EUROPEAN RESPIRATORY *journal*

FLAGSHIP SCIENTIFIC JOURNAL OF ERS

Early View

Original article

A genome-wide association study of severe asthma exacerbations in Latino children and adolescents

Maria Pino-Yanes, Cancan Qi, Raimon Rios, Yueh-Ying Han, Soyeon Kim, Sam Oh, Edna Acosta-Pérez, Rong Zhang, Donglei Hu, Celeste Eng, Scott Huntsman, Lydiana Avila, Nadia Boutaoui, Michelle M. Cloutier, Manuel E. Soto-Quiros, Cheng-jian Xu, Scott T. Weiss, Jessica Lasky-Su, Megan R. Kiedrowski, Camila Figueiredo, Jennifer Bomberger, Mauricio L. Barreto, Glorisa Canino, Wei Chen, Gerard H. Koppelman, Esteban G. Burchard, Juan C. Celedón

Please cite this article as: Pino-Yanes M, Qi C, Rios R, *et al*. A genome-wide association study of severe asthma exacerbations in Latino children and adolescents. *Eur Respir J* 2020; in press (https://doi.org/10.1183/13993003.02693-2020).

A genome-wide association study of severe asthma exacerbations in Latino children and adolescents

Qi Yan, PhD[1#], Erick Forno, MD, MPH[1#], Esther Herrera-Luis, PhD[2#], Maria Pino-Yanes, PhD[2,3], Cancan Qi, MsC[4,5], Raimon Rios, MSc[6], Yueh-Ying Han, PhD[1], Soyeon Kim, PhD[1], Sam Oh, PhD[7], Edna Acosta-Pérez, PhD[8], Rong Zhang, PhD[1], Donglei Hu, PhD[7], Celeste Eng[7], Scott Huntsman, MS[7], Lydiana Avila, MD[9], Nadia Boutaoui, PhD[1], Michelle M. Cloutier, MD[10], Manuel E. Soto-Quiros, MD, PhD[9], Cheng-jian Xu, PhD[11,12], Scott T. Weiss, MD, MS[13], Jessica Lasky-Su, DSc[13], Megan R. Kiedrowski[14], Camila Figueiredo, PhD[6], Jennifer Bomberger, PhD[14], Mauricio L. Barreto, MD, PhD[15], Glorisa Canino, PhD[8], Wei Chen, PhD[1], Gerard H. Koppelman, MD PhD[4,5], Esteban G. Burchard, MD, MPH[7^], Juan C. Celedón, MD, DrPH[1^*]

[1]Division of Pediatric Pulmonary Medicine, UPMC Children's Hospital of Pittsburgh, University of Pittsburgh, Pittsburgh, PA, USA. [2]Genomics and Health Group, Department of Biochemistry, Microbiology, Cell Biology and Genetics, Universidad de La Laguna, La Laguna, Santa Cruz de Tenerife, Spain. [3]CIBER de Enfermedades Respiratorias, Instituto de Salud Carlos III, Madrid, Spain. [4]University of Groningen, University Medical Center Groningen, Dept. of Pediatric Pulmonology and Pediatric Allergy, Beatrix Children's Hospital, and [5]University of Groningen, University Medical Center Groningen, GRIAC Research Institute, Groningen, The Netherlands. [6]Instituto de Ciências da Saúde, Universidade Federal da Bahia, Vale do Canela, Salvador, Bahia, Brazil. [7]Department of Medicine, University of California San Francisco, San Francisco, CA, USA. [8]Behavioral Sciences Research Institute, University of Puerto Rico, San Juan, Puerto Rico. [9]Department of Pediatrics, Hospital Nacional de Niños, San José, Costa Rica. [10]Department of Pediatrics, University of Connecticut, Farmington, CT, USA. [11]CiiM and TWINCORE, joint ventures between the Hannover Medical School and the Helmholtz Centre for Infection Research, Hannover, Germany. [12]Department of Internal Medicine and Radboud

Center for Infectious Diseases, Radboud University Medical Center, Nijmegen, the Netherlands. [13]*Channing Division of Network Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA.* [14]*Department of Microbiology and Molecular Genetics, University of Pittsburgh, Pittsburgh, PA, USA.* [15]*Instituto de Saúde Coletiva, Federal University of Bahia, Salvador, Brazil.*

[#]*Shared first authors. ^Shared senior authors.*

***Corresponding author:** Juan C. Celedón, MD, DrPH
Division of Pulmonary Medicine
UPMC Children's Hospital of Pittsburgh
4401 Penn Avenue, Pittsburgh, PA 15224
Phone: 412.692.8429; Fax 412.692.7636
Email: juan.celedon@chp.edu

Author contributions: Q.Y., E.F., and J.C.C. conceived and designed the study. E.G.B., G.K., and J.C.C. obtained funding. Q.Y., E.H.L., M.P-Y., and R.R. conducted the primary analysis and interpreted data. C.Q., Y-Y.H., S.K., S.O., E.A-P., R.Z., D.H., C.E., S.H., L.A., N.B., M.C., M.S-Q., C.X., S.W., J.L-S., M.K., C.F., J.B., M.B., G.C., W.C., and E.G.B. participated in data collection and data analysis. Q.Y., E.F., and J.C.C. prepared the first draft of the manuscript. All authors reviewed the draft for intellectual content, and approved submission of the final version of the manuscript.

Funding/Support: This work was supported by grants HL079966, HL117191, and MD011764 from the U.S. National Institutes of Health (NIH) to J.C.C. Dr. Yan's contribution was supported by grant HL138098 from the U.S. NIH. Dr. Forno's contribution was supported by grant HL149693 from the U.S. NIH. The GALA II study was supported by U.S. NIH grants to E.G.B:

HL088133, HL004464, HL117004, ES015794, ES24844, TRDRP 24RT 0025, MD006902, and GM007546. M.P.Y. was supported by the Ramón y Cajal Program (RYC-2015-17205) and by grant SAF2017-83417R from the Spanish Ministry of Economy, Industry and Competitiveness. The PIAMA study was supported by The Netherlands Organization for Health Research and Development; The Netherlands Organization for Scientific Research; the Lung Foundation of the Netherlands (with methylation and gene expression studies supported by AF 4.1.14.001); The Netherlands Ministry of Spatial Planning, Housing, and the Environment; and The Netherlands Ministry of Health, Welfare, and Sport. C.Q. was supported by a grant from the China Scholarship Council.

Running head: GWAS, severe asthma exacerbations, Latino children

Subject Category Descriptor Number: 1.18 Asthma (Genetics), 2.09 **Racial, Ethnic, or Social Disparities in Lung Disease and Treatment**

ABSTRACT

Severe asthma exacerbations are a major cause of school absences and healthcare costs in children, particularly those in high-risk racial/ethnic groups. To identify susceptibility genes for severe asthma exacerbations in Latino children and adolescents, we conducted a meta-analysis of genome-wide association studies (GWAS) in 4,010 Latino youth with asthma in four independent cohorts, including 1,693 Puerto Ricans, 1,019 Costa Ricans, 640 Mexicans, 256 Brazilians, and 402 members of other Latino subgroups. We then conducted methylation quantitative trait locus (mQTL), expression quantitative trait locus (eQTL), and expression quantitative trait methylation (eQTM) analyses to assess whether the top SNP in the meta-analysis is linked to DNA methylation and gene expression in nasal (airway) epithelium in separate cohorts of Puerto Rican and Dutch children and adolescents. In the meta-analysis of GWAS, a SNP in *FLJ22447* (rs2253681) was significantly associated with 1.55 increased odds of severe asthma exacerbations (95% confidence interval= 1.34 to 1.79, $P=6.3\times10^{-9}$). This SNP was significantly associated with DNA methylation of a CpG site (cg25024579) at the *FLJ22447* locus, which was in turn associated with increased expression of *KCNJ2-AS1* in nasal airway epithelium from Puerto Rican children and adolescents ($\beta=0.10$, $P=2.18 \times 10^{-7}$). Thus, SNP rs2253681 was significantly associated with both DNA methylation of a cis-CpG in *FLJ22447* and severe asthma exacerbations in Latino youth. This may be partly explained by changes in airway epithelial expression of a gene recently implicated in atopic asthma in Puerto Rican children and adolescents (*KCNJ2-AS1*).

INTRODUCTION

Asthma is the most common chronic respiratory disease among children [1]. In the United States (U.S.), total costs related to asthma exceed $81 billion per year [2]. Severe asthma exacerbations (SAEs), defined as episodes of disease worsening requiring a change in treatment to prevent a serious outcome [3], are a major cause of school or work absences and healthcare costs. Of the ~6.1 million children with asthma in the U.S., 2.1% report ≥1 asthma-related hospitalization and 10.7% report ≥1 asthma-related visit to the emergency department (ED) in the previous year [4]. Despite recent advances, the best predictor of SAEs remains having had one in the previous year [5].

Although genome-wide association studies (GWASs) have identified susceptibility loci for asthma [6-8], little is known about genetic determinants of asthma exacerbations, which may be distinct from those for asthma *per se.* A GWAS in Danish children with asthma (ages 2 to 6 years) identified *CDHR3* (cadherin-related family member 3), a gene not previously associated with asthma, as a susceptibility locus for recurrent severe exacerbations [9]. In a combined GWAS of two cohorts of non-Hispanic white children with asthma, four intronic single nucleotide polymorphisms (SNPs) in cadherin-associated protein alpha 3 (*CTNNA3*) were significantly associated with SAEs [10], but such association was not replicated in an independent cohort including 786 children and adults with asthma. Moreover, a GWAS of SAEs among 806 non-Hispanic white children and adults with asthma who were being treated with inhaled corticosteroids found no genome-wide significant results [11].

The burden of asthma varies across racial or ethnic groups in the U.S. and Latin America. For example, Puerto Rican children have a greater prevalence, morbidity and mortality from asthma than non-Hispanic white children in the U.S. [12], and Costa Rican adolescents have a greater burden of asthma than those in other Latin American countries [13]. Moreover, recent evidence

suggests that some susceptibility variants for asthma-related outcomes are ethnic-specific [14, 15]. Thus, we hypothesized that there would be susceptibility variants for SAEs that would be more common or exert a greater effect in Latino subgroups at risk for morbidity from asthma. To test this hypothesis, we conducted a meta-analysis of GWAS of SAEs among Latino youth with asthma in four independent studies.

METHODS

Please see the Online Supplement for more details.

Study populations included in the meta-analysis of GWAS of severe exacerbations

Hartford-Puerto Rico study (HPR): HPR is a case-control study of childhood asthma in Puerto Ricans [6]. SAEs were defined as ≥1 hospitalization for asthma or ≥1 ED/urgent care visit for asthma requiring treatment with systemic corticosteroids in the previous year, or ≥1 course of systemic corticosteroids for asthma in the previous year. After quality control (QC) measures, 554 independent children with asthma (236 of whom had ≥1 SAE in the previous year) were included in the genome-wide association analysis, which was conducted using logistic regression under an additive genetic model, adjusting for age, sex, inhaled steroid use, and the first two principal components (PCs, calculated using smartPCA) [16].

The Genetics of Asthma in Latino Americans study (GALA II): GALA II is a case-control study of asthma in Latino children and youth [17]. A SAE was defined as having one or more of the following events in the previous year: hospitalizations for asthma, emergency room visits or unscheduled and urgent doctor's visits because of asthma, or treatment with systemic corticosteroids for asthma. The current analysis focused on 2181 children with asthma (1283 of whom had ≥1 SAE) and self-reported Latino ethnicity (1,139 Puerto Rican, 640 Mexican, and

402 from other groups). Association testing was conducted using logistic regression under an additive genetic model, adjusting for age, sex, inhaled steroid use, ethnicity, and two PCs.

The Genetics of Asthma in Costa Rica Study (GACRS): GACRS is a genetic study of nuclear families of children with asthma in Costa Rica [18, 19]. A SAE was defined as ≥1 ED visit for asthma or ≥1 hospitalization for asthma in the previous year. After QC, 1019 independent children with asthma (851 of whom had ≥1 SAE) were included in the analysis, which was also conducted using logistic regression under an additive genetic model, adjusting for age, sex, inhaled steroid use, and the first two PCs.

The Social Changes, Asthma and Allergy in Latin America study (SCAALA) – Bahia, Brazil: SCAALA is a longitudinal study of asthma and allergic diseases of Brazilian children [20]. Children who reported asthma symptoms and had data on SAEs (n=256) were included in this analysis. A SAE was defined as ≥1 ED visit or ≥1 hospitalization due to wheeze in the previous year. The analysis was conducted using logistic regression under an additive genetic model, with adjustment for age, sex, and the first two PCs from genotypic data.

Study populations included in molecular quantitative trait analyses in nasal epithelium

The Epigenetic Variation and childhood Asthma in Puerto Rico study (EVA-PR): Nasal (airway) epithelium can serve as a surrogate marker for DNA methylation and gene expression in bronchial (airway) epithelium [21]. In EVA-PR, whole-genome methylation assays were performed using HumanMethylation450 BeadChips (Illumina, San Diego, CA). After QC, 227 901 CpG probes remained for the analysis of nasal epithelium, and M-values were used in all downstream analyses. RNA-Seq was performed with the Illumina NextSeq 500 platform, paired-end reads at 75 cycles, and 80 million reads/sample. After QC, 16 737 genes were retained for the analysis. The R function *sva* was used to estimate latent factors (LFs) that capture unknown

data heterogeneity [22]. Of the 543 study participants, 457 had complete genome-wide data for genotypes, methylation, and transcriptomics in nasal epithelium (see Supplementary Figure 1).

