II. In particular, the authors thank study coordinator Sandra Salazar; the recruiters who obtained the data: Duanny Alva, MD, Gaby Ayala-Rodriguez, Lisa Caine, Elizabeth Castellanos, Jaime Colon, Denise DeJesus, Blanca Lopez, Brenda Lopez, MD, Louis Martos, Vivian Medina, Juana Olivo, Mario Peralta, Esther Pomares, MD, Jihan Quraishi, Johanna Rodriguez, Shahdad Saeedi, Dean Soto, Ana Taveras; and the lab researcher Celeste Eng who processed the biospecimens.

The SAGE study collaborators include Harold J. Farber, Texas Children's Hospital; Emerita Brigino-Buenaventura, Kaiser Permanente; Michael A. LeNoir, Bay Area Pediatrics; Kelley Meade, UCSF Benioff Children's Hospital, Oakland; Luisa N. Borrell, City University of

New York; Adam Davis, UCSF Benioff Children's Hospital, Oakland and Fred Lurmann, Sonoma Technologies, Inc.

The authors acknowledge the families and patients for their participation and thank the numerous health care providers and community clinics for their support and participation in SAGE. In particular, the authors thank study coordinator Sandra Salazar; the recruiters who obtained the data: Lisa Caine, Elizabeth Castellanos, Brenda Lopez, MD, Shahdad Saeedi; and the lab researcher Celeste Eng who processed the biospecimens.

FINANCIAL SUPPORT STATEMENT

This study was supported by the awards (AC15/00015 and AC15/00058) funded by the Instituto de Salud Carlos III (ISCIII) through Strategic Action for Health Research (AES) and European Community (EC) within the Active and Assisted Living (AAL) Programme framework (MP-Y, OS-P), the SysPharmPedia grant from the ERACoSysMed 1st Joint Transnational Call from the European Union under the Horizon 2020, and the grant SAF2017-

83417R by the Spanish Ministry of Science, Innovation and Universities. The PACMAN study was funded by a strategic alliance between GlaxoSmithKline and Utrecht Institute for Pharmaceutical Sciences. The SLOVENIA study was financially supported by the Slovenian Research Agency (research core funding No. P3-0067) and from SysPharmPedia grant, co-financed by Ministry of Education, Science and Sport Slovenia (MIZS) (contract number C3330-16-500106). GALA II was supported by the National Heart, Lung, and Blood Institute of the National Institute of Health (NIH) grants R01HL117004 and X01HL134589; study enrolment supported by the Sandler Family Foundation, the American Asthma Foundation, the RWJF Amos Medical Faculty Development Program, Harry Wm. and Diana V. Hind Distinguished Professor in Pharmaceutical Sciences II and the National Institute of Environmental Health Sciences grant R01ES015794. SAGE was funded by the National Heart, Lung, and Blood Institute of the National Institute of Health (NIH) grants R01HL117004 and X01HL134589; study enrolment supported by the Sandler Family Foundation, the American Asthma Foundation, the RWJF Amos Medical Faculty Development Program, Harry Wm. and Diana V. Hind Distinguished Professor in Pharmaceutical Sciences II. The SHARE Bioresource (GoSHARE) and SHARE have ongoing funding from NHS Research Scotland and established by funding from The Wellcome Trust Biomedical Resource [Grant No. 099177/Z/12/Z]. Genotyping of samples from BREATHE-PAGES, GoSHARE, and SCSGES was carried out at CeGen-PRB3-ISCIII; supported by ISCIII and European Regional Development Fund (ERDF) (PT17/0019). ALSPAC was supported by the UK Medical Research Council and Wellcome (102215/2/13/2) and the University of Bristol. The Swedish Heart-Lung Foundation, the Swedish Research Council and Region Stockholm (ALF project and database maintenance) funded the BAMSE study. ESTATe was funded by an independent research grant by ZonMw project (113201006). CAMP was supported by grants from NIH (R01HL127332 and R01NR013391). The PASS

study was funded by the NHS Chair of Pharmacogenetics via the UK Department of Health. MP is Emeritus NIHR Senior Investigator.

REFERENCES

1. Global strategy for asthma management and prevention. Global Initiative for Asthma (GINA) 2020. http://ginasthma.org/.

2. Cerasoli F, Jr. Developing the ideal inhaled corticosteroid. Chest 2006;130(1 Suppl):54S-64S.
3. Szefler SJ, Phillips BR, Martinez FD, Chinchilli VM, Lemanske RF, Strunk RC, Zeiger RS, Larsen G, Spahn JD, Bacharier LB, Bloomberg GR, Guilbert TW, Heldt G, Morgan WJ, Moss MH, Sorkness CA, Taussig LM. Characterization of within-subject responses to fluticasone and montelukast in childhood asthma. J Allergy Clin Immunol 2005;115:233-242.
4. Mersha TB. Mapping asthma-associated variants in admixed populations. Front Genet 2015;6:292.
5. Hernandez-Pacheco N, Pino-Yanes M, Flores C. Genomic Predictors of Asthma Phenotypes and Treatment Response. Front Pediatr. 2019;7:6.
6. Ramadan AA, Gaffin JM, Israel E, Phipatanakul W. Asthma and Corticosteroid Responses in Childhood and Adult Asthma. Clin Chest Med 2019;40:163-177.
7. Park HW, Tantisira KG, Weiss ST. Pharmacogenomics in asthma therapy: where are we and where do we go? Annu Rev Pharmacol Toxicol. 2015;55:129-47.
8. Farzan N, Vijverberg SJ, Arets HG, Raaijmakers JA, Maitland-van der Zee AH. Pharmacogenomics of inhaled corticosteroids and leukotriene modifiers: a systematic review. Clin Exp Allergy 2017;47:271-293.
9. Levin AM, Gui H, Hernandez-Pacheco N, Yang M, Xiao S, Yang JJ, Xiao S, Yang JJ, Hochstadt S, Barczak AJ, Eckalbar WL, Rynkowski D, Samedy LA, Kwok PY, Pino-Yanes M, Erle DJ, Lanfear DE, Burchard EG, Williams LK. Integrative approach identifies corticosteroid response variant in diverse populations with asthma. J Allergy Clin Immunol 2019;143:1791-1802.

10. Keskin O, Farzan N, Birben E, Akel H, Karaaslan C, Maitland-van der Zee AH, et al. Genetic associations of the response to inhaled corticosteroids in asthma: a systematic review. Clin Transl Allergy 2019;9:2.

11. Tantisira KG, Damask A, Szefler SJ, Schuemann B, Markezich A, Su J, Klanderman B, Sylvia J, Wu R, Martinez F, Boushey HA, Chinchilli VM, Mauger D, Weiss ST, Israel E. Genome-wide association identifies the T gene as a novel asthma pharmacogenetic locus. Am J Respir Crit Care Med 2012;185:1286-1291.

12. Herrera-Luis E, Hernandez-Pacheco N, Vijverberg SJ, Flores C, Pino-Yanes M. Role of genomics in asthma exacerbations. Curr Opin Pulm Med 2019;25:101-112.

13. Hall R, Hall IP, Sayers I. Genetic risk factors for the development of pulmonary disease identified by genome-wide association. Respirology 2019;24:204-214.

14. Farzan N, Vijverberg SJ, Andiappan AK, Arianto L, Berce V, Blanca-Lopez N, Bisgaard H, Bonnelykke K, Burchard EG, Campo P, Canino G, Carleton B, Celedon JC, Chew FT, Chiang WC, Cloutier MM, Daley D, Den Dekker HT, Dijk FN, Duijts L, Flores C, Forno E, Hawcutt DB, Hernandez-Pacheco N, de Jongste JC, Kabesch M, Koppelman GH, Manolopoulos VG, Melen E, Mukhopadhyay S, Nilsson S, Palmer CN, Pino-Yanes M, Pirmohamed M, Potocnik U, Raaijmakers JA, Repnik K, Schieck M, Sio YY, Smyth RL, Szalai C, Tantisira KG, Turner S, van der Schee MP, Verhamme KM, Maitland-van der Zee AH. Rationale and design of the multiethnic Pharmacogenomics in Childhood Asthma consortium. Pharmacogenomics 2017;18:931-943.

15. Hernandez-Pacheco N, Farzan N, Francis B, Karimi L, Repnik K, Vijverberg SJ, Soares P, Schieck M, Gorenjak M, Forno E, Eng C, Oh SS, Perez-Mendez L, Berce V, Tavendale R, Samedy LA, Hunstman S, Hu D, Meade K, Farber HJ, Avila PC, Serebrisky D, Thyne SM, Brigino-Buenaventura E, Rodriguez-Cintron W, Sen S,

Kumar R, Lenoir M, Rodriguez-Santana JR, Celedon JC, Mukhopadhyay S, Potocnik U, Pirmohamed M, Verhamme KM, Kabesch M, Palmer CAN, Hawcutt DB, Flores C, Maitland-van der Zee AH, Burchard EG, Pino-Yanes M. Genome-wide association study of inhaled corticosteroid response in admixed children with asthma. Clin Exp Allergy 2019;49:789-798.

