

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

To the Editor,

An increasing number of therapies are available to treat asthma, but inhaled corticosteroids (ICS) are still the most commonly prescribed and effective controller asthma medication.¹ Both environmental and genetic factors are involved in ICS response. However, few biomarkers have been associated with response to asthma treatment with ICS.² Here, we aimed to identify novel markers of ICS response combining transcriptomic and genomic data.

A full description of the methods is provided in the Supplementary Methods at the Supporting Information, and it is also summarized in Figure S1. In the discovery stage, RNA sequencing (RNA-seq) data from airway smooth muscle (ASM) cells isolated from four non-asthmatic donors publicly available at the Sequence Read Archive (SRA, SRP033351) (<https://www.ncbi.nlm.nih.gov/sra>) were analyzed.³ Briefly, this study performed RNA-seq of cell lines from four non-asthmatic lung donors treated with control solution or with 1 μ M dexamethasone for 18 h³. Raw sequencing data were downloaded and processed according to the procedures described in the Supplementary Methods. Differential gene expression of cells treated with glucocorticosteroids (GCs) compared to those treated with control solution was evaluated using linear models. Differentially dysregulated genes were identified after a false-discovery rate (FDR) adjustment of 5% (q -value ≤ 0.05).

Differentially expressed genes in the discovery were examined to determine whether they also showed changes in peripheral blood mononuclear cells (PBMCs) obtained from ICS non-responders ($n = 3$) and ICS responders ($n = 3$) from the SLOVENIA study,⁴ defined based on the presence or absence of asthma exacerbations despite ICS therapy. RNA libraries were sequenced using the BGISEQ-500 instrument (BGI Inc), and data were analyzed following the same methodology as for the ASM cells. Gene expression levels were compared in responders to ICS and ICS non-responder patients, adopting a q -value ≤ 0.05 to declare significance. Validation of significant differentially expressed genes found in the previous datasets was sought using three additional transcriptomic data of ASM cells after GCs exposure (GSE13168, GSE34313, and SRP098649), which were combined in a random-effects meta-analysis. Evidence of replication was considered for genes with consistent effects and q -value ≤ 0.05 .

Genetic variants located within ± 100 kilobases (kb) from the genes with significant changes in expression levels across all transcriptomic datasets were examined for association with asthma exacerbations despite ICS. For that, ten studies participating in the Pharmacogenomics in Childhood of Asthma (PiCA) consortium were analyzed, including 2681 European and 1347 Hispanics/Latinos and African-American children and youth treated with ICS with genome-wide genotyping data available (Table S1). Details about each study are described at the Supporting Information and elsewhere.⁴

Individuals with asthma exacerbations despite ICS treatment were considered as ICS non-responders and those without asthma exacerbations as ICS responders. Asthma exacerbations were defined by the need for emergency care, hospitalizations, administration of systemic corticosteroids, unscheduled doctor visits, and/or school absences because of asthma (Table S1). Association between imputed single-nucleotide polymorphisms (SNPs) and asthma exacerbations despite ICS treatment was tested for each study through logistic regression models including age, sex, and principal components as covariates. Two separate meta-analyses were performed in Europeans and non-Europeans. A Bonferroni-like corrected threshold for significance was obtained for each population as $\alpha = 0.05/\text{total number of independent variants}$.

In the discovery, a total of 14707 genes were analyzed and 4718 of them were found to be differentially expressed in cells treated with GCs (q -value ≤ 0.05) (Figure 1A). From these genes, 24 showed significant changes in expression levels in PBMCs from asthma patients depending on ICS response status (q -value ≤ 0.05), with consistent alteration across both datasets based on $\log_2$ fold change (FC) values (Figure 1B, Table S2). Moreover, six out of the 24 genes were replicated with a consistent effect of up-regulation in the combined analysis of the three additional transcriptomic datasets of ASM cells treated with GCs: *LTBP1* (q -value = 7.46×10^{-4}), *MTURN* (q -value = 3.92×10^{-3}), *NAMPT* (q -value = 3.77×10^{-7}), *CALD1* (q -value = 5.22×10^{-5}), *MMD* (q -value = 5.84×10^{-4}), and *COL18A1* (q -value = 1.93×10^{-3}) (Table S3). The potential implication of these genes on asthma severity was assessed by evaluating their expression in PBMCs according to baseline lung function, showing no significant changes (Table S4). Additionally, only *LTBP1* remained significantly differentially expressed in response to ICS treatment after accounting for baseline lung function (Table S4).