PIAMA: PIAMA is a birth cohort study of children born in the Netherlands in 1996 and 1997 [23, 24]. A total of 479 nasal epithelial samples were hybridized to the Infinium HumanMethylation450 BeadChip arrays. After QC, 455 samples and 436 824 probes remained, and 432 samples had matched genotype data. RNA-Seq was performed with the Illumina HiSeq 2500 platform, paired-end sequencing. After stringent QC, 17 156 genes and 326 samples remained, with 233 samples having matched genotype data [25].

Meta-analysis of GWAS of SAEs

METAL [26] software was used to perform the meta-analysis of GWAS of SAEs, using data from HPR, GALA II, GACRS, and SCAALA.

Molecular quantitative trait locus analyses

To estimate the effects of our top SNP on DNA methylation and gene expression, we conducted a methylation quantitative trait locus (mQTL) analysis, to test for association between our top SNP and genome-wide DNA methylation in nasal epithelium, as well as an expression quantitative trait locus (eQTL) analysis, to test for association between our top SNP and genome-wide gene expression in nasal epithelium. We then conducted an expression quantitative trait methylation (eQTM) analysis, to test whether the top CpG site identified in the mQTL analysis was associated with genome-wide gene expression in nasal epithelium. All analyses were adjusted for age, sex, asthma status, atopy status, the top five PCs from genotypic data, and latent factors estimated from *sva* [22]. In addition, RNA-Seq batch and RNA sample sorting protocol (i.e., whole cells or CD326-positive nasal epithelial cells) [21] were

adjusted for in the eQTM analyses, and methylation batch was adjusted for in the mQTL and eQTM analyses; both *cis*- and *trans*- effects were considered.

Pathway analysis

A pathway analysis was performed with MAGMA [27], which conducts SNP-wise gene analysis of summary statistics with correction for LD between variants and genes, to test whether sets of genes are jointly associated with a phenotype (i.e. asthma exacerbation), compared to other genes across the genome. Adaptive permutation was used to produce an empirical *P*-value and FDR. Gene-sets used in the analyses were from GO [28, 29], KEGG [30, 31], REACTOME [32, 33], and BIOCARTA pathways.

RESULTS

The characteristics of the 4,010 participants in the four studies included in the meta-analysis of GWAS of SAEs are shown in **Table 1.** In total, there are 2,509 children with asthma and ≥1 SAE (cases) and 1,501 children with asthma but no SAEs (controls). Compared to subjects who participated in the HPR or SCAALA studies, those in GALA II were older and those in the GACRS were younger. Compared to subjects in the other studies, those in the GACRS were more likely to be male and to have had ≥1 severe asthma exacerbation in the previous year.

Approximately 6 million genotyped and imputed SNPs with MAF ≥0.05 were included in the meta-analysis of GWAS of SAEs. In this meta-analysis, one SNP (rs2253681, in *FLJ22447* on chromosome 14q23.2) was significantly associated with SAEs at $P < 5\times10^{-8}$ (**Figure 1a**). This SNP was genotyped in HPR, GALA II and SCAALA, and imputed in GACRS with imputation quality r^2=0.96. Each copy of the minor allele (A) of SNP rs2253681 was associated with 1.55 times increased odds of SAEs (95% confidence interval [CI]=1.34 to 1.79, $P=6.3\times10^{-9}$, **Figure 1b**). The Q-Q plot (**Figure 1c**) showed no inflation for the results for each of the four individual

cohorts or for the pooled analysis (**Figure 2**). There was no significant interaction between SNP rs2253681 and inhaled steroid use on SAEs in the GWAS conducted in the HPR, GALA II, and GACRS cohorts (P for interaction ≥0.15 in all instances).

We then examined whether SNPs previously associated with asthma in either a multi-ancestry meta-analysis [7] or a meta-analysis from United Kingdom Biobank [34] are associated with SAEs in our analysis (Supplementary Table 1). None of the previously reported asthma-susceptibility SNPs were significantly associated with SAEs in our meta-analysis of Latino youth. Moreover, no SNPs associated with SAEs or asthma hospitalizations in previous candidate-gene studies (e.g., *IL13*, *IL4RA)* or GWAS (e.g., *CDHR3*, *CTNNA3*) [9, 10, 18, 35-38] were significantly associated with SAEs in our meta-analysis (Meta-*P*≥0.05 in all instances, see Supplementary Table 2). Although the previously reported SNP rs7216389 in *ORMDL3* on chromosome 17q21 was nominally associated with SAEs in HPR ($P=1.6\times10^{-3}$), such SNP did not reach statistical significance in our meta-analysis (Meta-*P*=0.06, see Supplementary Table 2).

To examine whether SNP rs2253681 affects methylation of the *FLJ22447* locus in nasal epithelium, we first conducted a mQTL analysis. In this analysis, rs2253681 was a significant *cis*-acting mQTL in nasal epithelium for cg25024579 in *FLJ22447* (Beta=0.55, $P=3.6\times10^{-16}$ and *FDR-P*$=8.1\times10^{-11}$ in EVA-PR; Beta=0.34, $P=1.2\times10^{-11}$ in PIAMA; and Meta-*P*$=9.5\times10^{-25}$ for a combined analysis of EVA-PR and PIAMA; **Table 2**). In addition, among the top 20 mQTL, cg05223396 in *SH3PXD2B*, cg13127574 in *ADSSL1* and cg21115391 in *CCDC67* were associated with rs2253681 at *P*<0.05 in PIAMA, in the same direction as in EVA-PR (**Table 2**). Next, we conducted an eQTL analysis of SNP rs2253681 in the *FLJ22447* locus and gene expression in nasal epithelium, finding no *cis*- or *trans*- genome-wide significant results

(Supplementary Table 3). Among the top 20 eQTL, *SRF* was associated with rs2253681 at $P<0.05$ in PIAMA, in the same direction as in EVA-PR (Supplementary Table 3).

Given the association between SNP rs2253681 and methylation of cg25024579, we then examined the effect of that CpG on gene expression in nasal epithelium, by conducting an eQTM analysis (**Table 3**). In this analysis, methylation of cg25024579 was significantly associated in *trans* with expression of the gene *KCNJ2-AS1* (KCNJ2 antisense RNA 1) on chromosome 17q24.3 (Beta=0.10, $P=2.2\times10^{-7}$ and *FDR-P*$=3.2\times10^{-3}$) in EVA-PR. Although this was not replicated in PIAMA, the observed association remained significant in the combined analysis of the two cohorts (Beta=0.09, $P=1.4\times10^{-6}$). The top *cis*-eQTM gene in the current analysis in EVA-PR was protein kinase C eta (*PRKCH*; Beta=0.07, $P=4.1\times10^{-5}$ and *FDR-P*$=1.5\times10^{-1}$). *PRKCH* expression was also associated with cg2504579 methylation in PIAMA, in the same direction of association as in EVA-PR (Beta=0.11, $P=1.4\times10^{-2}$; and $P=2.4\times10^{-6}$ in the combined analysis of EVA-PR and PIAMA). Among the top 20 eQTM in EVA-PR, *SLC27A2* expression was associated with cg25024579 methylation in PIAMA, in the same direction of association as in EVA-PR (Beta=0.22, $P=4.2\times10^{-5}$ in PIAMA, and Beta=0.09, $P=4.9\times10^{-7}$ in the combined analysis of the two cohorts). In addition, expression of *FOCAD*, *PIGF* and *ERLEC1* was associated with cg25024579 methylation in PIAMA at $P<0.05$, in the same direction as in EVA-PR.

To further assess the biological relevance of the *FLJ22447* locus, we conducted a SNP-wise pathway analysis of SAEs using the meta-analysis results and evaluated public repositories and databases. Although there were no pathways associated with SAEs after adjusting for multiple testing, there were a number of nominally significant pathways that included genes on chromosome 14q23.2, near *FLJ22447* (Supplementary Table 4).

In a sensitivity analysis, we repeated the GWAS of SAEs after imputing genotypes using the 1,000 Genomes Ad Mixed American (AMR) reference panel instead of the Haplotype Reference Consortium (HRC) r1.1 2016 reference panel (see Online Supplement), obtaining very similar results (see Supplementary Figure 2 and Supplementary Table 5).

DISCUSSION

Our combined analysis including 4,010 youth in four study cohorts showed that a novel SNP in *FLJ22447* (rs2253681) is significantly associated with SAEs (P=6.3×10^{-9}) among children and adolescents in Latino subgroups affected with asthma (Puerto Ricans, Costa Ricans, Mexicans and Brazilians) [12]. In an mQTL analysis in nasal epithelium, this SNP was significantly associated with DNA methylation of a CpG site at the *FLJ22447* locus (cg25024579), which was in turn significantly associated with the expression of gene *KCNJ2-AS1* (KCNJ2 antisense RNA 1) in nasal epithelium.

Gene *FLJ22447* codes for a long non-coding RNA (lncRNA), a type of RNA that does not get translated to protein but may serve pre- and post-transcriptional regulatory functions. *FLJ22447* partially overlaps with *AL355916.3*, which encodes a protein kinase C paralog expressed in epithelial tissues. Of note, a SNP in *AL355916.3* has been associated with FEV_1/FVC and bronchodilator response in adults [34]. *FLJ22447* mediates IL-33 up-regulation in activated fibroblasts; together, *FLI22447* and IL-33 can increase fibroblast expression of α-*SMA* (α smooth muscle actin), vimentin, and N-cadherin [35], which have been associated with myofibroblast differentiation and airway remodeling [36, 37].

KCNJ2-AS1 also encodes an lncRNA. Expression of *KCNJ2-AS1* (log2[fold-change]=0.34; P=3.9×10^{-6}; *FDR-P*=3.1×10^{-5}) was significantly associated with atopic asthma in our recent transcriptome-wide association study (TWAS) of atopic asthma in Puerto Rican children and adolescents [38]. SNPs rs8066985 and rs312750 near *KCNJ2-AS1* have been associated with

body mass index (BMI) and waist-to-hip ratio [39, 40], which have been associated with worse asthma outcomes in children and adults. To our knowledge, this is the first study linking *FLJ22447* or *KCNJ2-AS1* to SAEs in children and adolescents.

While not genome-wide significant, the top *cis*-eQTM gene in EVA-PR was *PRKCH*, which is adjacent to *FLJ22447* and most highly expressed in the lungs [41]. Both methylation and transcription of this gene in nasal (airway) epithelium were recently associated with atopic asthma in Puerto Rican children and adolescents [21]. Protein kinase C eta (PKCη), encoded by *PRKCH*, plays a key role in the assembly and maintenance of epithelial tight junctions. PKCη phosphorylates occludin on threonine residues (T403 and T404), and such phosphorylation is required for the assembly and/or maintenance of occludin in epithelial tight junctions that are key to the integrity and function of the airway epithelial barrier [42].

Recent GWAS of exacerbations in non-Hispanic white children identified SNPs in two genes, *CDHR3* and *CTNNA3* [9, 10], neither of which replicated in our analysis, despite having similar or larger sample sizes than those in the original studies. Similarly, a previously reported association between a SNP on chromosome 17q21 (rs7216389) and SAEs was not statistically significant in our meta-analysis (P=0.06). While an association between the 17q21 locus and asthma is widely recognized, whether such locus is linked to severe disease exacerbations among children with asthma is less clear. The initial report in pediatric asthma analyzed a cohort of 376 children and reported 80 with recurrent wheezing and 66 with asthma [43]. The authors then analyzed the rate of severe exacerbations *within the full cohort* (57 among the 376 participants), rather than comparing children with asthma and an exacerbation to those with asthma but no exacerbation. A recent meta-analysis reported a significant association between rs7216389 and asthma hospitalizations and ED visits in children, but -while the pooled

estimates were significant- only 4 of the 13 cohorts showed significant results [44]. Although lack of an association between *CDHR3* and SAEs in the current study may be due to differences in the age of participants and outcome definitions across studies, our results for *FLJ22447* are novel and further highlight the importance of studying asthma outcomes in children and adolescents of diverse races and ethnicities.

We recognize several study limitations. First, we lack data on viral infections or air pollution, which may interact with genetic or epigenetic mechanisms on causing SAEs in children. Second, we cannot assess temporal relationships between DNA methylation or gene expression and severe asthma exacerbations in our cross-sectional analysis. Third, we had insufficient statistical power to detect either uncommon risk alleles or alleles with modest genetic effects on SAEs. Moreover, we had limited statistical power to detect an interaction between our top SNP and inhaled steroid use on SAEs or for our molecular quantitative trait analyses, and lacked a replication cohort for analyses of DNA methylation or gene expression in nasal epithelium and SAEs (as PIAMA lacks adequate data on asthma exacerbations). Fourth, the definitions of SAEs varied across the study cohorts. However, we obtained similar results in a sensitivity analysis for the HPR and GALA II cohorts, in which we re-ran the GWAS after excluding subjects who received systemic corticosteroids but did not report an unscheduled and acute visit for asthma (data not shown).

In summary, we identified a novel SNP in *FLJ22447* that is significantly associated with both SAEs in Latino children and adolescents and DNA methylation of a *cis*-CpG in *FLJ22447* in nasal epithelium. This CpG is, in turn, linked to nasal epithelial expression of a gene recently implicated in atopic asthma in children and adolescents (*KCNJ2-AS1*). Future longitudinal studies of asthma omics should assess whether and how genetic variants affect airway epithelial function and SAEs in childhood.

