Multi-omic approach associates blood methylome with bronchodilator drug response in pediatric asthma

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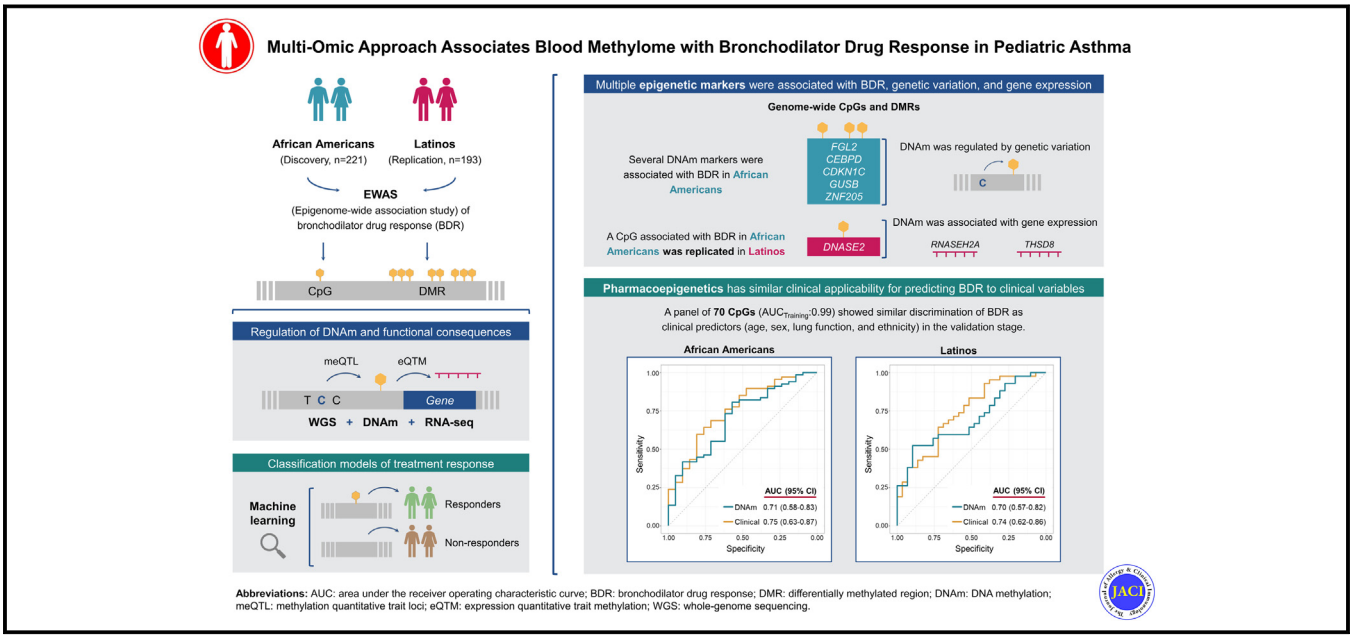
GRAPHICAL ABSTRACT



Background: Albuterol is the drug most widely used as asthma treatment among African Americans despite having a lower bronchodilator drug response (BDR) than other populations. Although BDR is affected by gene and environmental factors, the influence of DNA methylation is unknown.

Objective: This study aimed to identify epigenetic markers in whole blood associated with BDR, study their functional consequences by multi-omic integration, and assess their clinical applicability in admixed populations with a high asthma burden.

Methods: We studied 414 children and young adults (8-21 years old) with asthma in a discovery and replication design. We performed an epigenome-wide association study on 221 African Americans and replicated the results on 193 Latinos. Functional consequences were assessed by integrating epigenomics with genomics, transcriptomics, and environmental exposure data. Machine learning was used to develop a panel of epigenetic markers to classify treatment response.

Results: We identified 5 differentially methylated regions and 2 CpGs genome-wide significantly associated with BDR in African Americans located in *FGL2* (cg08241295, $P = 6.8 \times 10^{-9}$) and *DNASE2* (cg15341340, $P = 7.8 \times 10^{-8}$), which were regulated by genetic variation and/or associated with gene expression of nearby genes (false discovery rate < 0.05). The CpG cg15341340 was replicated in Latinos ($P = 3.5 \times 10^{-3}$). Moreover, a panel of 70 CpGs showed good classification for those with response and

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nonresponse to albuterol therapy in African American and Latino children (area under the receiver operating characteristic curve for training, 0.99; for validation, 0.70-0.71). The DNA methylation model showed similar discrimination as clinical predictors ($P > .05$).
Conclusions: We report novel associations of epigenetic markers with BDR in pediatric asthma and demonstrate for the first time the applicability of pharmacoepigenetics in precision medicine of respiratory diseases. (J Allergy Clin Immunol 2023;■■■:■■■-■■■.)