16. Reddel HK, Taylor DR, Bateman ED, Boulet LP, Boushey HA, Busse WW, Casale TB, Chanez P, Enright PL, Gibson PG, de Jongste JC, Kerstjens HA, Lazarus SC, Levy ML, O'Byrne PM, Partridge MR, Pavord ID, Sears MR, Sterk PJ, Stoloff SW, Sullivan SD, Szefler SJ, Thomas MD, Wenzel SE. An official American Thoracic Society/European Respiratory Society statement: asthma control and exacerbations: standardizing endpoints for clinical asthma trials and clinical practice. Am J Respir Crit Care Med. 2009;180:59-99.

17. McCarthy S, Das S, Kretzschmar W, Delaneau O, Wood AR, Teumer A, Kang HM, Fuchsberger C, Danecek P, Sharp K, Luo Y, Sidore C, Kwong A, Timpson N, Koskinen S, Vrieze S, Scott LJ, Zhang H, Mahajan A, Veldink J, Peters U, Pato C, van Duijn CM, Gillies CE, Gandin I, Mezzavilla M, Gilly A, Cocca M, Traglia M, Angius A, Barrett J C, Boomsma D, Branham K, Breen G, Brummett CM, Busonero F, Campbell H, Chan A, Chen S, Chew E, Collins FS, Corbin LJ, Smith GD, Dedoussis G, Dorr M, Farmaki AE, Ferrucci L, Forer L, Fraser RM, Gabriel S, Levy S, Groop L, Harrison T, Hattersley A, Holmen OL, Hveem K, Kretzler M, Lee JC, McGue M, Meitinger T, Melzer D, Min JL, Mohlke KL, Vincent JB, Nauck M, Nickerson D, Palotie A, Pato M, Pirastu N, McInnis M, Richards JB, Sala C, Salomaa V, Schlessinger D, Schoenherr S, Slagboom PE, Small K, Spector T, Stambolian D, Tuke M, Tuomilehto J, Van den Berg LH, Van Rheenen W, Volker U, Wijmenga C, Toniolo D, Zeggini E, Gasparini P, Sampson M G, Wilson JF,

Frayling T, de Bakker PI, Swertz MA, McCarroll S, Kooperberg C, Dekker A, Altshuler D, Willer C, Iacono W, Ripatti S, Soranzo N, Walter K, Swaroop A, Cucca F, Anderson CA, Myers RM, Boehnke M, McCarthy MI, Durbin R. A reference panel of 64,976 haplotypes for genotype imputation. Nat Genet 2016;48:1279-1283.

18. Abecasis GR, Auton A, Brooks LD, DePristo MA, Durbin RM, Handsaker RE, Kang HM, Marth GT, McVean GA. An integrated map of genetic variation from 1,092 human genomes. Nature 2012;491:56-65.
19. Kang HM. EPACTS (Efficient and Parallelizable Association Container Toolbox). http://genome.sph.umich.edu/wiki/EPACTS (2016).
20. Han B, Eskin E. Random-effects model aimed at discovering associations in meta-analysis of genome-wide association studies. Am J Hum Genet 2011;88:586-598.
21. Yang J, Ferreira T, Morris AP, Medland SE, Madden PA, Heath AC, Martin NG, Montgomery GW, Weedon MN, Loos RJ, Frayling TM, McCarthy MI, Hirschhorn JN, Goddard ME, Visscher PM. Conditional and joint multiple-SNP analysis of GWAS summary statistics identifies additional variants influencing complex traits. Nat Genet 2012;44:369-375, S1-S3.
22. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, Maller J, Sklar P, de Bakker PI, Daly MJ, Sham PC. PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet 2007;81:559-575.
23. Marchini J, Howie B. Genotype imputation for genome-wide association studies. Nat Rev Genet 2010;11:499-511.
24. British Thoracic Society and the Scottish Intercollegiate Guidelines Network. British guideline on the management of asthma. Thorax 2014;69 Suppl 1:1-192.

25. Ward LD, Kellis M. HaploReg v4: systematic mining of putative causal variants, cell types, regulators and target genes for human complex traits and disease. Nucleic Acids Res 2016;44:D877-D881.

26. GTEx Consortium. The Genotype-Tissue Expression (GTEx) project. Nat Genet 2013;45:580-585.

27. Staley JR, Blackshaw J, Kamat MA, Ellis S, Surendran P, Sun BB, Paul DS, Freitag D, Burgess S, Danesh J, Young R, Butterworth AS. PhenoScanner: a database of human genotype-phenotype associations. Bioinformatics 2016;32:3207-3209.

28. Kamat MA, Blackshaw JA, Young R, Surendran P, Burgess S, Danesh J, Butterworth AS, Staley JR. PhenoScanner V2: an expanded tool for searching human genotype-phenotype associations. Bioinformatics 2019;35:4851-4853.

29. Karolchik D, Hinrichs AS, Furey TS, Roskin KM, Sugnet CW, Haussler D, Kent WJ. The UCSC Table Browser data retrieval tool. Nucleic Acids Res 2004;32:D493-D496.

30. Barreto-Luis A, Corrales A, Acosta-Herrera M, Gonzalez-Colino C, Cumplido J, Martinez-Tadeo J, Carracedo A, Villar J, Carrillo T, Pino-Yanes M, Flores C. A pathway-based association study reveals variants from Wnt signalling genes contributing to asthma susceptibility. Clin Exp Allergy 2017;47:618-626.

31. Srikanth K, Lee SH, Chung KY, Park JE, Jang GW, Park MR, Kim NY, Kim TH, Chai HH, Park WC, Lim D. A Gene-Set Enrichment and Protein-Protein Interaction Network-Based GWAS with Regulatory SNPs Identifies Candidate Genes and Pathways Associated with Carcass Traits in Hanwoo Cattle. Genes (Basel) 2020;11:316.

32. Kuleshov MV, Jones MR, Rouillard AD, Fernandez NF, Duan Q, Wang Z, Koplev S, Jenkins SL, Jagodnik KM, Lachmann A, McDermott MG, Monteiro CD, Gundersen

GW, Ma'ayan A.Enrichr: a comprehensive gene set enrichment analysis web server 2016 update. Nucleic Acids Res 2016;44:W90-W97.

33. The ENCODE Project Consortium. An integrated encyclopedia of DNA elements in the human genome. Nature 2012;489:57-74.

34. Sun BB, Maranville JC, Peters JE, Stacey D, Staley JR, Blackshaw J, Burgess S, Jiang T, Paige E, Surendran P, Oliver-Williams C, Kamat MA, Prins BP, Wilcox SK, Zimmerman ES, Chi A, Bansal N, Spain SL, Wood AM, Morrell NW, Bradley JR, Janjic N, Roberts DJ, Ouwehand WH, Todd JA, Soranzo N, Suhre K, Paul DS, Fox CS, Plenge RM, Danesh J, Runz H, Butterworth AS. Genomic atlas of the human plasma proteome. Nature 2018;558:73-79.

35. Stelzer G, Rosen N, Plaschkes I, Zimmerman S, Twik M, Fishilevich S, Stein TI, Nudel R, Lieder I, Mazor Y, Kaplan S, Dahary D, Warshawsky D, Guan-Golan Y, Kohn A, Rappaport N, Safran M, Lancet D. The GeneCards Suite: From Gene Data Mining to Disease Genome Sequence Analyses. Curr Protoc Bioinformatics 2016;54:1301-1313.

36. Bonder MJ, Luijk R, Zhernakova DV, Moed M, Deelen P, Vermaat M, van Iterson M, van Dijk F, van Galen M, Bot J, Slieker RC, Jhamai PM, Verbiest M, Suchiman HE, Verkerk M, van der Breggen R, van Rooij J, Lakenberg N, Arindrarto W, Kielbasa SM, Jonkers I, van 't Hof P, Nooren I, Beekman M, Deelen J, van Heemst D, Zhernakova A, Tigchelaar EF, Swertz MA, Hofman A, Uitterlinden AG, Pool R, van Dongen J, Hottenga JJ, Stehouwer CD, van der Kallen CJ, Schalkwijk CG, van den Berg LH, van Zwet EW, Mei H, Li Y, Lemire M, Hudson TJ, Slagboom PE, Wijmenga C, Veldink JH, van Greevenbroek MM, van Duijn CM, Boomsma DI, Isaacs A, Jansen R, van Meurs JB, t Hoen PA, Franke L, Heijmans BT. Disease

variants alter transcription factor levels and methylation of their binding sites. Nat Genet 2017;49:131-138.

