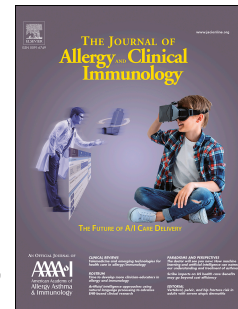


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A Deoxyribonuclease 1 Like 3 genetic variant associates with asthma exacerbations

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Word count: 1,098; Figures: 1; Tables: 1

ABBREVIATIONS

ALPL: alkaline phosphatase, liver/bone/kidney
 CI: Confidence interval
 CLC: Charcot-Leyden crystal galectin
 CPA3: carboxypeptidase 3
 CXCR2: C-X-C Motif chemokine receptor 2
 DNASE1L3: Deoxyribonuclease 1-like 3
 eQTL: Expression quantitative trait locus
 FEV₁: Forced expiratory volume in one second
 FLNB: Filamin B
 FVC: Forced vital capacity
 GALA II: Genes, Environments & Admixture in Latino Americans II
 GTEx: Genotype-tissue expression
 IgE: Immunoglobulin E
 IL1B: Interleukin 1 Beta
 LD: Linkage disequilibrium
 OR: Odds ratio
 NLRC4: NLR family CARD domain containing 4
 NLRP3: NLR family, pyrin domain containing 3
 RNA: Ribonucleic acid
 SAGE: Study of African Americans, Asthma, Genes & Environments
 SNP: Single nucleotide polymorphism
 sQTL: splicing quantitative trait locus

CONFLICT OF INTERESTS

EH-L reports a fellowship from the Spanish Ministry of Science, Innovation, and Universities. MP-Y and FLD report grants from the Spanish Ministry of Science, Innovation, and Universities, the State Research Agency, and the European Regional Development Funds from the European Union (MICIU/AEI/FEDER, UE). EGB reports grants from the National Institutes of Health, the Tobacco-Related Disease Research Program, the Sandler Family Foundation, the American Asthma Foundation, the Amos Medical Faculty Development Program from the Robert Wood Johnson Foundation, and from the Harry Wm. and Diana V. Hind Distinguished Professorship in Pharmaceutical Sciences II. The rest of the authors declare no conflict of interest.

CAPSULE SUMMARY

The analysis of genetic variants from six genes whose expression is predictive of asthma exacerbations revealed a novel association of a regulatory polymorphism from *DNASE1L3* in African-Americans and Latinos.

KEYWORDS

Asthma exacerbations; *DNASE1L3*, African-Americans, Latinos; Hispanics; gene; polymorphism; minority populations

88 To the Editor,

89 Recently, Fricker et al.¹ reported that an expression signature in the sputum predicted
 90 efficiently exacerbations among asthma patients, showing the greatest performance for frequent
 91 exacerbators. This signature was based on six genes (*ALPL*, *CLC*, *CPA3*, *CXCR2*, *DNASE1L3*, and
 92 *IL1B*) that can differentiate airway asthmatic inflammatory phenotypes. Thus, we hypothesized that
 93 genetic variation within those genes may contribute to susceptibility to exacerbations. In the United
 94 States, African-descent minority populations show the highest rates of asthma exacerbations. In
 95 fact, African ancestry is a risk factor not only for asthma in Puerto Ricans and African-Americans
 96 but also for exacerbations among African-Americans^{2,3}. Hence, we performed a candidate-gene
 97 association study of asthma exacerbations in African-Americans and Latinos.

98 Details on the study populations and methods are described in the Online Repository.
 99 Genotype dosages with an imputation quality (R^2) ≥ 0.3 , minor allele frequency $\geq 5\%$, and located
 100 within the gene limits ± 100 kilobases were examined for association with asthma exacerbations. In
 101 the discovery, 1,002 African-American asthma patients from the Study of African Americans,
 102 Asthma, Genes & Environments (SAGE), 470 with exacerbations, were analyzed (**Table 1**). Asthma
 103 exacerbations were defined as a dichotomous variable (presence/absence) based on oral
 104 corticosteroids use, hospitalizations, or acute care due to asthma in the 12 months prior to study
 105 enrollment. Association testing was performed by logistic regression models with correction for
 106 age, sex, and two principal components (PCs). To account for multiple comparisons, we established
 107 a Bonferroni-corrected threshold for significance based on the number of independent single
 108 nucleotide polymorphisms (SNPs) within each genomic region by linkage disequilibrium (LD)
 109 pruning ($r^2 < 0.2$). Replication was evaluated in 2,181 Latinos with asthma from the Genes,
 110 Environments & Admixture in Latino Americans (GALA II) study, including 1,283 exacerbators
 111 (**Table 1**). This two-stage design provided $>80\%$ statistical power to detect the association of
 112 common variants for different scenarios (**Table E1**).