Key words: *Epigenomics, precision medicine, albuterol, African Americans, Hispanic Americans*

Albuterol is the most commonly prescribed reliever medication to treat asthma symptoms.[1-3] Also, it is often the only medication used to treat asthma in low-income minority populations regardless of asthma severity.[2] Response to bronchodilators is usually measured in terms of bronchodilator drug response (BDR).[1,3] Interindividual variation in BDR results from underlying factors, such as asthma severity, age, genetics, or environmental and social factors.[1-3] Furthermore, significant differences in BDR have been observed among populations.[1,4,5] African Americans have lower BDR along with higher asthma morbidity and mortality compared to individuals of European descent.[1,3] It is therefore essential to understand how genetic and environmental factors interact to influence lower BDR among African Americans and contribute to health disparities in asthma outcomes.

Epigenetic changes are a reflection of genetics, environment, and gene-environment interactions, and are known to vary across populations.[6] Existing epigenetic variations may be explained by epigenetic heritability, population-specific environmental factors, or methylation quantitative trait loci.[6] Epigenetics involves several mechanisms that regulate gene expression without

Abbreviations used

AUC:	Area under the receiver operating characteristic curve
BDR:	Bronchodilator drug response
CEBPD:	CCAAT/enhancer-binding protein δ
CI:	Confidence interval
CpG:	Cytosine–phosphate–guanine–3′ dinucleotide
DMR:	Differentially methylated region
DNAm:	DNA methylation
EWAS:	Epigenome-wide association study
FDR:	False discovery rate
FGL2:	Fibrinogen-like protein 2
GALA II:	Genes-environments & Admixture in Latino Americans
meQTL:	*cis*-Methylation quantitative trait loci
SAGE:	Study of African Americans, Asthma, Genes & Environments
SNP:	Single nucleotide polymorphism

The first 2 authors contributed equally to this article, and both should be considered first author. The last 2 authors contributed equally to this article, and both should be considered senior author.

All data necessary to evaluate the conclusions of this report are reported in the main text and/or the Online Repository (available at www.jacionline.org). TOPMed whole-genome sequencing data and RNA sequencing data from GALA II and SAGE are available in the database of Genotypes and Phenotypes (dbGaP) under accession numbers phs000920.v4.p2 and phs000921.v4.p1, respectively. The summary statistics of the epigenome-wide association study are available at the Zenodo repository (https://doi.org/10.5281/zenodo.5494396).

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modifying the underlying genetic sequence. Epigenetics might explain a portion of complex disease heritability that cannot be accounted for by genome-wide association studies.[7] The study of the epigenome provides an opportunity to understand how genetics and the environment interact to influence BDR.

DNA methylation (DNAm), the most studied epigenetic marker, consists of the addition of a methyl group to a cytosine that occurs with a higher frequency among 5′-cytosine-phosphate-guanine-3′ dinucleotide (CpG) sites.[6] DNAm has been reported to be a useful asthma biomarker.[8-13] Epigenome-wide association studies (EWAS) allow screening DNAm across the whole genome and provide new insights into the discovery of biomarkers of asthma.[11] To the best of our knowledge, only 1 study has assessed the association between whole-genome DNAm and BDR. Cardenas et al[11] conducted an EWAS in nasal samples of different asthma-related traits, reporting 130 CpGs associated with BDR and no differentially methylated region (DMR). Nevertheless, this study comprised 88% of children without asthma and 60.7% were of European ancestry. Thus, the role of epigenetics on BDR in high asthma-burden minority populations is unknown. On the other hand, whole blood has been proposed as a useful biomarker for different diseases since up to 80% of human genes are expressed in this tissue. Moreover, blood cells have been demonstrated to be a potentially less invasive proxy for the dysregulation of gene expression in lower airway cells in patients with severe asthma.[14,15] However, the relation between whole blood genome-wide DNAm and BDR has not been studied.

We hypothesize that DNAm in whole blood is associated with BDR among African Americans and Latinos with asthma. Therefore, we studied 414 children and young adults in a discovery and replication design, conducting an EWAS of BDR in 221 African Americans from the Study of African Americans, Asthma, Genes & Environments (SAGE) and 193 Latinos from the Genes-environments & Admixture in Latino Americans (GALA II) study. We assessed the contribution of genetic variation to the epigenetic markers identified and the functional consequences of DNAm in gene expression. In addition, we developed epigenetic classification models as proof of concept for the clinical applicability of pharmacoepigenetics in respiratory diseases.