37. Carvalho-Silva D, Pierleoni A, Pignatelli M, Ong C, Fumis L, Karamanis N, Carmona M, Faulconbridge A, Hercules A, McAuley E, Miranda A, Peat G, Spitzer M, Barrett J, Hulcoop DG, Papa E, Koscielny G, Dunham I et al. Open Targets Platform: new developments and updates two years on. Nucleic Acids Res. 2019;47:D1056-D1065.

38. Davies A, Hendrich J, Van Minh AT, Wratten J, Douglas L, Dolphin AC. Functional biology of the alpha(2)delta subunits of voltage-gated calcium channels. Trends Pharmacol Sci 2007;28:220-228.

39. P Parkash J, Asotra K. Calcium wave signaling in cancer cells. Life Sci 2010;87:587-595.

40. Grossman NL, Ortega VE, King TS, Bleecker ER, Ampleford EA, Bacharier LB, Cabana MD, Cardet JC, Carr TF, Castro M, Denlinger LC, Denson JL, Fandino N, Fitzpatrick AM, Hawkins GA, Holguin F, Krishnan JA, Lazarus SC, Nyenhuis SM, Phipatanakul W, Ramratnam SK, Wenzel S, Peters SP, Meyers DA, Wechsler ME, Israel E. Exacerbation-prone asthma in the context of race and ancestry in Asthma Clinical Research Network trials. J Allergy Clin Immunol 2019;144:1524-1533.

41. Grossman NL, Doros GD, Fandino N, Fuhlbrigge AL, Pace WD, Wechsler ME, Yawn BP, Israel E. Susceptibility to exacerbations in Black adults with asthma. J Asthma 2019;56:704-710.

42. Kichaev G, Bhatia G, Loh PR, Gazal S, Burch K, Freund MK, Schoech A, Pasaniuc B, Price AL. Leveraging Polygenic Functional Enrichment to Improve GWAS Power. Am J Hum Genet 2019;104:65-75.

43. Shrine N, Guyatt AL, Erzurumluoglu AM, Jackson VE, Hobbs BD, Melbourne CA, Batini C, Fawcett KA, Song K, Sakornsakolpat P, Li X, Boxall R, Reeve NF, Obeidat M, Zhao JH, Wielscher M, Weiss S, Kentistou KA, Cook JP, Sun BB, Zhou J, Hui J,

Karrasch S, Imboden M, Harris SE, Marten J, Enroth S, Kerr SM, Surakka I, Vitart V, Lehtimaki T, Allen RJ, Bakke PS, Beaty TH, Bleecker ER, Bosse Y, Brandsma CA, Chen Z, Crapo JD, Danesh J, DeMeo DL, Dudbridge F, Ewert R, Gieger C, Gulsvik A, Hansell AL, Hao K, Hoffman JD, Hokanson JE, Homuth G, Joshi PK, Joubert P, Langenberg C, Li L, Lin K, Lind L, Locantore N, Luan J, Mahajan A, Maranville JC, Murray A, Nickle DC, Packer R, Parker MM, Paynton ML, Porteous DJ, Prokopenko D, Qiao D, Rawal R, Runz H, Sayers I, Sin DD, Smith BH, Soler Artigas M, Sparrow D, Tal-Singer R, Timmers Prhj, Van den Berge M, Whittaker JC, Woodruff PG, Yerges-Armstrong LM, Troyanskaya OG, Raitakari OT, Kahonen M, Polasek O, Gyllensten U, Rudan I, Deary IJ, Probst-Hensch NM, Schulz H, James AL, Wilson JF, Stubbe B, Zeggini E, Jarvelin MR, Wareham N, Silverman EK, Hayward C, Morris AP, Butterworth AS, Scott RA, Walters RG, Meyers DA, Cho, MH, Strachan DP, Hall IP, Tobin MD, Wain LV. New genetic signals for lung function highlight pathways and chronic obstructive pulmonary disease associations across multiple ancestries. Nat Genet 2019;51:481-493.

44. Lutz SM, Cho MH, Young K, Hersh CP, Castaldi PJ, McDonald ML, Regan E, Mattheisen M, DeMeo DL, Parker M, Foreman M, Make BJ, Jensen RL, Casaburi R, Lomas DA, Bhatt SP, Bakke P, Gulsvik A, Crapo JD, Beaty TH, Laird NM, Lange C, Hokanson JE, Silverman EK. A genome-wide association study identifies risk loci for spirometric measures among smokers of European and African ancestry. BMC Genet 2015;16:138.

45. van der Valk RJ, Duijts L, Timpson NJ, Salam MT, Standl M, Curtin JA, Genuneit J, Kerhof M, Kreiner-Moller E, Caceres A, Gref A, Liang LL, Taal HR, Bouzigon E, Demenais F, Nadif R, Ober C, Thompson EE, Estrada K, Hofman A, Uitterlinden AG, van Duijn C, Rivadeneira F, Li X, Eckel SP, Berhane K, Gauderman WJ,

Granell R, Evans DM, St Pourcain B, McArdle W, Kemp JP, Smith GD, Tiesler CM, Flexeder C, Simpson A, Murray CS, Fuchs O, Postma DS, Bonnelykke K, Torrent M, Andersson M, Sleiman P, Hakonarson H, Cookson WO, Moffatt MF, Paternoster L, Melen E, Sunyer J, Bisgaard H, Koppelman GH, Ege M, Custovic A, Heinrich J, Gilliland FD, Henderson AJ, Jaddoe VW, de Jongste JC. Fraction of exhaled nitric oxide values in childhood are associated with 17q11.2-q12 and 17q12-q21 variants. J Allergy Clin Immunol 2014;134:46-55.

46. Kaplan A, Hardjojo A, Yu S, Price D. Asthma Across Age: Insights From Primary Care. Front Pediatr 2019;7:162.

47. Smith AD, Cowan JO, Brassett KP, Herbison GP, Taylor DR. Use of exhaled nitric oxide measurements to guide treatment in chronic asthma. N Engl J Med 2005;352:2163-2173.

48. van Amerongen R, Mikels A, Nusse R. Alternative wnt signaling is initiated by distinct receptors. Sci Signal 2008;1:re9.

49. Baarsma HA, Konigshoff M. 'WNT-er is coming': WNT signalling in chronic lung diseases. Thorax 2017;72:746-759.

50. Sharma S, Tantisira K, Carey V, Murphy AJ, Lasky-Su J, Celedon JC, Lazarus R, Klanderman B, Rogers A, Soto-Quiros M, Avila L, Mariani T, Gaedigk R, Leeder S, Torday J, Warburton D, Raby B, Weiss ST. A role for Wnt signaling genes in the pathogenesis of impaired lung function in asthma. Am J Respir Crit Care Med 2010;181:328-336.

51. Reuter S, Beckert H, Taube C. Take the Wnt out of the inflammatory sails: modulatory effects of Wnt in airway diseases. Lab Invest 2016;96:177-185.

52. Choy DF, Modrek B, Abbas AR, Kummerfeld S, Clark HF, Wu LC, Fedorowicz G, Modrusan Z, Fahy JV, Woodruff PG, Arron JR. Gene expression patterns of Th2

inflammation and intercellular communication in asthmatic airways. J Immunol 2011;186:1861-1869.

53. Januskevicius A, Vaitkiene S, Gosens R, Janulaityte I, Hoppenot D, Sakalauskas R, Malakauskas K. Eosinophils enhance WNT-5a and TGF-beta1 genes expression in airway smooth muscle cells and promote their proliferation by increased extracellular matrix proteins production in asthma. BMC Pulm Med 2016;16:94.

54. Bousquet J, Chanez P, Lacoste JY, Barneon G, Ghavanian N, Enander I, Venge P, Ahlstedt S, Simony-Lafontaine J, Godard P, Michel FB. Eosinophilic inflammation in asthma. N Engl J Med 1990;323:1033-1039.

55. Kay AB, Phipps S, Robinson DS. A role for eosinophils in airway remodelling in asthma. Trends Immunol 2004;25:477-482.

56. Skronska-Wasek W, Gosens R, Konigshoff M, Baarsma HA. WNT receptor signalling in lung physiology and pathology. Pharmacol Ther 2018;187:150-166.

57. El-Sahli S, Xie Y, Wang L, Liu S. Wnt Signaling in Cancer Metabolism and Immunity. Cancers (Basel) 2019;11.

58. Zhu Z, Lee PH, Chaffin MD, Chung W, Loh PR, Lu Q, Christiani DC, Liang L. A genome-wide cross-trait analysis from UK Biobank highlights the shared genetic architecture of asthma and allergic diseases. Nat Genet 2018;50:857-864.

59. Sun R, Hui S, Bader GD, Lin X, Kraft P. Powerful gene set analysis in GWAS with the Generalized Berk-Jones statistic. PLoS Genet 2019;15:e1007530.

60. Mogal A, Abdulkadir SA. Effects of Histone Deacetylase Inhibitor (HDACi); Trichostatin-A (TSA) on the expression of housekeeping genes. Mol Cell Probes 2006;20:81-86.

