University of Groningen

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

Yan, Qi; Forno, Erick; Herrera-Luis, Esther; Pino-Yanes, Maria; Qi, Cancan; Rios, Raimon; Han, Yueh-Ying; Kim, Soyeon; Oh, Sam; Acosta-Pérez, Edna

Published in:
European Respiratory Journal

DOI:
[10.1183/13993003.02693-2020](https://doi.org/10.1183/13993003.02693-2020)

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2021

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):

Yan, Q., Forno, E., Herrera-Luis, E., Pino-Yanes, M., Qi, C., Rios, R., Han, Y.-Y., Kim, S., Oh, S., Acosta-Pérez, E., Zhang, R., Hu, D., Eng, C., Huntsman, S., Avila, L., Boutaoui, N., Cloutier, M. M., Soto-Quiros, M. E., Xu, C.-J., ... Celedón, J. C. (2021). A genome-wide association study of severe asthma exacerbations in Latino children and adolescents. *European Respiratory Journal*, 57(4), [2002693]. <https://doi.org/10.1183/13993003.02693-2020>

Copyright

Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license. More information can be found on the University of Groningen website: <https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment>.

Take-down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): <http://www.rug.nl/research/portal>. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.

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

Qi Yan^{1,16}, Erick Forno^{1,16}, Esther Herrera-Luis^{2,16}, Maria Pino-Yanes^{2,3}, Cancan Qi^{4,5}, Raimon Rios⁶, Yueh-Ying Han¹, Soyeon Kim¹, Sam Oh⁷, Edna Acosta-Pérez⁸, Rong Zhang¹, Donglei Hu⁷, Celeste Eng⁷, Scott Huntsman⁷, Lydiana Avila⁹, Nadia Boutaoui¹, Michelle M. Cloutier¹⁰, Manuel E. Soto-Quiros⁹, Cheng-Jian Xu^{11,12}, Scott T. Weiss¹³, Jessica Lasky-Su¹³, Megan R. Kiedrowski¹⁴, Camila Figueiredo⁶, Jennifer Bomberger¹⁴, Mauricio L. Barreto¹⁵, Glorisa Canino⁸, Wei Chen¹, Gerard H. Koppelman^{4,5}, Esteban G. Burchard^{7,17} and Juan C. Celedón^{1,17}

@ERSpublications

A novel SNP in *FLJ22447* is significantly associated with both severe asthma exacerbations in Latino youth and DNA methylation of a cis-CpG in nasal epithelium. This CpG is linked to nasal epithelial expression of a gene implicated in atopic asthma. <https://bit.ly/33D9Joc>

Cite this article as: Yan Q, Forno E, Herrera-Luis E, *et al.* A genome-wide association study of severe asthma exacerbations in Latino children and adolescents. *Eur Respir J* 2021; 57: 2002693 [<https://doi.org/10.1183/13993003.02693-2020>].

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 4010 Latino youth with asthma in four independent cohorts, including 1693 Puerto Ricans, 1019 Costa Ricans, 640 Mexicans, 256 Brazilians and 402 members of other Latino subgroups. We then conducted methylation quantitative trait locus, expression quantitative trait locus and expression quantitative trait methylation analyses to assess whether the top single nucleotide polymorphism (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, an SNP in *FLJ22447* (rs2253681) was significantly associated with 1.55 increased odds of severe asthma exacerbation (95% CI 1.34–1.79, $p=6.3\times 10^{-9}$). 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 ($\beta=0.10$, $p=2.18\times 10^{-7}$).

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*).

This article has supplementary material available from erj.ersjournals.com

Received: 15 July 2020 | Accepted after revision: 30 Sept 2020

Copyright ©ERS 2021

Introduction

Asthma is the most common chronic respiratory disease among children [1]. In the USA, total costs related to asthma exceed \$81 billion per year [2]. Severe asthma exacerbations (SAEs), defined as episodes of disease worsening that require 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 USA, 2.1% report ≥ 1 asthma-related hospitalisation 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 is having had an SAE 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 2–6 years) identified cadherin-related family member 3 (*CDHR3*), a gene not previously associated with asthma, as a susceptibility locus for recurrent SAEs [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 the 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 and ethnic groups in the USA and Latin America. For example, Puerto Rican children have a greater prevalence of and morbidity and mortality from asthma than non-Hispanic white children in the USA [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 ethnicity-specific [14, 15]. We hypothesised 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 refer to the supplementary material for more details.