FIGURE LEGENDS

Figure 1. 1a) Manhattan plot of meta-analysis results: Manhattan plot showing the summary meta-analysis results of HPR, GALA II, GACRS, and SCAALA. The chromosomal position of each SNP is displayed along the X-axis and the negative logarithm of the association *P*-value is displayed on the Y-axis. The blue line represents the suggestive significance line ($P < 1\times10^{-6}$). The red line represents the genome-wide significance line ($P < 5\times10^{-8}$). HPR: Hartford-Puerto Rico cohort. GALA II: Genetics of Asthma in Latino Americans II. GACRS: Genetics of Asthma in Costa Rica Study, and the SCAALA (Social Changes, Asthma and Allergy in Latin America) Study. **1b) Results of the meta-analysis on the chromosome 14 region:** The relative location of genes and the direction of transcription are shown in the lower portion of the figure, and the chromosomal position is shown on the *x* axis. The light blue line shows the recombination rate across the region (right *y* axis), and the left *y* axis shows the significance of the associations. The purple diamond shows the *P*-value for rs2253681 that is the most significant SNP in the meta-analysis. The circles show the *P*-values for all other SNPs and are color coded according to the level of LD with rs2253681 in the 1000 Genome Project Admixed American (AMR) population. This plot was generated at http://locuszoom.org/. **1c) QQ plots for the meta-analysis.** λ is the genomic control value.

Figure 2. Forest plots of odds ratio and 95% confidence interval for the association with asthma: Forest plots for rs2253681, the most significant SNP in the meta-analysis. HPR:

Hartford-Puerto Rico cohort. GALA II: Genetics of Asthma in Latino Americans II. GACRS: Genetics of Asthma in Costa Rica Study, SCAALA: Social Changes, Asthma and Allergy in Latin America. The heterogeneity measure I^2=0 that implies no heterogeneity among the ORs from the four studies (see supplementary text for details).

Table 1: Summary of main characteristics of study participants

	HPR (n=554)	GALA II (n=2181)	GACRS (n=1019)	SCAALA (n=256)
Age in years (mean ± SD)	10.0±2.7	12.7±3.3	9.2±1.9	7.2±1.9
Male gender (n, %)	300 (54.2)	1,196 (54.8)	598 (58.7)	139 (54.3)
Asthma exacerbation (n, %)	236 (42.6)	1,283 (58.8)	851 (83.5)	139 (54.3)
Inhaled steroid use (n, %)	187 (33.8)	996 (45.7)	521 (51.1)	Not available
FEV_1 % predicted (mean ± SD)	86.8±16.0	90.6±16.3	99.1±17.3	Not available
FEV_1/FVC % predicted (mean ± SD)	91.8±9.8	96.3± 8.8	94.5±8.7	Not available
Study sites	Hartford (CT) and San Juan (Puerto Rico)	Chicago (IL), Bronx (NY), Houston (TX), San Francisco (CA) and Puerto Rico	Costa Rica	Salvador (Bahia), Brazil
Genotyping platform	Illumina 2.5M	Affymetrix Axiom® LAT1	Illumina Human Omni Express-12v1_A	Illumina Human Omni 2.5-8v1

HPR: Hartford-Puerto Rico Study. GALA II: Genetics of Asthma in Latino Americans II Study. GACRS: Genetics of Asthma in Costa Rica Study. SCAALA: Social Changes, Asthma and Allergy in Latin America Study. For comparability, all percent predicted values across the three studies were calculated using reference values for Mexican American youth (Hankinson J et al, Am J Respir Crit Care Med 1999;159(1):179-187.

Table 2: Top 20 mQTLs for rs2253681 in EVA-PR nasal epithelial cells and replication results from PIAMA

			Nearest	Distance	EVA-PR			PIAMA		Meta	
CpG	Chr	Position	gene	to gene	Effect	*P*-value	*FDR*[*]	Effect	*P*-value	Effect	*P*-value
cg25024579	**14**	**62 076 297**	***FLJ22447***	**0**	**0.5520**	**3.55E-16**	**8.08E-11**	**0.3365**	**1.16E-11**	**0.4114**	**9.45E-25**
cg02787615	2	99 434 688	*KIAA1211L*	0	-0.1080	9.68E-07	1.10E-01	0.0415	1.73E-02	-0.0161	2.41E-01
cg05223396	5	171 830 155	*SH3PXD2B*	0	-0.2175	1.89E-06	1.44E-01	-0.0641	2.52E-02	-0.1074	9.56E-06
cg24718015	17	40 489 721	*STAT3*	0	-0.1581	5.01E-06	2.80E-01	0.0215	5.00E-01	-0.0609	9.48E-03
cg21785067	1	54 587 182	*TCEANC2*	8989	0.2305	7.39E-06	2.80E-01	0.0824	6.42E-02	0.1458	1.48E-05
cg21917349	15	29 213 860	*APBA2*	0	-0.2427	7.89E-06	2.80E-01	-0.0441	2.22E-01	-0.1050	4.82E-04
cg15904664	14	104 569 653	*ASPG*	0	-0.2093	9.96E-06	2.80E-01	-0.0574	1.24E-01	-0.1156	8.11E-05
cg11374933	8	9 766 172	*MIR124-1*	5189	-0.1660	1.18E-05	2.80E-01	0.0357	2.55E-01	-0.0464	5.49E-02
cg15375424	5	131 823 451	*IRF1*	0	-0.2272	1.37E-05	2.80E-01	-0.0623	9.38E-02	-0.1177	1.02E-04
cg07975634	1	147 232 608	*GJA5*	0	-0.2236	1.49E-05	2.80E-01	0.0396	6.77E-02	0.0002	9.92E-01
cg08487909	6	30 904 981	*DPCR1*	3794	-0.1356	1.59E-05	2.80E-01	-0.0364	8.15E-02	-0.0668	1.23E-04
cg00203736	7	55 022 567	*EGFR*	64156	-0.2440	1.86E-05	2.80E-01	0.0034	9.20E-01	-0.0614	3.54E-02
cg23696248	19	45 260 501	*BCL3*	0	-0.3099	2.01E-05	2.80E-01	0.0230	7.09E-01	-0.1164	1.33E-02
cg13127574	14	105 196 523	*ADSSL1*	0	-0.2116	2.11E-05	2.80E-01	-0.0445	9.01E-03	-0.0621	1.18E-04
cg08403064	11	44 068 709	*ACCSL*	820	-0.2011	2.23E-05	2.80E-01	0.0472	1.78E-01	-0.0406	1.50E-01
cg21115391	11	93 143 810	*CCDC67*	0	-0.2452	2.23E-05	2.80E-01	-0.0945	9.51E-03	-0.1374	8.40E-06
cg01220257	19	45 260 935	*BCL3*	0	-0.1474	2.30E-05	2.80E-01	0.0269	5.78E-01	-0.0879	1.87E-03
cg12765028	4	13 526 659	*LINC01097*	1282	0.1566	2.34E-05	2.80E-01	-0.0063	7.70E-01	0.0349	6.07E-02
cg11136041	1	158 111 350	*LOC646268*	919	-0.2204	2.39E-05	2.80E-01	0.0249	4.28E-01	-0.0404	1.33E-01
cg01735277	15	75 077 691	*CSK*	0	-0.2291	2.46E-05	2.80E-01	-0.0460	1.30E-01	-0.0897	7.22E-04

[*] *FDR* is adjusted for the whole genome in EVA-PR

Table 3: Top 20 eQTMs for cg25024579 in EVA-PR nasal epithelial cells and replication results from PIAMA

Gene	Chr	Start	End	EVA-PR			PIAMA		Meta-analysis	
				Effect	*P*-value	*FDR*[*]	Effect	*P*-value	Effect	*P*-value
KCNJ2-AS1	**17**	**68 163 101**	**68 165 543**	**0.1019**	**2.18×10^{-7}**	**3.18×10^{-3}**	-0.1265	1.95×10^{-1}	0.0930	1.40×10^{-6}
CCDC125	5	68 576 518	68 616 410	0.0669	2.11×10^{-5}	1.22×10^{-1}	0.0439	4.03×10^{-1}	0.0650	1.60×10^{-5}
FARS2	6	5 261 583	5 771 816	0.0563	2.50×10^{-5}	1.22×10^{-1}	0.0262	6.10×10^{-1}	0.0544	2.58×10^{-5}
PRKCH	14	61 788 514	62 017 698	0.0705	4.08×10^{-5}	1.49×10^{-1}	0.1119	1.35×10^{-2}	0.0757	2.44×10^{-6}
TBL2	7	72 983 276	72 993 013	0.0567	5.81×10^{-5}	1.53×10^{-1}	-0.0624	1.06×10^{-1}	0.0427	1.27×10^{-3}
FOCAD	9	20 658 307	20 995 954	0.0764	7.38×10^{-5}	1.53×10^{-1}	0.1647	2.70×10^{-2}	0.0819	1.13×10^{-5}
ERLIN1	10	101 909 846	101 945 734	0.0596	9.62×10^{-5}	1.53×10^{-1}	0.0530	2.01×10^{-1}	0.0588	4.10×10^{-5}
LOC401052	3	10 048 101	10 052 779	0.0001	9.75×10^{-5}	1.53×10^{-1}	NA	NA	NA	NA
SMIM14	4	39 552 545	39 640 481	0.0752	9.97×10^{-5}	1.53×10^{-1}	-0.0787	8.53×10^{-2}	0.0519	3.55×10^{-3}
SLC27A2	15	50 474 392	50 528 589	0.0772	1.19×10^{-4}	1.53×10^{-1}	**0.2221**	**4.23×10^{-5}**	0.0947	4.92×10^{-7}
BLOC1S6	15	45 879 416	45 901 909	0.0445	1.43×10^{-4}	1.53×10^{-1}	0.0104	7.89×10^{-1}	0.0417	1.99×10^{-4}
TRMT12	8	125 463 047	125 465 266	0.0526	1.46×10^{-4}	1.53×10^{-1}	0.0887	9.43×10^{-2}	0.0549	4.17×10^{-5}
CDK19	6	110 931 180	111 136 412	0.0728	1.48×10^{-4}	1.53×10^{-1}	-0.1092	7.73×10^{-2}	0.0568	1.93×10^{-3}
ACSS1	20	24 986 865	25 013 342	0.0720	1.48×10^{-4}	1.53×10^{-1}	0.0173	6.87×10^{-1}	0.0630	2.81×10^{-4}
SEC22A	3	122 920 773	122 992 982	0.0485	1.74×10^{-4}	1.53×10^{-1}	-0.0870	1.15×10^{-1}	0.0415	9.79×10^{-4}
PIGF	2	46 808 412	46 844 251	0.0624	1.76×10^{-4}	1.53×10^{-1}	0.1262	4.12×10^{-2}	0.0667	3.29×10^{-5}
NME6	3	48 335 588	48 342 848	0.0474	1.89×10^{-4}	1.53×10^{-1}	0.0335	3.94×10^{-1}	0.0461	1.36×10^{-4}
ERLEC1	2	54 014 067	54 045 956	0.0532	2.07×10^{-4}	1.53×10^{-1}	0.0675	4.21×10^{-2}	0.0554	2.53×10^{-5}
KATNAL2	18	44 526 786	44 628 614	0.0783	2.27×10^{-4}	1.53×10^{-1}	0.0687	3.38×10^{-1}	0.0775	1.41×10^{-4}
PKHD1L1	8	110 374 705	110 543 500	0.1190	2.43×10^{-4}	1.53×10^{-1}	-0.3652	1.87×10^{-1}	0.1125	4.82×10^{-4}

[*] *FDR* is adjusted for the whole genome in EVA-PR

REFERENCES

1. Center for Disease Control and Prevention. Asthma. Data, statistics and surveillance. https://www.cdc.gov/asthma/most_recent_data.htm. Accessed on September 18, 2020.

2. Nurmagambetov T, Kuwahara R, Garbe P. The Economic Burden of Asthma in the United States, 2008-2013. *Ann Am Thorac Soc* 2018: 15(3): 348-356.

3. Fuhlbrigge A, Peden D, Apter AJ*, et al.* Asthma outcomes: exacerbations. *J Allergy Clin Immunol* 2012: 129(3 Suppl): S34-48.

4. Akinbami LJ, Moorman JE, Liu X. Asthma prevalence, health care use, and mortality: United States, 2005-2009. *Natl Health Stat Report* 2011(32): 1-14.

5. Puranik S, Forno E, Bush A*, et al.* Predicting Severe Asthma Exacerbations in Children. *Am J Respir Crit Care Med* 2017: 195(7): 854-859.

6. Yan Q, Brehm J, Pino-Yanes M*, et al.* A meta-analysis of genome-wide association studies of asthma in Puerto Ricans. *Eur Respir J* 2017: 49(5).

7. Demenais F, Margaritte-Jeannin P, Barnes KC*, et al.* Multiancestry association study identifies new asthma risk loci that colocalize with immune-cell enhancer marks. *Nat Genet* 2018: 50(1): 42-53.

8. Torgerson DG, Ampleford EJ, Chiu GY*, et al.* Meta-analysis of genome-wide association studies of asthma in ethnically diverse North American populations. *Nat Genet* 2011: 43(9): 887-892.

9. Bonnelykke K, Sleiman P, Nielsen K*, et al.* A genome-wide association study identifies CDHR3 as a susceptibility locus for early childhood asthma with severe exacerbations. *Nat Genet* 2014: 46(1): 51-55.

10. McGeachie MJ, Wu AC, Tse SM*, et al.* CTNNA3 and SEMA3D: Promising loci for asthma exacerbation identified through multiple genome-wide association studies. *J Allergy Clin Immunol* 2015: 136(6): 1503-1510.

11. Dahlin A, Denny J, Roden DM*, et al.* CMTR1 is associated with increased asthma exacerbations in patients taking inhaled corticosteroids. *Immunity, inflammation and disease* 2015: 3(4): 350-359.