METHODS

Study populations

An extensive Methods section is reported in the Online Repository available at www.jacionline.org. The discovery and replication phases of this study included African Americans and Latinos with asthma from the SAGE and GALA II studies, respectively. Full information regarding the enrollment of patients is reported elsewhere.[16,17] All participants were children and young adults (aged 8-21 years old) who self-identified as African American and had 4 African American grandparents in SAGE, and as Latinos in GALA II. Asthma cases were defined by (1) physician diagnosis of asthma, (2) recent receipt of asthma controller/reliever medication, or (3) occurrence of ≥2 asthma symptoms (cough, wheeze, or shortness of breath) in the 2 years before enrollment. Full information regarding spirometry and BDR measurement is available in the Methods section in the Online Repository available at www.jacionline.org. Subject selection for each analysis based on available data is summarized in Fig E1, also in the Online Repository.

DNAm and BDR association

Genome-wide DNAm at more than 850,000 CpGs was profiled in whole blood cells with the Infinium Illumina MethylationEPIC array (Illumina, San Diego, Calif). Quality control of methylation data is described in the Online Repository available at www.jacionline.org (see Table E1 there). Tissue heterogeneity was captured using a non–reference-based method (see Fig E2 in the Online Repository). Covariates included in subsequent analyses were selected on the basis of a principal component regression analysis (see Fig E3 in the Online Repository).

The association between DNAm (*M* values) and BDR was tested through robust linear regression models adjusted for age, sex, ancestry (genotype principal components), and tissue heterogeneity. A false discovery rate (FDR) of 5% was used to control for false positives, and the $P < 9 \times 10^{-8}$ threshold was used to declare genome-wide significance as previously empirically determined for the EPIC array.[18] Genome-wide significant CpGs were followed up in all subsequent analyses. Replication was performed on Puerto Ricans, Mexican Americans, and a meta-analysis of the 2 populations was conducted. On the basis of the epigenome-wide association study (EWAS) results, DMRs were identified using 3 independent software packages (comb-p, DMRcate, and dmrff), conserving those regions with adjusted $P < .05$. To minimize false-positive results, only DMRs that overlapped among the 3 software were followed up in subsequent analyses.

Genetic regulation of the identified DNAm changes

We conducted *cis*-methylation quantitative trait loci (meQTL) analysis to test the association between DNAm and nearby single nucleotide polymorphisms (SNPs) (±250 kb) detected through whole-genome sequencing. meQTL analyses were adjusted by age, sex, ancestry, and tissue heterogeneity. Independent signals were identified through conditional regression analysis and tested for association with BDR. *In silico* meQTL analyses were performed using public databases. In the case of DMRs, we summarized the global DNAm of the genomic regions using a principal component analysis including the M-values of all CpGs located within the DMR (see Fig E4 in the Online Repository available at www.jacionline.org). The first principal component was included as a predictor in the meQTL analysis.

Functional and enrichment analyses

Whole blood transcriptomes were generated using RNA sequencing as described in the Methods in the Online Repository available at www.jacionline.org. *cis*-Expression quantitative trait methylation analysis was performed to assess the association between DNAm and normalized gene expression levels of nearby genes (transcription start site within 250 kb). Linear regression models were adjusted by age, sex, ancestry, tissue heterogeneity, and surrogate variables of gene expression data (see Fig E5 in the Online Repository). Moreover, a trait methylation analysis of DMRs was conducted summarizing the global DNAm of these regions as previously described.

Furthermore, enrichment analyses were performed on the basis of the summary results of the discovery phase. CpGs associated with BDR at FDR ≤ 0.2 were included in CpG-set and gene-set enrichment analyses that were based on traits, biological pathways, and gene ontologies. In addition, protein–protein interaction networks were also assessed.

Classification models of BDR

The development of classification models of BDR is extensively detailed in the Methods section in the Online Repository available at www.jacionline.org. Briefly, a BDR threshold of 12% was used to classify response as good (BDR ≥ 12%) and poor (BDR < 12%). We used a machine learning approach applying an elastic net regression model for variable selection and regularization,[13] as explained in the Online Repository. African Americans from SAGE and Latinos from GALA II were split into 2 subsets equally balanced with regard to good or poor response to train the model (60%) and

TABLE I. Clinical and demographic characteristics of study populations stratified by ethnicity