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

Hartford-Puerto Rico study

The Hartford-Puerto Rico (HPR) study is a case-control study of childhood asthma in Puerto Ricans [6]. SAEs were defined as ≥ 1 hospitalisation 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

The Genetics of Asthma in Latino Americans (GALA II) study is a case-control study of asthma in Latino children and youth [17]. An SAE was defined as having had ≥ 1 of the following events in the previous

Affiliations: ¹Division of Pediatric Pulmonary Medicine, University of Pittsburgh Medical Centre, Children's Hospital of Pittsburgh, University of Pittsburgh, Pittsburgh, PA, USA. ²Genomics and Health Group, Dept of Biochemistry, Microbiology, Cell Biology and Genetics, Universidad de La Laguna, La Laguna, Spain. ³CIBER de Enfermedades Respiratorias, Instituto de Salud Carlos III, Madrid, Spain. ⁴Dept of Pediatric Pulmonology and Pediatric Allergy, Beatrix Children's Hospital, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands. ⁵GRIAC Research Institute, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands. ⁶Instituto de Ciências da Saúde, Universidade Federal da Bahia, Salvador, Brazil. ⁷Dept of Medicine, University of California San Francisco, San Francisco, CA, USA. ⁸Behavioral Sciences Research Institute, University of Puerto Rico, San Juan, Puerto Rico. ⁹Dept of Pediatrics, Hospital Nacional de Niños, San José, Costa Rica. ¹⁰Dept of Pediatrics, University of Connecticut, Farmington, CT, USA. ¹¹CiiM and TWINCORE, joint ventures between the Hannover Medical School and the Helmholtz Centre for Infection Research, Hannover, Germany. ¹²Dept of Internal Medicine and Radboud Center for Infectious Diseases, Radboud University Medical Center, Nijmegen, The Netherlands. ¹³Channing Division of Network Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA. ¹⁴Dept of Microbiology and Molecular Genetics, University of Pittsburgh, Pittsburgh, PA, USA. ¹⁵Instituto de Saúde Coletiva, Federal University of Bahia, Salvador, Brazil. ¹⁶Shared first authors. ¹⁷Shared senior authors.

Correspondence: Juan C. Celedón, Division of Pulmonary Medicine, UPMC Children's Hospital of Pittsburgh, 4401 Penn Avenue, Pittsburgh, PA 15224, USA. E-mail: juan.celedon@chp.edu

year: hospitalisations for asthma, ED 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 (1139 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 the first two PCs.

The Genetics of Asthma in Costa Rica Study

The Genetics of Asthma in Costa Rica Study (GACRS) is a genetic study of nuclear families of children with asthma in Costa Rica [18, 19]. An SAE was defined as ≥ 1 ED visit for asthma or ≥ 1 hospitalisation for asthma in the previous year. After QC, 1019 independent children with asthma (851 with ≥ 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, Bahia, Brazil

The Social Changes, Asthma and Allergy in Latin America (SCAALA) study 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. An SAE was defined as ≥ 1 ED visit or ≥ 1 hospitalisation 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

Nasal (airway) epithelium can serve as a surrogate marker for DNA methylation and gene expression in bronchial (airway) epithelium [21]. In the Epigenetic Variation and Childhood Asthma in Puerto Rico (EVA-PR) study, whole-genome methylation assays were performed using HumanMethylation450 BeadChips (Illumina, San Diego, CA, USA). After QC, 227 901 CpG probes remained for the analysis of nasal epithelium, and M-values were used in all downstream analyses. RNA sequencing was performed with the Illumina NextSeq 500 platform, with paired-end reads at 75 cycles and 80 million reads per sample. After QC, 16 737 genes were retained for the analysis. The R function *sva* was used to estimate latent factors 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 (supplementary figure S1).