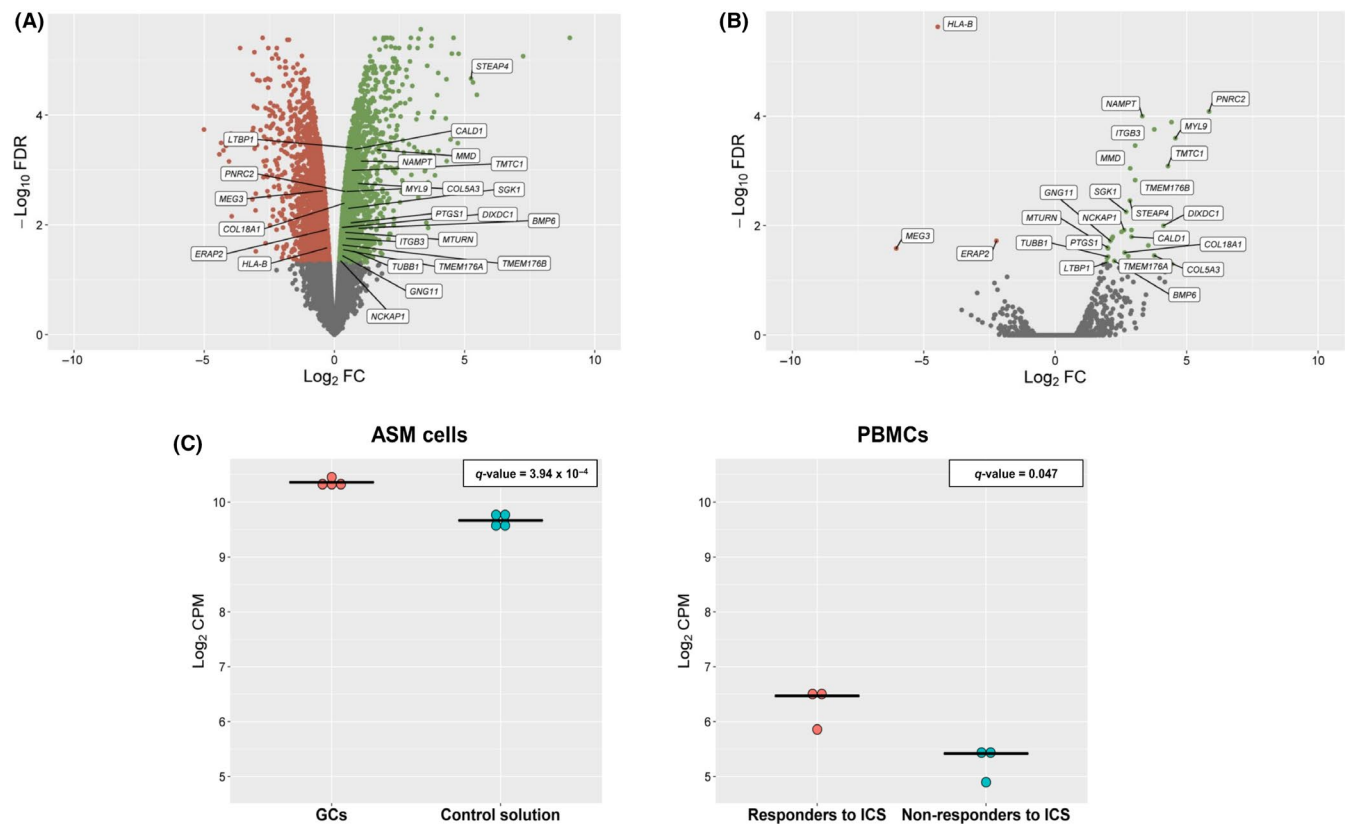


FIGURE 1 Results of differential expression in response to GCs in ASM cells and PBMCs. Volcano plot of differential expression results in ASM cells after exposure to GCs compared to a control solution (panel A). Results of differential expression in PBMCs are shown for genes with significant changes in expression levels in ASM cells in response to GCs after adjusting with a false-discovery rate (FDR) of 5% (q -value ≤ 0.05) (panel B). Results are represented in terms of $\log_2$ fold change ($\log_2$ FC) (x-axis) and the logarithmic transformation of FDR ($-\log_{10}$ FDR) (y-axis). Genes significantly (q -value ≤ 0.05) found to be up-regulated ($\log_2$ FC > 0) or down-regulated ($\log_2$ FC < 0) are represented by means of green or red dots, respectively. Genes with consistent alteration of expression levels in ASM cells and PBMCs are labeled into white boxes. Dots plots of differential expression for *LTBP1* in ASM cells and PBMCs (panel C). Gene expression levels are represented in terms of $\log_2$ counts per million (CPM) in the y-axis as dots for cases (red) and control (blue) samples. The median expression level is represented for each sample group by a black horizontal line. P -values adjusted by false-discovery rate are shown (q -value)

Association of 7042 SNPs with asthma exacerbations despite ICS use within ± 100 kilobases from the six genes validated was tested in Europeans. After applying a Bonferroni-like correction ($P \leq 4.96 \times 10^{-5}$ for 1007 independent variants tested), the SNP rs11681246 located within *LTBP1* (Figure 1C) was significantly associated with asthma exacerbations despite ICS use in Europeans (OR for G allele: 0.72, 95% CI: 0.63, 0.83, $P = 3.28 \times 10^{-6}$) (Figure 2A,B).

The effect of this association was similar after adjusting by treatment step, as a proxy of disease severity, in a subset of 2282 patients with complete information about asthma medication (OR for G allele: 0.74, 95% CI: 0.63, 0.86, $P = 1.13 \times 10^{-4}$). However, this SNP was not replicated in non-Europeans. Nonetheless, we assessed the association of alternative polymorphisms in non-Europeans, revealing the association of six intronic *LTBP1* variants in high linkage disequilibrium ($r^2 > .95$), all dependent from the association of rs76390075 (OR for C allele: 0.40, 95% CI: 0.26, 0.63, $P = 6.76 \times 10^{-5}$), after adjustment for 234 independent variants tested ($P \leq 2.14 \times 10^{-4}$) (Figure 2C,D). Moreover, this association was robust to the adjustment by medication step, OR for C allele: 0.41, 95% CI: 0.26, 0.65, $P = 1.12 \times 10^{-4}$.

However, the association signals detected in European and admixed populations are not in LD ($r^2 < .01$). A description of the potential functional implications of these variants is available at the Data Supporting Information.

This study describes the results of transcriptomic analyses of several datasets revealing *LTBP1* as a gene involved in ICS response among asthma patients. GCs were found to increase *LTBP1* expression levels in four datasets of ASM cells experimentally exposed to different types of GCs. Similar effects were detected in PBMCs obtained from asthma patients that were ICS responders, but not in non-responders. This suggests that ICS treatment may not induce *LTBP1* expression in PBMCs from non-responders. Additionally, the association of two variants within this gene with asthma exacerbations despite ICS use was found in diverse populations.

LTBP1 encodes a member of the family of latent-transforming growth factor-beta (TGF- β) binding proteins. *LTBP1* is involved in the regulation of the TGF- β 1 activity, including its activation from a precursor form, folding, secretion out from the cell, and deposition at the extracellular matrix through interactions with fibrillin