61. Ma J, Li N, Zhao J, Lu J, Ma Y, Zhu Q, Dong Z, Liu K, Ming L. Histone deacetylase inhibitor trichostatin A enhances the antitumor effect of the oncolytic adenovirus

H101 on esophageal squamous cell carcinoma in vitro and in vivo. Oncol Lett 2017;13:4868-4874.

62. Royce SG, Karagiannis TC. Histone deacetylases and their role in asthma. J Asthma 2012;49:121-128.

63. Banerjee A, Trivedi CM, Damera G, Jiang M, Jester W, Hoshi T, Epstein JA, Panettieri RA. Trichostatin A abrogates airway constriction, but not inflammation, in murine and human asthma models. Am J Respir Cell Mol Biol 2012;46:132-138.

64. Toki S, Goleniewska K, Reiss S, Zhou W, Newcomb DC, Bloodworth MH, Stier MT, Boyd KL, Polosukhin VV, Subramaniam S, Peebles RS. The histone deacetylase inhibitor trichostatin A suppresses murine innate allergic inflammation by blocking group 2 innate lymphoid cell (ILC2) activation. Thorax 2016;71:633-645.

65. Adcock IM, Tsaprouni L, Bhavsar P, Ito K. Epigenetic regulation of airway inflammation. Curr Opin Immunol. 2007;19:694-700.

66. Choi JH, Oh SW, Kang MS, Kwon HJ, Oh GT, Kim DY. Trichostatin A attenuates airway inflammation in mouse asthma model. Clin Exp Allergy 2005;35:89-96.

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.

Table 2. Summary of the conditional regression models for each locus suggestively associated with asthma exacerbations in patients treated with ICS 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}	rs113364932	NA
	rs72805125	2	56684554	T/C	0.063	2.09 (1.53-2.85)	3.11×10^{-6}		0.888
CNTNAP5	rs76496334	2	125427606	T/C	0.022	2.29 (1.64-3.19)	9.69×10^{-7}	rs144289311	0.491
	rs146921813	2	125432412	C/G	0.022	2.26 (1.63-3.16)	1.34×10^{-6}		0.534
	rs141194780	2	125432413	A/G	0.022	2.26 (1.63-3.16)	1.34×10^{-6}		0.534
	rs144289311	2	125432440	A/G	0.022	2.33 (1.67-3.25)	6.73×10^{-7}		NA
	rs145694710	2	125434780	T/C	0.022	2.28 (1.63-3.17)	1.21×10^{-6}		0.515
	rs17011852	2	125440426	G/A	0.022	2.32 (1.66-3.24)	7.27×10^{-7}		NA

[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.

Table 2 (continuation). Summary of the conditional regression models for each locus suggestively associated with asthma exacerbations in patients treated with ICS use 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**

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]	rs7587871	NA
	rs7420798	2	201506713	G/A	0.318	1.09 (0.75-1.58)	3.24×10^{-6} [e]		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.

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.

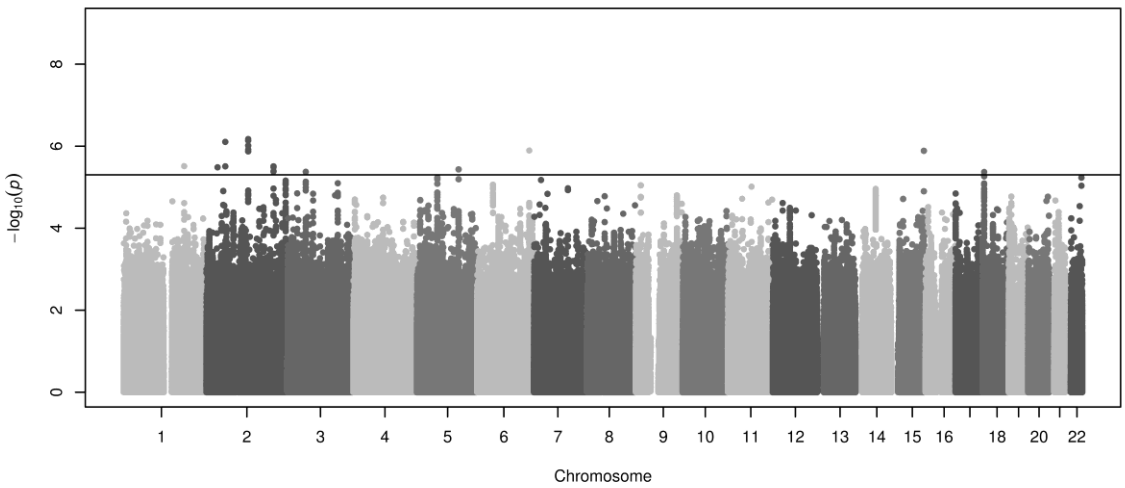
SNPs with evidence of replication in independent European populations are in boldface.