Prevention and Incidence of Asthma and Mite Allergy study

The Prevention and Incidence of Asthma and Mite Allergy (PIAMA) study 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 hybridised to the Infinium HumanMethylation450 BeadChip arrays. After QC, 455 samples and 436 824 probes remained, and 432 samples had matched genotype data. RNA sequencing was performed with the Illumina HiSeq 2500 platform with 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 an association between our top SNP and genome-wide DNA methylation in nasal epithelium, and an expression quantitative trait locus (eQTL) analysis to test for an 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 sequencing 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 linkage disequilibrium 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 false discovery rate (FDR). Gene sets used in the analyses were from Gene Ontology (GO) [28, 29], Kyoto Encyclopedia of Genes and Genomes (KEGG) [30, 31], Reactome [32, 33] and BioCarta pathways.

Results

The characteristics of the 4010 participants in the four studies included in the meta-analysis of GWAS of SAEs are shown in table 1. In total, there were 2509 children with asthma and ≥ 1 SAE (cases) and 1501 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 GACRS were younger. Compared to subjects in the other studies, those in GACRS were more likely to be male and to have had ≥ 1 severe asthma exacerbation in the previous year.

Approximately six million genotyped and imputed SNPs with a minor allele frequency (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 ($p < 5 \times 10^{-8}$) (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% CI 1.34–1.79, $p = 6.3 \times 10^{-9}$, 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 or 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 the UK Biobank [34] were associated with SAEs in our analysis (supplementary table S1). None of the previously reported asthma-susceptibility SNPs was significantly associated with SAEs in our meta-analysis of Latino youth. Moreover, no SNPs associated with SAEs or asthma hospitalisations 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- $p \geq 0.05$ in all instances, supplementary table S2). Although the previously reported SNP rs7216389 in *ORMDL3* on chromosome 17q21 was nominally associated with SAEs in HPR ($p = 1.6 \times 10^{-3}$), it did not reach statistical significance in our meta-analysis (meta- $p = 0.06$, supplementary table S2).

To examine whether SNP rs2253681 affects methylation of the *FLJ22447* locus in nasal epithelium, we first conducted an mQTL analysis. In this analysis, rs2253681 was a significant *cis*-acting mQTL in nasal epithelium for cg25024579 in *FLJ22447* ($\beta = 0.55$, $p = 3.6 \times 10^{-16}$ and FDR- $p = 8.1 \times 10^{-11}$ in EVA-PR; $\beta = 0.34$,

TABLE 1 Summary of main characteristics of study participants

	HPR	GALA II	GACRS	SCAALA
Subjects n	554	2181	1019	256
Age years	10.0 $\pm$ 2.7	12.7 $\pm$ 3.3	9.2 $\pm$ 1.9	7.2 $\pm$ 1.9
Male sex	300 (54.2)	1196 (54.8)	598 (58.7)	139 (54.3)
Asthma exacerbation	236 (42.6)	1283 (58.8)	851 (83.5)	139 (54.3)
Inhaled steroid use	187 (33.8)	996 (45.7)	521 (51.1)	Not available
FEV₁ % pred[#]	86.8 $\pm$ 16.0	90.6 $\pm$ 16.3	99.1 $\pm$ 17.3	Not available
FEV₁/FVC % pred[#]	91.8 $\pm$ 9.8	96.3 $\pm$ 8.8	94.5 $\pm$ 8.7	Not available
Study sites	Hartford (CT, USA) and San Juan (Puerto Rico)	Chicago (IL, USA), Bronx (NY, USA), Houston (TX, USA), San Francisco (CA, USA) 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

Data are presented as n (%) or mean $\pm$ sd, unless otherwise indicated. HPR: Hartford-Puerto Rico study; 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; FEV₁: forced expiratory volume in 1 s; FVC: forced vital capacity. [#]: for comparability, all % predicted values across the three studies were calculated using reference values for Mexican American youth [45].