113 A total of 4,089 SNPs was evaluated (**Table E2**). Among African Americans, an intronic
 114 variant in the deoxyribonuclease 1 Like 3 (*DNASE1L3*) region (**Figure 1**), rs67622929, exceeded
 115 the significance threshold corrected by multiple comparisons (0.05/57 independent variants, $p =$
 116 8.77×10^{-4}) [odds ratio (OR) (95% confidence interval [CI]) for the C allele: 1.48 (1.18–1.87),
 117 $p = 7.9 \times 10^{-4}$, imputation $R^2 = 0.99$]. The association of rs67622929 was replicated in Latinos [OR
 118 (95% CI): 1.18 (1.01–1.37), $p = 0.03$, imputation $R^2 = 0.97$]. An inverse-variance weighted fixed-
 119 effect meta-analysis of effects was performed since no heterogeneity was detected (Cochran's Q
 120 $p = 0.103$), revealing that the C allele was significantly associated with increased odds of
 121 exacerbations [OR (95% CI) 1.26 (1.11–1.43), $p = 2.6 \times 10^{-4}$]. Population stratification adjustment by

African ancestry in African-Americans and African and Native ancestry in Latinos, instead of PCs, revealed similar results (**Table E3**). Furthermore, the C allele of rs67622929 was also associated with increased risk of exacerbations when adjusted by disease severity in a subset of African-Americans with data available [OR (95% CI): 1.40 (1.10–1.78), $p=6.6 \times 10^{-3}$] and Latinos [OR (95% CI): 1.18 (1.01–1.38), $p=0.034$], as well as in the meta-analysis [OR (95% CI): 1.24 (1.09–1.41), $p=1.2 \times 10^{-3}$, Cochran's Q $p=0.250$]. To explore whether this association was population-specific, the SNP rs67622929 was evaluated for validation in European-descent individuals through online databases, and no evidence of association was found ($p>0.05$) (**Table E4**). However, the definition of the control group differed from our study, given that individuals without asthma were analyzed instead of non-exacerbators asthma patients. Therefore, the association of this SNP with severe asthma exacerbations among European-descent populations cannot be fully ruled out.

Next, we assessed the association of rs67622929 with asthma-related traits, including IgE levels and normalized lung function values at baseline. The linear regression models for normalized lung function values were adjusted by the 2 PCs. For $\log_{10}$ IgE levels, age and sex were also included as covariates. The SNP rs67622929 was not associated ($p>0.05$) with FEV₁, FVC, FEV₁/FVC, or IgE (**Table E3**).

According to the GTEx database⁴, the C allele of rs67622929 acts as an expression quantitative trait locus (eQTL) that increases the expression of *DNASE1L3* in several tissues, including cultured fibroblasts ($p_{\text{GTEx}}=7.1 \times 10^{-16}$) (**Figure E1A, Table E5**). This SNP is also a cis-splicing quantitative trait locus (sQTL) for *DNASE3L1* in the lung ($p_{\text{GTEx}}=9.4 \times 10^{-9}$) (**Figure E1B**) and other tissues (**Table E6**). Furthermore, rs67622929 has been linked to *FLNB* in naive CD8 cells through promoter capture Hi-C, according to OpenTargets⁵. *FLNB* is located close to *DNASE1L3* (**Figure 1**) and it is transcribed in the opposite direction. *FLNB* is involved in actin, cadherin, and RNA binding, as well as many biological processes including antiviral mechanisms, cellular response to interferon-gamma, epithelial cell morphogenesis, and actin cytoskeleton organization. Interestingly, *Flnb* deficient mice show skeletal abnormalities and decreased cellular adhesion⁵.

DNASE1L3 encodes an endonuclease that participates in apoptosis, clearing DNA from circulating apoptotic bodies, and has an important role in autoimmunity protection⁶. Additionally, *DNASE1L3* participates in different catabolic and metabolic processes, neutrophil activation, cell development, regulation of inflammasome activation, and cytokine secretion^{5,6}. Genetic variants from *DNASE1L3* have been previously associated with neutrophil counts and systemic rheumatic diseases^{5,6}, and this is the first report of an association with asthma exacerbations. Interestingly, the allele associated with risk for asthma exacerbations in our study is associated with higher *DNASE1L3* expression, an effect that is in the same direction as the one described for this gene in

the expression signature associated with asthma exacerbations in the previous study. Additionally, *DNASE1L3* is overexpressed in induced sputum from patients with eosinophilic asthma^{1,7}, and in endobronchial samples of asthma patients compared to healthy controls⁸. Despite all this evidence, the mechanistic implication of *DNASE1L3* in asthma remains unclear, and functional studies are needed to disentangle it.

We acknowledge some limitations of our study. First, the association of rs67622929 would not be considered significant after applying a more restrictive correction for multiple comparisons accounting for all polymorphisms tested across the six genomic regions ($0.05/384$ independent variants= 1.30×10^{-4}). Second, the sample size of this study is modest compared to genetic studies of asthma. However, to our knowledge, this is one of the largest candidate-gene association studies of severe asthma exacerbations published to date. Third, heterogeneity in genetic admixture exists in the populations analyzed. While African-Americans are descendants of African and European ancestors, Latinos also have Native American admixture, with varying proportions of each component depending on the Latino subgroup⁹. In this regard, ethnicity-stratified analyses revealed that the effect in Latinos was driven mainly by Mexicans and other Latinos (**Table E7**).

In conclusion, the SNP rs67622929 was associated with severe asthma exacerbations in African-Americans and Latinos and showed regulatory effects over *DNASE1L3* expression.