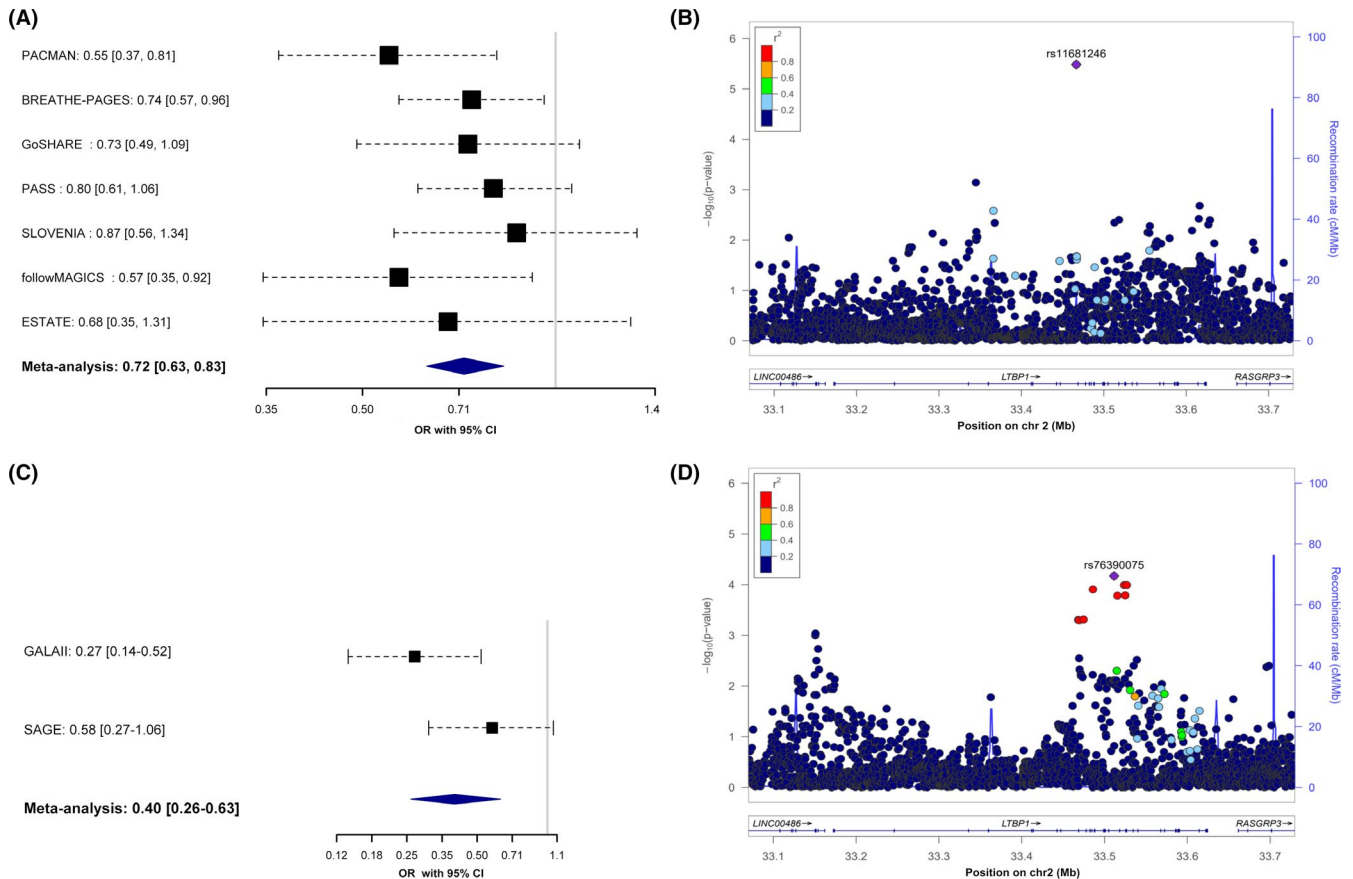


FIGURE 2 Association results with asthma exacerbations despite ICS treatment for *LTBP1*. Forest plot of association effects of the SNPs rs11681246 across the European studies (panel A) and rs76390075 in non-European studies (panel C). The effects are shown in terms of OR for the effect alleles (G and C, respectively) for each study and after performing a meta-analysis of the results by black boxes and a blue diamond, respectively. The 95% CI are represented by black dash lines. Results are not provided for BREATHE since this SNP did not pass quality control checks in this study. Regional plot of association results with asthma exacerbations despite ICS use among Europeans (panel B) and non-Europeans (panel D). The y-axis represents the logarithmic transformation of the association results ($-\log_{10} p\text{-value}$) by chromosome position (x-axis) for each SNP as a dot. The diamond corresponds to the most significant variant after Bonferroni-like correction; the remaining SNPs are color-coded based on pairwise linkage disequilibrium (r^2 values) with that SNP for Europeans (panel B) or Admixed American populations (panel D) from 1KGP (GRCh37/hg19 build)

molecules.⁵ Interestingly, TGF- β 1 has been proposed to play a key role in cell growth and differentiation, immune response, and airway remodeling.⁶ Specifically, increased levels of the active form of TGF- β 1 have been detected in asthma patients, which has been suggested to recruit myofibroblasts triggering an increased collagen deposition and the development of subepithelial fibrosis in asthma.⁷ *LTBP1* has also been proposed to be involved in allergic diseases and idiopathic pulmonary fibrosis (IPF), where *LTBP1* interacts with fibulin 1c (FBLN1), modulating lung remodeling and fibrosis through the regulation of TGF- β 1 activation.⁸ In fact, the inhibition of FBLN1 binding to *LTBP1* has been proposed as a therapeutic strategy to reduce fibrotic processes.⁸ Interestingly, genetic variants near or within *LTBP1* are also associated with lung function measurements among participants from the UK Biobank.⁹

We acknowledge the limited sample size of the transcriptomic datasets and the lack of functional experiments as major limitations

of our study. Therefore, validation of the association in independent populations analyzing larger sample sizes and functional studies are needed. These will provide insights about the biological mechanisms implicating *LTBP1* in ICS response and to test its clinical relevance predicting the treatment response. Additionally, a different gene expression profile would be obtained if the analyses would have been restricted only to ASM cells, as indicated by Kan et al.¹⁰

In summary, our study revealed *LTBP1* as a potential novel locus for ICS response in asthma patients. These results indicate that combining publicly available data from different omic sources could be a powerful approach to provide novel insights about the mechanisms involved in the ICS treatment response.

KEYWORDS

asthma treatment, childhood asthma, exacerbations, genetic polymorphism, pharmacogenomics

ACKNOWLEDGMENTS

The authors would like to thank the patients, families, recruiters, healthcare 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, SA) to the results of this research and also to 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. 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-Cintrón, VA Hospital, Puerto Rico; Pedro C. Avila, Northwestern University; Jose R. Rodriguez-Santana, Centro de Neumología Pediátrica; 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 healthcare 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 laboratory 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 healthcare 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 laboratory researcher Celeste Eng who processed the biospecimens.

CONFLICT OF INTEREST

NH-P declares funding from Instituto de Salud Carlos III (ISCIII) and the European Social Funds. SM reports funding from The Gannochy Trust, Perth and Kinross City Council, and Scottish Enterprises Tayside. MP declares funding from the MRC Clinical Pharmacology Training Scheme, EPSRC, Astra Zeneca, and Bristol Myers Squibb. KV reports funding from ZonMw, Yamanouchi, Pfizer/Boehringer Ingelheim, Novartis, and GSK. MK declares funding from the

European Union, German Ministry of Education, Research, German Research Foundation, and other sources. EGB reports funding from the National Institute of Health, National Institute of Health and Environmental Health Sciences, National Institute on Minority Health and Health Disparities, National Institute of General Medical Sciences, National Human Genome Research Institute, Sandler Family Foundation, American Asthma Foundation, and Robert Wood Johnson Foundation. A-HM declares funding from GlaxoSmithKline, Boehringer Ingelheim, and Astra Zeneca. UP reports funding from the Slovenian Research Agency and the Ministry of Education, Science, and Sport of Slovenia. MP-Y declares funding from ISCIII, the Spanish Ministry of Science, Innovation, and Universities, and Spanish Ministry of Economy, Industry, and Competitiveness.

