PRKCH and severe asthma exacerbations in Latino children

Qi Yan[1#], Erick Forno[1#], Esther Herrera-Luis[2#], Maria Pino-Yanes[2,3], Raimon Rios[4], Yueh-Ying Han[1], Sam Oh[5], Edna Acosta-Pérez[6], Rong Zhang[1], Donglei Hu[5], Celeste Eng[5], Scott Huntsman[5], Lydiana Avila[7], Nadia Boutaoui[1], Michelle M. Cloutier[8], Manuel E. Soto-Quiros[7], Scott T. Weiss[9], Jessica Lasky-Su[9], Camila Figueiredo[4], Mauricio L. Barreto[10], Glorisa Canino[6], Wei Chen[1], Esteban G. Burchard[5^], Juan C. Celedón[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]Instituto de Ciências da Saúde, Universidade Federal da Bahia, Vale do Canela, Salvador, Bahia, Brazil. [5]Department of Medicine, University of California San Francisco, San Francisco, CA, USA. [6]Behavioral Sciences Research Institute, University of Puerto Rico, San Juan, Puerto Rico. [7]Department of Pediatrics, Hospital Nacional de Niños, San José, Costa Rica. [8]Department of Pediatrics, University of Connecticut, Farmington, CT, USA. [9]Channing Division of Network Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA. [10] Instituto de Saúde Coletiva, Federal University of Bahia, Salvador, Brazil.
[#]Shared first authors. [^]Shared senior authors.

ABSTRACT

•*Background*: Severe asthma exacerbations are a major cause of healthcare costs in children, particularly those in high-risk subgroups such as Latinos. Here we aim to identify susceptibility genes for severe asthma exacerbations in Latino children at high risk.

•*Methods*: We conducted a meta-analysis of genome-wide association studies (GWAS) of severe asthma exacerbations. We then conducted a methylation quantitative trait locus (mQTL) analysis and an expression quantitative trait locus (eQTL) analysis, to test for association between our top SNP in the GWAS meta-analysis and genome-wide DNA methylation and gene expression in nasal (airway) epithelium in Puerto Rican children. Next, we tested whether expression of a gene linked to our top SNP through the mQTL and eQTL analysis (*PRKCH,* encoding protein kinase C eta) is associated with severe asthma exacerbations in Puerto Ricans.

•*Findings*: In the meta-analysis of GWAS, a SNP in *FLJ22447* (rs2253681) was significantly associated with 1.55 increased odds of severe asthma exacerbations (P=6·3×10^{-9}). This SNP was significantly associated with reduced expression of *PRKCH* (protein kinase C eta) in nasal airway epithelium, through methylation of a CpG site at the *FLJ22447* locus (cg25024579). In turn, reduced nasal epithelial expression of *PRKCH* was associated with severe asthma exacerbations in Puerto Rican children. Moreover, primary asthma airway epithelial cells treated with poly I:C had significantly lower *PRKCH* gene expression (P=0.0006) than those treated with sham.

•*Interpretation*: Protein kinase C eta (PKCη), encoded by *PRKCH*, plays a key role in the assembly and maintenance of epithelial tight junctions. We have identified a novel SNP associated with an increased risk of severe asthma exacerbations through reduced *PRKCH* expression in airway epithelium, likely leading to defective function of the epithelial barrier against viral pathogens or air pollutants.

INTRODUCTION

•Asthma is the most common respiratory disease among children and affects approximately 235 million people worldwide[1]. Severe asthma exacerbations, defined as episodes of disease worsening requiring a change in treatment to prevent a serious outcome[2], are a major cause of school absences and healthcare costs.

•Although GWAS have identified susceptibility loci for asthma[3-5], little is known about genetic determinants of asthma exacerbations, which may be distinct from those for asthma *per se.*

•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.[6]. Moreover, recent evidence suggests that some susceptibility variants for asthma-related outcomes are ethnic-specific[7]. Thus, we hypothesized that there would be susceptibility variants for severe exacerbations that are specific to Latino subgroups at high risk of asthma morbidity, as they would be more common or exert a greater effect in such subgroups. To test this hypothesis, we conducted a meta-analysis of GWAS of severe exacerbations among children with asthma in four studies of Puerto Ricans, Costa Ricans, and Brazilians.