12. Rosser FJ, Forno E, Cooper PJ*, et al.* Asthma in Hispanics. An 8-year update. *Am J Respir Crit Care Med* 2014: 189(11): 1316-1327.

13. Forno E, Gogna M, Cepeda A*, et al.* Asthma in Latin America. *Thorax* 2015: 70(9): 898-905.

14. Burkart KM, Sofer T, London SJ*, et al.* A Genome-Wide Association Study in Hispanics/Latinos Identifies Novel Signals for Lung Function. The Hispanic Community Health Study/Study of Latinos. *Am J Respir Crit Care Med* 2018: 198(2): 208-219.

15. Mak ACY, White MJ, Eckalbar WL*, et al.* Whole-Genome Sequencing of Pharmacogenetic Drug Response in Racially Diverse Children with Asthma. *Am J Respir Crit Care Med* 2018: 197(12): 1552-1564.

16. Price AL, Patterson NJ, Plenge RM*, et al.* Principal components analysis corrects for stratification in genome-wide association studies. *Nat Genet* 2006: 38(8): 904-909.

17. Nishimura KK, Galanter JM, Roth LA*, et al.* Early-life air pollution and asthma risk in minority children. The GALA II and SAGE II studies. *American journal of respiratory and critical care medicine* 2013: 188(3): 309-318.

18. Hunninghake GM, Soto-Quiros ME, Avila L*, et al.* Polymorphisms in IL13, total IgE, eosinophilia, and asthma exacerbations in childhood. *J Allergy Clin Immunol* 2007: 120(1): 84-90.

19. Hunninghake GM, Soto-Quiros ME, Avila L*, et al.* Sensitization to Ascaris lumbricoides and severity of childhood asthma in Costa Rica. *J Allergy Clin Immunol* 2007: 119(3): 654-661.

20. Barreto ML, Cunha SS, Alcantara-Neves N*, et al.* Risk factors and immunological pathways for asthma and other allergic diseases in children: background and methodology of a

longitudinal study in a large urban center in Northeastern Brazil (Salvador-SCAALA study). *BMC Pulm Med* 2006: 6: 15.

21. Forno E, Wang T, Qi C*, et al.* DNA methylation in nasal epithelium, atopy, and atopic asthma in children: a genome-wide study. *Lancet Respir Med* 2019: 7(4): 336-346.

22. Leek JT, Johnson WE, Parker HS*, et al.* The sva package for removing batch effects and other unwanted variation in high-throughput experiments. *Bioinformatics* 2012: 28(6): 882-883.

23. Brunekreef B, Smit J, de Jongste J*, et al.* The prevention and incidence of asthma and mite allergy (PIAMA) birth cohort study: design and first results. *Pediatr Allergy Immunol* 2002: 13(s15): 55-60.

24. Wijga A, Smit HA, Brunekreef B*, et al.* Are children at high familial risk of developing allergy born into a low risk environment? The PIAMA Birth Cohort Study. Prevention and Incidence of Asthma and Mite Allergy. *Clin Exp Allergy* 2001: 31(4): 576-581.

25. Qi C, Jiang Y, Yang IV*, et al.* Nasal DNA methylation profiling of asthma and rhinitis. *J Allergy Clin Immunol* 2020; 145(6):1655-63..

26. Willer CJ, Li Y, Abecasis GR. METAL: fast and efficient meta-analysis of genomewide association scans. *Bioinformatics* 2010: 26(17): 2190-2191.

27. de Leeuw CA, Mooij JM, Heskes T*, et al.* MAGMA: generalized gene-set analysis of GWAS data. *PLoS computational biology* 2015: 11(4): e1004219.

28. Ashburner M, Ball CA, Blake JA*, et al.* Gene ontology: tool for the unification of biology. The Gene Ontology Consortium. *Nat Genet* 2000: 25(1): 25-29.

29. Gene Ontology C. Gene Ontology Consortium: going forward. *Nucleic Acids Res* 2015: 43(Database issue): D1049-1056.

30. Kanehisa M, Sato Y, Kawashima M*, et al.* KEGG as a reference resource for gene and protein annotation. *Nucleic acids research* 2016: 44(D1): D457-462.

31. Ogata H, Goto S, Sato K*, et al.* KEGG: Kyoto Encyclopedia of Genes and Genomes. *Nucleic acids research* 1999: 27(1): 29-34.

32. Fabregat A, Sidiropoulos K, Garapati P*, et al.* The Reactome pathway Knowledgebase. *Nucleic acids research* 2016: 44(D1): D481-487.

33. Croft D, O'Kelly G, Wu G*, et al.* Reactome: a database of reactions, pathways and biological processes. *Nucleic acids research* 2011: 39(Database issue): D691-697.

34. Zhu Z, Lee PH, Chaffin MD, et al. A genome-wide cross-trait analysis from UK Biobank highlights the shared genetic architecture of asthma and allergic diseases. Nat Genet 2018: 50(6): 857-864.35.

35. Ding L, Ren J, Zhang D, *et al*. A novel stromal lncRNA signature reprograms fibroblasts to promote the growth of oral squamous cell carcinoma via LncRNA-CAF/interleukin-33. Carcinogenesis 2018;39(3):397-406.

36. Hackett T-L, Warner S, Stefanowicz D, *et al*. Induction of epithelial-mesenchymal transition in primary airway epithelial cells from patients with asthma by transforming growth factor-beta1. Am J Respir Crit Care Med 2009;180(2):122-33.

37. Liu T, Liu Y, Miller M, *et al.* Autophagy plays a role in FSTL1-induced epithelial mesenchymal transition and airway remodeling in asthma. Am J Physiol Lung Cell Mol Physiol. 2017 Jul 1;313(1):L27-L40.

38. Forno E, Zhang R, Jiang Y*, et al.* Transcriptome-wide and differential expression network analyses of childhood asthma in nasal epithelium. *J Allergy Clin Immunol* 2020; 146(3):671-675..

39. Shungin D, Winkler TW, Croteau-Chonka DC*, et al.* New genetic loci link adipose and insulin biology to body fat distribution. *Nature* 2015: 518(7538): 187-196.

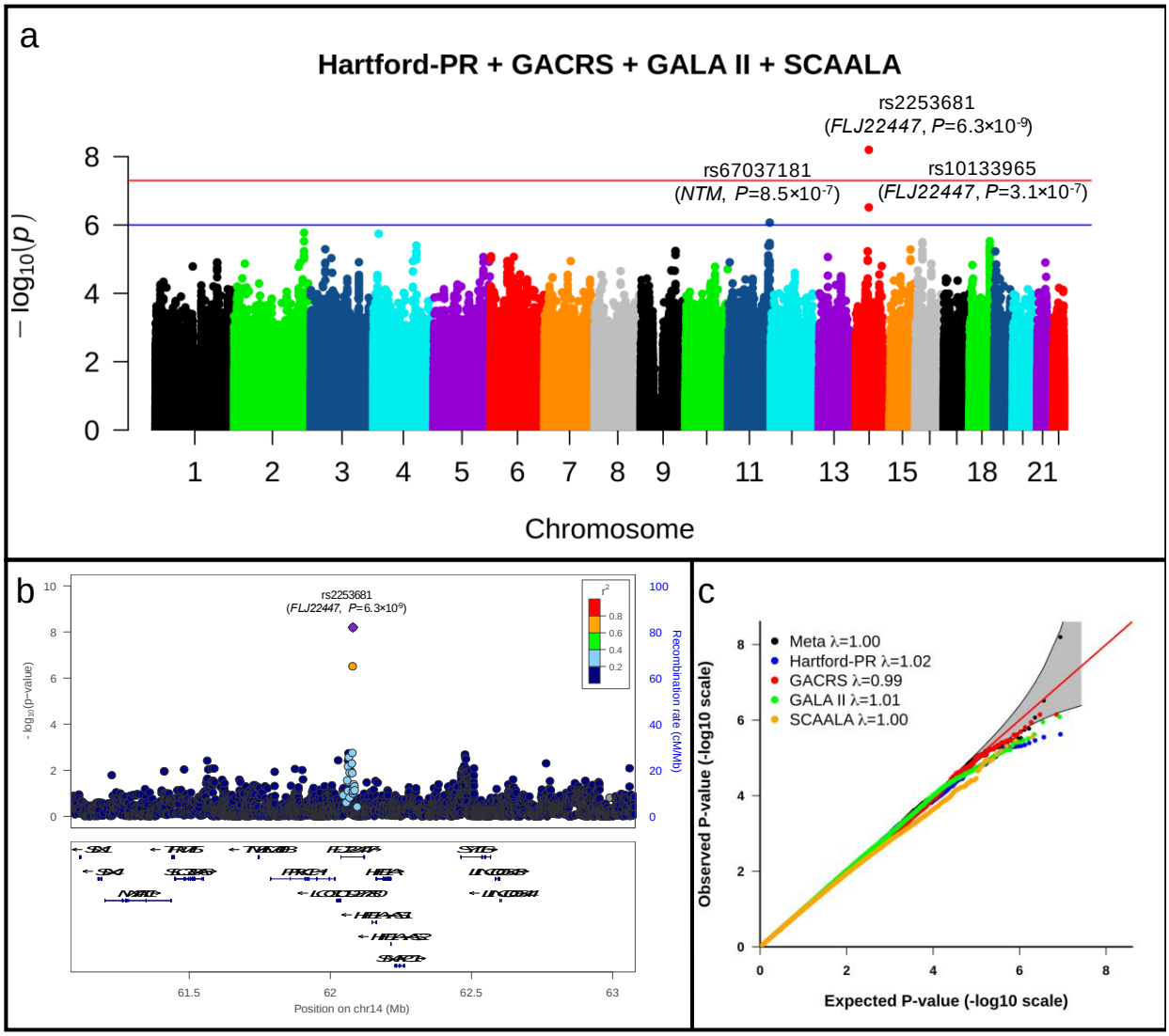
40. Winkler TW, Justice AE, Graff M*, et al.* The Influence of Age and Sex on Genetic Associations with Adult Body Size and Shape: A Large-Scale Genome-Wide Interaction Study. *PLoS Genet* 2015: 11(10): e1005378.

41. The Genotype-Tissue Expression (GTEx) project. *Nat Genet* 2013: 45(6): 580-585.

42. Suzuki T, Elias BC, Seth A*, et al.* PKC eta regulates occludin phosphorylation and epithelial tight junction integrity. *Proc Natl Acad Sci U S A* 2009: 106(1): 61-66.

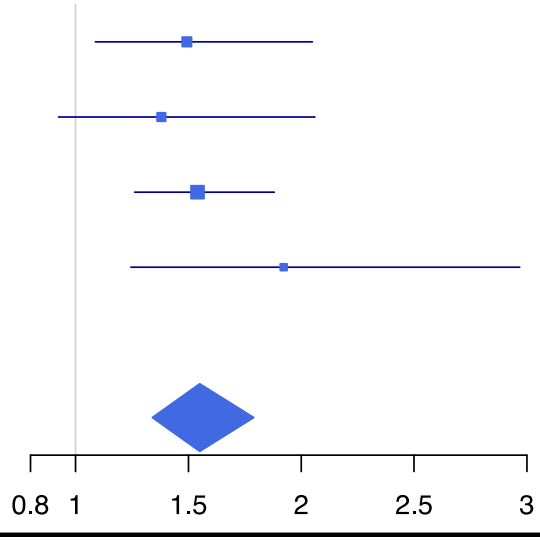
43. Bisgaard H, Bonnelykke K, Sleiman PM*, et al.* Chromosome 17q21 gene variants are associated with asthma and exacerbations but not atopy in early childhood. *Am J Respir Crit Care Med* 2009: 179(3): 179-185.