FUNDING INFORMATION

This study was supported by the award (AC15/00015) 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), and the SysPharmPedia grant from the ERACoSysMed 1st Joint Transnational Call from the European Union under the Horizon 2020. This study was also funded by the Spanish Ministry of Science, Innovation, and Universities, the State Research Agency, and the European Regional Development Funds from the European Union (MICIU/AEI/FEDER, UE, SAF2017-83417R). N.H-P was supported by a fellowship (FI16/00136) from Instituto de Salud Carlos III (ISCIII) and co-funded by the European Social Funds from the European Union (ESF) "ESF invests in your future." MP-Y was funded by the Ramón y Cajal Program (RYC-2015-17205) by the Spanish Ministry of Economy, Industry, and Competitiveness. 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 the SysPharmPedia grant, co-financed by the 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 enrollment 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 enrollment 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]. ESTATE was funded by an independent research

grant by ZonMw project (113201006). The PASS study was funded by the NHS Chair of Pharmacogenetics via the UK Department of Health. MP is Emeritus NIHR Senior Investigator. Genotyping of samples from BREATHE-PAGES, GoSHARE, and SCSGES was carried out at CeGen-PRB3-ISCIII, and supported by ISCIII and European Regional Development Fund (ERDF) (PT17/0019).

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

SUPPORTING INFORMATION

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

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SUPPLEMENTARY METHODS

Sample description and sequencing details of the SLOVENIA study

The SLOVENIA study includes children and young adults (5-18 years old) with a physician's diagnosis of mild or moderate persistent asthma of Slovenian origin. This study has been approved by the Slovenian National Medical Ethics Committee (Ljubljana, Slovenia), ethical approval number: 0120-569/2017/4. Detailed information about the study design has been described elsewhere^{S1,S2}. Part of the asthmatic patients included in the study were under regular treatment with inhaled corticosteroids (ICS). Adherence to ICS therapy was confirmed in consequent follow-ups based on parents/patient self-reports and prescription refills. For the current analyses, a total of six patients were selected based on data on emergency asthma care, hospitalizations, and/or administration of oral corticosteroids because of asthma symptoms in the past 12 months while treated with ICS according to PRACTALL^{S3} and NAEPP^{S4} guidelines. None of the patients were on high dose ICS therapy and no differences in ICS dose were found between cases and controls (p -value=0.317). Patients were classified as ICS non-responders ($n=3$, mean age=10.7 $\pm$ 3.1) if they had a history of any of such events, or responders to ICS ($n=3$, mean age=11.3 $\pm$ 0.6) if they did not experience any of those types of events.

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

RNA-seq processing and statistical analyses of differential expressed genes in airway smooth muscle cells and PBMCs

RNA-seq raw FASTA files from airway smooth muscle (ASM) cells were downloaded from Sequence Read Archive and converted to fastq format using a perl script code assuming a quality score of 40 (raw fastq format was already available for PBMCs). Subsequent analyses were performed on fastq files with a pipeline in R 3.4.4^{S5}, including initial quality control (QC) analysis based on parameters obtained with the FastQC 0.11.8 software^{S6} to detect overrepresented sequences, GC contents, length distributions, and duplication levels. Alignment to the GRCh37/hg19 reference genome was performed for paired-end reads using the *Rsubread* package^{S7,S8}. Mapped sequence reads were assigned to genomic features at gene-level using the *featureCounts* function implemented in *Rsubread*^{S9}. Counts per million (CPM) were obtained using *edgeR*^{S10}. Very low expressed genes were filtered out based on CPM values corresponding to 10 or fewer read counts. Retained genes were normalized according to library sizes using the trimmed mean of M values (TMM) method implemented in *edgeR*^{S11}. Subsequently, mean-variance modeling at the observational level (VOOM) transformation was applied to filter and normalize counts^{S12}. Differential gene expression levels in ASM cells treated with glucocorticosteroids (GCs) compared to those treated with control solution or in PBMCs from ICS responders compared to ICS non-responders were evaluated using the package LIMMA^{S13}. For ASM cells, differential gene expression was evaluated through moderated paired t-tests in linear models allowing for sample-pair effects. Cell composition was estimated through the CIBERSORT method using LM22 PBMC signature matrix^{S14} before fitting the linear models in PBMCs. Subsequent regression analyses were adjusted by the estimated proportion of lymphocytes B and T cells, natural killer cells, and monocytes. Differentially dysregulated genes in response to GCs treatment were identified after multiple comparison adjustment with a false discovery rate (FDR) of 5% (q -value $\leq$ 0.05)^{S15}.

Validation of transcriptome changes in additional datasets from ASM cells

Validation of significant differentially expressed genes found in both ASM cells treated with GCs and PBMCs from ICS responder asthma patients was sought using additional datasets of gene expression profiles of

ASM cells after GCs exposure compared to control solutions (GSE13168, GSE34313, and SRP098649). The first dataset, GSE13168, comprised ASM cells from tracheae from 3 non-asthmatic lung transplant donors treated or not with fluticasone propionate (10 nM)^{S16}. The second dataset, GSE34313, also included ASM cells from tracheae but both from 2 asthmatic and 4 healthy lung transplant donors treated with dexamethasone (1 µM)^{S17}. Finally, the third dataset, SRP098649, focused on ASM cells obtained from 8 non-asthmatic lung transplant donors that were experimentally treated by budesonide (100 nM)^{S18}. Transcriptome analysis was performed by microarray profiling in GSE13168 and GSE34313, and by means of RNAseq in SRP098649.

Differential expression analyses comparing ASM cells exposed to GCs treatment or control solutions were performed using Reproducible Analysis and Validation of Expression Data (RAVED) (<https://github.com/HimesGroup/raved>) using the online tool Reducing Associations by Linking Genes And omics Results (REALGAR) (<http://realgar.org/>)^{S19,S20}. Differential expression results obtained from these three independent ASM transcriptomic datasets were combined in a meta-analysis using a random-effects model accounting for the variance among studies through the *metaVolcanoR* package^{S21}. Evidence of replication was considered for genes with significantly consistent changes in expression levels in cells treated with GCs relative to the controls ($q\text{-value} \leq 0.05$).