FIGURE 1 a) Manhattan plot showing the summary meta-analysis results of Hartford-Puerto Rico (HPR), Genetics of Asthma in Latino Americans II (GALA II), Genetics of Asthma in Costa Rica Study (GACRS) and Social Changes, Asthma and Allergy in Latin America (SCAALA) studies. The chromosomal position of each single nucleotide polymorphism (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 ($p < 1 \times 10^{-6}$). The red line represents the genome-wide significance line ($p < 5 \times 10^{-8}$). b) 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 vertical 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, which is the most significant SNP in the meta-analysis. The circles show the p-values for all other SNPs and are colour-coded according to the level of linkage disequilibrium with rs2253681 in the 1000 Genome Project Admixed American (AMR) population. This plot was generated at www.locuszoom.org/. c) QQ plots for the meta-analysis. λ is the genomic control value.

$p = 1.2 \times 10^{-11}$ in PIAMA; and meta- $p = 9.5 \times 10^{-25}$ 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, and found no *cis* or *trans* genome-wide significant results (supplementary table S3). 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 S3).

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 *KCNJ2* antisense RNA 1 (*KCNJ2-AS1*) on chromosome 17q24.3 ($\beta = 0.10$, $p = 2.2 \times 10^{-7}$ and FDR- $p = 3.2 \times 10^{-3}$) in EVA-PR. Although this was not replicated in PIAMA, the observed association remained significant in the combined analysis of the two cohorts ($\beta = 0.09$, $p = 1.4 \times 10^{-6}$). The top *cis*-eQTM gene in the current analysis in EVA-PR was protein kinase C- η (PKC η), encoded by *PRKCH* ($\beta = 0.07$, $p = 4.1 \times 10^{-5}$ and

rs2253681

FIGURE 2 Forest plots of odds ratio and 95% CI for the association with asthma for rs2253681, the most significant single nucleotide polymorphism in the meta-analysis. The heterogeneity measure $I^2=0$ implies no heterogeneity among the odds ratios from the four studies [see supplementary material for details]. HPR: Hartford-Puerto Rico cohort; GACRS: Genetics of Asthma in Costa Rica Study; GALA II: Genetics of Asthma in Latino Americans II; SCAALA: Social Changes, Asthma and Allergy in Latin America; MAF: minor allele frequency.

FDR= 1.5×10^{-1}). *PRKCH* expression was also associated with cg2504579 methylation in PIAMA, in the same direction of association as in EVA-PR ($\beta=0.11$, $p=1.4 \times 10^{-2}$; and $p=2.4 \times 10^{-6}$ 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 ($\beta=0.22$, $p=4.2 \times 10^{-5}$ in PIAMA; and $\beta=0.09$, $p=4.9 \times 10^{-7}$ in the combined analysis of the two cohorts). In addition, expression of *FOCAD*, *PIGF* and *ERLECI* was associated with cg25024579 methylation in PIAMA at $p<0.05$, in the same direction as in EVA-PR.

TABLE 2 Top 20 methylation quantitative trait locus for rs2253681 in EVA-PR nasal epithelial cells and replication results from PIAMA