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REFERENCES

1. Fricker M, Gibson PG, Powell H, Simpson JL, Yang IA, Upham JW, et al. A sputum 6-gene signature predicts future exacerbations of poorly controlled asthma. *J Allergy Clin Immunol.* 2019;144:51-60.e11.
2. Grossman NL, Ortega VE, King TS, Bleecker ER, Ampleford EA, Bacharier LB, et al. Exacerbation-prone asthma in the context of race and ancestry in Asthma Clinical Research Network trials. *J Allergy Clin Immunol.* 2019;144:1524-33.
3. Hernandez-Pacheco N, Flores C, Oh SS, Burchard EG, Pino-Yanes M. What ancestry can tell us about the genetic origins of inter-ethnic differences in asthma expression. *Curr Allergy Asthma Rep.* 2016;16:53.
4. Lonsdale J, Thomas J, Salvatore M, Phillips R, Lo E, Shad S, et al. The Genotype-Tissue Expression (GTEx) project. *Nat Genet.* 2013;45:580-5.
5. Koscielny G, An P, Carvalho-Silva D, Cham JA, Fumis L, Gasparian R, et al. Open Targets: a platform for therapeutic target identification and validation. *Nucleic Acids Res.* 2017;45: D985–94.
6. Shi G, Abbott KN, Wu W, Salter RD, Keyel PA. Dnase1L3 regulates inflammasome-dependent cytokine secretion. *Front Immunol.* 2017;8:522.
7. Baines KJ, Simpson JL, Wood LG, et al. Sputum gene expression signature of 6 biomarkers discriminates asthma inflammatory phenotypes. *J Allergy Clin Immunol.* 2014;133:997-1007.
8. Sánchez-Ovando S, Baines KJ, Barker D, Wark PA, Simpson JL. Six gene and TH2 signature expression in endobronchial biopsies of participants with asthma. *Immunity, Inflamm Dis.* 2020;8:40-9.
9. Bryc K, Durand EY, Macpherson JM, Reich D, Mountain JL. The genetic ancestry of African Americans, Latinos, and European Americans across the United States. *Am J Hum Genet.* 2015;96:37-53.

Table 1. Characteristics of the subjects included in the analyses.

	SAGE (n=1,002)		GALA II (n=2,181)	
	Exacerbators (n=470)	Non-exacerbators (n=532)	Exacerbators (n=1,283)	Non-exacerbators (n=898)
Gender (% female)	229 (48.7)	286 (50.4)	582 (45.4)	403 (44.9)
Mean age in years $\pm$ SD	14.3 $\pm$ 5.6 ^a	14.7 $\pm$ 4.1	12.2 $\pm$ 3.1 ^a	13.4 $\pm$ 3.5
African ancestry (%)	79.4 $\pm$ 10.7	78.4 $\pm$ 12.1	18.9 $\pm$ 13.2 ^a	12.6 $\pm$ 11.9
Native American ancestry (%)	NA	NA	22.9 $\pm$ 22.6 ^b	37.4 $\pm$ 25.5
Asthma control, n (%)				
Well controlled	60 (15.7)	182 (36.8)	210 (16.4) ^a	431 (48.0)
Partially controlled	112 (29.4)	150 (30.4)	533 (41.5) ^a	282 (31.4)
Poorly controlled	209 (54.9)	162 (32.8)	540 (42.1) ^a	185 (20.6)
Treatment category, n (%)				
Step 1	111 (23.6)	249 (47.1)	210 (16.4) ^a	431 (48.0)
Step 2	183 (39.0)	210 (39.7)	533 (41.5) ^a	282 (31.4)
Step 3	176 (37.4)	70 (13.2)	540 (42.1) ^a	185 (20.6)
Baseline lung function				
FEV ₁ (%) predicted	91.2 $\pm$ 11.9	92.4 $\pm$ 11.6	97.9 $\pm$ 15.7 ^a	103.6 $\pm$ 14.4
FVC (%) predicted	96.5 $\pm$ 11.8	97.0 $\pm$ 10.9	102.0 $\pm$ 16.0 ^a	107.3 $\pm$ 15.3
FEV ₁ /FVC (%) predicted	94.3 $\pm$ 8.3	95.3 $\pm$ 7.6	96.4 $\pm$ 7.6 ^a	97.2 $\pm$ 7.2
Total serum IgE (IU/ml)	168.3 $\pm$ 4.9 ^a	139.7 $\pm$ 4.6	254.0 $\pm$ 4.5 ^a	147.4 $\pm$ 4.7

Abbreviations: FEV₁, forced expiratory volume in one second; FVC, forced vital capacity; NA: Not available/applicable.

For continuous variables, the mean and standard deviation are displayed. For total serum IgE levels, the geometrical mean and standard deviation are reported. For categorical variables, the number and percentage of subjects in each category are shown.