Studies from the Pharmacogenomics in Childhood Asthma Consortium (PiCA) included in the association analyses of genetic variants within the differentially expressed genes

The association of genetic variants located within 100 kilobases (kb) upstream or downstream from the genes with evidence of differential expression in all the transcriptomic datasets with asthma exacerbations despite ICS use was assessed in ten studies participating in the Pharmacogenomics in Childhood of Asthma (PiCA) consortium^{S22}. A total of eight studies in European descent populations were included: the Pharmacogenetics of Asthma Medication in Children: Medication with Anti-inflammatory effects (PACMAN); the Paediatric Asthma Gene-Environment Study (PAGES); BREATHE; Genetics of the Scottish Health Research Register (GoSHARE); the Pharmacogenetics of Adrenal Suppression study (PASS); SLOVENIA; the follow-up stage of the Multicenter Asthma Genetics in Childhood Study (followMAGICS); Effectiveness and Safety of Treatment with Asthma Therapy in Children (ESTATe). Additionally, two studies in non-European populations were also analyzed: Latinos/Hispanics from the Genes-Environment and Admixture in Latino Americans (GALA II) study, and African Americans included in the Study of African Americans, Asthma, Genes and Environments (SAGE)^{S2}. Additional information about each study is described elsewhere^{S2}.

All studies included were approved by their local institutional review boards and written informed consent was provided by participants or their parents/caregivers. The Medical Ethics Committee of the University Medical Centre Utrecht (Utrecht, the Netherlands) approved PACMAN (protocol number: 08/023). PAGES was approved by the Cornwall and Plymouth Research Ethics Committee (Plymouth, United Kingdom). GoSHARE and BREATHE were approved by the Tayside Committee on Medical Research Ethics (Dundee, United Kingdom). The Liverpool Paediatric Research Ethics Committee (Liverpool, United Kingdom) (reference number: 08/H1002/56) approved PASS. The Slovenian National Medical Ethics Committee (Ljubljana, Slovenia) approved SLOVENIA (ethical approval number: 0120-569/2017/4). followMAGICS was approved by the Ethik-Kommission der Bayerischen Landesärztekammer (Munich, Germany) (ethics reference number: 01218). The Medische Ethische Toetsings Commissie, Erasmus Medical Center (Rotterdam, the Netherlands) (ethics approval number: MEC-2011-474) approved ESTATE. The Human Research Protection Program Institutional Review Board of the University of California, San Francisco (San Francisco, United States) approved GALA II and SAGE (ethics approval numbers: 217802 and 210362, respectively).

Children and young adults with asthma (2-25 years old) with available genome-wide genotype data, reported use of ICS, and data related to the ICS response based on the occurrence of asthma exacerbations during the 6 or 12 months preceding the study enrollment were included. Asthma exacerbations were defined by the need for emergency care, hospitalizations, or administration of systemic corticosteroids because of asthma (**Table S1**). Alternative definitions were used for those studies without available information regarding any of these events,

such as unscheduled general practitioner or respiratory system specialist visits and school absences (**Table S1**). Individuals with asthma exacerbations despite ICS treatment were considered as ICS non-responders and those without asthma exacerbations as ICS responders^{S2}.

PACMAN (n=654)

The Pharmacogenetics of Asthma Medication in Children: Medication with Anti-inflammatory effects (PACMAN) study is an observational cohort including children (4-12 years old) with a declared use of any asthma medications. This information is based on records from community pharmacies in the Netherlands^{S23}.

PAGES (n=437)

The Paediatric Asthma Gene-Environment Study (PAGES) is a cross-sectional study that recruited children and young adults (2-16 years old) with a pediatrician's diagnosis of asthma attending secondary care clinics in the United Kingdom. Clinical assessment through questionnaires and saliva sample collection was carried out. Candidates with any respiratory diseases apart from asthma or significant health problems were excluded^{S24}.

BREATHE (n=288)

The BREATHE study recruited participants (3-22 years old) with a physician diagnosis of asthma from the United Kingdom^{S25-S27}. From the BREATHE samples included, genotypes from 182 patients had been obtained using the Illumina Infinium CoreExome-24 BeadChip (Illumina) array, whereas 103 samples were genotyped using the Axiom™ Precision Medicine Research Array (Affymetrix Inc.). Association analyses were performed for the latter together with PAGES samples due to similarities of study design and sample characteristics, denoted as BREATHE-PAGES.

GoSHARE (n=472)

Children and young adults aged 3 to 18 years old participating in the Genetic of Scottish Health Research Register (GoSHARE) study were recruited in Tayside (Scotland) using databases from the National Health Service. Further details have been reported in previous publications^{S28}.

PASS (n=402)

Participants (5-18 years old) with a physician diagnosis of asthma and under treatment with inhaled corticosteroids (ICS) from the Pharmacogenetics of Adrenal Suppression study (PASS) were included. This study was initially designed to assess associations between the use of corticosteroids and adrenal suppression so that, clinical concern about adrenal suppression was also considered as an inclusion criterion^{S29,S30}.

SLOVENIA (n=182)

Children and young adults (5-18 years old) with mild and moderate persistent asthma recruited from tertiary health centers in Slovenia were included in the SLOVENIA study. Lung function was measured as the forced expiratory volume in 1 second (FEV₁) before and after 6 weeks after treatment with ICS using the Vitalograph 2150 spirometer (Compact, Buckingham, UK) according to ERS/ATS guidelines^{S1}.

followMAGICS (n=147)

Participants from the observational Multicenter Asthma Genetics in Childhood Study (MAGICS) were included in the follow-up phase of this study (followMAGICS), including patients with persistent asthma symptoms, now aged from 7 to 25 years. Recruitment was carried out at secondary and tertiary centers from Germany and Austria^{S31-S34}.

ESTATe (n=102)

The Effectiveness and Safety of Treatment with Asthma Therapy in children (ESTATe) is a case-control study including children and young adults (4-19 years old). Patients with a physician diagnosis of asthma were

recruited at primary care units in the Netherlands based on electronic medical records. Only those individuals with reported use of asthma controller therapy were included^{S2}.

GALA II (n=854)

Genes-Environment and Admixture in Latino Americans (GALA II) is a case-control study including patients with a physician diagnosis of asthma and controls (8-21 years old) with four Latino grandparents. Participants were recruited from five different centers in the United States and Puerto Rico^{S35}.

SAGE (n=493)

The Study of African Americans, Asthma, Genes and Environments (SAGE) is a cross-sectional asthma study, whose participants show similar characteristics as GALA II, although only those with four grandparents of African American ancestry were included in this study. In this case, subjects were recruited in centers from the United States^{S35,S36}.