	SAGE		GALA II			
Characteristic	**No.**	**African American**	**No.**	**Latino***	**Puerto Rican**	**Mexican**
Sex (% male)	221	112 (50.7)	193	106 (54.9)	86 (55.8)	20 (51.3)
Age (years)	221	13.8 (10.9-17.2)	193	13.1 (11.2-16.4)	12.8 (11.2-15.9)	14.6 (12.0-17.5)
Pre-FEV_1 (predicted %)	221	100.2 (92.9-108.6)	193	92.7 (82.9-101.3)	89.9 (80.4-99.4)	99.4 (93.0-107.0)
Pre-FEV_1 (L)	221	2.5 (1.9-3.1)	193	2.7 (2.1-3.3)	2.6 (2.1-3.2)	3.1 (2.6-3.7)
BDR (%)	221	8.4 (4.6-11.7)	193	10.0 (6.9-13.2)	11.1 (8.1-14.4)	5.6 (2.6-9.3)
BMI category	221		192			
Underweight		0		3 (1.6)	3 (2.0)	0
Normal		99 (44.8)		89 (46.3)	76 (49.7)	13 (33.3)
Overweight		50 (22.6)		38 (19.8)	29 (18.9)	9 (23.1)
Obese		72 (32.6)		62 (32.3)	45 (29.4)	17 (43.6)
Smoking						
In utero exposure	216	46 (21.3)	193	10 (5.2)	9 (5.8)	1 (2.6)
Secondhand exposure	172	48 (27.9)	186	34 (18.3)	30 (20.4)	4 (10.3)
Controller medication receipt	221	95 (43.0)	193	44 (22.8)	31 (21.2)	13 (13.3)
Insurance status	217		191			
None		2 (0.9)		9 (4.7)	4 (2.6)	5 (13.5)
Public		112 (51.6)		116 (60.8)	94 (61.0)	22 (59.5)
Private		103 (47.5)		66 (34.5)	56 (36.4)	10 (27.0)

Descriptive statistics are represented as medians (interquartile ranges) or means (standard deviations) for continuous variables, and counts (proportions) for categorical variables.
BMI, body mass index.
*A total of 154 Puerto Ricans and 39 Mexican Americans were included.

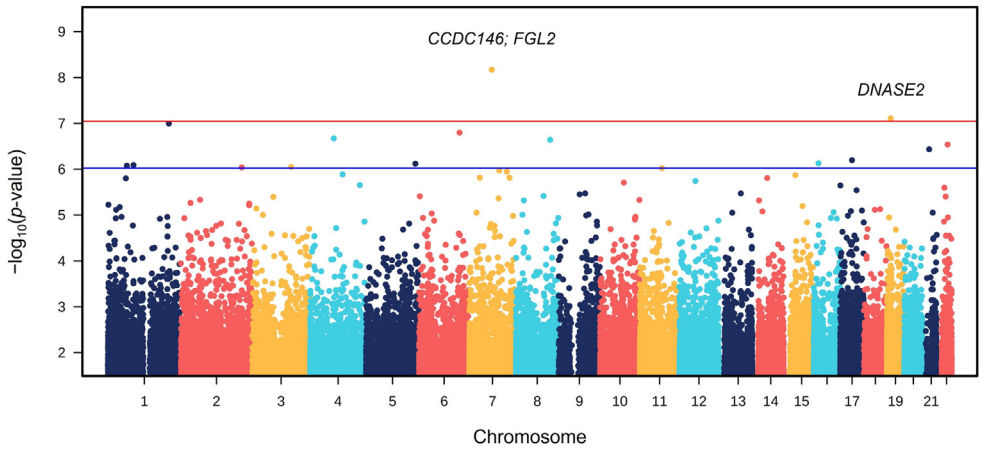

FIG 1. Manhattan plot of EWAS of BDR in African Americans. *Blue* and *red lines* represent FDR < 0.05 and genome-wide thresholds, respectively.

validate it (40%). A 5-time-repeated 10-fold cross-validation was performed to train the models, and the trained model was validated in the 2 independent validation subsets of African Americans and Latinos. Diagnostic test performance measures were computed, including sensitivity, specificity, positive predictive value, negative predictive value, and accuracy. We built an epigenetic classification panel on the basis of EWAS data and compared its performance with classification models generated according to (1) clinical data, (2) candidate SNPs previously reported in the literature to be associated with BDR, and (3) SNPs selected from the results of a meta–genome-wide association study of BDR in African Americans and Latinos

RESULTS

Study populations

The main characteristics of 221 asthma patients from SAGE and 193 from GALA II analyzed in the discovery and replication phases, respectively, are summarized in Table I. The median age of African American children was 13.8 years (interquartile range, 10.9-17.2), 50.7% were male, and less than 30% had been exposed to environmental smoking. Regarding Latinos, 154 were Puerto Ricans and 39 were Mexican Americans. Their median age was 13.1 years (interquartile range, 11.2-16.4), 54.9% were male, and less than 21% had been exposed to secondhand smoke. Median BDR was 8.4% (interquartile range, 4.6-11.7) in African Americans and 10.0% (interquartile range, 6.9-13.2) in Latinos.