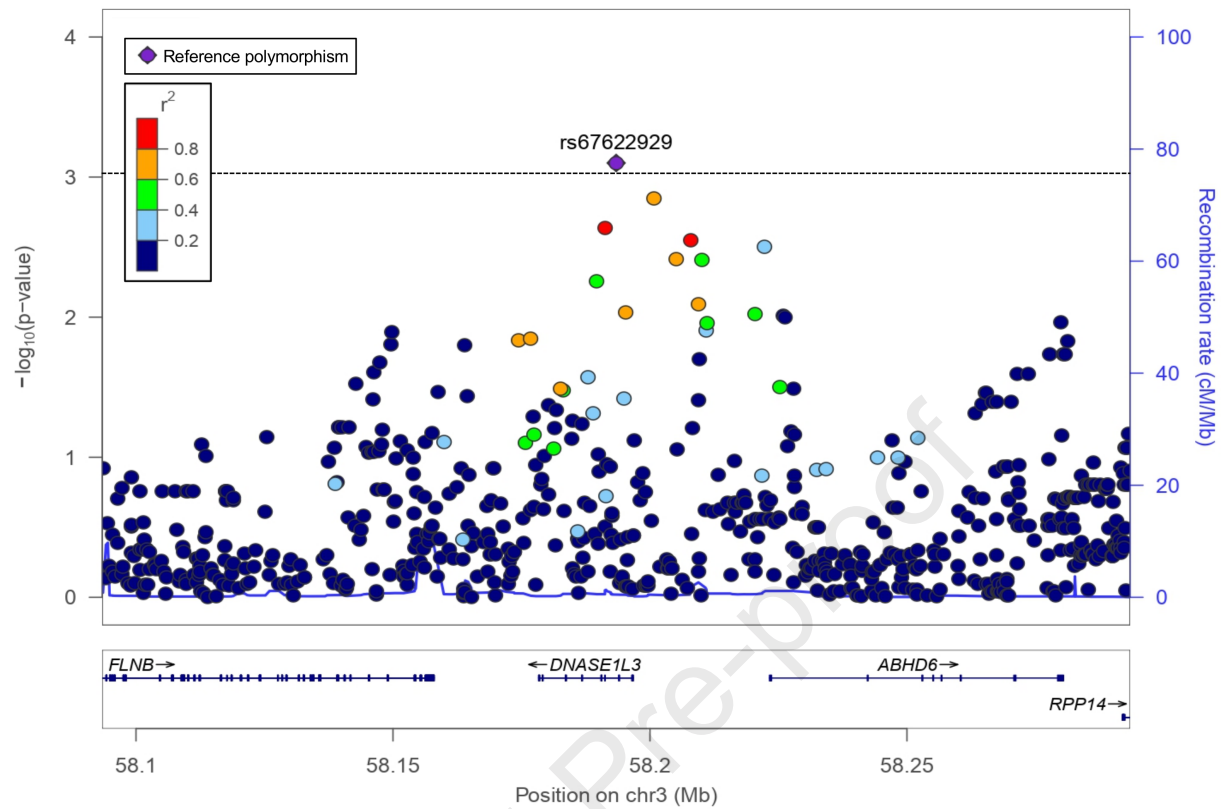
^a $p < 0.01$ for the comparison between asthma cases with exacerbations and controls.

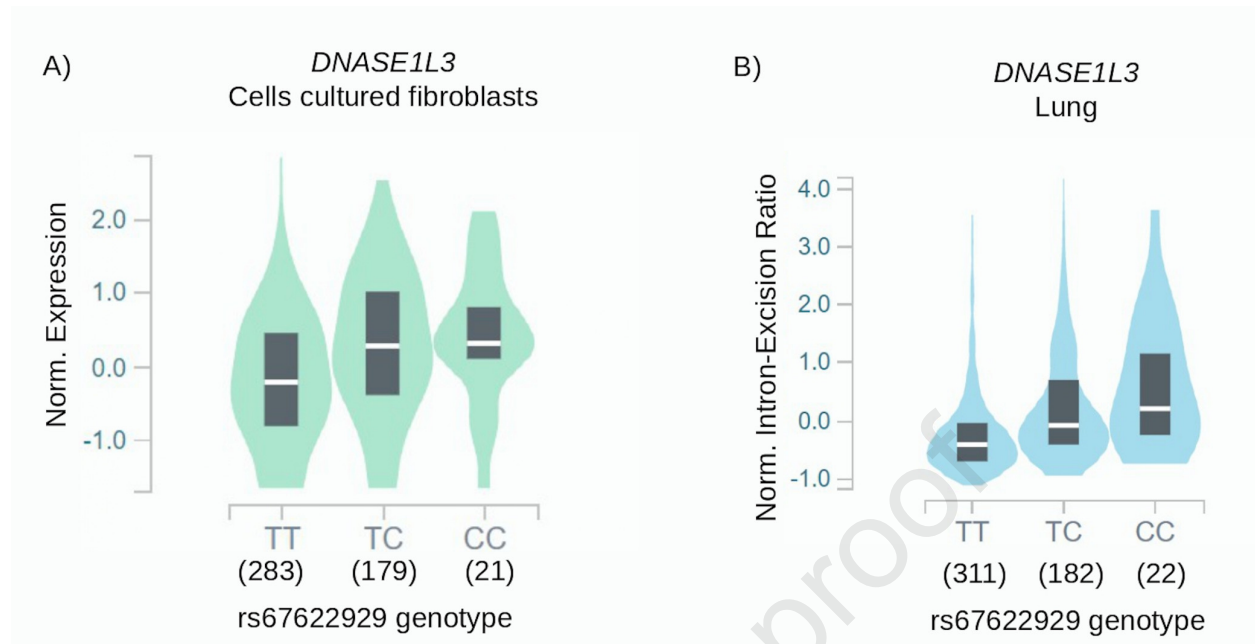
The severity medication regime was categorized into three levels based on the use of short beta agonists (step 1), one inhaled corticosteroid, leukotriene inhibitor, or theophylline tablet (step 2), more than one or a combination of an inhaled corticosteroid, leukotriene inhibitor, or theophylline tablet or a combination of inhaled corticosteroids and long-beta agonists (step 3).

Asthma control and treatment category were available for 875 and 999 individuals from SAGE, respectively. Asthma control and severity were available for 2,181 and 2,170 individuals from GALA II, respectively. FEV₁ was available for 807 and 2,068 SAGE and GALA II subjects. FVC was available for 821 participants from SAGE and 2,077 subjects from GALA II. FEV₁/FVC was available for 804 and 2,068 SAGE and GALA II subjects. Total serum IgE levels were available for 784 and 1,856 SAGE and GALA II subjects.