Association analyses of genetic variants within the differentially expressed genes in different studies

Association between imputed genetic variants and asthma exacerbations despite ICS treatment was tested for each study through Wald logistic regressions using EPACTS 3.2.6^{S37}. Principal components (PCs) of genetic ancestry were calculated to account for population stratification using PLINK v1.9^{S38,S39}, and EIGENSOFT 6.14^{S40}. Subsequently, age, gender, and PCs were included as covariates in the regression models. The number of PCs used as covariates was chosen comparing different regression models including different numbers of PCs and that showing the best lambda fit ($\lambda_{GC} \sim 1$), estimated through the *Gap* v1.2.2 R package, was selected. Further details have been described elsewhere^{S2}. Two separate meta-analyses were performed based on the ancestry of the studies included: European and non-European. Single nucleotide polymorphisms (SNPs) with a minor allele frequency (MAF) $\geq 1\%$ and imputation quality (Rsq) ≥ 0.3 shared among the studies included in each group were meta-analyzed applying fixed-effects or random-effects models depending on the Cochran Q-test implemented on METASOFT^{S41}. Independent variants within each gene region evaluated were separately estimated for Europeans and non-Europeans through empirical autocorrelations based on the $-\log_{10} p$ -value of each SNP analyzed using *coda* R package^{S42}. A Bonferroni-like correction was applied accounting for the total number of independent variants tested for each population group. Evidence of association with ICS response was considered for those variants reaching the threshold established as $\alpha = 0.05/\text{total number of independent variants}$.

Sensitivity analyses were carried out for the variants identified through the same association models but also adjusting by treatment step as a proxy of asthma severity^{S43}. Only patients with available data about the medications included in the definition of medication steps were included. Logistic regressions were carried out with R 3.4.4^{S5} including a covariate classifying individuals as follows: *Step 1*, as-needed use of short-acting $\beta 2$ agonists (SABA); *Step 2*, as-needed use of SABA plus regular ICS; *Step 3*, as-needed use of SABA plus regular ICS and long-acting $\beta 2$ agonists (LABA); *Step 4*, as-needed use of SABA plus regular ICS, LABA and leukotriene receptor antagonists (LTRA). Alternatively, patients with reported use of SABA as needed plus combinations of ICS and LABA; as-needed SABA plus ICS and combinations of ICS and LABA; or as-needed SABA plus ICS and LTRA were also classified into *Step 3*. *Step 4* was also defined as the use of SABA as needed plus LABA, combinations of ICS and LABA, and LTRA; as-needed SABA plus ICS, combinations of ICS and LABA, and LTRA; or as-needed SABA plus combinations of ICS and LABA, and LTRA. All the patients were classified into *Step 2* or above since ICS use was considered as one of the inclusion criteria in our study.

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

Functional evaluation of the most significant variants within differentially expressed genes in response to GCs in ASM cells and PBMCs with evidence of association with asthma exacerbations despite ICS use was carried out using publicly available information from the Encyclopedia of DNA Elements (ENCODE)^{S44} using HaploReg v4.1^{S45} and, the Portal for the Genotype-Tissue Expression (GTEx)^{S46}.

SUPPLEMENTARY RESULTS

Functional evaluation of variants associated with ICS in different populations

According to the ENCODE project, the SNP rs11681246, associated with ICS response in European populations, is located within the monomethylation of histone H3 at lysine 4 (H3K4me1) and acetylation of histone H3 at lysine 27 (H3K27ac) histone marks in several pulmonary cell lines, such as fetal fibroblasts and adult lung fibroblast primary cells. It has been also suggested to be a DNase hypersensitivity site in pulmonary fibroblast primary cells. Furthermore, the most significantly associated variant with asthma exacerbations despite ICS use (rs76390075) is in LD ($r^2=1$) in admixed populations with variants that had been evidenced to play a potential functional role in pulmonary tissues, including epigenetic modifications such as the acetylation of histone H3 at lysine 9 and, H3K4me1 and H3K27ac marks (rs75486357 and rs3820912), and the location at DNase hypersensitivity sites in fetal fibroblasts and pulmonary fibroblast primary cells (rs3820912)^{S44,S45}. Nonetheless, no evidence of implications as expression quantitative trait loci (eQTL) was found for any of these variants^{S46}.

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Table S1. Clinical and demographic characteristics of the studies included in the association analyses with asthma exacerbations despite ICS use for the genes differentially regulated in response to GCs treatment.

	PACMAN (n=654)	BREATHE -PAGES (n=540)	GoSHARE (n=472)	PASS (n=402)	SLOVENIA (n=182)	BREATHE (n=182)	followMAGICS (n=147)	ESTATe (n=102)	GALA II (n=854)	SAGE (n=493)
Gender (% male)	61.6	60.4	24.8	55.0	57.1	59.3	59.9	58.8	57.3	60.4
Mean age $\pm$ SD (years)	8.7 $\pm$ 2.3	10.2 $\pm$ 3.5	11.3 $\pm$ 5.7	12.0 $\pm$ 2.0	10.8 $\pm$ 3.4	8.9 $\pm$ 4.0	17.2 $\pm$ 3.0	10.6 $\pm$ 4.2	12.1 $\pm$ 3.2	10.2 $\pm$ 3.5
Recruitment country	Netherlands	United Kingdom	United Kingdom	United Kingdom	Slovenia	United Kingdom	Germany/Austria	Netherlands	United States	United States
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	66.4	51.9
Definition	ER/OCS	HOSP/ OCS/SA	HOSP/ OCS	OCS	ER/ HOSP/OCS	OCS/ HOSP/SA	ER/ HOSP/GP/SV	ER/ HOSP/OCS	ER/ HOSP/ OCS	ER/ HOSP/ OCS
ER visits (%) ^b	6.1	NA	NA	NA	28.0	NA	7.5	NA	56.6	43.2
OCS use (%) ^c	6.7	35.0	13.8	51.7	12.6	48.4	NA	35.3	40.2	29.4
Hospitalizations (%) ^d	NA	13.5	0.21	NA	9.9	46.7	3.4	12.7 ^h	12.6	5.7
GP visits (%) ^e	NA	NA	NA	NA	NA	NA	49.0	NA	NA	NA
Specialist visits (%) ^f	NA	NA	NA	NA	NA	NA	21.8	NA	NA	NA
School absences (%) ^g	NA	43.1	NA	NA	NA	47.2	NA	NA	NA	NA

^a Asthma exacerbations-related data was available for the 6 precedent months of the study enrollment; ^b Proportion of patients with any exacerbations who sought emergency care due to asthma; ^c Proportion of patients with any exacerbations who needed the use oral corticosteroids because of asthma; ^d Proportion of patients with any exacerbations who needed to be hospitalized because of asthma; ^e Proportion of patients with any exacerbations who needed any unscheduled general practitioner visits because of asthma; ^f Proportion of patients with any exacerbations who needed any respiratory system specialist visits because of asthma; ^gProportion of patients with any exacerbations who were absent from school because of asthma; ^h ER visits and hospitalizations were considered as a single variable.