Epigenome-wide association study

No genomic inflation was observed ($\lambda = 1.17$; see Fig E6 in the Online Repository available at www.jacionline.org), and a total of 16 CpGs were significantly associated with BDR (FDR ≤ 0.05) (Fig 1, Table II). Two CpGs surpassed the genome-wide significance threshold: cg08241295 located in the promoter region of *FGL2* ($P = 6.75 \times 10^{-9}$) and cg15341340 located in the first exon of *DNASE2* ($P = 7.84 \times 10^{-8}$). The CpG cg15341340 was replicated in the meta-analysis of Latinos

TABLE II. Summary of the CpGs associated with BDR at FDR ≤ 0.05 in African Americans

CpG	Chromosome	Position*	Nearest gene(s)	Coefficient	Standard error	*P* value
cg08241295	7	77200662	*CCDC146, FGL2*	−0.026	0.004	6.75×10^{-9}
cg15341340	19	12881423	*DNASE2*	0.018	0.003	7.84×10^{-8}
cg00352785	1	208037126	*PLXNA2*	−0.022	0.004	1.01×10^{-7}
cg18341600	6	137777476	*TNFAIP3, OLIG3*	−0.025	0.005	1.60×10^{-7}
cg07124719	4	79827398	*GDEP*	−0.022	0.004	2.13×10^{-7}
cg14382954	8	116058442	*LINC00536*	0.014	0.003	2.29×10^{-7}
cg09339568†	22	36900271	*CSF2RB, NCF4*	−0.023	0.004	2.90×10^{-7}
cg14355482	21	21000721	*NCAM2*	−0.026	0.005	3.69×10^{-7}
cg00823480	17	42016313	*NKIRAS2, DNAJC7*	−0.024	0.005	6.38×10^{-7}
cg24304533	16	16049176	*ABCC1*	−0.027	0.005	7.45×10^{-7}
cg13569779‡	5	168829246	*SLIT3*	−0.022	0.004	7.60×10^{-7}
cg01445399	1	87131251	*LOC339524*	−0.021	0.004	8.15×10^{-7}
cg10271974	1	64453953	*CACHD1, UBE2U*	−0.023	0.005	8.39×10^{-7}
cg00975929§	3	133545342	*CDV3, BFSP2*	−0.018	0.004	8.95×10^{-7}
cg06689758	2	206982696	*CPO, KFL7*	−0.016	0.003	9.15×10^{-7}
cg01167694	11	75698560	*MAP6, MOGAT2*	−0.021	0.004	9.51×10^{-7}

*Position based on GRCh38/hg38 build.
†SNP at CpG site.
‡SNP within probe.
§Cross-reactive probe.

TABLE III. Replication of genome-wide significant CpGs associated with BDR in Latinos

				Puerto Rican		Mexican American		Meta-analysis†		
CpG	Chr	Position*	Gene	Coef (SE)	*P* value	Coef (SE)	*P* value	Coef (SE)	*P* value	Cochran *Q P* value
cg08241295	7	77200662	*FGL2*	0.002 (0.007)	.743	0.013 (0.019)	.503	0.004 (0.007)	.584	.604
cg15341340	19	12881423	*DNASE2*	0.013 (0.004)	3.05×10^{-3}	0.003 (0.013)	.849	0.012 (0.004)	3.54×10^{-3}	.435

Chr, Chromosome; *Coef,* coefficient; *SE,* standard error.
*Position based on GRCh38/hg38 build.
†Meta-analysis of Latinos including Puerto Ricans and Mexican Americans.

($P = 3.54 \times 10^{-3}$; FDR $= 7.07 \times 10^{-3}$; Cochran Q $P = .435$; $I^2 = 0.00$), most likely driven by its association in Puerto Ricans (Table III). These associations were robust to the adjustment for socioeconomic, clinical, and environmental factors (see Table E2 in the Online Repository). The robustness of these findings was also demonstrated using an alternative approach to correct for latent confounders (see Table E3 in the Online Repository). In addition, we identified multiple DMRs associated with BDR in African Americans, with adjusted $P < .05$ (see Tables E4 and E5 in the Online Repository), and a total of 5 DMRs overlapped among the 3 independent software (Table IV, and see Fig E7 in the Online Repository). The top-hit is a region of 446 bp located near *CEBPD* that includes 8 CpGs (adjusted $P = 6.07 \times 10^{-9}$). The other DMRs were annotated to *ZNF205, CDK1NC, FGL2,* and *GUSB* genes.