FIGURE LEGENDS

Figure 1. Regional plot for *DNASE1L3* in the discovery sample. The top hit (rs67622929) is shown as a purple diamond, and the rest of the genetic variants are colored based on their linkage disequilibrium (LD) with it using pairwise r^2 values from African populations from the 1000 Genomes Project. Chromosomal positions, the $-\log_{10}$ transformed p -values and the recombination rate are plotted in the x-axis, right and left y-axis, respectively. The Bonferroni-corrected threshold for significance is indicated by the dashed line.





ONLINE REPOSITORY MATERIALS

A Deoxyribonuclease 1 Like 3 genetic variant associates with asthma exacerbations

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Study populations

The Study of African Americans, Asthma, Genes and Environments (SAGE) and the Genes-Environment and Admixture in Latino Americans (GALA II) study are two ongoing case-control studies approved by the Human Research Protection Program Institutional Review Board of the University of California, San Francisco (California, United States).^{E1} Ethics approval numbers are 210362 (SAGE) and 217802 (GALA II). While GALA II recruited Hispanic/Latinos, SAGE II focused on African Americans. The participants (aged 8-21 years) declared to have all four grandparents of Hispanic/Latino or African American ancestry, for GALA II and SAGE respectively. All participants/parents provided written assent/consent. The same sample collection procedures, protocols, and questionnaires were used for both studies. SAGE participants were recruited in San Francisco Bay Area (United States). GALA II participants were recruited in different centers of the United States as well as Puerto Rico. Asthma was diagnosed by the attending physician and reports of symptoms or use of controller or rescue medication in the two years preceding enrollment. Asthma exacerbations were defined as the administration of oral corticosteroids, emergency asthma care, or hospitalizations in the last 12 months prior to study enrolment.

Spirometry was performed with a KoKo® PFT Spirometer (nSpire Health Inc., Louisville, CO) according to American Thoracic Society recommendations (1995). Subjects with asthma were instructed to withhold their bronchodilator medications for at least 8 hours prior to pulmonary function testing. Baseline lung function values were normalized to obtain predicted values with the Global Lung Initiative 2012 reference equations^{E2}. Measurements of immunoglobulin E (IgE) levels were conducted in duplicate using the ImmunoCAP™ 100 system (Phadia, Kalamazoo, MI).

Asthma severity was defined following the Expert Panel Report 3 (EPR-3) guidelines for managing asthma based on the medication treatment.^{E3} Classification of asthma control was based on the items included in the Childhood Asthma Control Test (C-ACT)^{E4} and the Asthma Control Questionnaire (ACQ)^{E5} based asthma nighttime symptoms, daytime symptoms, activity limitations, rescue medication use, and lung function measurements. The following questions were used to collect the data on each category: a) In general during the past week how much shortness of breath did the child experience because of asthma?; b) On average during the past week how often was the child woken by his/her asthma during the night?; c) In general during the past week how limited was the child in his/her activities because of asthma?; d) How many times did the patient report short-acting beta-agonist use in the last two weeks?; e) Percent predicted forced expiratory volume in 1 second and FEV₁/FVC ratio; f) Most recent time the child was prescribed oral steroids for asthma? The first five questions were scored as 0 (best asthma control), 1, or 2 (worst asthma control). Additionally, prescription of oral steroids for asthma was scored as 0 (more than 6 months ago) or 1 (6 months ago or less). The maximum score across the five domains was used to define asthma control with a score of 0 (very well controlled), 1 (not well controlled), or 2 (very poorly controlled).

Genotyping and statistical analysis

Genotyping was performed with the Axiom LAT1 array (World Array 4, Affymetrix, Santa Clara, United States), as previously described^{E6,E7}, and a subset of additional samples was also genotyped

with the Axiom LAT1 Array Plus T1EA^{E6,E7}. Quality control was performed as described elsewhere^{E6,E7}. Imputation was carried out using the Haplotype Reference Consortium (HRC)^{E9} as the reference population in the Michigan Imputation Server. Global ancestry proportions were estimated with the software ADMIXTURE^{E10} assuming African and European parental populations for African Americans and European, African and Native American populations for Hispanics/Latinos. Reference haplotypes from parental populations were estimated from genotyping data from European (CEU) and African (YRI) samples from the HapMap Project Phase III downloaded from the Axiom Genotype Data Set (<https://www.thermofisher.com/es/es/home/life-science/microarray-analysis/microarray-data-analysis/microarray-analysis-sample-data/axiom-genotype-data-set.html>). For the Native American population, 71 individuals genotyped with Axiom® LAT1 array (Affymetrix Inc.) were used as reference, as described elsewhere^{E7}. Association of genetic variants and phenotypes was tested through the logistic Wald test for binary traits and linear Wald test for quantitative measures using EPACTS v3.2.6^{E11}. In the discovery stage, the number of independent variants was determined using PLINK^{E12} v1.9 through a pruning procedure to exclude variants in linkage disequilibrium retaining those with $r^2 < 0.2$ within a 50 kb window and 5 SNPs window-shift for each candidate region. This r^2 threshold was selected based Sobota et al.^{E13} findings for populations of African ancestry that indicate that other thresholds are overly conservative and result in a reduced statistical power to detect associations in these populations. Meta-analyses were performed with METASOFT^{E14}. Genetic heterogeneity was assessed by means of the I^2 and Cochran's Q. Power calculations were performed using the software CATs v0.0.2^{E15} for a two-stage case-control study assuming a severe asthma exacerbation prevalence of 55%, as observed in the populations analysed, and different values of minor allele frequency (MAF, ranging from 10% to 50%) and genotype relative risk (GRR, ranging from 1.2 to 2.0).