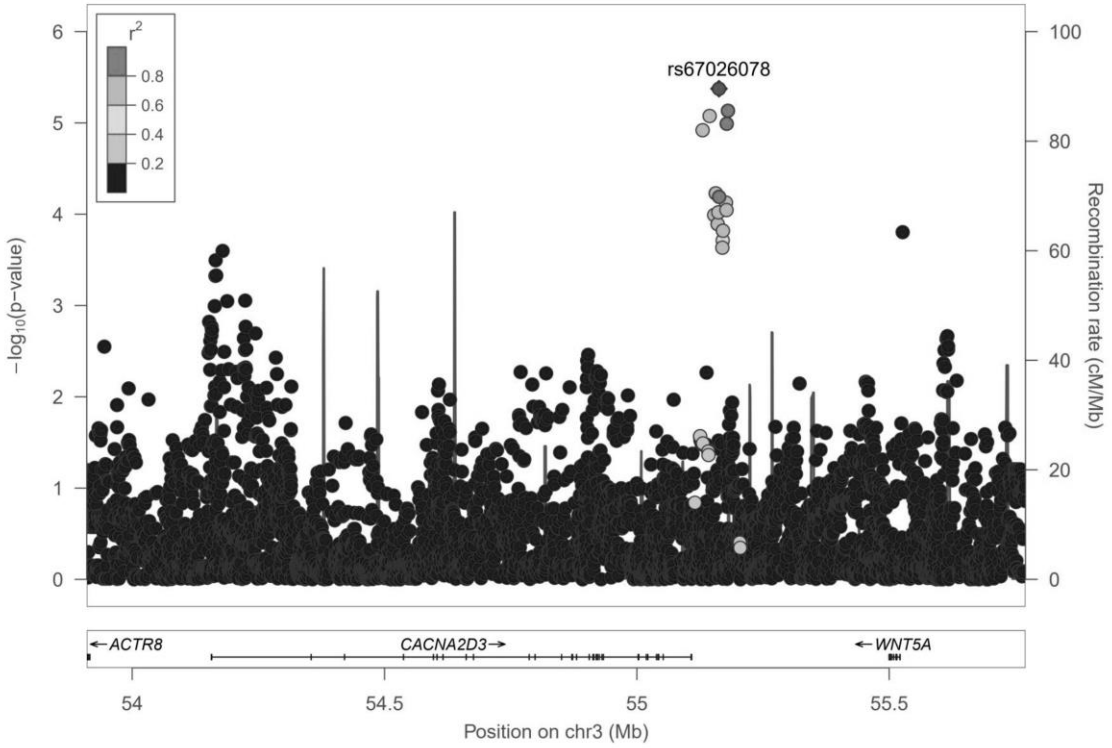
FIGURE LEGENDS

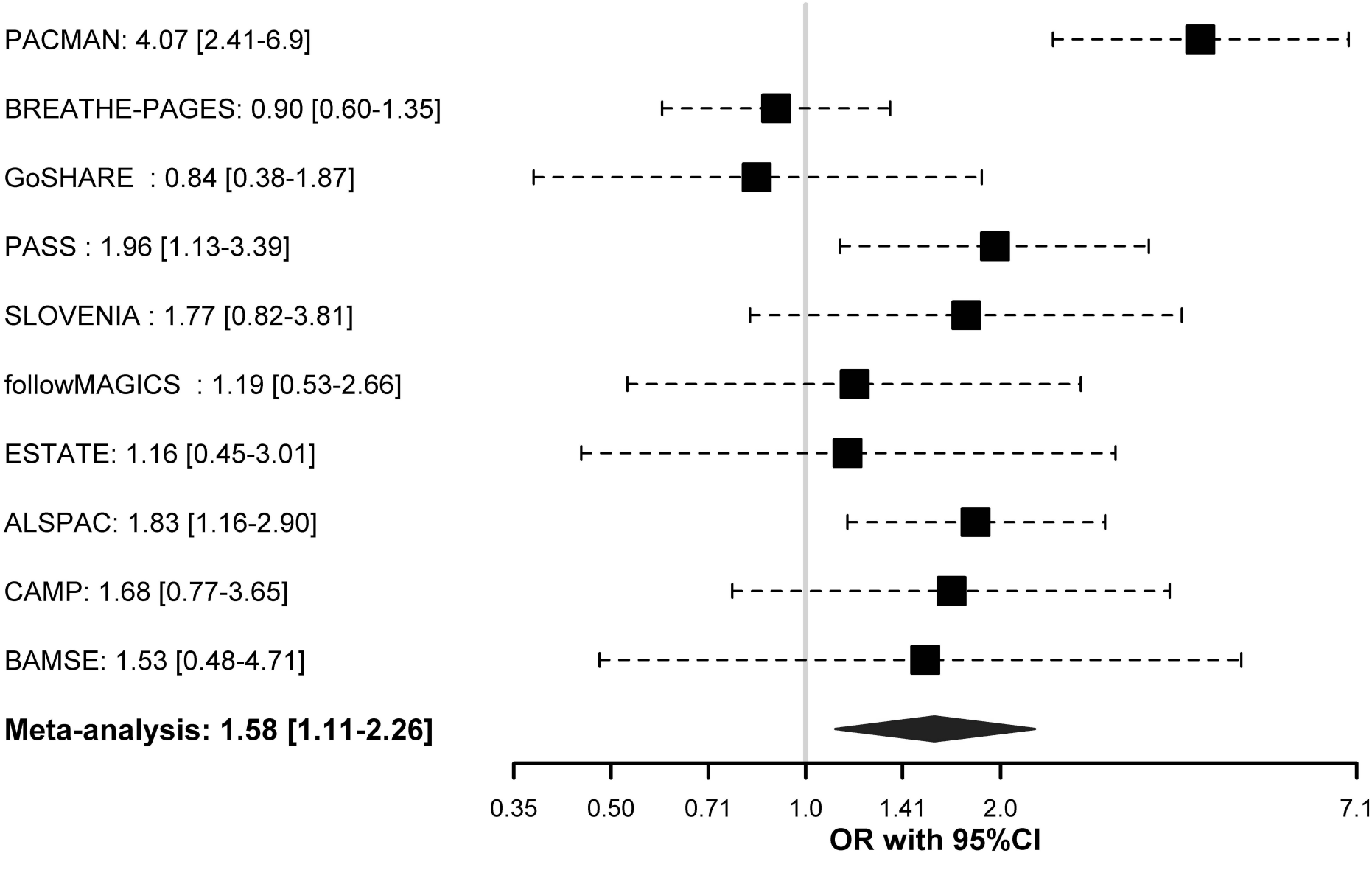
Figure 1. Manhattan plot of association results of asthma exacerbations in ICS users included in the discovery phase. Association results are represented as -$\log_{10}$ *p*-value on the *y*-axis along the chromosomes (*x*-axis). The horizontal black line shows the suggestive significance threshold for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

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.

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-analysing 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. Effect of association results is not given for BREATHE since rs67026078 did not pass quality control checks.


−log10(p)
0
2
4
6
8
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
18
20
22
Chromosome


r2
0.8
0.6
0.4
0.2
rs67026078
−log10(p−value)
Recombination rate (cM/Mb)
ACTR8
CACNA2D3
WNT5A
Position on chr3 (Mb)


PACMAN: 4.07 [2.41-6.9]
BREATHE-PAGES: 0.90 [0.60-1.35]
GoSHARE : 0.84 [0.38-1.87]
PASS : 1.96 [1.13-3.39]
SLOVENIA : 1.77 [0.82-3.81]
followMAGICS : 1.19 [0.53-2.66]
ESTATE: 1.16 [0.45-3.01]
ALSPAC: 1.83 [1.16-2.90]
CAMP: 1.68 [0.77-3.65]
BAMSE: 1.53 [0.48-4.71]
Meta-analysis: 1.58 [1.11-2.26]
0.35
0.50
0.71
1.0
1.41
2.0
7.1
OR with 95%CI

SUPPLEMENTARY MATERIAL

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

Natalia Hernandez-Pacheco[1,2], Susanne J Vijverberg[3,4,5], Esther Herrera-Luis[2], Jiang Li[6], Yang Yie SIO[7], Raquel Granell[8], Almudena Corrales[1,9], Cyrielle Maroteau[10], Ryan Lethem[8], Javier Perez-Garcia[2], Niloufar Farzan[3,4,11], Katja Repnik[12,13], Mario Gorenjak[12], Patricia Soares[14,15], Leila Karimi[16], Maximilian Schieck[17,18], Lina Pérez-Méndez[1,9,19], Vojko Berce[12,20], Roger Tavendale[21], Celeste Eng[22], Olaia Sardon[23,24], Inger Kull[25], Somnath Mukhopadhyay[14,21], Munir Pirmohamed[26], Katia MC Verhamme[16], Esteban G Burchard[22,27], Michael Kabesch[17], Daniel B Hawcutt[28,29], Erik Melén[25,30], Uroš Potočnik[12,13], Fook Tim CHEW[7], Kelan G Tantisira[6,31], Steve Turner[32], Colin N Palmer[21], Carlos Flores[1,9,33,34], Maria Pino-Yanes[1,2,9,34*], Anke H Maitland-van der Zee[3,4,5*], on behalf of the PiCA and SysPharmPedia consortia

[1]Research Unit, Hospital Universitario N.S. de Candelaria, Universidad de La Laguna, Spain.

[2]Genomics and Health Group, Department of Biochemistry, Microbiology, Cell Biology and Genetics, Universidad de La Laguna, San Cristóbal de La Laguna, Santa Cruz de Tenerife, Spain.

[3]Department of Respiratory Medicine, Amsterdam UMC, University of Amsterdam, Amsterdam, The Netherlands.

[4]Division of Pharmacoepidemiology and Clinical Pharmacology, Faculty of Science, Utrecht University, Utrecht, The Netherlands.

[5]Department of Paediatric Respiratory Medicine and Allergy, Emma's Children Hospital, Amsterdam UMC, University of Amsterdam, Amsterdam, The Netherlands.

[6]The Channing Division of Network Medicine, Department of Medicine, Brigham & Women's Hospital and Harvard Medical School, Boston, Massachusetts, United States.

[7]Department of Biological Sciences, National University of Singapore, Singapore.

[8]MRC Integrative Epidemiology Unit, Population Health Sciences, Bristol Medical School, University of Bristol, Bristol, United Kingdom.

[9]CIBER de Enfermedades Respiratorias, Instituto de Salud Carlos III, Madrid, Spain.

[10]Division of Population Health and Genomics, School of Medicine, University of Dundee, Dundee, United Kingdom.

[11]Breathomix B.V., El Reeuwijk, The Netherlands.

[12]Center for Human Molecular Genetics and Pharmacogenomics, Faculty of Medicine, University of Maribor, Maribor, Slovenia.

[13]Laboratory for Biochemistry, Molecular Biology and Genomics, Faculty for Chemistry and Chemical Engineering, University of Maribor, Maribor, Slovenia.

[14]Academic Department of Paediatrics, Brighton and Sussex Medical School, Royal Alexandra Children's Hospital, Brighton, United Kingdom.

[15]Escola Nacional de Saúde Pública, Lisboa, Portugal

[16]Department of Medical Informatics, Erasmus University Medical Center, Rotterdam, The Netherlands.

[17]Department of Paediatric Pneumology and Allergy, University Children's Hospital Regensburg (KUNO), Regensburg, Germany.

[18]Department of Human Genetics, Hannover Medical School, Hannover, Germany.

[19]Department of Clinic Epidemiology and Biostatistics, Research Unit, Hospital Universitario N.S. de Candelaria, Gerencia de Atención Primaria, Santa Cruz de Tenerife, Spain.

[20]Department of Paediatrics, University Medical Centre Maribor, Maribor, Slovenia.

[21]Population Pharmacogenetics Group, Biomedical Research Institute, Ninewells Hospital and Medical School, University of Dundee, Dundee, United Kingdom.

[22]Department of Medicine, University of California, San Francisco, San Francisco, California, United States.

[23]Division of Paediatric Respiratory Medicine, Hospital Universitario Donostia, San Sebastián, Guipúzcoa, Spain.

[24]Department of Paediatrics, University of the Basque Country (UPV/EHU), San Sebastián, Guipúzcoa, Spain.

[25]Department of Clinical Sciences and Education Södersjukhuset, Karolinska Institutet and Sachs' Children's Hospital, Stockholm, Sweden.

[26]Department of Molecular and Clinical Pharmacology, Institute of Translational Medicine, University of Liverpool, Liverpool, United Kingdom.

[27]Department of Bioengineering and Therapeutic Sciences, University of California, San Francisco, San Francisco, California, United States.

[28]Department of Women's and Children's Health, University of Liverpool, Liverpool, United Kingdom.

[29]Alder Hey Children's Hospital, Liverpool, United Kingdom.

[30]Institute of Environmental Medicine, Karolinska Institutet, Solna, Sweden.