44. Farzan N, Vijberger S, Hernandez-Pacheco N, *et al*. 17q21 variant increases the risk of exacerbations in asthmatic children despite inhaled corticosteroid use. Allergy 2018; 73(10):2083-88.


a
Hartford-PR + GACRS + GALA II + SCAALA
rs2253681
(FLJ22447, P=6.3×10-9)
rs67037181
(NTM, P=8.5×10-7)
rs10133965
(FLJ22447, P=3.1×10-7)
− log10(p)
Chromosome
b
rs2253681
(FLJ22447, P=6.3×10-9)
r2
Recombination rate (cM/Mb)
Position on chr14 (Mb)
c
Meta λ=1.00
Hartford-PR λ=1.02
GACRS λ=0.99
GALA II λ=1.01
SCAALA λ=1.00
Observed P-value (-log10 scale)
Expected P-value (-log10 scale)

rs2253681

Study	Minor allele	MAF	P–value	OR
HPR	A	0.20	1.3E–2	1.49
GACRS	A	0.11	0.12	1.38
GALA II	A	0.16	2.3E–5	1.54
SCAALA	A	0.24	3.2E–3	1.92
Meta			**6.3E–9**	**1.55**



Online Supplement

A genome-wide association study of severe asthma exacerbations in Latino children and adolescents

Qi Yan, PhD[1#], Erick Forno, MD, MPH[1#], Esther Herrera-Luis, PhD[2#], Maria Pino-Yanes, PhD[2,3], Cancan Qi, MsC[4,5], Raimon Rios, MSc[6], Yueh-Ying Han, PhD[1], Soyeon Kim, PhD[1], Sam Oh, PhD[7], Edna Acosta-Pérez, PhD[8], Rong Zhang, PhD[1], Donglei Hu, PhD[7], Celeste Eng[7], Scott Huntsman, MS[7], Lydiana Avila, MD[9], Nadia Boutaoui, PhD[1], Michelle M. Cloutier, MD[10], Manuel E. Soto-Quiros, MD, PhD[9], Cheng-jian Xu, PhD[11,12], Scott T. Weiss, MD, MS[13], Jessica Lasky-Su, DSc[13], Megan R. Kiedrowski[14], Camila Figueiredo, PhD[6], Jennifer Bomberger, PhD[14], Mauricio L. Barreto, MD, PhD[15], Glorisa Canino, PhD[8], Wei Chen, PhD[1], Gerard H. Koppelman, MD PhD[4,5], Esteban G. Burchard, MD, MPH[7^], Juan C. Celedón, MD, DrPH, ATSF[1^*]

[1]Division of Pediatric Pulmonary Medicine, UPMC Children's Hospital of Pittsburgh, University of Pittsburgh, Pittsburgh, PA, USA. [2]Genomics and Health Group, Department of Biochemistry, Microbiology, Cell Biology and Genetics, Universidad de La Laguna, La Laguna, Santa Cruz de Tenerife, Spain. [3]CIBER de Enfermedades Respiratorias, Instituto de Salud Carlos III, Madrid, Spain. [4]University of Groningen, University Medical Center Groningen, Dept. of Pediatric Pulmonology and Pediatric Allergy, Beatrix Children's Hospital, and [5]University of Groningen, University Medical Center Groningen, GRIAC Research Institute, Groningen, The Netherlands. [6]Instituto de Ciências da Saúde, Universidade Federal da Bahia, Vale do Canela, Salvador, Bahia, Brazil. [7]Department of Medicine, University of California San Francisco, San Francisco, CA, USA. [8]Behavioral Sciences Research Institute, University of Puerto Rico, San Juan, Puerto Rico. [9]Department of Pediatrics, Hospital Nacional de Niños, San José, Costa Rica. [10]Department of Pediatrics, University of Connecticut, Farmington, CT, USA. [11]CiiM and TWINCORE, joint ventures between the Hannover Medical School and the Helmholtz Centre for

Infection Research, Hannover, Germany. [12]*Department of Internal Medicine and Radboud Center for Infectious Diseases, Radboud University Medical Center, Nijmegen, the Netherlands.* [13]*Channing Division of Network Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA.* [14]*Department of Microbiology and Molecular Genetics, University of Pittsburgh, Pittsburgh, PA, USA.* [15]*Instituto de Saúde Coletiva, Federal University of Bahia, Salvador, Brazil.*

[#]*Shared first authors. ^Shared senior authors.*

***Corresponding author:** Juan C. Celedón, MD, DrPH, ATSF
Division of Pulmonary Medicine
UPMC Children's Hospital of Pittsburgh
4401 Penn Avenue, Pittsburgh, PA 15224
Phone: 412.692.8429; Fax 412.692.7636;
Email: juan.celedon@chp.edu

METHODS

Study populations included in the meta-analysis of GWAS of severe exacerbations

Hartford-Puerto Rico study (HPR): From September 2003 to June 2010, children with and without asthma were recruited in Hartford, (CT) and San Juan (PR), as reported elsewhere [1]; only children with asthma (n=618) were considered for the current analysis. All participants were 6 to 14 years old and had four Puerto Rican grandparents, and asthma was defined as physician-diagnosed asthma and ≥1 episode of wheeze in the prior year. Genome-wide genotyping was conducted using the HumanOmni2.5 BeadChip platform (Illumina Inc., San Diego, CA), as previously described [2]. Genotype imputation was performed with the Michigan Imputation Server [3], using the Haplotype Reference Consortium (HRC) r1.1 2016 [4] as the reference panel. Genotyped or imputed SNPs with imputation quality r^2 <0.3 or Hardy-Weinberg equilibrium (HWE) P $<1\times10^{-4}$ or minor allele frequency MAF <0.05 were excluded from the analysis. Written parental consent was obtained for participating children, from whom written assent was also obtained. The study was approved by the Institutional Review Boards of the University of Puerto Rico (San Juan, PR), Brigham and Women's Hospital (Boston, MA) and the University of Pittsburgh (Pittsburgh, PA).

The Genetics of Asthma in Latino Americans study (GALA II): Subjects were recruited using a combination of community and clinic-based approaches from centers throughout the U.S. (Chicago [IL], Bronx [NY], Houston [TX], San Francisco Bay Area [CA] and Puerto Rico). Subjects were eligible if they were aged 8 to 21 years, had <10 pack-years of smoking history and were not current smokers, and had four grandparents of Hispanic or Latino ethnicity. Asthma was defined based on a physician's diagnosis and self-reported symptoms and medication use for asthma within the last two years. Genotyping was conducted with the Axiom® LAT1 array (World Array 4, Affymetrix, Santa Clara, CA), and QC was performed as previously

described [5]. Imputation was performed using the Michigan Imputation Server [3], using HRC r1.1 2016 [4] as reference, and only SNPs with MAF ≥0.05 and imputation quality r^2≥0.3 were kept. The study was approved by the Institutional Review Boards of UCSF and at each participating center. All subjects and their parents provided written informed assent and written informed consent, respectively.

The Genetics of Asthma in Costa Rica Study (GACRS): Subject recruitment and study procedures in the GACRS have been described elsewhere [6, 7]. In brief, Costa Rican children ages 6 to 14 years were recruited from February 2001 to July 2011. Children were included in the study if they had asthma (defined as physician-diagnosed asthma and at least two respiratory symptoms [wheezing, cough, or dyspnea] or a history of asthma attacks in the previous year) and a high probability of having at least 6 great-grandparents born in the Central Valley of Costa Rica. Genome-wide genotyping was conducted using the HumanOmniExpress-12v1_A chip [6]. Genotype imputation was performed with the Michigan Imputation Server [3], using the Haplotype Reference Consortium (HRC) r1.1 2016 [4] as the reference panel, with QC measures as in the HPR study. In addition, since the original data were for nuclear families, SNPs with Mendelian error rate ≥0.01 were excluded from the analysis. The study was approved by the Institutional Review Boards of the Hospital Nacional de Niños (San Jose, Costa Rica) and Brigham and Women's Hospital (Boston, Mass).

The Social Changes, Asthma and Allergy in Latin America study (SCAALA) – Bahia, Brazil: Children ages 5 to 12 years were recruited in 2005. Subject recruitment and the study protocol have been described in detail [8]. Genotyping was carried out using the Illumina HumanOmni2.5-8v1 Kit BeadChip (Illumina, San Diego, CA) platform. QC measures for the genotypic data were similar to those in the HPR study. Written informed consent was obtained

from the legal guardian of each subject. The study was approved by the ethics committees at the Federal University of Bahia and National Council for Ethics in Research.

Study populations included in molecular quantitative trait analyses in nasal epithelium

The Epigenetic Variation and childhood Asthma in Puerto Rico study (EVA-PR): In EVA-PR, children with and without asthma (aged 9-20 years) were recruited in San Juan (PR) from February 2014 to May 2017, using a similar approach to that used in the HPR study [9]. DNA and RNA were extracted from nasal specimens collected from the inferior turbinate, as reported elsewhere [9]. For whole-genome methylation QC, the R package *ENmix* was used to filter CpG probes with obvious multimodal distributions [10]. Cross-reactive and SNP-containing probes [11], sex chromosomal probes, and low-quality probes (>10% of samples with detection p-values >0.01) were removed. We further removed CpG probes with mean β-value <0.1 or >0.9 [12]. Methylation β-values were calculated as a percentage: $\beta=M/(M+U+\alpha)$, where M and U represent methylated and unmethylated signal intensities, respectively, and α is an arbitrary offset to stabilize β-values where fluorescent intensities are low. β-values were then transformed to M-values as $\log_2(\beta/(1-\beta))$. For RNA-Seq QC, FastQC was used to check read quality in raw fastq files [13]. Low quality reads and 3' adapters were trimmed with Trim Galore! and Cutadapt [14, 15]. Saved reads were aligned to reference human genome (hg19) with STAR [16] and TPM (Transcripts Per Kilobase Million) was used as proxy for gene expression level. Samples with low alignment percentage were removed from downstream analyses. Furthermore, low expressed genes with mean TPM <0.5 were removed. The study was approved by the institutional review boards of the University of Puerto Rico (San Juan, PR) and the University of Pittsburgh (Pittsburgh, PA). Written parental consent and assent were obtained from participants <18 years old, and consent was obtained from participants ≥18 years old.

PIAMA: Details of the study design and protocol have been previously published [17, 18]. The Medical Ethical Committees of the participating institutes approved the study, and the parents and legal guardians of all participants, as well as the participants themselves, gave written informed consent. At the age of 16 years, nasal epithelial cells were collected at two study centers (Groningen and Utrecht) [19] by brushing the lateral area underneath the right inferior turbinate. DNA methylation data were pre-processed with Bioconductor package *minfi* [20], using the original IDAT files from the HiScanSQ scanner. Samples with call rate <99% were removed. 65 SNP probes were used to check for concordance between paired DNA samples (nasal and blood DNA samples from the same subjects were hybridized in the same experiments); paired samples with Pearson correlation coefficient <0.9 were excluded, as were probes on sex chromosomes, probes that mapped to multiple loci, 65 SNP-probes, and probes containing SNPs at the target CpG sites with a MAF>0.05 [11]. "DASEN" [21] was used to perform signal correction and normalization. After QC, 455 samples and 436 824 probes remained, and 432 samples had matched genotype data.

Meta-analysis of GWAS of SAEs

METAL [22] takes *P*-values across independent studies as input, with MAF, sample size and effect direction considered. After the test allele was determined, a Z-score was calculated in each study:

$$Z_i = \Phi^{-1}\left(1 - {}^{P_i}/_{2}\right) \times \mathrm{sign}(\Delta_i),$$

where Z_i is the Z-score for study *i*, P_i is the *P*-value for study *i*, Δ_i is the direction of effect for study *i*, and Φ^{-1} gives the percentile of a standard normal distribution. Then, the meta Z-score and *P*-value can be calculated,

$$Z = \frac{\sum_i Z_i w_i}{\sqrt{\sum_i w_i^2}}, \qquad P = 2\Phi(|-Z|)$$

where Z is the meta Z-score, P is the meta *P*-value, and w_i is the weight for study *i*,

$$w_i = \frac{MAF_i(1 - MAF_i)N_i^{cas}N_i^{con}}{(N_i^{cas} + N_i^{con})}$$

where MAF_i is the minor allele frequency for study *i*, N_i^{cas} is the number of cases for study *i* and N_i^{con} is the number of controls for study *i*. This weighting is intended to assign larger weights to studies with larger sample size, more balanced case-control numbers and higher MAF [23]. Summary odds ratios (ORs) were calculated by averaging the study-specific log-odds ratios, with weights reflecting the standard errors from the study-specific ORs. Specifically,

$$\mathrm{OR} = exp\left(\sum_i \frac{log(\mathrm{OR}_i)}{(\mathrm{SE}_i)^2} \Big/ \sum_i \frac{1}{(\mathrm{SE}_i)^2}\right)$$

where SE_i is the standard error of log(OR) for study *i*.

Higgin's & Thompson's I^2

We first need to calculate Cochran's *Q*-statistic, which is calculated as the weighted sum of squared differences between individual study effects and the pooled effect across studies, with the weights being those used in the pooling method.

$$Q = \sum_i \frac{1}{(\mathrm{SE}_i)^2}\left(log(\mathrm{OR}_i) - \sum_i \frac{log(\mathrm{OR}_i)}{(\mathrm{SE}_i)^2} \Big/ \sum_i \frac{1}{(\mathrm{SE}_i)^2}\right)^2$$

where SE_i is the standard error of log(OR) for study *i*. We then calculated I^2 by using

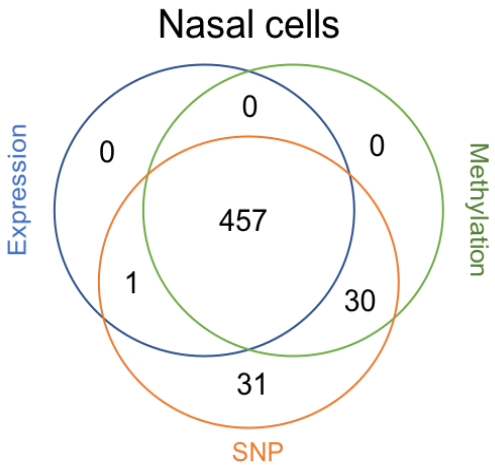
$$I^2 = max\left\{0, \quad \frac{Q - (K - 1)}{Q}\right\}$$

where *K* is the number of studies, which is 4.