Expression quantitative trait methylation analysis

The CpG cg15341340 *(DNASE2)* exhibited association with *RNASEH2A* ($\beta = 0.78$; $P = 1.2 \times 10^{-3}$, FDR $= 0.034$) and *THSD8* expression in whole blood ($\beta = 0.78$; $P = 1.7 \times 10^{-3}$, FDR $= 0.034$) (see Table E6 in the Online Repository available at www.jacionline.org). Furthermore, the global DNAm at the DMR located at *ZNF205* showed to be negatively associated with the gene expression in whole blood of that gene ($\beta = -0.29$; $P = 1.8 \times 10^{-5}$; FDR $= 1.1 \times 10^{-3}$) (see Table E7 in the Online Repository).

Methylation quantitative trait loci analysis

From the 3,234 SNPs included in this analysis, 124 meQTLs (5 independent meQTL signals) were significantly associated (FDR ≤ 0.05) with the methylation status of cg08241295 *(FGL2)* (Table V, and see Table E8 in the Online Repository available at www.jacionline.org). The C allele of the SNP rs77056943 (top-hit meQTL) was associated with lower methylation at this CpG ($\beta = -0.612$, $P = 2.5 \times 10^{-15}$, FDR $= 8.08 \times 10^{-12}$) (Fig 2). Four of those 5 independent meQTLs were available for *in silico* analyses, and all were replicated in other populations (min $P = 5.75 \times 10^{-266}$; max $P = 3.86 \times 10^{-6}$). Additionally, all of the identified DMRs were significantly associated with genetic variation (FDR ≤ 0.05): *CEBPD* (2 independent meQTLs, min $P = 2.0 \times 10^{-7}$), *ZNF205* (4 independent meQTLs, min $P = 1.0 \times 10^{-13}$), *CDKN1C* (2 independent meQTLs, min $P = 4.7 \times 10^{-5}$), *FGL2* (6 independent meQTLs, min $P = 7.9 \times 10^{-18}$), and *GUSB* (3 independent meQTLs, min $P = 3.0 \times 10^{-4}$) (Fig 2, Table V, and see Table E9 in the Online Repository). Among independent meQTLs, the G allele of rs3093273 (top-hit meQTL of the DMR at *FGL2*) was significantly associated with higher BDR after multiple

TABLE IV. DMRs associated with BDR in African Americans using 3 independent software packages

Chromosome	Start*	End*	Width (bp)	No. of CpGs	Corrected *P* value	Gene
8	47763086	47763532	446	7	6.07×10^{-9}	*CEBPD*
16	3112225	3113228	1003	9	1.05×10^{-7}	*ZNF205*
11	2869159	2869496	337	20	1.22×10^{-7}	*CDKN1C*
7	77200528	77200663	135	2	1.31×10^{-6}	*FGL2*
7	65954198	65954275	77	6	7.41×10^{-6}	*GUSB*

*Position based on GRCh38/hg38 build.

TABLE V. Independent meQTLs identified in African Americans

rsID	Chr	Position*	A1	A2	MAF	Beta	SE	*P* value	FDR
cg08241295									
rs77056943	7	77222983	C	T	0.05	−0.61	0.07	2.50×10^{-15}	8.08×10^{-12}
rs3093273	7	77199958	G	A	0.05	−0.61	0.07	1.25×10^{-14}	1.35×10^{-11}
rs3108424	7	77253084	A	G	0.47	0.20	0.03	4.64×10^{-9}	6.82×10^{-7}
rs76887620	7	77423839	C	A	0.07	−0.26	0.07	2.14×10^{-4}	7.29×10^{-3}
rs115458846†	7	77182811	T	G	0.06	−0.24	0.07	1.45×10^{-3}	4.30×10^{-2}
DMR annotated to *CEBPD*									
rs10113241	8	47567227	A	T	0.33	−1.26	0.23	1.95×10^{-7}	1.57×10^{-5}
rs7820803	8	47861366	G	A	0.05	−1.61	0.53	2.40×10^{-3}	3.84×10^{-2}
DMR annotated to *ZNF205*									
rs731045	16	3102232	T	C	0.30	−1.42	0.13	1.17×10^{-22}	4.54×10^{-19}
rs2735537	16	3141355	A	G	0.35	1.06	0.13	1.04×10^{-13}	2.53×10^{-11}
rs1053997	16	3142672	C	T	0.39	0.85	0.13	1.09×10^{-9}	1.23×10^{-7}
rs250479	16	3332844	C	T	0.14	−0.67	0.21	1.56×10^{-3}	2.94×10^{-2}
DMR annotated to *CDKN1C*									
rs450852	11	2735084	A	G	0.39	−1.21	0.29	4.74×10^{-5}	1.90×10^{-3}
rs12282506	11	2894144	A	G	0.07	1.75	0.58	2.88×10^{-3}	4.46×10^{-2}
DMR annotated to *FGL2*									
rs3093273	7	77199958	G	A	0.05	−2.03	0.22	7.91×10^{-18}	5.58×10^{-15}
rs77056943	7	77222983	C	T	0.05	−1.94	0.21	5.44×10^{-17}	3.24×10^{-14}
rs3093269	7	77200552	G	A	0.09	1.34	0.17	4.85×10^{-14}	1.45×10^{-11}
rs111326973	7	77243843	T	C	0.09	1.26	0.17	1.10×10^{-12}	2.13×10^{-10}
rs3108413	7	77247195	T	C	0.33	0.45	0.12	1.76×10^{-4}	5.39×10^{-3}
rs12704927	7	77390549	C	A	0.25	0.40	0.13	3.17×10^{-3}	4.84×10^{-2}
DMR annotated to *GUSB*									
rs148158941	7	65971458	A	G	0.04	1.15	0.31	2.97×10^{-4}	8.51×10^{-3}
rs4729415	7	77444550	A	G	0.08	0.69	0.19	4.09×10^{-4}	1.09×10^{-2}
rs3852244	7	65757230	T	A	0.14	−0.62	0.19	1.09×10^{-3}	2.19×10^{-2}