CpG	Chr	Position	Nearest gene	Distance to gene	EVA-PR			PIAMA		Meta	
					Effect	p-value	FDR [#]	Effect	p-value	Effect	p-value
cg25024579	14	62 076 297	FLJ22447	0	0.5520	3.55×10^{-16}	8.08×10^{-11}	0.3365	1.16×10^{-11}	0.4114	9.45×10^{-25}
cg02787615	2	99 434 688	KIAA1211L	0	-0.1080	9.68×10^{-07}	1.10×10^{-01}	0.0415	1.73×10^{-02}	-0.0161	2.41×10^{-01}
cg05223396	5	171 830 155	SH3PXD2B	0	-0.2175	1.89×10^{-06}	1.44×10^{-01}	-0.0641	2.52×10^{-02}	-0.1074	9.56×10^{-06}
cg24718015	17	40 489 721	STAT3	0	-0.1581	5.01×10^{-06}	2.80×10^{-01}	0.0215	5.00×10^{-01}	-0.0609	9.48×10^{-03}
cg21785067	1	54 587 182	TCEANC2	8989	0.2305	7.39×10^{-06}	2.80×10^{-01}	0.0824	6.42×10^{-02}	0.1458	1.48×10^{-05}
cg21917349	15	29 213 860	APBA2	0	-0.2427	7.89×10^{-06}	2.80×10^{-01}	-0.0441	2.22×10^{-01}	-0.1050	4.82×10^{-04}
cg15904664	14	104 569 653	ASPG	0	-0.2093	9.96×10^{-06}	2.80×10^{-01}	-0.0574	1.24×10^{-01}	-0.1156	8.11×10^{-05}
cg11374933	8	9 766 172	MIR124-1	5189	-0.1660	1.18×10^{-05}	2.80×10^{-01}	0.0357	2.55×10^{-01}	-0.0464	5.49×10^{-02}
cg15375424	5	131 823 451	IRF1	0	-0.2272	1.37×10^{-05}	2.80×10^{-01}	-0.0623	9.38×10^{-02}	-0.1177	1.02×10^{-04}
cg07975634	1	147 232 608	GJA5	0	-0.2236	1.49×10^{-05}	2.80×10^{-01}	0.0396	6.77×10^{-02}	0.0002	9.92×10^{-01}
cg08487909	6	30 904 981	DPCR1	3794	-0.1356	1.59×10^{-05}	2.80×10^{-01}	-0.0364	8.15×10^{-02}	-0.0668	1.23×10^{-04}
cg00203736	7	55 022 567	EGFR	64156	-0.2440	1.86×10^{-05}	2.80×10^{-01}	0.0034	9.20×10^{-01}	-0.0614	3.54×10^{-02}
cg23696248	19	45 260 501	BCL3	0	-0.3099	2.01×10^{-05}	2.80×10^{-01}	0.0230	7.09×10^{-01}	-0.1164	1.33×10^{-02}
cg13127574	14	105 196 523	ADSSL1	0	-0.2116	2.11×10^{-05}	2.80×10^{-01}	-0.0445	9.01×10^{-03}	-0.0621	1.18×10^{-04}
cg08403064	11	44 068 709	ACCSL	820	-0.2011	2.23×10^{-05}	2.80×10^{-01}	0.0472	1.78×10^{-01}	-0.0406	1.50×10^{-01}
cg21115391	11	93 143 810	CCDC67	0	-0.2452	2.23×10^{-05}	2.80×10^{-01}	-0.0945	9.51×10^{-03}	-0.1374	8.40×10^{-06}
cg01220257	19	45 260 935	BCL3	0	-0.1474	2.30×10^{-05}	2.80×10^{-01}	0.0269	5.78×10^{-01}	-0.0879	1.87×10^{-03}
cg12765028	4	13 526 659	LINC01097	1282	0.1566	2.34×10^{-05}	2.80×10^{-01}	-0.0063	7.70×10^{-01}	0.0349	6.07×10^{-02}
cg11136041	1	158 111 350	LOC646268	919	-0.2204	2.39×10^{-05}	2.80×10^{-01}	0.0249	4.28×10^{-01}	-0.0404	1.33×10^{-01}
cg01735277	15	75 077 691	CSK	0	-0.2291	2.46×10^{-05}	2.80×10^{-01}	-0.0460	1.30×10^{-01}	-0.0897	7.22×10^{-04}

Chr: chromosome; EVA-PR: Epigenetic Variation and Childhood Asthma in Puerto Rico; PIAMA: Prevention and Incidence of Asthma and Mite Allergy; FDR: false discovery rate. [#]: FDR is adjusted for the whole genome in EVA-PR.