[31]Division of Pulmonary and Critical Care Medicine, Department of Medicine, Brigham and Women’s Hospital, and Harvard Medical School, Boston, Massachusetts, United States.

[32]Child Health, University of Aberdeen, Aberdeen, United Kingdom.

[33]Genomics Division, Instituto Tecnológico y de Energías Renovables (ITER), Santa Cruz de Tenerife, Spain.

[34]Instituto de Tecnologías Biomédicas (ITB), Universidad de La Laguna, San Cristóbal de La Laguna, Santa Cruz de Tenerife, Spain.

** These authors contributed equally to this work*

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 paediatric supervision. Clinical concern about adrenal suppression was also considered as eligibility criterion since this study was initially designed to explore the clinical and pharmacogenomic associations between the use of corticosteroids and adrenal suppression. A detailed description is available in previous publications [S7, S8].

SLOVENIA (n = 182)

SLOVENIA recruited children and young adults (5-18 years old) with mild and moderate persistent asthma from tertiary health centres 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 centres 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 foetuses, 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 foetuses. 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 centres in the United States and Puerto Rico (Chicago, Illinois; New York City, New York; Houston, Texas; San Francisco, California; and San Juan, Puerto Rico). Subjects with a physician diagnosis of asthma were defined as cases. A detailed description of the eligibility and exclusion criteria has been previously described [S24, S25].

SAGE (n = 493)

The Study of African Americans, Asthma, Genes and Environments (SAGE) is a cross-sectional asthma study with similar characteristics to GALA II but focused on individuals with four grandparents of African American ancestry. Subjects were recruited in the San Francisco Bay Area, California, United States. Further details about the study design have been published elsewhere [S24, S25].

SCSGES (n = 425)

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

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

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

Samples from PAGES, goSHARE and part of BREATHE were genotyped for the current study with the Axiom™ Precision Medicine Research Array (Affymetrix Inc.) by Centro Nacional de Genotipado (CeGen; www.cegen.org). Genotyping assays were successfully performed for 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 information available about daily ICS dosage, were included in the analyses. This information was based on the daily dosages of equivalents to budesonide described in the last prescription for ICS inhaler refilling before study enrolment that was recorded in pharmacy electronic systems [S31]. The association model applied included the information about the occurrence of asthma exacerbations as a dependent variable, and allele dosages of the SNP rs67026078 as an independent variable plus age, gender, principal components and a quantitative variable related to daily ICS dose as covariates. This analysis was also carried out adjusting by a categorical variable derived from the daily ICS dosage taking into account that different ICS dosages are recommended by international guidelines based on the age group and asthma severity of the patients. Therefore, ICS dosage was categorized into low, medium or high

depending on whether the individuals were <12 years old (100-200 mcg, 200-400 mcg, >400 mcg) or ≥12 years old (200-400 mcg, 400-800 mcg, >800 mcg) [S32].

Additionally, association analyses were carried out for the SNP rs67026078 accounting for asthma severity based on the classification into treatment steps based on a modification of the guidelines established by the British Thoracic Society and the Scottish Intercollegiate Guidelines Network (BTS/SIGN) [S33]. Only those individuals from the studies included in the discovery stage with available information about the use of the medications included in the classification into treatment steps were selected. SLOVENIA was not included since information about any of the medications included in the definition of treatment steps was not available. BREATHE was also excluded because rs67026078 did not pass quality control checks. Therefore, individuals were classified as follows: *Step 1*, as-needed use of short-acting 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).

SUPPLEMENTARY REFERENCES

S1. Koster ES, Raaijmakers JA, Koppelman GH, Postma DS, van der Ent CK, Koenderman L, Bracke M, Maitland-van der Zee AH. Pharmacogenetics of anti-inflammatory treatment in children with asthma: rationale and design of the PACMAN cohort. Pharmacogenomics 2009;10:1351-1361.

S2. Turner SW, Ayres JG, Macfarlane TV, Mehta A, Mehta G, Palmer CN, Cunningham S, Adams T, Aniruddhan K, Bell C, Corrigan D, Cunningham J, Duncan A, Hunt G, Leece R, MacFadyen U, McCormick J, McLeish S, Mitra A, Miller D, Waxman E, Webb A, Wojcik S, Mukhopadhyay S, Macgregor D. A methodology to establish a database to study gene environment interactions for childhood asthma. BMC Med Res Methodol 2010;10:107.

S3. Palmer CN, Doney AS, Lee SP, Murrie I, Ismail T, Macgregor DF, Mukhopadhyay S. Glutathione S-transferase M1 and P1 genotype, passive smoking, and peak expiratory flow in asthma. Pediatrics 2006;118:710-716.

S4. Palmer CN, Lipworth BJ, Lee S, Ismail T, Macgregor DF, Mukhopadhyay S. Arginine-16 beta2 adrenoceptor genotype predisposes to exacerbations in young asthmatics taking regular salmeterol. Thorax 2006;61:940-944.

S5. Tavendale R, Macgregor DF, Mukhopadhyay S, Palmer CN. A polymorphism controlling ORMDL3 expression is associated with asthma that is poorly controlled by current medications. J Allergy Clin Immunol 2008;121:860-863.

S6. McKinstry B, Sullivan FM, Vasishta S, Armstrong R, Hanley J, Haughney J, Philip S, Smith BH, Wood A, Palmer CN. Cohort profile: the Scottish Research register

SHARE. A register of people interested in research participation linked to NHS data sets. BMJ Open 2017;7:e013351.

S7. Hawcutt DB, Jorgensen AL, Wallin N, Thompson B, Peak M, Lacy D, Newland P, Didi M, Couriel J, Blair J, Pirmohamed M, Smyth RL. Adrenal responses to a low-dose short synacthen test in children with asthma. Clin Endocrinol 2015;82:648-656.

S8. Hawcutt DB, Francis B, Carr DF, Jorgensen AL, Yin P, Wallin N, et al. Susceptibility to corticosteroid-induced adrenal suppression: a genome-wide association study. Lancet Respir Med. 2018 Mar 15.

S9. Berce V, Kozmus CE, Potocnik U. Association among ORMDL3 gene expression, 17q21 polymorphism and response to treatment with inhaled corticosteroids in children with asthma. Pharmacogenomics J 2013;13:523-529.

S10. Moffatt MF, Kabesch M, Liang L, Dixon AL, Strachan D, Heath S, Depner M, von Berg A, Bufe A, Rietschel E, Heinzmann A, Simma B, Frischer T, Willis-Owen SA, Wong KC, Illig T, Vogelberg C, Weiland SK, von Mutius E, Abecasis GR, Farrall M, Gut IG, Lathrop GM, Cookson WO. Genetic variants regulating ORMDL3 expression contribute to the risk of childhood asthma. Nature 2007;448:470-473.

S11. Pandey RC, Michel S, Schieck M, Binia A, Liang L, Klopp N, Franke A, von Berg A, Bufe A, Rietschel E, Heinzmann A, Laub O, Simma B, Frischer T, Genuneit J, Illig T, Kabesch M. Polymorphisms in extracellular signal-regulated kinase family influence genetic susceptibility to asthma. J Allergy Clin Immunol 2013;131:1245-1247.

S12. Schieck M, Michel S, Suttner K, Illig T, Zeilinger S, Franke A, Vogelberg C, von Berg A, Bufe A, Heinzmann A, Laub O, Rietschel E, Simma B, Frischer T, Genuneit

J, Kerzel S, Kabesch M. Genetic variation in TH17 pathway genes, childhood asthma, and total serum IgE levels. J Allergy Clin Immunol 2014;133:888-891.

S13. Nieuwenhuis MA, Siedlinski M, van den Berge M, Granell R, Li X, Niens M, van der Vlies P, Altmuller J, Nurnberg P, Kerkhof M, van Schayck OC, Riemersma RA, van der Molen T, de Monchy JG, Bosse Y, Sandford A, Bruijnzeel-Koomen CA, Gerth van Wijk R, Ten Hacken NH, Timens W, Boezen HM, Henderson J, Kabesch M, Vonk JM, Postma DS, Koppelman GH. Combining genomewide association study and lung eQTL analysis provides evidence for novel genes associated with asthma. Allergy 2016;71:1712-1720.

S14. Hernandez-Pacheco N, Farzan N, Francis B, Karimi L, Repnik K, Vijverberg SJ, Soares P, Schieck M, Gorenjak M, Forno E, Eng C, Oh SS, Perez-Mendez L, Berce V, Tavendale R, Samedy LA, Hunstman S, Hu D, Meade K, Farber HJ, Avila PC, Serebrisky D, Thyne SM, Brigino-Buenaventura E, Rodriguez-Cintron W, Sen S, Kumar R, Lenoir M, Rodriguez-Santana JR, Celedon JC, Mukhopadhyay S, Potocnik U, Pirmohamed M, Verhamme KM, Kabesch M, Palmer CAN, Hawcutt DB, Flores C, Maitland-van der Zee AH, Burchard EG, Pino-Yanes M. Genome-wide association study of inhaled corticosteroid response in admixed children with asthma. Clin Exp Allergy 2019;49:789-798.