A1, Effect allele; *A2,* non–effect allele; *Chr,* chromosome; *MAF,* minor allele frequency (effect allele); *rsID,* reference SNP cluster ID; *SE,* standard error.
*Position based on GRCh38/hg38 build.
†This CpG was not reported in PhenoScannerV2 as meQTL.

comparisons adjustment ($\beta = 0.278$, $P = .001$, FDR < 0.05) (see Table E10 in the Online Repository).

Enrichment analyses

A CpG-set analysis based on 263 CpGs associated with BDR at FDR ≤ 0.2 showed significant enrichment in previous EWAS associations with multiple asthma-related traits. The fractional exhaled nitric oxide (153 CpGs, $P < 2.23 \times 10^{-308}$), allergic asthma (53 CpGs, $P = 9.95 \times 10^{-190}$), and allergic sensitization (37 CpGs, $P = 3.18 \times 10^{-115}$) showed the strongest associations. Additionally, we also identified an enrichment in CpGs previously associated with air pollutants (NO_2 and PM_{10}) (see Fig E8 in the Online Repository available at www.jacionline.org). Moreover, the gene-set analysis revealed enrichment in biological processes and molecular functions mainly related to phosphorylation, regulation of intracellular processes, and pro-inflammatory cytokines pathways, such as interleukin 2 and tumor necrosis factor (adjusted $P < .05$; see Tables E11 and E12 in the Online Repository). In addition, the proteins encoded by these genes established a significant functional and physical protein–protein interaction network (confidence score >0.7, $P = 7.2 \times 10^{-3}$) (see Fig E9 in the Online Repository) enriched in protein phosphorylation and immune response gene ontologies (adjusted $P < .05$) (see Table E13 in the Online Repository).

Epigenetic models to classify treatment response

A panel consisting of 70 CpGs was identified as the best epigenetic classifier of BDR (see Fig E10 and Table E14 in the Online Repository available at www.jacionline.org). This model had an excellent classification performance of BDR during the training stage ($AUC_{training}$: 0.99, 95% confidence interval [CI]: 0.98-1.00) and was validated independently in 2 subsets of African Americans and Latinos (Fig 3). This model showed