ER: emergency room visits; GCs: glucocorticosteroids; GP: general practitioner visits; HOSP: hospitalizations; NA: not available; OCS: systemic corticosteroids use; SA: school absences; SD: standard deviation; SV: specialist visits.

Table S2. Genes differentially expressed in ASM cells and PBMCs obtained from asthma patients in response to GCs.

Gene	Chr. ^a	Position begin 5' ^b	Position end 3' ^b	ASM cells ¹			PBMCs		
				log ₂ FC (95% CI) ^c	p-value	q-value ^d	log ₂ FC (95% CI) ^e	p-value	q-value ^f
<i>PNRC2</i>	1	24285599	24289952	0.41 (0.26, 0.57)	2.54 x 10 ⁻⁴	2.47 x 10 ⁻³	5.85 (3.46, 8.24)	1.45 x 10 ⁻⁵	8.19 x 10 ⁻⁵
<i>LTBP1</i>	2	33172039	33624576	0.69 (0.5, 0.87)	1.82 x 10 ⁻⁵	3.94 x 10 ⁻⁴	1.95 (0.52, 3.38)	8.62 x 10 ⁻³	0.047
<i>NCKAP1</i>	2	183773843	183903586	0.25 (0.06, 0.43)	0.014	0.045	2.62 (-1.61, 1.13)	2.21 x 10 ⁻³	0.012
<i>ERAP2</i>	5	96211643	96255420	-0.29 (-0.45, -0.14)	2.35 x 10 ⁻³	0.012	-2.24 (-3.70, -0.78)	3.49 x 10 ⁻³	0.019
<i>BMP6</i>	6	7727011	7881961	0.94 (0.45, 1.44)	2.16 x 10 ⁻³	0.012	2.25 (0.62, 3.88)	8.00 x 10 ⁻³	0.044
<i>HLA-B</i>	6	31321649	31324989	-0.30 (-0.5, -0.11)	6.69 x 10 ⁻³	0.026	-4.47 (-5.96, -2.98)	4.08 x 10 ⁻⁷	2.31 x 10 ⁻⁶
<i>SGK1</i>	6	134490384	134639250	0.55 (0.31, 0.78)	6.96 x 10 ⁻⁴	5.00 x 10 ⁻³	2.70 (1.17, 4.24)	9.96 x 10 ⁻⁴	5.56 x 10 ⁻³
<i>MTURN</i>	7	30174426	30202381	0.43 (0.2, 0.67)	2.75 x 10 ⁻³	0.014	1.99 (0.65, 3.33)	4.56 x 10 ⁻³	0.025
<i>STEAP4</i>	7	87905744	87936228	5.25 (4.5, 5.99)	1.27 x 10 ⁻⁷	2.16 x 10 ⁻⁵	2.84 (1.29, 4.38)	6.21 x 10 ⁻⁴	3.47 x 10 ⁻³
<i>GNG11</i>	7	93551011	93557922	0.32 (0.1, 0.55)	0.010	0.037	2.10 (0.74, 3.47)	3.41 x 10 ⁻³	0.019
<i>NAMPT</i>	7	105888731	105926772	1.04 (0.73, 1.34)	4.20 x 10 ⁻⁵	6.90 x 10 ⁻⁴	3.31 (1.94, 4.69)	1.76 x 10 ⁻⁵	9.93 x 10 ⁻⁵
<i>CALD1</i>	7	134429003	134655480	0.78 (0.57, 0.99)	1.99 x 10 ⁻⁵	4.18 x 10 ⁻⁴	2.90 (1.05, 4.74)	2.97 x 10 ⁻³	0.016
<i>TMEM176B</i>	7	150488373	150498448	0.32 (0.12, 0.52)	5.60 x 10 ⁻³	0.023	3.04 (1.51, 4.57)	2.63 x 10 ⁻⁴	1.47 x 10 ⁻³
<i>TMEM176A</i>	7	150497491	150502208	0.35 (0.12, 0.58)	7.39 x 10 ⁻³	0.028	2.78 (0.82, 4.74)	6.62 x 10 ⁻³	0.036
<i>PTGS1</i>	9	125132824	125157982	0.64 (0.32, 0.96)	1.59 x 10 ⁻³	9.15 x 10 ⁻³	2.01 (0.65, 3.37)	4.73 x 10 ⁻³	0.026
<i>DIXDC1</i>	11	111797868	111893308	0.30 (0.14, 0.45)	2.06 x 10 ⁻³	0.011	4.12 (1.64, 6.61)	1.77 x 10 ⁻³	0.010

Table S2 (continuation). Genes differentially expressed in ASM cells and PBMCs obtained from asthma patients in response to GCs.

Gene	Chr. ^a	Position begin 5' ^b	Position end 3' ^b	ASM cells ¹			PBMCs		
				log ₂ FC (95% CI) ^c	p-value	q-value ^d	log ₂ FC (95% CI) ^e	p-value	q-value ^f
<i>TMTC1</i>	12	29653746	29937692	0.68 (0.47, 0.9)	7.31 x 10 ⁻⁵	1.02 x 10 ⁻³	4.29 (2.23, 6.35)	1.44 x 10 ⁻⁴	8.08 x 10 ⁻⁴
<i>MEG3</i>	14	101245747	101327368	-0.46 (-0.64, -0.29)	2.44 x 10 ⁻⁴	2.40 x 10 ⁻³	-6.05 (-10.14, -1.96)	4.74 x 10 ⁻³	0.026
<i>ITGB3</i>	17	45331208	45421658	0.46 (0.19, 0.73)	3.87 x 10 ⁻³	0.018	3.04 (1.67, 4.42)	6.09 x 10 ⁻⁵	3.42 x 10 ⁻⁴
<i>MMD</i>	17	53469974	53499353	1.67 (1.22, 2.11)	2.08 x 10 ⁻⁵	4.28 x 10 ⁻⁴	2.85 (1.47, 4.23)	1.60 x 10 ⁻⁴	8.97 x 10 ⁻⁴
<i>COL5A3</i>	19	10070237	10121147	0.92 (0.59, 1.25)	1.57 x 10 ⁻⁴	1.76 x 10 ⁻³	3.77 (1.12, 6.41)	6.42 x 10 ⁻³	0.035
<i>MYL9</i>	20	35169887	35178228	0.48 (0.3, 0.67)	2.53 x 10 ⁻⁴	2.46 x 10 ⁻³	4.57 (2.56, 6.59)	4.48 x 10 ⁻⁵	2.52 x 10 ⁻⁴
<i>TUBB1</i>	20	57594309	57601709	0.75 (0.25, 1.24)	8.11 x 10 ⁻³	0.030	2.01 (0.59, 3.43)	6.72 x 10 ⁻³	0.037
<i>COL18A1</i>	21	46825052	46933634	0.37 (0.21, 0.53)	5.11 x 10 ⁻⁴	4.03 x 10 ⁻³	2.64 (0.81, 4.46)	5.71 x 10 ⁻³	0.031

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

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

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

Table S3. Validation of identified differentially expressed genes in publicly available transcriptomic datasets evaluating the effect of GCs.