***In silico* functional evaluation of the variant associated**

Evidence of significant expression quantitative trait loci (eQTL) and *cis*-splicing quantitative trait loci (sQTL) was queried in the GTEx v8 database.^{E16} Lung tissue and fibroblasts were considered biologically relevant tissues and cells for asthma exacerbations^{E17}, given its involvement in airway inflammation and remodelling^{E18,E19} which has an important role in this phenotype.

ONLINE REPOSITORY FIGURE LEGEND

Figure E1. A) Expression quantitative trait loci (eQTL) analysis of SNP rs67622929 for *DNASE1L3* in cell-cultured fibroblasts from GTEx^{E16} v8 Portal ($p=7.1 \times 10^{-16}$). B) Splicing quantitative trait loci (sQTL) analysis of rs67622929 for *DNASE1L3* in lung from GTEx^{E16} v8 Portal. The splicing affects the intron 58204881:58208218:clu_47729 ($p=9.4 \times 10^{-9}$) .

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Table E1. Estimation of the statistical power for the genomic regions assessed under different genotype relative risk scenarios taking into account the number of independent SNPs tested within each genomic region in the two-stage case-control study.

Minor allele frequency	Stage	# independent SNPs	GRR=1.2	GRR=1.4	GRR=1.6	GRR=1.8	GRR=2.0
10%	Discovery	348	91	100	100	100	100
	Replication	1	47	100	100	100	100
	Joint analysis	1	47	100	100	100	100
20%	Discovery	348	100	100	100	100	100
	Replication	1	77	100	100	100	100
	Joint analysis	1	77	100	100	100	100
30%	Discovery	384	100	100	100	100	100
	Replication	1	87	100	100	100	100
	Joint analysis	1	87	100	100	100	100
40%	Discovery	348	100	100	100	100	100
	Replication	1	89	100	100	100	100
	Joint analysis	1	89	100	100	100	100
50%	Discovery	348	100	100	100	100	100
	Replication	1	88	100	100	100	100
	Joint analysis	1	88	100	100	100	100

Abbreviations: GRR: Genotype relative risk; SNP: Single nucleotide polymorphism.

Statistical power was obtained using CATs v0.0.2 comparing different minor allele frequency, genotype relative risk, for a mean prevalence of severe asthma exacerbations of 55%, as observed in the populations analysed in this case-control study.

Values of statistical power equal or higher than 80% are highlighted in boldface.

Table E2. Summary of association results for the six genes analysed in SAGE.

Gene	<i>ALPL</i>	<i>CLC</i>	<i>CPA3</i>	<i>CXCR2</i>	<i>DNASE1L3</i>	<i>IL1B</i>
#SNPs tested	789	743	709	582	710	556
#Independent SNPs	100	72	77	40	57	38
Bonferroni threshold	5.00×10^{-4}	6.94×10^{-4}	6.49×10^{-4}	1.25×10^{-3}	8.77×10^{-4}	1.32×10^{-3}
Significant SNPs	0	0	0	0	1	0
SNP with minimum <i>p</i> -value	rs10799704	rs1568101	rs2001228	rs56979819	rs67622929	rs3136557
Minimum <i>p</i> -value	5.08×10^{-4}	9.18×10^{-4}	1.43×10^{-4}	0.014	7.93×10^{-4}	8.8×10^{-3}

Abbreviations: SNP, single nucleotide polymorphism.

The Bonferroni-like threshold was calculated as the $0.05/\text{number of independent variants located within each gene limits } \pm 100$ kilobases.

Table E3. Association results (additive model) for the C allele of rs67622929 with different outcomes.

Trait	SAGE		GALA II		Meta-analysis		Cochran's Q <i>p</i> -value
	OR/Beta (95% CI) ^a	<i>p</i> -value	OR/Beta (95% CI) ^a	<i>p</i> -value	OR/Beta (95% CI) ^a	<i>p</i> -value	
Asthma exacerbations (adjusted age, sex, and global ancestry)	1.48 (1.17 – 1.86)	7.9 x 10 ⁻⁴	1.18 (1.01 – 1.37)	0.03	1.26 (1.11–1.43)	2.6x10 ⁻⁴	0.103
FEV ₁ predicted (%)	-1.17 (-1.90 – (-0.45))	0.104	-0.45 (-0.97 – 0.07)	0.391	-0.70 (-1.12 – (-0.27))	0.099	0.418
FVC predicted (%)	-0.93 (-1.62 – (-0.24))	0.178	0.05 (-0.48 – 0.58)	0.930	0.05 (-0.33 – 0.42)	0.900	1.000
FEV ₁ /FVC predicted (%)	-0.81 (0.17 – (-1.79))	0.107	-0.25 (-0.78 – 0.28)	0.345	-0.38 (-0.84 – 0.86)	0.110	0.331
Total serum IgE levels	0.03 (-0.02 – 0.07)	0.538	0.02 (0.00 – 0.04)	0.491	0.02 (0.00 – 0.04)	0.365	0.855

Abbreviations: CI, Confidence interval; FEV₁, forced expiratory volume in one second; FVC, forced vital capacity; IgE: Immunoglobulin E; OR: Odds ratio.

^a Beta estimates or odds ratio are reported, for quantitative measurement and binary traits, respectively.

Table E4. Association results between rs67622929 and susceptibility to asthma with exacerbations in public online databases including European descent individuals.