TABLE 3 Top 20 expression quantitative trait methylations 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
<i>KCNJ2-AS1</i>	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}
<i>CCDC125</i>	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}
<i>FARS2</i>	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}
<i>PRKCH</i>	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}
<i>TBL2</i>	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}
<i>FOCAD</i>	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}
<i>ERLIN1</i>	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}
<i>LOC401052</i>	3	10 048 101	10 052 779	0.0001	9.75×10^{-5}	1.53×10^{-1}	NA	NA	NA	NA
<i>SMIM14</i>	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}
<i>SLC27A2</i>	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}
<i>BLOC1S6</i>	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}
<i>TRMT12</i>	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}
<i>CDK19</i>	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}
<i>ACSS1</i>	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}
<i>SEC22A</i>	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}
<i>PIGF</i>	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}
<i>NME6</i>	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}
<i>ERLEC1</i>	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}
<i>KATNAL2</i>	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}
<i>PKHD1L1</i>	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}

Chr: chromosome; EVA-PR: Epigenetic Variation and Childhood Asthma in Puerto Rico; PIAMA: Prevention and Incidence of Asthma and Mite Allergy; FDR: false discovery rate. [#]: FDR is adjusted for the whole genome in EVA-PR.

To further assess the biological relevance of the *FLJ22447* locus, we conducted an 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 S4).

In a sensitivity analysis, we repeated the GWAS of SAEs after imputing genotypes using the 1000 Genomes Ad Mixed American (AMR) reference panel instead of the Haplotype Reference Consortium (HRC) r1.1 2016 reference panel (supplementary material), obtaining very similar results (supplementary figure S2 and supplementary table S5).

Discussion

Our combined analysis including 4010 youths in four study cohorts showed that a novel SNP in *FLJ22447* (rs2253681) is significantly associated with SAEs ($p=6.3 \times 10^{-9}$) 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 *KCNJ2-AS1* in nasal epithelium.

FLJ22447 codes for a long non-coding RNA, a type of RNA that does not get translated to a 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, an SNP in *AL355916.3* has been associated with forced expiratory volume in 1 s/forced vital capacity and bronchodilator response in adults [34]. *FLJ22447* mediates interleukin (IL)-33 upregulation in activated fibroblasts; together, *FLJ22447* and IL-33 can increase fibroblast expression of α -smooth muscle actin (α -SMA), vimentin and N-cadherin [35], which are associated with myofibroblast differentiation and airway remodelling [36, 37].

KCNJ2-AS1 also encodes a long non-coding RNA. Expression of *KCNJ2-AS1* ($\log_2(\text{fold-change})=0.34$, $p=3.9 \times 10^{-6}$, $\text{FDR-}p=3.1 \times 10^{-5}$) was significantly associated with atopic asthma in our recent transcriptome-wide association study of atopic asthma in Puerto Rican children and adolescents [38]. SNPs rs8066985 and rs312750 near *KCNJ2-AS1* are associated with body mass index and waist-to-hip ratio [39, 40], which are 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 significant genome wide, 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]. PKC η , encoded by *PRKCH*, plays a key role in the assembly and maintenance of epithelial tight junctions. PKC η 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 our having similar or larger sample sizes than those in the original studies. Similarly, a previously reported association between an 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 recognised, whether this locus is linked to severe disease exacerbations among children with asthma is less clear. The initial report in paediatric asthma analysed a cohort of 376 children and reported 80 with recurrent wheezing and 66 with asthma [43]. The authors then analysed 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 hospitalisations and ED visits in children, but, while the pooled estimates were significant, only four of the 13 cohorts showed significant results [44]. Although the 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 recognise several study limitations. First, we lack data on viral infections or air pollution, which may interact with genetic or epigenetic mechanisms causing SAEs in children. Second, we cannot assess temporal relationships between DNA methylation or gene expression and SAEs 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 (because 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 have 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.