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

Table S3 (continuation). Validation of identified differentially expressed genes in publicly available transcriptomic datasets evaluating the effect of GCs.

Gene	Chr. ^a	Position begin 5' ^b	Position end 3' ^b	GSE13168 ¹		GSE34313 ²		SRP098649 ³		Meta-analysis ^e	
				log ₂ FC (95% CI) ^c	q-value ^d	log ₂ FC (95% CI) ^c	q-value ^d	log ₂ FC (95% CI) ^c	q-value ^d	log ₂ FC (95% CI) ^c	q-value ^d
<i>PTGS1</i>	9	125132824	125157982	-0.17 (-0.45, 0.11)	0.708	-0.86 (-1.03, -0.67)	3.70 x 10 ⁻⁴	-0.34 (-0.54, -0.15)	1.83 x 10 ⁻³	-0.47 (-0.87, -0.06)	0.078
<i>DIXDC1</i>	11	111797868	111893308	-0.10 (-0.29, 0.08)	0.734	0.43 (0.20, 0.66)	0.024	-0.17 (-0.38, 0.04)	0.245	0.05 (-0.32, 0.42)	0.792
<i>TMTC1</i>	12	29653746	29937692	NA	NA	1.41 (1.23, 1.58)	2.54 x 10 ⁻⁵	0.16 (-0.04, 0.38)	0.262	0.79 (-0.44, 2.01)	0.625
<i>MEG3</i>	14	101245747	101327368	NA	NA	NA	NA	NA	NA	NA (NA, NA)	NA
<i>ITGB3</i>	17	45331208	45421658	-0.14 (-0.36, 0.10)	0.726	0.23 (0.01, 0.44)	0.150	-0.42 (-0.62, -0.23)	1.16 x 10 ⁻⁰⁴	-0.11 (-0.48, 0.26)	0.666
<i>MMD</i>	17	53469974	53499353	2.19 (1.95, 2.42)	6.36 x 10 ⁻⁵	0.84 (0.59, 1.08)	1.68 x 10 ⁻³	2.14 (1.88, 2.41)	1.67 x 10 ⁻⁵⁷	1.72 (0.86, 2.59)	5.84 x 10⁻⁴
<i>COL5A3</i>	19	10070237	10121147	-0.10 (-0.32, 0.11)	0.768	-0.22 (-0.51, 0.08)	0.342	2.29 (1.93, 2.66)	4.92 x 10 ⁻³⁴	0.65 (-0.95, 2.25)	0.530
<i>MYL9</i>	20	35169887	35178228	0.00 (-0.20, 0.19)	0.994	0.80 (0.59, 1.01)	9.86 x 10 ⁻⁴	0.28 (-0.09, 0.63)	0.271	0.36 (-0.12, 0.84)	0.206
<i>TUBB1</i>	20	57594309	57601709	-0.06 (-0.25, 0.14)	0.875	0.19 (0.01, 0.36)	0.151	NA	NA	0.07 (-0.18, 0.31)	0.582
<i>COL18A1</i>	21	46825052	46933634	0.25 (0.04, 0.45)	0.305	0.51 (0.33, 0.68)	3.39 x 10 ⁻³	0.43 (-0.25, 1.11)	0.381	0.39 (0.18, 0.61)	1.93 x 10⁻³

^a Chromosome; ^b Positions based on GRCh37/hg19 build; ^c Base 2 logarithmic transformation of fold change obtained for ASM cells exposed to GCs vs. control solution; ^d Adjusted p-value after adjusting for false discovery rate; ^e Random-effects model was applied taking into account the variance among studies. ASM: airway smooth muscle; CI: confidence interval; GCs: glucocorticosteroids; ICS: inhaled corticosteroids; NA: not available; PBMCs: peripheral blood mononuclear cells. Genes with significantly consistent changes in expression levels in ASM cells exposed to GCs vs. control solution after combining the publicly available datasets are in boldface. Results taken from www.realgar.org.

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

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

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

Table S4. Results of differential expression analyses in PBMCs for genes differentially expressed across datasets analyzed using different regression models.

Gene	<i>Asthma exacerbations + age + %FEV₁</i> ^{a, b}			<i>Predicted baseline FEV₁</i> ^{c, d}		
	log ₂ FC (95% CI) ^e	p-value	q-value ^f	log ₂ FC (95% CI) ^e	p-value	q-value ^f
<i>LTBP1</i>	1.05 (0.45, 1.65)	4.28 x 10 ⁻³	0.022	-0.07 (-0.26, 0.11)	0.369	0.576
<i>MTURN</i>	0.59 (-0.16, 1.34)	0.106	0.480	0.01 (-0.17, 0.19)	0.907	0.951
<i>NAMPT</i>	-1.21 (-3.17, 0.74)	0.186	0.700	0.07 (-0.21, 0.35)	0.561	0.731
<i>CALD1</i>	1.45 (0.45, 2.46)	0.011	0.061	-0.07 (-0.40, 0.26)	0.611	0.769
<i>MMD</i>	0.84 (-0.14, 1.82)	0.083	0.399	-0.08 (-0.33, 0.16)	0.439	0.634
<i>COL18A1</i>	0.58 (-0.35, 1.50)	0.186	0.700	0.00 (-0.21, 0.21)	0.993	0.996

^a Absence/presence of asthma exacerbations despite ICS use was evaluated as dependent variable; ^b The predicted FEV₁ measured at baseline before starting ICS therapy and age were included as covariates; ^c Predicted FEV₁ measured at baseline before starting ICS therapy was evaluated as dependent variable; ^d Estimated relative percentages of lymphocytes B and T, natural killer cells and monocytes were included as covariates; ^e Base 2 logarithmic transformation of fold change obtained for PBMCs from asthma patients with different patterns of ICS responsiveness based on the occurrence of asthma exacerbations; ^f *p*-value adjusted by false discovery rate accounting for the genes differentially expressed genes in the ASM analyzed in the discovery phase.

Genes with significant changes in expression levels are in boldface.

ASM: airway smooth muscle; CI: confidence interval; ICS: inhaled corticosteroids; FEV₁: forced expiratory volume in one second; PBMCs: peripheral blood mononuclear cells.

SUPPLEMENTARY FIGURE LEGENDS

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

Figure S1