Study	Phenotype ^a	Cases	Controls	Beta (C allele)	SE	p-value	Details
UK Biobank	Asthma with exacerbations compared to subjects without asthma	252	375,505	-0.05	0.11	0.67	Based on data imputed to the HRC panel and SAIGE analysis of ICD-derived traits. ^{E20}
UK Biobank	Asthma-related hospitalizations compared to subjects without asthma	1,986	359,208	-1.13x10 ⁻⁴	2.12x10 ⁻⁴	0.59	Imputed genotypes from HRC plus UK10K & KGP reference panels (Neale lab GWAS round 2). ^{E21}
Michigan Genomics Initiative	Asthma with exacerbations compared to subjects without asthma	1,506	32,282	-0.05	0.046	0.20	Based on MGI data (freeze2) imputed to the HRC panel. ^{E22}

^a The time period for which the presence / absence of exacerbations is determined is not detailed in the databases where the information was extracted.

Abbreviations: GWAS: Genome-wide association study; HRC: Haplotype Reference Consortium; ICD: International Classification of Diseases; MGI: Michigan Genomics Initiative; KGP: 1000 Genomes project; SE: standard error of the beta estimate.

Table E5. Cis-acting expression quantitative trait loci (eQTL) effects described in GTEx^{E16} v8 Portal for rs67622929.

Gene Symbol	p-value	NES	Tissue
<i>DNASE1L3</i>	7.10×10^{-16}	0.48	Cells - Cultured fibroblasts
<i>DNASE1L3</i>	3.90×10^{-15}	0.65	Testis
<i>DNASE1L3</i>	1.20×10^{-11}	0.36	Adipose - Subcutaneous
<i>DNASE1L3</i>	1.00×10^{-9}	0.35	Thyroid
<i>ABHD6</i>	6.70×10^{-8}	0.15	Skin - Sun Exposed (Lower leg)
<i>PDHB</i>	1.20×10^{-7}	0.34	Brain - Cortex
<i>RP11-456N14.4</i>	3.30×10^{-7}	0.35	Heart - Left Ventricle
<i>RP11-456N14.4</i>	7.00×10^{-7}	0.33	Adipose - Visceral (Omentum)
<i>DNASE1L3</i>	9.50×10^{-7}	0.32	Skin - Not Sun Exposed (Suprapubic)
<i>ABHD6</i>	1.20×10^{-6}	0.23	Testis
<i>DNASE1L3</i>	1.70×10^{-6}	0.27	Skin - Sun Exposed (Lower leg)
<i>ABHD6</i>	2.90×10^{-6}	0.21	Esophagus - Muscularis
<i>DNASE1L3</i>	4.70×10^{-6}	0.28	Breast - Mammary Tissue
<i>ABHD6</i>	4.80×10^{-6}	0.14	Skin - Not Sun Exposed (Suprapubic)
<i>RPP14</i>	1.70×10^{-5}	0.14	Muscle - Skeletal
<i>DNASE1L3</i>	2.20×10^{-5}	0.29	Nerve - Tibial
<i>PDHB</i>	3.30×10^{-5}	0.25	Brain - Cerebellar Hemisphere
<i>PDHB</i>	4.00×10^{-5}	0.08	Muscle - Skeletal
<i>FLNB-AS1</i>	4.40×10^{-5}	0.22	Heart - Atrial Appendage
<i>FLNB-AS1</i>	8.70×10^{-5}	0.13	Skin - Not Sun Exposed (Suprapubic)
<i>DNASE1L3</i>	1.00×10^{-4}	0.23	Heart - Atrial Appendage
<i>FLNB-AS1</i>	1.30×10^{-4}	0.34	Brain - Cerebellum
<i>FLNB-AS1</i>	2.10×10^{-4}	0.14	Thyroid
<i>DNASE1L3</i>	2.30×10^{-4}	0.26	Esophagus - Muscularis
<i>RP11-456N14.4</i>	2.50×10^{-4}	0.22	Adipose - Subcutaneous

Abbreviations: NES, Normalized Effect Size. The NES magnitude has no direct biological interpretation in GTEx^{E16}.

Table E6. Splicing quantitative trait (sQTL) effects described in GTEx^{E16} v8 Portal for rs67622929.

Gene Symbol	Intron Id	<i>p</i> -value	NES	Tissue
<i>DNASE1L3</i>	58204881:58208218:clu_48244	7.60x10 ⁻³⁴	0.75	Thyroid
<i>DNASE1L3</i>	58204881:58208218:clu_38886	2.00x10 ⁻²⁶	1.1	Spleen
<i>DNASE1L3</i>	58204881:58208218:clu_42898	2.50x10 ⁻²¹	0.95	Pituitary
<i>DNASE1L3</i>	58204881:58208218:clu_35842	1.50x10 ⁻¹⁶	0.83	Adrenal Gland
<i>DNASE1L3</i>	58204881:58208218:clu_30751	1.10x10 ⁻¹¹	0.52	Liver
<i>DNASE1L3</i>	58204881:58208218:clu_39805	2.90x10 ⁻⁹	0.43	Heart - Atrial Appendage
<i>DNASE1L3</i>	58204881:58208218:clu_47729	9.40x10 ⁻⁹	0.37	Lung
<i>DNASE1L3</i>	58204881:58208218:clu_45777	1.90x10 ⁻⁸	0.44	Breast - Mammary Tissue
<i>DNASE1L3</i>	58204881:58208218:clu_41846	3.70x10 ⁻⁸	0.35	Esophagus - Mucosa

Abbreviations: NES, Normalized Effect Size. The NES magnitude has no direct biological interpretation in GTEx^{E16}.

Table E7. Association results of rs67622929 (C allele) with asthma exacerbations stratified by ethnicity.