Author contributions: Q. Yan, E. Forno and J.C. Celedón conceived and designed the study. E.G. Burchard, G.H. Koppelman and J.C. Celedón obtained funding. Q. Yan, E. Herrera-Luis, M. Pino-Yanes and R. Rios conducted the primary analysis and interpreted data. C. Qi, Y.-Y. Han, S. Kim, S. Oh, E. Acosta-Pérez, R. Zhang, D. Hu, C. Eng, S. Huntsman, L. Avila, N. Boutaoui, M.M. Cloutier, M.E. Soto-Quiros, C.-J. Xu, S.T. Weiss, J. Lasky-Su, M.R. Kiedrowski, C. Figueiredo, J. Bomberger, M.L. Barreto, G. Canino, W. Chen and E.G. Burchard participated in data collection and data analysis. Q. Yan, E. Forno and J.C. Celedón 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.

Support statement: This work was supported by grants HL079966, HL117191 and MD011764 from the US National Institutes of Health (NIH) to J.C. Celedón. Q. Yan's contribution was supported by grant HL138098 from the US NIH. E. Forno's contribution was supported by grant HL149693 from the US NIH. The GALA II study was supported by US NIH grants to E.G. Burchard: HL088133, HL004464, HL117004, ES015794, ES24844, TRDRP 24RT 0025, MD006902 and GM007546. M. Pino-Yanes 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 Organisation for Health Research and Development; The Netherlands Organisation 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. Qi was supported by a grant from the China Scholarship Council. Funding information for this article has been deposited with the Crossref Funder Registry.

Conflict of interest: Q. Yan reports grants from National Institutes of Health (K01HL138098), during the conduct of the study. E. Forno has nothing to disclose. E. Herrera-Luis reports grants from the Spanish Ministry of Science, Innovation and Universities (PRE2018-083837), during the conduct of the study. M. Pino-Yanes reports grants from Spanish Ministry of Economy, Industry and Competitiveness, the State Research Agency and the European Regional

Development Funds from the European Union (MINECO/AEI/FEDER, UE, SAF2017-83417R), and grants from Spanish Ministry of Economy, Industry and Competitiveness (Ramón y Cajal grant RYC-2015-17205), during the conduct of the study. C. Qi has nothing to disclose. R. Rios has nothing to disclose. Y-Y. Han has nothing to disclose. S. Kim has nothing to disclose. S. Oh has nothing to disclose. E. Acosta-Pérez has nothing to disclose. R. Zhang has nothing to disclose. D. Hu has nothing to disclose. C. Eng has nothing to disclose. S. Huntsman has nothing to disclose. L. Avila has nothing to disclose. N. Boutaoui has nothing to disclose. M.M. Cloutier has nothing to disclose. M.E. Soto-Quiros has nothing to disclose. C-J. Xu has nothing to disclose. S.T. Weiss has nothing to disclose. J. Lasky-Su has nothing to disclose. M.R. Kiedrowski has nothing to disclose. C. Figueiredo has nothing to disclose. J. Bomberger has nothing to disclose. M.L. Barreto has nothing to disclose. G. Canino has nothing to disclose. W. Chen has nothing to disclose. G.H. Koppelman reports grants from the Lung Foundation of the Netherlands, during the conduct of the study; grants from Ubbo Emmius Foundation, TETRI Foundation, GSK, Vertex, TEVA, the Lung Foundation of the Netherlands and the European Union H2020 Program, outside the submitted work; and has served on advisory boards to GSK and PURE IMS (fees paid to institution). E.G. Burchard reports grants from National Heart, Lung, and Blood Institute, National Institute of Health, General Medical Sciences, National Institute on Minority Health and Health Disparities, Tobacco-Related Disease Research Program, Food and Drug Administration, and the Sandler Family Foundation, during the conduct of the study. J.C. Celedón has received research materials from GSK and Merck (inhaled steroids) and Pharmavite (vitamin D and placebo capsules), in order to provide medications free of cost to participants in NIH-funded studies, unrelated to the current work.