S15. Fraser A, Macdonald-Wallis C, Tilling K, Boyd A, Golding J, Davey Smith G, Henderson J, Macleod J, Molloy L, Ness A, Ring S, Nelson SM, Lawlor DA. Cohort Profile: the Avon Longitudinal Study of Parents and Children: ALSPAC mothers cohort. Int J Epidemiol 2013;42:97-110.

S16. Boyd A, Golding J, Macleod J, Lawlor DA, Fraser A, Henderson J, Molloy L, Ness A, Ring S, Davey Smith G. Cohort Profile: the 'children of the 90s'--the index

offspring of the Avon Longitudinal Study of Parents and Children. Int J Epidemiol 2013;42:111-127.

S17. Haag K, Fraser A, Hiller R, Seedat S, Zimmerman A, Halligan SL. The emergence of sex differences in PTSD symptoms across development: evidence from the ALSPAC cohort. Psychol Med 2019;14:1-6.

S18. Design and implementation of a patient education center for the Childhood Asthma Management Program. Childhood Asthma Management Program Research Group. Ann Allergy Asthma Immunol 1998;81:571-581.

S19. The Childhood Asthma Management Program (CAMP): design, rationale, and methods. Childhood Asthma Management Program Research Group. Control Clin Trials 1999;20:91-120.

S20. Zeiger RS, Dawson C, Weiss S. Relationships between duration of asthma and asthma severity among children in the Childhood Asthma Management Program (CAMP). J Allergy Clin Immunol 1999;103:376-387.

S21. Wickman M, Kull I, Pershagen G, Nordvall SL. The BAMSE project: presentation of a prospective longitudinal birth cohort study. Pediatr Allergy Immunol 2002;13:11-13.

S22. Ostblom E, Lilja G, Pershagen G, van Hage M, Wickman M. Phenotypes of food hypersensitivity and development of allergic diseases during the first 8 years of life. Clin Exp Allergy 2008;38:1325-1332.

S23. Kull I, Melen E, Alm J, Hallberg J, Svartengren M, van Hage M, Pershagen G, Wickman, M, Bergstrom A. Breast-feeding in relation to asthma, lung function, and sensitization in young schoolchildren. J Allergy Clin Immunol 2010;125:1013-1019.

S24. Nishimura KK, Galanter JM, Roth LA, Oh SS, Thakur N, Nguyen EA, Thyne S, Farber HJ, Serebrisky D, Kumar R, Brigino-Buenaventura E, Davis A, LeNoir MA, Meade K, Rodriguez-Cintron W, Avila PC, Borrell LN, Bibbins-Domingo K, Rodriguez-Santana JR, Sen S, Lurmann F, Balmes JR, Burchard EG. Early-life air pollution and asthma risk in minority children. The GALA II and SAGE II studies. Am J Respir Crit Care Med 2013;188:309-318.

S25. Neophytou AM, White MJ, Oh SS, Thakur N, Galanter JM, Nishimura KK, Pino-Yanes M, Torgerson DG, Gignoux CR, Eng C, Nguyen EA, Hu D, Mak AC, Kumar R, Seibold MA, Davis A, Farber HJ, Meade K, Avila PC, Serebrisky D, Lenoir MA, Brigino-Buenaventura E, Rodriguez-Cintron W, Bibbins-Domingo K, Thyne SM, Williams LK, Sen S, Gilliland FD, Gauderman WJ, Rodriguez-Santana JR, Lurmann F, Balmes JR, Eisen EA, Burchard EG. Air Pollution and Lung Function in Minority Youth with Asthma in the GALA II & SAGE II Studies. Am J Respir Crit Care Med 2016;193:1271-1280.

S26. Andiappan AK, Sio YY, Lee B, Suri BK, Matta SA, Lum J, Foo S, Koh G, Liu J, Zolezzi F, Poidinger M, Wang de Y, Rotzschke O, Chew FT. Functional variants of 17q12-21 are associated with allergic asthma but not allergic rhinitis. J Allergy Clin Immunol 2016;137:758-66 e3.

S27. Andiappan AK, Anantharaman R, Nilkanth PP, Wang de Y, Chew FT. Evaluating the transferability of Hapmap SNPs to a Singapore Chinese population. BMC Genet 2010;11:36.

S28. Andiappan AK, Wang de Y, Anantharaman R, Parate PN, Suri BK, Low HQ, Li Y, Zhao W, Castagnoli P, Liu J, Chew FT. Genome-wide association study for atopy and allergic rhinitis in a Singapore Chinese population. PLoS One 2011;6:e19719.

S29. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, Maller J, Sklar P, de Bakker PI, Daly MJ, Sham PC. PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet 2007;81:559-575.

S30. R Development Core Team. R: A Language and Environment for Statistical Computing. Vienna, Austria: R Foundation for Statistical Computing; 2013. http://www.R-project.org/.

S31. Vijverberg SJ, Tavendale R, Leusink M, Koenderman L, Raaijmakers JA, Postma DS, Koppelman GH, Turner SW, Mukhopadhyay S, Palmer CN, Maitland-van der Zee AH. Pharmacogenetic analysis of GLCCI1 in three north European pediatric asthma populations with a reported use of inhaled corticosteroids. Pharmacogenomics 2014;15:799-806.

S32. Global strategy for asthma management and prevention. Global Initiative for Asthma (GINA) 2018. http://ginasthma.org/.

S33. British Thoracic Society and the Scottish Intercollegiate Guidelines Network. British guideline on the management of asthma. Thorax 2014; 69 Suppl 1:1-192.

S34. Han B, Eskin E. Random-effects model aimed at discovering associations in meta-analysis of genome-wide association studies. Am J Hum Genet 2011;88:586-598.

Table S1. Clinical and demographic characteristics of the studies analysed 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.

Citations:

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

2. 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.

3. 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.

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.

5. 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.

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

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

8. Leusink M, et al. Genetic variation in uncontrolled childhood asthma despite ICS treatment. Pharmacogenomics J 2016;16:1158-1163.

9. Mosteller M, et al. No evidence of large genetic effects on steroid response in asthma patients. J Allergy Clin Immunol 2017;139:797-803 e7.

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

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 signalling 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, antitumour, 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	Tumour 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 FEV1 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:

1. Park TJ, *et al*. Genome-wide association study identifies *ALLC* polymorphisms correlated with FEV1 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 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
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	Leukaemia and other neoplasms.
Retinoic acid	0.011	0.714	27	Acne, photodamaged skin, keratinization disorders, acute promyelocytic leukaemia.
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, leukaemia, neuroblastomas, ovarian cancer).
Tioguanine	0.024	0.787	4	Acute leukaemia.
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 tumours).
Carmustine 4 mg	0.025	0.787	4	Brain tumours 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 tumours).
Busulfan	0.027	0.787	4	Chronic myeloid leukaemia.
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 tumours 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 tumours and other malignant neoplasms.
Daunorubicin	0.037	0.787	4	Leukaemia 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 (tumours, 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.

				GWAS of asthma exacerbations despite ICS use 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)	1.42×10^{-5}
FHIT	3	59735036	61237133	rs12489758	2.38 (1.56-3.63)	5.31×10^{-5}
ROBO2	3	75955845	77699115	rs72891545	4.79 (2.36-9.73)	1.44×10^{-5}
ARHGAP24	4	86396267	86923823	rs62315626	3.15 (1.90-5.21)	8.19×10^{-6}
BANK1	4	102332443	102995969	rs74934013	2.66 (0.75-9.44)	9.45×10^{-5}
SEMA5A	5	9035138	9546233	rs707637	1.44 (1.21-1.72)	4.96×10^{-5}
CDH10	5	24487209	24645087	rs17459974	1.35 (1.17-1.55)	3.63×10^{-5}
LAMA2	6	129204286	129837714	rs12527452	0.73 (0.59-0.90)	3.37×10^{-5}
PDE10A	6	165740776	166400091	rs57042153	1.43 (1.20-1.70)	5.97×10^{-5}
HERPUD2	7	35672269	35735181	rs79634971	1.29 (1.14-1.47)	7.84×10^{-5}
GSN	9	123970072	124095121	rs113561738	2.10 (1.49-2.96)	2.84×10^{-5}
JMJD1C	10	64926981	65225722	rs12780983	1.33 (1.15-1.53)	9.03×10^{-5}

[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 S11 (continuation). Genes enriched at trichostatin A in European children with asthma.