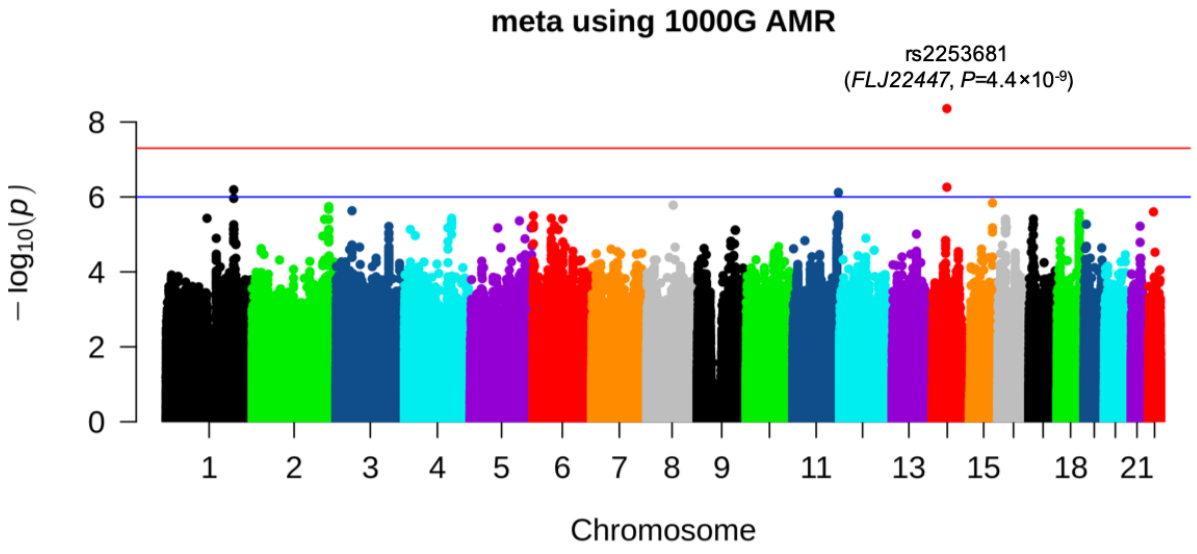
REFERENCES

1. Brehm JM, Acosta-Perez E, Klei L*, et al.* African ancestry and lung function in Puerto Rican children. *J Allergy Clin Immunol* 2012: 129(6): 1484-1490 e1486.
2. Brehm JM, Acosta-Perez E, Klei L*, et al.* Vitamin D insufficiency and severe asthma exacerbations in Puerto Rican children. *Am J Respir Crit Care Med* 2012: 186(2): 140-146.
3. Das S, Forer L, Schonherr S*, et al.* Next-generation genotype imputation service and methods. *Nat Genet* 2016: 48(10): 1284-1287.

4. McCarthy S, Das S, Kretzschmar W*, et al.* A reference panel of 64,976 haplotypes for genotype imputation. *Nat Genet* 2016: 48(10): 1279-1283.
5. Pino-Yanes M, Thakur N, Gignoux CR*, et al.* Genetic ancestry influences asthma susceptibility and lung function among Latinos. *J Allergy Clin Immunol* 2015: 135(1): 228-235.
6. Hunninghake GM, Soto-Quiros ME, Avila L*, et al.* Polymorphisms in IL13, total IgE, eosinophilia, and asthma exacerbations in childhood. *J Allergy Clin Immunol* 2007: 120(1): 84-90.
7. Hunninghake GM, Soto-Quiros ME, Avila L*, et al.* Sensitization to Ascaris lumbricoides and severity of childhood asthma in Costa Rica. *J Allergy Clin Immunol* 2007: 119(3): 654-661.
8. Barreto ML, Cunha SS, Alcantara-Neves N*, et al.* Risk factors and immunological pathways for asthma and other allergic diseases in children: background and methodology of a longitudinal study in a large urban center in Northeastern Brazil (Salvador-SCAALA study). *BMC Pulm Med* 2006: 6: 15.
9. Forno E, Wang T, Qi C*, et al.* DNA methylation in nasal epithelium, atopy, and atopic asthma in children: a genome-wide study. *Lancet Respir Med* 2019: 7(4): 336-346.
10. Xu Z, Niu L, Li L*, et al.* ENmix: a novel background correction method for Illumina HumanMethylation450 BeadChip. *Nucleic Acids Res* 2016: 44(3): e20.
11. Chen YA, Lemire M, Choufani S*, et al.* Discovery of cross-reactive probes and polymorphic CpGs in the Illumina Infinium HumanMethylation450 microarray. *Epigenetics* 2013: 8(2): 203-209.
12. Chen W, Wang T, Pino-Yanes M*, et al.* An epigenome-wide association study of total serum IgE in Hispanic children. *J Allergy Clin Immunol* 2017: 140(2): 571-577.
13. Bioinformatics B. FastQC A quality control tool for high throughput sequence data. *Cambridge, UK: Babraham Institute* 2011.
14. Martin M. Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet journal* 2011: 17(1): pp. 10-12.
15. Krueger F. Trim Galore!: A wrapper tool around Cutadapt and FastQC to consistently apply quality and adapter trimming to FastQ files. 2015.
16. Dobin A, Davis CA, Schlesinger F*, et al.* STAR: ultrafast universal RNA-seq aligner. *Bioinformatics* 2013: 29(1): 15-21.
17. Brunekreef B, Smit J, de Jongste J*, et al.* The prevention and incidence of asthma and mite allergy (PIAMA) birth cohort study: design and first results. *Pediatr Allergy Immunol* 2002: 13(s15): 55-60.
18. Wijga A, Smit HA, Brunekreef B*, et al.* Are children at high familial risk of developing allergy born into a low risk environment? The PIAMA Birth Cohort Study. Prevention and Incidence of Asthma and Mite Allergy. *Clin Exp Allergy* 2001: 31(4): 576-581.
19. Xu CJ, Soderhall C, Bustamante M*, et al.* DNA methylation in childhood asthma: an epigenome-wide meta-analysis. *Lancet Respir Med* 2018: 6(5): 379-388.
20. Aryee MJ, Jaffe AE, Corrada-Bravo H*, et al.* Minfi: a flexible and comprehensive Bioconductor package for the analysis of Infinium DNA methylation microarrays. *Bioinformatics* 2014: 30(10): 1363-1369.
21. Pidsley R, CC YW, Volta M*, et al.* A data-driven approach to preprocessing Illumina 450K methylation array data. *BMC Genomics* 2013: 14: 293.
22. Willer CJ, Li Y, Abecasis GR. METAL: fast and efficient meta-analysis of genomewide association scans. *Bioinformatics* 2010: 26(17): 2190-2191.
23. Yan Q, Brehm J, Pino-Yanes M*, et al.* A meta-analysis of genome-wide association studies of asthma in Puerto Ricans. *Eur Respir J* 2017: 49(5).



<u>Supplementary Figure 1</u> – The omics data distribution from the EVA-PR cohort
Expression and methylation in nasal airway epithelial cells



<u>Supplementary Figure 2</u> – Manhattan plot of meta-analysis results using 1000 Genome AMR as the imputation reference panel: Manhattan plot showing the summary meta-analysis results of HPR, GALA II, GACRS, and SCAALA. HPR, GALA II and GACRS were re-imputed using 1000 Genome AMR as the reference panel. SCAALA was not imputed.

Supplementary Table 1: Meta-analysis of GWAS of asthma exacerbation, for SNPs associated with asthma in two previous meta-analyses of multi-ancestry population and UK Biobank

SNP	CHR: BP	Alt	Ref	In Cited papers		HPR			GACRS			GALA II			SCAALA			Meta	Nearby genes
				OR*	P#	AAF	OR	*P*	AAF	OR	*P*	AAF	OR	*P*	AAF	OR	*P*	*P*	
Loci reported by Demenais, F. *et al*. *Nat Genet* 2018; 50: 42-53																			
rs7705042	5: 141 492 419	C	A	0.92	7.90E-09	0.31	1.18	0.22	0.33	0.98	0.89	0.31	1.02	0.79	NA	NA	NA	0.51	*NDFIP1,GNDPA1,SPRY4*
rs1233578	6: 28 712 247	G	A	1.09	5.90E-07	0.24	0.94	0.69	0.15	0.99	0.95	0.19	1.13	0.18	0.30	0.81	0.30	0.38	*GPX5,TRIM27*
rs2325291	6: 90 986 686	A	G	0.91	2.20E-12	0.24	0.96	0.78	0.25	1.09	0.53	0.26	1.06	0.41	NA	NA	NA	0.42	*BACH2,GJA10,MAP3K7*
rs167769	12: 57 503 775	T	C	1.08	3.90E-09	0.33	1.02	0.87	0.40	0.96	0.72	0.39	1.04	0.62	NA	NA	NA	0.75	*STAT6,NAB2,LRP1*
rs17637472	17: 47 461 433	A	G	1.08	6.60E-09	0.29	0.86	0.30	0.25	1.08	0.60	0.23	1.05	0.57	NA	NA	NA	0.83	*ZNF652,PHB*
rs2855812	6: 31 472 720	T	G	1.10	8.90E-12	0.19	1.08	0.63	0.12	1.02	0.91	0.17	1	1.00	0.18	0.83	0.48	0.81	*MICB,HCP5,MCCD1*
rs2589561	10: 9 046 645	A	G	1.10	3.50E-09	0.13	1.02	0.92	0.11	1.14	0.52	0.12	1.13	0.26	0.18	1.17	0.48	0.23	*GATA3,CELF2*
rs12543811	8: 81 278 885	G	A	1.09	1.10E-10	0.42	1.01	0.96	0.45	1.05	0.68	0.47	1.13	0.07	NA	NA	NA	0.10	*TPD52,ZBTB10*
rs17806299	16: 11 199 980	A	G	0.91	2.70E-10	0.16	0.74	0.09	0.16	0.98	0.91	0.12	1.03	0.79	0.08	0.95	0.87	0.57	*CLEC16A,DEXI,SOCS1*
rs1420101	2: 102 957 716	T	C	1.12	3.90E-21	0.33	1.15	0.31	0.31	1.17	0.24	0.30	1.04	0.57	0.35	1.27	0.24	0.17	*IL1RL1,IL1RL2,IL18R1*
rs10455025	5: 110 404 999	C	A	1.15	9.40E-26	0.28	0.98	0.90	0.32	1.09	0.51	0.26	1.06	0.46	0.18	1.29	0.30	0.40	*SLC25A46,TSLP*
rs20541	5: 131 995 964	A	G	1.12	5.00E-16	0.23	0.92	0.58	0.31	0.85	0.23	0.36	1	0.98	0.20	1.24	0.33	0.47	*IL13,RAD50,IL4*
rs9272346	6: 32 604 372	G	A	0.86	5.70E-24	0.39	1.02	0.89	0.37	0.94	0.62	0.33	0.91	0.19	NA	NA	NA	0.23	*HLA-DRB1,HLA-DQA1*
rs992969	9: 6 209 697	A	G	0.86	7.20E-20	0.28	1.00	0.99	0.22	1.12	0.44	0.24	0.99	0.92	0.31	0.84	0.40	0.81	*RANBP6,IL33*
rs7927894	11: 76 301 316	T	C	1.10	2.20E-14	0.37	0.79	0.08	0.30	1.30	0.06	0.29	1	0.96	NA	NA	NA	0.99	*EMSY,LRRC32*
rs11071558	15: 61 069 421	G	A	0.89	1.30E-09	0.23	1.07	0.64	NA	NA	NA	0.18	1.06	0.51	NA	NA	NA	0.42	*RORA,NARG2,VPS13C*
rs2033784	15: 67 449 660	G	A	1.10	7.40E-15	0.37	0.98	0.87	0.39	0.58	1.19E-5	0.37	1	0.97	0.39	0.99	0.96	0.05	*SMAD3,SMAD6,AAGAB*
rs2952156	17: 37 876 835	A	G	1.15	2.20E-30	0.45	1.23	0.09	0.52	1.02	0.85	0.44	1.02	0.78	0.42	1.28	0.17	0.32	*ERBB2,PGAP3,MIEN1*
Loci reported by Zhu, *Z. et al*. *Nat Genet* 2018; 50: 857-864																			
rs7936070	11: 76 293 527	T	G	1.08	2.81E-28	0.47	0.92	0.53	0.42	1.23	0.09	0.45	1	0.98	NA	NA	NA	0.64	*C11orf30,LOC100506127,PRKRIR*
rs72823641	2: 102 936 159	A	T	0.89	1.58E-27	0.11	0.79	0.27	0.08	0.94	0.77	0.08	1.1	0.45	NA	NA	NA	0.98	*IL1R1,IL1RL1,IL1RL2,IL18R1,IL18RAP,MIR4772,SLC9A2, SLC9A4*
rs56062135	15: 67 455 630	T	C	1.16	1.56E-22	0.18	1.20	0.26	0.18	0.61	0.0005	0.15	1.21	0.04	0.11	1.16	0.62	0.58	*SMAD3*
rs36045143	16: 11 224 966	G	A	0.93	1.83E-21	0.20	0.82	0.20	0.18	0.93	0.62	0.16	0.96	0.67				0.27	*CLEC16A,DEXI*
rs1837253	5: 110 401 872	T	C	0.93	4.38E-21	0.23	0.90	0.50	0.23	0.99	0.96	0.27	0.89	0.14	0.24	0.90	0.62	0.13	*TSLP*
rs7705653	5: 110 142 816	G	A	1.14	1.12E-19	0.26	0.83	0.19	0.21	1.16	0.34	0.25	0.92	0.33	NA	NA	NA	0.34	*SLC25A46,TMEM232*
rs28393318	4: 38 784 267	G	A	0.92	2.14E-19	0.39	0.88	0.32	0.25	1.00	0.98	0.28	1	0.95	NA	NA	NA	0.69	*FAM114A1,MIR574,TLR1,TLR6,TLR10*
rs869402	17: 38 068 043	T	C	0.89	4.15E-17	0.29	0.66	0.003	0.34	1.01	0.96	0.31	0.96	0.59	NA	NA	NA	0.11	*ERBB2,GRB7,GSDMA,GSDMB,IKZF3,LRRC3C,MIEN1, MIR4728,ORMDL3,PGAP3,PNMT,STARD3,TCAP,ZPBP2*
rs34290285	2: 242 698 640	A	G	0.93	5.17E-17	0.26	1.16	0.33	NA	NA	NA	0.21	0.92	0.30	NA	NA	NA	0.66	*D2HGDH,GAL3ST2*
rs10174949	2: 8 442 248	A	G	0.94	1.70E-16	0.24	1.11	0.46	0.23	0.89	0.41	0.26	0.93	0.37	NA	NA	NA	0.44	*LINC00299*
rs9911533	17: 3 877 5476	C	T	0.92	9.70E-16	0.33	1.09	0.55	0.35	1.11	0.42	0.31	0.95	0.52	NA	NA	NA	0.94	*KRT24,KRT222,SMARCE1*
rs12413578	10: 9 049 253	T	C	0.91	1.09E-14	0.10	0.72	0.15	0.10	0.99	0.96	0.07	1.02	0.88	NA	NA	NA	0.58	*HV745896*
rs6881270	5: 35 879 095	T	C	0.91	1.53E-14	0.18	0.97	0.87	0.17	0.93	0.63	0.18	1.05	0.58	NA	NA	NA	0.85	*CAPSL,IL7R,LOC100506406,SPEF2,UGT3A1*
rs10876864	12: 56 401 085	G	A	1.05	1.41E-13	0.50	0.96	0.73	0.33	1.25	0.09	0.37	1.07	0.37	0.60	0.95	0.77	0.20	*CDK2,ERBB3,IKZF4,PA2G4,RAB5B,RPL41,RPS26,SUOX, ZC3H10*
rs1059513	12: 57 489 709	C	T	0.92	7.65E-13	0.11	0.98	0.93	0.08	1.06	0.79	0.09	1.16	0.23	0.10	0.72	0.27	0.30	*GPR182,MYO1A,NAB2,RDH16,SDR9C7,STAT6,TAC3, TMEM194A,ZBTB39*
rs56267605	4: 123 363 109	C	A	1.05	2.56E-12	0.30	1.02	0.90	0.44	0.89	0.33	0.37	0.94	0.42	NA	NA	NA	0.31	*ADAD1,IL2,IL21,IL21-AS1,KIAA1109*
rs61839660	10: 6 094 697	T	C	1.12	2.30E-11	0.05	1.13	0.67	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.67	*IL2RA,RBM17*
rs2706362	5: 131 925 187	C	T	1.06	3.75E-11	0.32	0.95	0.73	0.19	1.19	0.27	0.26	1.04	0.60	NA	NA	NA	0.49	*IL13,RAD50*
rs1214598	1: 167 426 424	A	G	0.95	5.14E-11	0.28	1.26	0.10	0.27	1.06	0.65	0.25	1.05	0.52	NA	NA	NA	0.16	*CD247*
rs659529	11: 11 143 6896	T	A	0.95	6.03E-11	0.42	1.13	0.36	0.27	1.12	0.40	0.31	1.06	0.43	NA	NA	NA	0.17	*ALG9,BTG4,C11orf1,C11orf88,FDXACB1,LAYN,MIR34B, MIR34C,PPP2R1B,SIK2*
rs2766664	20: 52 171 241	A	G	1.08	8.07E-11	0.20	0.94	0.68	0.23	1.03	0.85	0.22	0.83	0.02	0.26	0.92	0.71	0.05	*LOC101927770,ZNF217*
rs2169282	9: 6 350 235	A	G	1.09	1.80E-10	0.49	0.97	0.80	0.40	1.13	0.34	0.41	1	0.97	NA	NA	NA	0.80	*GLDC,UHRF2*
rs10414065	19: 33 721 455	T	C	0.91	2.63E-10	0.05	0.96	0.90	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.90	*SLC7A10*
rs6461503	7: 20 560 996	C	T	0.95	3.19E-10	0.49	0.99	0.93	0.37	0.91	0.45	0.44	1.02	0.80	0.61	0.77	0.14	0.89	*ITGB8*