References

- Center for Disease Control and Prevention. Asthma. Data, statistics and surveillance. www.cdc.gov/asthma/most_recent_data.htm. Date last accessed: September 18, 2020.
- Nurmagambetov T, Kuwahara R, Garbe P. The economic burden of asthma in the United States, 2008–2013. *Ann Am Thorac Soc* 2018; 15: 348–356.
- Fuhlbrigge A, Peden D, Apter AJ, et al. Asthma outcomes: exacerbations. *J Allergy Clin Immunol* 2012; 129: Suppl. 3, S34–S48.
- 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.
- Puranik S, Forno E, Bush A, et al. Predicting severe asthma exacerbations in children. *Am J Respir Crit Care Med* 2017; 195: 854–859.
- 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: 1601505.
- 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: 42–53.
- 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: 887–892.
- 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: 51–55.
- 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: 1503–1510.
- Dahlin A, Denny J, Roden DM, et al. CMTR1 is associated with increased asthma exacerbations in patients taking inhaled corticosteroids. *Immun Inflamm Dis* 2015; 3: 350–359.
- Rosser FJ, Forno E, Cooper PJ, et al. Asthma in Hispanics. An 8-year update. *Am J Respir Crit Care Med* 2014; 189: 1316–1327.
- Forno E, Gogna M, Cepeda A, et al. Asthma in Latin America. *Thorax* 2015; 70: 898–905.
- 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: 208–219.
- 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: 1552–1564.
- Price AL, Patterson NJ, Plenge RM, et al. Principal components analysis corrects for stratification in genome-wide association studies. *Nat Genet* 2006; 38: 904–909.
- 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. *Am J Respir Crit Care Med* 2013; 188: 309–318.
- 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: 84–90.
- 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: 654–661.
- 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.
- 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: 336–346.
- 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: 882–883.
- 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: 55–60.
- 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. *Clin Exp Allergy* 2001; 31: 576–581.
- Qi C, Jiang Y, Yang IV, et al. Nasal DNA methylation profiling of asthma and rhinitis. *J Allergy Clin Immunol* 2020; 145: 1655–1663.
- Willer CJ, Li Y, Abecasis GR. METAL: fast and efficient meta-analysis of genomewide association scans. *Bioinformatics* 2010; 26: 2190–2191.

- 27 de Leeuw CA, Mooij JM, Heskes T, *et al.* MAGMA: generalized gene-set analysis of GWAS data. *PLoS Comput Biol* 2015; 11: e1004219.
- 28 Ashburner M, Ball CA, Blake JA, *et al.* Gene ontology: tool for the unification of biology. *Nat Genet* 2000; 25: 25–29.
- 29 Gene Ontology Consortium. Gene Ontology Consortium: going forward. *Nucleic Acids Res* 2015; 43: D1049–D1056.
- 30 Kanehisa M, Sato Y, Kawashima M, *et al.* KEGG as a reference resource for gene and protein annotation. *Nucleic Acids Res* 2016; 44: D457–D462.
- 31 Ogata H, Goto S, Sato K, *et al.* KEGG: Kyoto Encyclopedia of Genes and Genomes. *Nucleic Acids Res* 1999; 27: 29–34.
- 32 Fabregat A, Sidiropoulos K, Garapati P, *et al.* The Reactome pathway knowledgebase. *Nucleic Acids Res* 2016; 44: D481–D487.
- 33 Croft D, O’Kelly G, Wu G, *et al.* Reactome: a database of reactions, pathways and biological processes. *Nucleic Acids Res* 2011; 39: D691–D697.
- 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: 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: 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- β 1. *Am J Respir Crit Care Med* 2009; 180: 122–133.
- 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; 313: 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: 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: 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: e1005378.
- 41 GTEx Consortium. The Genotype-Tissue Expression (GTEx) project. *Nat Genet* 2013; 45: 580–585.
- 42 Suzuki T, Elias BC, Seth A, *et al.* PKC η regulates occludin phosphorylation and epithelial tight junction integrity. *Proc Natl Acad Sci USA* 2009; 106: 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: 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: 2083–2088.
- 45 Hankinson JL, Odencrantz JR, Fedan KB. Spirometric reference values from a sample of the general U.S. population. *Am J Respir Crit Care Med* 1999; 159: 179–187.