				GWAS of asthma exacerbations despite ICS use 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**
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

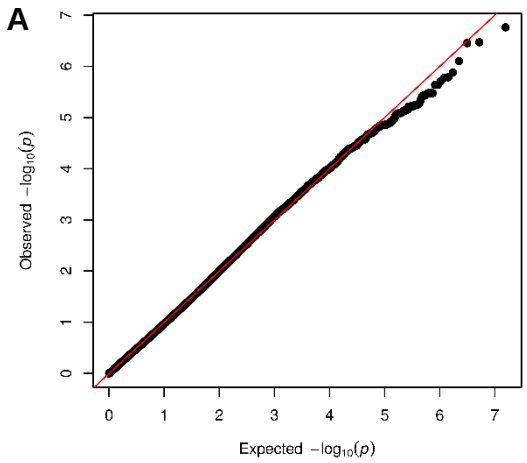
Table S12 (continuation). Genes enriched at trichostatin A in European children with previous evidence of potential implication in asthma-related traits or treatment response.

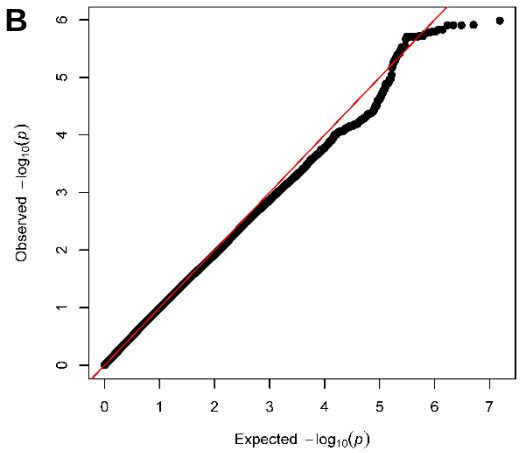
Gene	Main(s) function(s) of protein encoded	Asthma-related traits with evidence of association	Reference
LAMA2	Cell attachment, migration and organization into tissues	Lung function measurements	Kichaev *et al. Am J Hum Genet* 2019; 104:65-75 Shrine *et al. Nat Genet* 2019; 51:481-493
GSN	Assembly and 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	Almoguera *et al. Am J Respir Crit Care Med* 2017; 195:456-463
		Lung function measurements	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

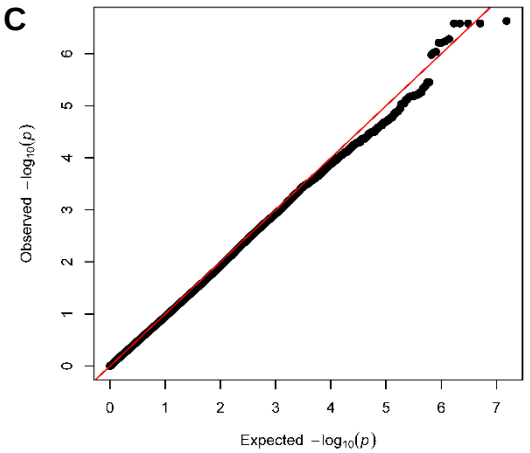
TGF-β1: transforming growth factor β1

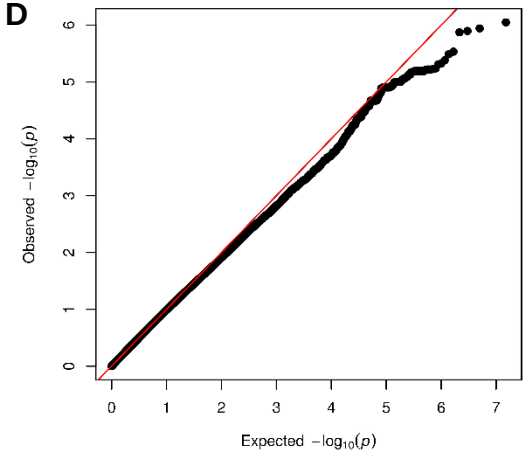
SUPPLEMENTARY FIGURE LEGENDS

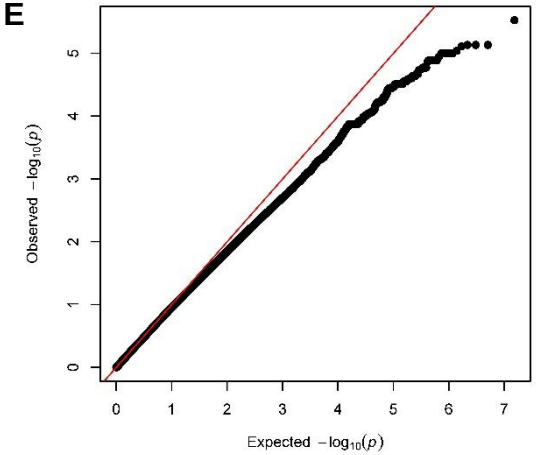
Figure S1. Quantile-quantile plots of association results of asthma exacerbations in patients treated with ICS from the European studies analysed in the discovery phase. Observed and expected association results are represented as -log10 *p*-value on the *y*-axis and *x*-axis, respectively. Figures S1A-H 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).

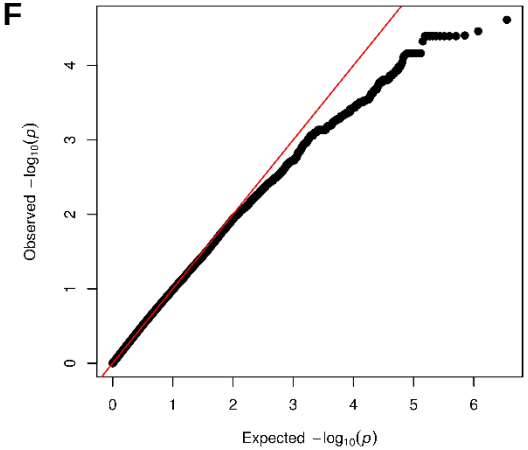

A
Observed $-\log_{10}(p)$
Expected $-\log_{10}(p)$


B
Observed $-\log_{10}(p)$
Expected $-\log_{10}(p)$

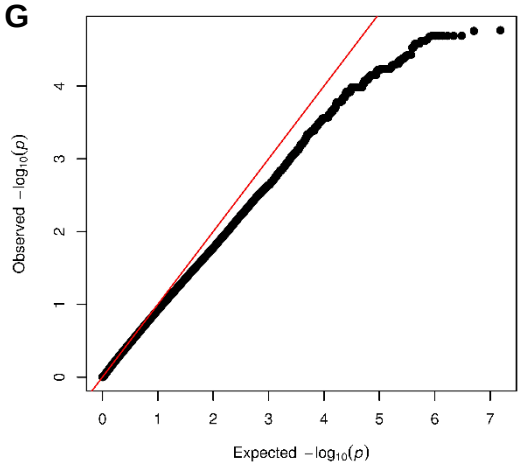

C
Observed $-\log_{10}(p)$
Expected $-\log_{10}(p)$


D
Observed $-\log_{10}(p)$
Expected $-\log_{10}(p)$

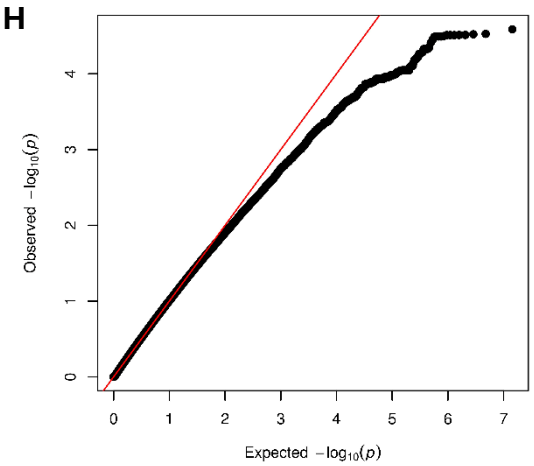

E
Observed $-\log_{10}(p)$
Expected $-\log_{10}(p)$


F
Observed $-\log_{10}(p)$
Expected $-\log_{10}(p)$

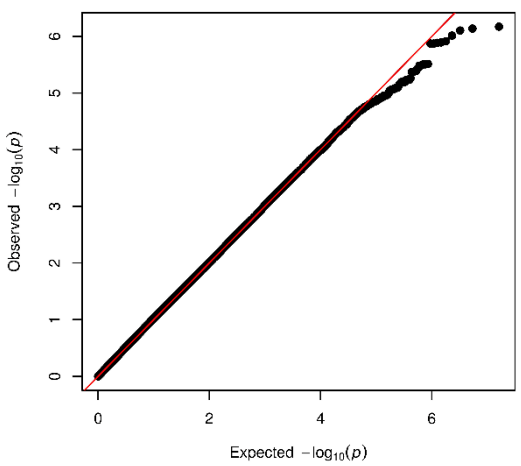
G


Observed $-\log_{10}(p)$
Expected $-\log_{10}(p)$
0
1
2
3
4
5
6
7

H


Observed $-\log_{10}(p)$
Expected $-\log_{10}(p)$
0
1
2
3
4
5
6
7

I


Observed $-\log_{10}(p)$
Expected $-\log_{10}(p)$
0
1
2
3
4
5
6
0
2
4
6