REFERENCES

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RESULTS

Study Populations in GWAS

Table 1. Summary of main characteristics of study participants in the meta-analysis of GWAS

	HPR (n=554)	GALA II (n=2,181)	GACRS (n=1,019)	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)
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;
- 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.

GWAS results

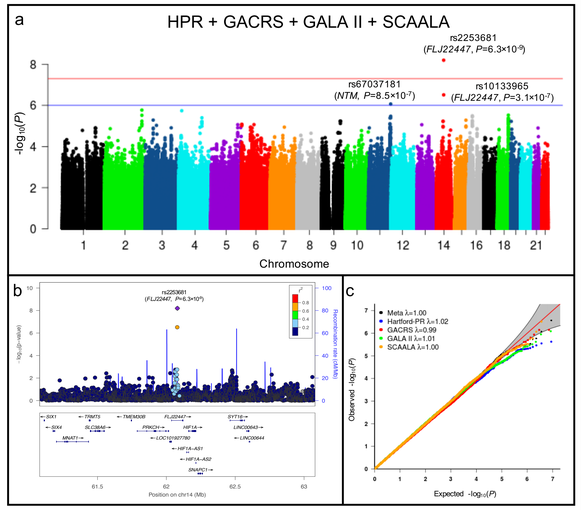


Figure 1. 1a) Manhattan plot of meta-analysis results: Manhattan plot showing the summary meta-analysis results of HPR, GALA II, GACRS, and SCAALA. The chromosomal position of each SNP is displayed along the X-axis and the negative logarithm of the association *P*-value is displayed on the *Y*-axis. 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. **1c) QQ plots for the meta-analysis.** λ is the genomic control value.

RESULTS (cont)

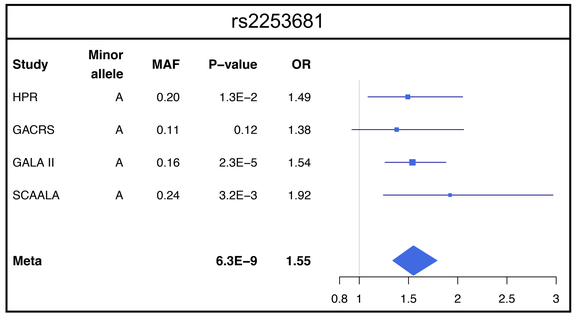
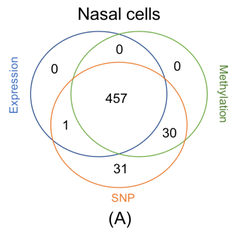


Figure 2. Forest plots of odds ratio and 95% confidence interval for the association with asthma: Forest plots for rs2253681, the most significant SNP in the meta-analysis.

Study Population in gene expression and methylation



(A)

Figure 3 – The omics data distribution from the EVA-PR cohort (Puerto Ricans). Expression and methylation in nasal airway epithelial cells

eQTL, mQTL and eQTM

To examine whether SNP rs2253681 affects methylation or transcription of *PRKCH* in airway epithelium:

1. We first conducted a methylation quantitative trait locus (mQTL) analysis. In this analysis, rs2253681 was a significant *cis*-acting mQTL in nasal epithelium for cg25024579 in *FLJ22447* (genome-wide corrected FDR=6.1×10^{-11}).

CpG	Effect	*P*-value	Chr	Position	Distance to gene	Gene	FDR
cg25024579	0.6715	2.70E-16	14	62076297	0	*FLJ22447*	6.15E-11

2. Next, we conducted an expression quantitative trait locus (eQTL) analysis of SNP rs2253681 and *PRKCH* expression in nasal epithelium. In this analysis, SNP rs2253681 was a significant *cis*-acting eQTL for lowering *PRKCH* expression (FDR$_{locus}$-corrected P=0.038 corrected for four genes; genome-wide FDR=1).

Gene	Effect	*P*-value	Chr	Start	End	FDR
PRKCH	0.0749	0.0095	14	61788514	62017698	1

3. We then conducted an expression quantitative trait methylation (eQTM) analysis of cg25024579 in *FLJ22447* and *PRKCH*, finding that this CpG has a significant *cis*-effect on lowering *PRKCH* expression in nasal epithelium (P=1.5×10^{-7}; genome-wide FDR=0.003).