Ref: reference allele; Alt: alternative allele; AAF: alternative allele frequency; OR: odds ratio. Human Genome version: hg19.

*: This column is odds ratio from multi-ancestry meta-analysis in Demenais, F. *et al*. *Nat Genet* 2018; 50: 42-53 and from asthma/allergy meta-analysis in Zhu, Z. *et al*. *Nat Genet* 2018; 50: 857-864.

#: This column is *P*-value from multi-ancestry meta-analysis in Demenais, F. *et al*. *Nat Genet* 2018; 50: 42-53 and from asthma/allergy meta-analysis in Zhu, Z. *et al*. *Nat Genet* 2018; 50: 857-864.

<u>Supplementary Table 2</u>: Analysis of association between previously published SNPs for asthma exacerbations or hospitalizations and severe asthma exacerbations in the current meta-analysis of GWAS

SNP	CHR: BP	Gene	Alt	Ref	HPR			GACRS			GALA II			SCAALA			Meta	Ref
					AAF	OR	*P*	AAF	OR	*P*	AAF	OR	*P*	AAF	OR	*P*	*P*	
rs1800925	5: 131 992 809	*IL13*	T	C	0.28	0.86	0.31	0.20	0.98	0.88	0.28	1.03	0.71	NA	NA	NA	*0.86*	*1*
rs1805011	16: 27 373 872	*IL4RA*	C	A	0.24	1.00	1.00	0.15	0.81	0.22	0.20	1.01	0.89	NA	NA	NA	*0.73*	*2*
rs1801275	16: 27 374 400	*IL4RA*	G	A	0.37	0.91	0.50	0.29	0.91	0.46	0.35	1.05	0.50	0.51	1.02	0.92	*0.99*	*2*
rs4950928	1: 203 155 882	*CHI3L1*	G	C	0.18	0.73	0.08	0.17	0.92	0.60	0.17	1.09	0.35	NA	NA	NA	*0.09*	*3*
rs7216389	17: 38 069 949	*ORMDL3*	C	T	0.30	0.63	1.6E-3	0.34	0.99	0.97	0.31	1.04	0.59	0.28	0.84	0.38	*0.06*	*4*
rs6967330	7: 105 658 451	*CDHR3*	A	G	0.26	1.20	0.21	0.16	1.13	0.49	0.23	1.06	0.49	0.26	0.98	0.92	*0.20*	*5*
rs1099729	12: 97 251 586	*CTNNA3*	C	T	0.06	1.11	0.69	NA	NA	NA	0.06	1.16	0.30	NA	NA	NA	*0.46*	*6*
rs9587342	13: 107 936 790	*FAM155A*	A	G	0.41	0.93	0.59	0.47	0.97	0.82	0.45	0.99	0.84	0.40	1.33	0.13	*0.95*	*7**
rs6426881	1: 164 816 726	*PBX1*	T	C	0.11	1.20	0.36	0.12	0.99	0.96	0.13	0.94	0.55	0.09	1.06	0.86	*0.92*	*7*
rs1074119	7: 3 196 333	*CARD11*	T	C	0.44	0.99	0.92	0.41	1.07	0.58	0.44	0.95	0.48	NA	NA	NA	*0.44*	*7*
rs858928	2: 50 892 009	*NRXN1*	A	C	0.18	0.96	0.80	0.15	1.28	0.18	0.18	1.03	0.77	NA	NA	NA	*0.51*	*7*
rs12201938	6: 7 026 562	*RREB1*	A	G	0.09	0.82	0.40	0.08	0.64	0.03	0.08	1.09	0.52	0.05	1.09	0.83	*0.52*	*7*
rs9325122	5:148 202 936	*ADRβ2*	C	T	0.28	0.98	0.92	0.23	1.07	0.66	0.22	0.92	0.32	NA	NA	NA	*0.51*	*8*
rs1432622	5:148203762	*ADRβ2*	T	C	0.37	1.09	0.53	0.26	1.09	0.54	0.30	0.92	0.26	NA	NA	NA	*0.70*	*8*
rs1432623	5:148204008	*ADRβ2*	C	T	0.37	1.09	0.53	0.26	1.09	0.54	0.30	0.92	0.26	NA	NA	NA	*0.70*	*8*
rs11168068	5:148204121	*ADRβ2*	C	T	0.37	1.09	0.53	0.26	1.09	0.54	0.30	0.92	0.26	NA	NA	NA	*0.70*	*8*
rs17778257	5:148204577	*ADRβ2*	T	A	0.37	1.18	0.20	0.42	1.05	0.70	0.37	1.03	0.66	NA	NA	NA	*0.29*	*8*
rs2400706	5:148204864	*ADRβ2*	T	C	0.26	0.72	0.03	0.31	0.88	0.32	0.32	1.04	0.55	NA	NA	NA	*0.43*	*8*
rs2895795	5:148204966	*ADRβ2*	A	T	0.26	0.72	0.03	0.31	0.88	0.32	0.33	1.05	0.54	NA	NA	NA	*0.45*	*8*
rs2400707	5:148205052	*ADRβ2*	A	G	0.37	1.10	0.49	0.26	1.09	0.54	0.29	0.92	0.27	NA	NA	NA	*0.73*	*8*
rs2053044	5:148205372	*ADRβ2*	A	G	0.37	1.10	0.49	0.26	1.09	0.52	0.29	0.92	0.25	NA	NA	NA	*0.71*	*8*
rs12654778	5:148205741	*ADRβ2*	A	G	0.37	1.18	0.20	0.42	1.04	0.72	0.37	1.02	0.74	0.31	0.65	0.02	*0.75*	*8*
rs11168070	5:148205927	*ADRβ2*	G	C	0.28	0.98	0.89	0.23	1.06	0.70	0.22	0.92	0.30	NA	NA	NA	*0.47*	*8*
rs11959427	5:148206028	*ADRβ2*	C	T	0.29	0.97	0.86	0.23	1.06	0.70	0.22	0.92	0.30	NA	NA	NA	*0.46*	*8*
rs1801704	5:148206375	*ADRβ2*	C	T	0.29	0.98	0.90	0.23	1.04	0.78	0.22	0.92	0.27	0.23	1.17	0.45	*0.56*	*8*
rs1042713	5:148206440	*ADRβ2*	A	G	0.45	1.29	0.05	0.46	1.07	0.58	0.44	1.01	0.92	0.46	0.89	0.52	*0.36*	*8*
rs1042714	5:148206473	*ADRβ2*	G	C	0.29	0.97	0.86	0.23	1.04	0.78	0.22	0.92	0.27	0.23	1.16	0.48	*0.53*	*8*
rs1042717	5:148206646	*ADRβ2*	A	G	0.26	0.70	0.02	0.31	0.90	0.39	0.33	1.06	0.43	0.31	0.97	0.89	*0.54*	*8*
rs1042718	5:148206917	*ADRβ2*	A	C	0.24	0.67	0.01	0.28	0.88	0.36	0.31	1.12	0.13	0.29	1.01	0.97	*0.91*	*8*
rs1042720	5:148207633	*ADRβ2*	A	G	0.43	0.79	0.06	NA	NA	NA	0.46	1.00	0.98	0.46	1.25	0.22	*0.69*	*8*

Ref: reference allele; Alt: alternative allele; AAF: alternative allele frequency; OR: odds ratio. Human Genome version: hg19.

* Top 5 out of 160 SNPs used in asthma exacerbations prediction in 7 are shown.

1. *Hunninghake GM, Soto-Quiros ME, Avila L, Su J, Murphy A, Demeo DL, et al. Polymorphisms in IL13, total IgE, eosinophilia, and asthma exacerbations in childhood. J Allergy Clin Immunol 2007; 120:84-90.*
2. *Wenzel SE, Balzar S, Ampleford E, Hawkins GA, Busse WW, Calhoun WJ, et al. IL4R alpha mutations are associated with asthma exacerbations and mast cell/IgE expression. Am J Respir Crit Care Med 2007; 175:570-6.*
3. *Cunningham J, Basu K, Tavendale R, Palmer CN, Smith H, Mukhopadhyay S. The CHI3L1 rs4950928 polymorphism is associated with asthma-related hospital admissions in children and young adults. Ann Allergy Asthma Immunol 2011; 106:381-6.*
4. *Bisgaard H, Bonnelykke K, Sleiman PM, Brasholt M, Chawes B, Kreiner-Moller E, et al. Chromosome 17q21 gene variants are associated with asthma and exacerbations but not atopy in early childhood. Am J Respir Crit Care Med 2009; 179:179-85.*
5. *Bonnelykke K, Sleiman P, Nielsen K, Kreiner-Moller E, Mercader JM, Belgrave D, et al. A genome-wide association study identifies CDHR3 as a susceptibility locus for early childhood asthma with severe exacerbations. Nat Genet 2014; 46:51-5.*
6. *McGeachie MJ, Wu AC, Tse SM, Clemmer GL, Sordillo J, Himes BE, et al. CTNNA3 and SEMA3D: Promising loci for asthma exacerbation identified through multiple genome-wide association studies. J Allergy Clin Immunol 2015; 136:1503-10.*
7. *Xu M, Tantisira KG, Wu A, Litonjua AA, Chu JH, Himes BE, et al. Genome Wide Association Study to predict severe asthma exacerbations in children using random forests classifiers. BMC Med Genet 2011; 12:90.*
8. *Hawkins GA, Tantisira K, Meyers DA, Ampleford EJ, Moore WC, Klanderman B, et al. Sequence, Haplotype, and Association Analysis of ADRβ2 in a Multiethnic Asthma Case-Control Study. Am J Respir Crit Care Med 2006; 174:1101–9.*

Supplementary Table 3: Top 20 eQTLs for rs2253681 in EVA-PR nasal epithelial cells and replication results from PIAMA