Population	MAF cases (%)	MAF controls (%)	OR (95% CI)	<i>p</i> -value
African American SAGE	21.16	15.61	1.48 (1.18 – 1.87)	7.93 x 10 ⁻⁴
Puerto Ricans GALA II	21.08	21.47	0.97 (0.77 – 1.23)	0.823
Mexicans GALA II	33.96	27.96	1.30 (1.00 – 1.67)	0.043
Other Latinos GALA II	30.30	27.26	1.35 (0.97 – 1.88)	0.076

MAF: minor allele frequency, OR: odds ratio.

ONLINE REPOSITORY REFERENCES

- E1. Nishimura KK, Galanter JM, Roth LA, Oh SS, Thakur N, Nguyen EA, 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;188:309-18.
- E2. Quanjer PH, Stanojevic S, Cole TJ, Baur X, Hall GL, Culver BH, et al. Multi-ethnic reference values for spirometry for the 3-95-yr age range: The global lung function 2012 equations. *Eur Respir J* 2012;40:1324-43.
- E3. National Asthma Education and Prevention Program. Expert Panel Report 3 (EPR-3): Guidelines for the Diagnosis and Management of Asthma-Summary Report 2007. *J Allergy Clin Immunol* 2007;120:S94-138.
- E4. Liu AH, Zeiger R, Sorkness C, Mahr T, Ostrom N, Burgess S, et al. Development and cross-sectional validation of the Childhood Asthma Control Test. *J Allergy Clin Immunol* 2007;119:817-25.
- E5. Juniper EF, O'Byrne PM, Guyatt GH, Ferrie PJ, King DR. Development and validation of a questionnaire to measure asthma control. *Eur Respir J* 1999;14:902-907.
- E6. White MJ, Risse-Adams O, Goddard P, Contreras MG, Adams J, Hu D, et al. Novel genetic risk factors for asthma in African American children: Precision Medicine and the SAGE II Study. *Immunogenetics* 2016;68:391-400.
- E7. Pino-Yanes M, Gignoux CR, Galanter JM, Levin AM, Campbell CD, Eng C, et al. Genome-wide association study and admixture mapping reveal new loci associated with total IgE levels in Latinos. *J Allergy Clin Immunol* 2015;135:1502-10.
- E8. Genes-environments and Admixture in Latino Asthmatics (GALA II) GWAS Study; 2015. Available at: https://www.ncbi.nlm.nih.gov/projects/gap/cgi-bin/study.cgi?study_id=phs001274.v1.p1. Accessed May 12, 2019.
- E9. McCarthy S, Das S, Kretzschmar W, Delaneau O, Wood AR, Teumer A, et al. A reference panel of 64,976 haplotypes for genotype imputation. *Nat Genet* 2016;48:1279-83.
- E10. Alexander DH, Novembre J, Lange K. Fast model-based estimation of ancestry in unrelated individuals. *Genome Res* 2009;19:1655-1664.
- E11. Kang HM. EPACTS (Efficient and Parallelizable Association Container Toolbox); 2016. Available at: <https://genome.sph.umich.edu/wiki/EPACTS>. Accessed August 10, 2019.
- E12. Chang CC, Chow CC, Tellier LCAM, Vattikuti S, Purcell SM, Lee JJ. Second-generation PLINK: Rising to the challenge of larger and richer datasets. *Gigascience* 2015;4.
- E13. Sobota RS, Shriner D, Kodaman N, Goodloe R, Zheng W, Gao YT, et al. Addressing population-specific multiple testing burdens in genetic association studies. *Ann Hum Genet* 2015;792:136-147.
- E14. Han B, Eskin E. Random-effects model aimed at discovering associations in meta-analysis of genome-wide association studies. *Am J Hum Genet* 2011;88:586-98.
- E15. Skol AD, Scott LJ, Abecasis GR, Boehnke M. Joint analysis is more efficient than replication-based analysis for two-stage genome-wide association studies *Nat Genet* 2006;38:209-213.

- E10. Lonsdale J, Thomas J, Salvatore M, Phillips R, Lo E, Shad S, et al. The Genotype-Tissue Expression (GTEx) project. *Nat Genet* 2013;45:580-5.
- E17. Westergren-Thorsson G, Larsen K, Nihlberg K, Andersson-Sjöland A, Hallgren O, Marko-Varga G, et al. Pathological airway remodelling in inflammation. *Clin Respir J* 2010;4S1:1-8.
- E18. Descalzi D, Folli C, Scordamaglia F, Riccio AM, Gamalero C, Canonica GW. Importance of fibroblasts-myofibroblasts in asthma-induced airway remodeling. *Recent Pat Inflamm Allergy Drug Discov* 2007;1:237-241.
- E19. Reeves SR, Barrow KA, Kolstad TK, White MP, Rich LM, Wight TN, et al. Fibroblast gene expression following asthmatic bronchial epithelial cell conditioning correlates with epithelial donor lung function and exacerbation history. *Sci Rep* 2018;8:15768.
- E20. UKBiobank ICD PheWeb: Analysis of 1403 ICD-based traits using SAIGE; 2020. Available at: <http://pheweb.sph.umich.edu/SAIGE-UKB/pheno/495.2>. Accessed June 11, 2020.
- E21. GWAS round 2 results. Neale lab; 2018. Available at: <http://www.nealelab.is/uk-biobank>. Accessed June 11, 2020.
- E22. MGI freeze2 PheWeb; 2020. Available at: <http://pheweb.sph.umich.edu/pheno/495.2>. Accessed June 11, 2020.