Gene	Effect	*P*-value	Chr	Start	End	FDR
PRKCH	0.0819	1.46E-07	14	61788514	62017698	0.0023

RESULTS (cont)

Table 2. Mediation results of rs2253681, cg25024579 and *PRKCH*

Step	Description	*P*-value*
Step1	cg25024579 - rs2253681	2.70E-16
Step2	*PRKCH* - rs2253681	0.0095
Step3	*PRKCH* - cg25024579	1.46E-07
Step4	*PRKCH* - rs2253681 (adjusted for cg25024579)	0.3964

Mediation analysis using data from nasal airway epithelium in EVA-PR.
*Results indicated a full mediation.

Table 3. Mediation results of rs2253681, *PRKCH* and asthma exacerbations

Step	Description	P-value*
Step1	*PRKCH* - rs2253681 (EVA-PR)	0.0095
Step2	asthma exacerbation - rs2253681 (Hartford-PR)	0.0132
Step3	asthma exacerbation - *PRKCH* (EVA-PR)	0.0298
Step4	asthma exacerbation - rs2253681 (adjusted for *PRKCH*, EVA-PR)	0.3242

Mediation analysis using data from nasal airway epithelium in EVA-PR.
*Results indicated a full mediation.

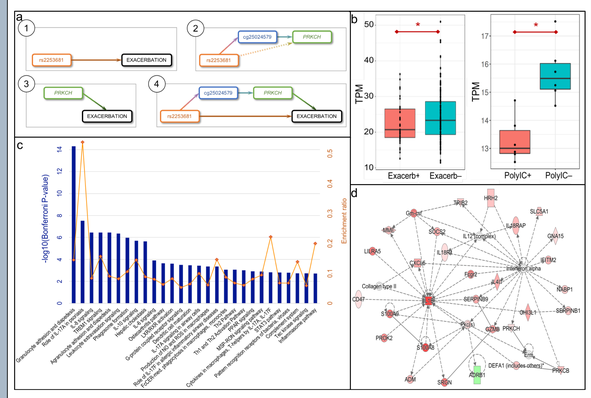


Figure 4. 3a) Overview of the mediation analysis: Step 1 depicts the original association between SNP rs2253681 and asthma exacerbations. Step 2 represents the mediation analysis between eQTM (SNP rs2253681 and CpG cg25024579), mQTL (CpG and *PRKCH* gene expression), and eQTL (SNP and *PRKCH*); in this step, the association between SNP and expression is fully mediated by methylation. Step 3 shows the association between *PRKCH* expression and asthma exacerbations. Step 4 shows the full pathway, whereby the original association between SNP and exacerbations is fully mediated by DNA methylation and gene expression. **3b) *PRKCH* gene expression:** Expression of *PRKCH* in nasal airway epithelium (left) was lower in children with exacerbations than in those without exacerbations. Likewise, *PRKCH* expression was lower in asthma airway epithelial cells (right) treated with poly I:C as a surrogate for viral infection. **3c) Pathway analysis:** Canonical pathways associated with the 298 significant eQTMs of cg25024579 in nasal epithelium. Blue bars show -log(*P*-value); orange line shows the enrichment ratios. Only the top pathways shown. **3d) Network analysis:** Top network using the same 298 significant nasal epithelium eQTMs. Red nodes are downregulated and green nodes are upregulated. Pathway and network analysis performed using Ingenuity Pathway Analysis

CONCLUSION

In summary, our combined analysis including 4,010 participants in four cohorts showed that a novel SNP in *FLJ22447* (rs2253681) was significantly associated with severe asthma exacerbations (P=6·3×10^{-9}) among children in Latino subgroups that are heavily affected with asthma (Puerto Ricans, Costa Ricans, and Brazilians)[12]. This SNP was significantly associated with expression of *PRKCH* in nasal airway epithelium, through methylation of a CpG site at the *FLJ22447* locus (cg25024579). In turn, nasal epithelial expression of *PRKCH* was associated with severe asthma exacerbations, likely by affecting airway epithelial integrity.