Gene	Chr	Start	End	EVA-PR			PIAMA		Meta-analysis	
				Effect	*P*-value	*FDR*[*]	Effect	*P*-value	Effect	*P*-value
SRF	6	43 139 032	43 149 244	-0.0723	5.84×10^{-4}	1.00	-0.0921	8.26×10^{-3}	-0.0776	1.64×10^{-5}
OR2A20P	7	143 947 766	143 948 696	-0.1858	6.27×10^{-4}	1.00	NA	NA	NA	NA
RASIP1	19	49 223 841	49 243 970	-0.1083	9.93×10^{-4}	1.00	-0.1064	1.40×10^{-1}	-0.1080	3.08×10^{-4}
LOC100128398	19	58 514 261	58 518 574	-0.0845	1.56×10^{-3}	1.00	NA	NA	NA	NA
SNORA53	12	98 993 412	98 993 662	0.2324	1.56×10^{-3}	1.00	NA	NA	NA	NA
HPGDS	4	95 219 706	95 264 027	0.1359	2.14×10^{-3}	1.00	0.0868	7.27×10^{-1}	0.1344	2.05×10^{-3}
NPTXR	22	39 214 455	39 240 017	-0.0864	2.41×10^{-3}	1.00	-0.1737	5.39×10^{-2}	-0.0943	5.13×10^{-4}
ASCC1	10	73 855 789	73 975 867	0.0352	2.42×10^{-3}	1.00	0.0562	1.44×10^{-1}	0.0369	8.83×10^{-4}
SOX4	6	21 593 971	21 598 849	-0.0840	2.59×10^{-3}	1.00	-0.0367	3.78×10^{-1}	-0.0694	2.76×10^{-3}
ZNF675	19	23 835 707	23 870 017	0.0674	2.82×10^{-3}	1.00	-0.0524	4.63×10^{-1}	0.0565	8.62×10^{-3}
PPIP5K1	15	43 825 659	43 877 090	-0.0356	2.85×10^{-3}	1.00	0.0207	4.89×10^{-1}	-0.0279	1.19×10^{-2}
TCF7L2	10	114 710 008	114 927 436	-0.0598	3.05×10^{-3}	1.00	-0.0266	4.01×10^{-1}	-0.0502	3.19×10^{-3}
SSR4P1	21	46 490 869	46 493 126	0.0693	3.27×10^{-3}	1.00	0.0078	9.56×10^{-1}	0.0676	3.63×10^{-3}
SNORD17	20	17 943 352	17 943 589	0.1407	3.34×10^{-3}	1.00	NA	NA	NA	NA
LOC100130691	2	178 148 235	178 257 419	0.0726	3.41×10^{-3}	1.00	NA	NA	NA	NA
TNNI3	19	55 663 135	55 669 100	-0.1318	3.49×10^{-3}	1.00	NA	NA	NA	NA
ARHGEF5	7	144 052 488	144 077 725	-0.0946	3.61×10^{-3}	1.00	-0.0399	3.49×10^{-1}	-0.0745	3.94×10^{-3}
LOC100132077	9	97 094 757	97 123 230	0.1005	4.04×10^{-3}	1.00	NA	NA	NA	NA
HACE1	6	105 175 967	105 307 794	0.0405	4.08×10^{-3}	1.00	0.0634	1.92×10^{-1}	0.0423	1.79×10^{-3}
NDUFS6	5	1 801 495	1 816 167	-0.0441	4.12×10^{-3}	1.00	0.0262	5.71×10^{-1}	-0.0371	1.09×10^{-2}

[*] *FDR* is adjusted for the whole genome in EVA-PR

<u>Supplementary Table 4</u>: Nominally significant pathways associated with severe asthma exacerbations with genes in the *FLJ22447* locus

Gene*	Pathway	*P*-value
PRKCH	REACTOME_SIGNALING_BY_GPCR	0.0084
PRKCH	REACTOME_PLATELET_ACTIVATION_SIGNALING_AND_AGGREGATION	0.0168
PRKCH	REACTOME_GPCR_DOWNSTREAM_SIGNALING	0.0315
PRKCH	GO_REGULATION_OF_IMMUNE_SYSTEM_PROCESS	0.0004
PRKCH	GO_REGULATION_OF_EPITHELIAL_CELL_DIFFERENTIATION	0.0024
PRKCH	GO_REGULATION_OF_CELL_PROLIFERATION	0.0035
PRKCH	GO_POSITIVE_REGULATION_OF_CELL_PROLIFERATION	0.0055
PRKCH	GO_PROTEIN_PHOSPHORYLATION	0.0153
PRKCH	GO_ENZYME_BINDING	0.0158
PRKCH	GO_POSITIVE_REGULATION_OF_GLIOGENESIS	0.0193
PRKCH	GO_REGULATION_OF_MULTICELLULAR_ORGANISMAL_DEVELOPMENT	0.0205
PRKCH	GO_POSITIVE_REGULATION_OF_EPITHELIAL_CELL_DIFFERENTIATION	0.0209
PRKCH	GO_PLATELET_ACTIVATION	0.0255
PRKCH	GO_POSITIVE_REGULATION_OF_DEVELOPMENTAL_PROCESS	0.0303
PRKCH	GO_POSITIVE_REGULATION_OF_CELL_DIFFERENTIATION	0.0340
PRKCH	GO_POSITIVE_REGULATION_OF_EPIDERMAL_CELL_DIFFERENTIATION	0.0384
PRKCH	GO_RAL_GTPASE_BINDING	0.0398
PRKCH	GO_REGULATION_OF_CELL_DIFFERENTIATION	0.0444
PRKCH	GO_TRANSFERASE_ACTIVITY_TRANSFERRING_PHOSPHORUS_CONTAINING_GROUPS	0.0448
PRKCH	GO_POSITIVE_REGULATION_OF_IMMUNE_SYSTEM_PROCESS	0.0460
PRKCH	GO_ADENYL_NUCLEOTIDE_BINDING	0.0483
SNAPC1	GO_NUCLEAR_TRANSCRIPTION_FACTOR_COMPLEX	0.0028
SNAPC2	GO_TRANSCRIPTION_FACTOR_COMPLEX	0.0295
HIF1A	KEGG_MTOR_SIGNALING_PATHWAY	0.0341
HIF1A	GO_REGULATION_OF_IMMUNE_SYSTEM_PROCESS	0.0004
HIF1A	GO_REGULATION_OF_HEMOPOIESIS	0.0005
HIF1A	GO_COLUMNAR_CUBOIDAL_EPITHELIAL_CELL_DEVELOPMENT	0.0011
HIF1A	GO_CELLULAR_COMPONENT_MORPHOGENESIS	0.0012
HIF1A	GO_CELLULAR_RESPONSE_TO_INTERLEUKIN_1	0.0016
HIF1A	GO_ESTABLISHMENT_OF_LOCALIZATION_BY_MOVEMENT_ALONG_MICROTUBULE	0.0020
HIF1A	GO_RESPONSE_TO_INTERLEUKIN_1	0.0028
HIF1A	GO_NUCLEAR_TRANSCRIPTION_FACTOR_COMPLEX	0.0028
HIF1A	GO_CELL_DEVELOPMENT	0.0029
HIF1A	GO_REGULATION_OF_CELL_PROLIFERATION	0.0035
HIF1A	GO_REGULATION_OF_MYELOID_CELL_DIFFERENTIATION	0.0045
HIF1A	GO_ORGANELLE_TRANSPORT_ALONG_MICROTUBULE	0.0054
HIF1A	GO_POSITIVE_REGULATION_OF_CELL_PROLIFERATION	0.0055
HIF1A	GO_CYTOPLASMIC_REGION	0.0055
HIF1A	GO_EPITHELIAL_CELL_DEVELOPMENT	0.0068
HIF1A	GO_CYTOSKELETON_DEPENDENT_INTRACELLULAR_TRANSPORT	0.0079
HIF1A	GO_EMBRYONIC_HEART_TUBE_MORPHOGENESIS	0.0085
HIF1A	GO_CELL_PROJECTION_CYTOPLASM	0.0104
HIF1A	GO_HEART_MORPHOGENESIS	0.0108
HIF1A	GO_UBIQUITIN_LIKE_PROTEIN_LIGASE_BINDING	0.0117
HIF1A	GO_MORPHOGENESIS_OF_AN_EPITHELIUM	0.0127
HIF1A	GO_POSITIVE_REGULATION_OF_HEMOPOIESIS	0.0128
HIF1A	GO_ACUTE_INFLAMMATORY_RESPONSE	0.0138
HIF1A	GO_RECEPTOR_BINDING	0.0145
HIF1A	GO_RNA_POLYMERASE_II_TRANSCRIPTION_FACTOR_COMPLEX	0.0148
HIF1A	GO_REGULATION_OF_VASCULAR_ENDOTHELIAL_GROWTH_FACTOR_RECEPTOR_SIGNALING_PATHWAY	0.0154
HIF1A	GO_ENZYME_BINDING	0.0158
HIF1A	GO_TISSUE_MORPHOGENESIS	0.0160
HIF1A	GO_COLUMNAR_CUBOIDAL_EPITHELIAL_CELL_DIFFERENTIATION	0.0170
HIF1A	GO_REGULATION_OF_VASCULAR_ENDOTHELIAL_GROWTH_FACTOR_PRODUCTION	0.0177
HIF1A	GO_AXON_PART	0.0186
HIF1A	GO_DIGESTIVE_SYSTEM_DEVELOPMENT	0.0195
HIF1A	GO_REGULATION_OF_EPITHELIAL_CELL_PROLIFERATION	0.0196
HIF1A	GO_AXO_DENDRITIC_TRANSPORT	0.0201
HIF1A	GO_REGULATION_OF_MULTICELLULAR_ORGANISMAL_DEVELOPMENT	0.0205
HIF1A	GO_REGULATION_OF_TRANSCRIPTION_FROM_RNA_POLYMERASE_II_PROMOTER	0.0217
HIF1A	GO_POSITIVE_REGULATION_OF_NUCLEOTIDE_METABOLIC_PROCESS	0.0225

HIF1A	GO_NEGATIVE_REGULATION_OF_MULTICELLULAR_ORGANISMAL_PROCESS	0.0227
HIF1A	GO_CELL_MORPHOGENESIS_INVOLVED_IN_DIFFERENTIATION	0.0229
HIF1A	GO_HEART_DEVELOPMENT	0.0244
HIF1A	GO_TUBE_MORPHOGENESIS	0.0248
HIF1A	GO_POSITIVE_REGULATION_OF_NEUROBLAST_PROLIFERATION	0.0270
HIF1A	GO_EMBRYONIC_HEART_TUBE_DEVELOPMENT	0.0270
HIF1A	GO_CELLULAR_RESPONSE_TO_OXYGEN_LEVELS	0.0277
HIF1A	GO_TRANSCRIPTION_FACTOR_COMPLEX	0.0295
HIF1A	GO_POSITIVE_REGULATION_OF_DEVELOPMENTAL_PROCESS	0.0303
HIF1A	GO_CELLULAR_RESPONSE_TO_STRESS	0.0321
HIF1A	GO_POSITIVE_REGULATION_OF_CELL_DIFFERENTIATION	0.0340
HIF1A	GO_DOPAMINERGIC_NEURON_DIFFERENTIATION	0.0341
HIF1A	GO_STEM_CELL_DIFFERENTIATION	0.0381
HIF1A	GO_POSITIVE_REGULATION_OF_TRANSCRIPTION_FROM_RNA_POLYMERASE_II_PROMOTER	0.0391
HIF1A	GO_CELLULAR_RESPONSE_TO_CYTOKINE_STIMULUS	0.0399
HIF1A	GO_AXON	0.0416
HIF1A	GO_OUTFLOW_TRACT_MORPHOGENESIS	0.0424
HIF1A	GO_TISSUE_REMODELING	0.0432
HIF1A	GO_REGULATION_OF_CELL_DIFFERENTIATION	0.0444
HIF1A	GO_DEVELOPMENTAL_MATURATION	0.0447
HIF1A	GO_POSITIVE_REGULATION_OF_IMMUNE_SYSTEM_PROCESS	0.0460
HIF1A	GO_REGULATION_OF_SMOOTH_MUSCLE_CELL_PROLIFERATION	0.0467
HIF1A	GO_TRANSCRIPTION_FACTOR_ACTIVITY_RNA_POLYMERASE_II_TRANSCRIPTION_FACTOR_BINDING	0.0471
HIF1A	GO_RNA_POLYMERASE_II_TRANSCRIPTION_FACTOR_ACTIVITY_SEQUENCE_SPECIFIC_DNA_BINDING	0.0480

* The genes (*TMEM30B, PRKCH, LOC101927780, HIF1A-AS1, HIF1A-AS2, SNAPC1, FLJ22447* and *HIF1A*) in *FLJ22447* locus and also included in the nominally significant pathways.

Supplementary Table 5: Results for SNP rs2253681 (minor allele = A) and severe asthma exacerbations using different imputation reference panels

Reference panels	HPR (genotyped)			GACRS (imputed)			GALA II† (genotyped)			SCAALA* (genotyped)			Meta	
	MAF	OR	*P*	MAF	OR	*P*	MAF	OR	*P*	MAF	OR	*P*	OR	*P*
HRC	0.20	1.49	0.013	0.11	1.38	0.12	0.16	1.54	2.3E-5	0.24	1.92	3.2E-3	1.55	6.3E-9
1000G AMR	0.20	1.49	0.013	0.11	1.37	0.12	0.16	1.55	1.5E-5				1.55	4.4E-9

SNP rs2253681 was genotyped in HPR, GALA II and SCAALA, and only imputed in GACRS.

†Although rs2253681 was a genotyped SNP in GALA II, there was one sample with missing genotype. This sample had different imputed genotypes for rs2253681 between HRC and 1000G AMR, which caused the small discrepancy in p-values and ORs.

*SCAALA data were not imputed. Thus, SCAALA results were not affected by reference panels.