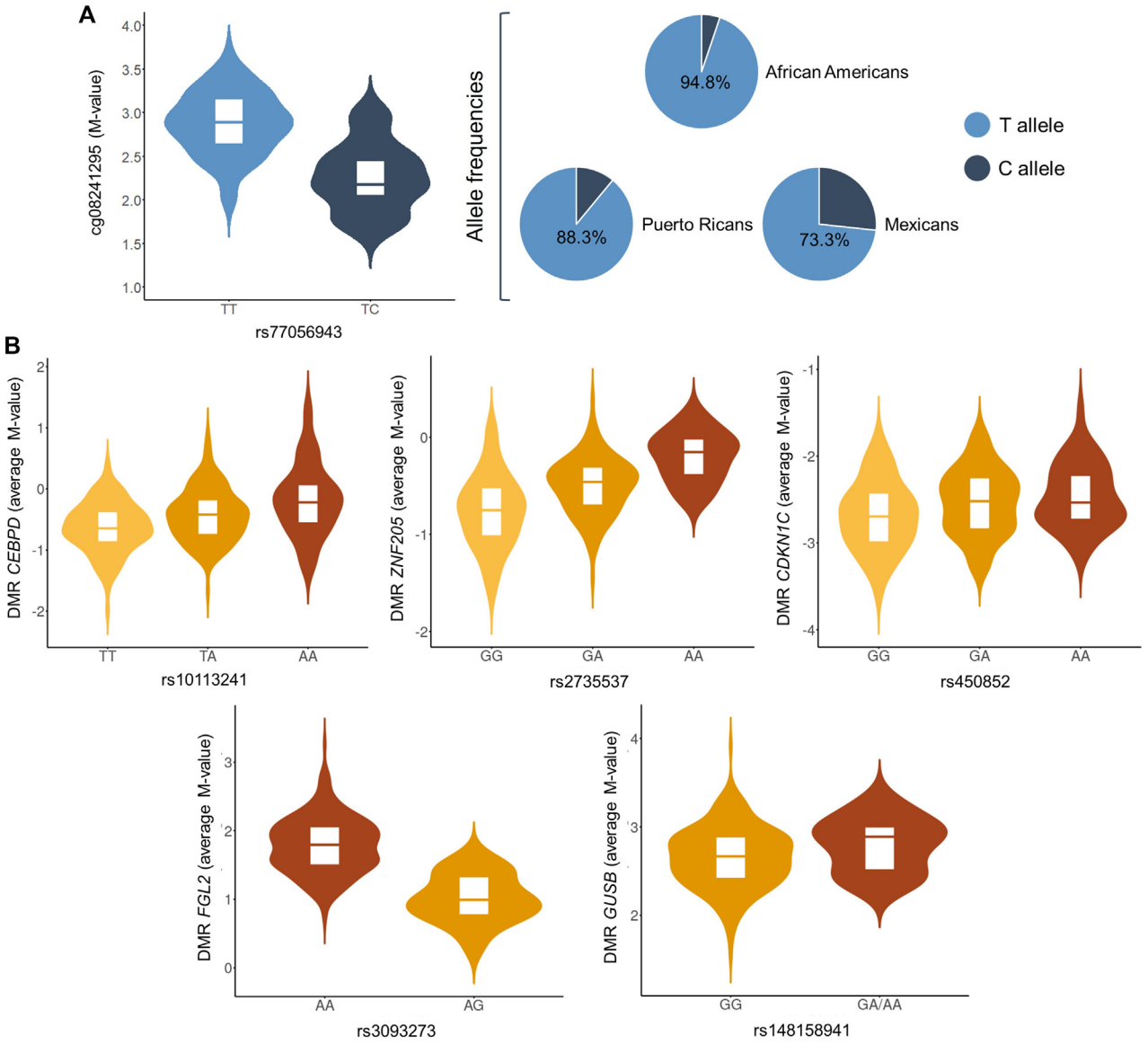

FIG 2. Violin plot of methylation levels and top-hit meQTLs identified in African Americans for each locus. **(A)** Top-hit meQTL for CpG cg08241295 near *FGL2* and its allele frequencies in African Americans and Latinos. **(B)** Top-hit meQTLs for DMRs.

similar discrimination of albuterol response in the validation stage as a clinical classification model built according to age, sex, ethnicity, height, and lung function in African Americans (AUC_{DNAm} [95% CI]: 0.71 [0.58-0.83] vs $AUC_{clinical}$ [95% CI]: 0.75 [0.63-0.87], $P = .611$) and Latinos (AUC_{DNAm} [95% CI]: 0.70 [0.57-0.82] vs $AUC_{clinical}$ [95% CI]: 0.74 [0.62-0.86], $P = .583$). We attempted to generate genetic classification models according to candidate SNPs and genome-wide data (see Table E15 and Fig E11 in the Online Repository). However, in the validation, the lower limit of the AUC confidence intervals of these models crossed AUC:0.50 both in African Americans and Latinos, indicating a null classification performance (see Table E16 in the Online Repository).

DISCUSSION

To our knowledge, this is the first integrative omic study in pediatric asthma reporting associations between whole blood DNAm and BDR. We identified 2 novel genome-wide associations of epigenetic markers with BDR: the CpG cg08241295 *(FGL2),* which displayed a population-specific effect in African Americans and was regulated by genetic variation; and the CpG cg15341340 *(DNASE2),* with a shared effect in African Americans and Latinos and associated with gene expression of nearby genes. In addition, we reported for the first time DMRs associated with BDR with evidence of regulation by meQTLs and associated with gene expression of nearby genes. Finally, we showed the potential role of epigenetics

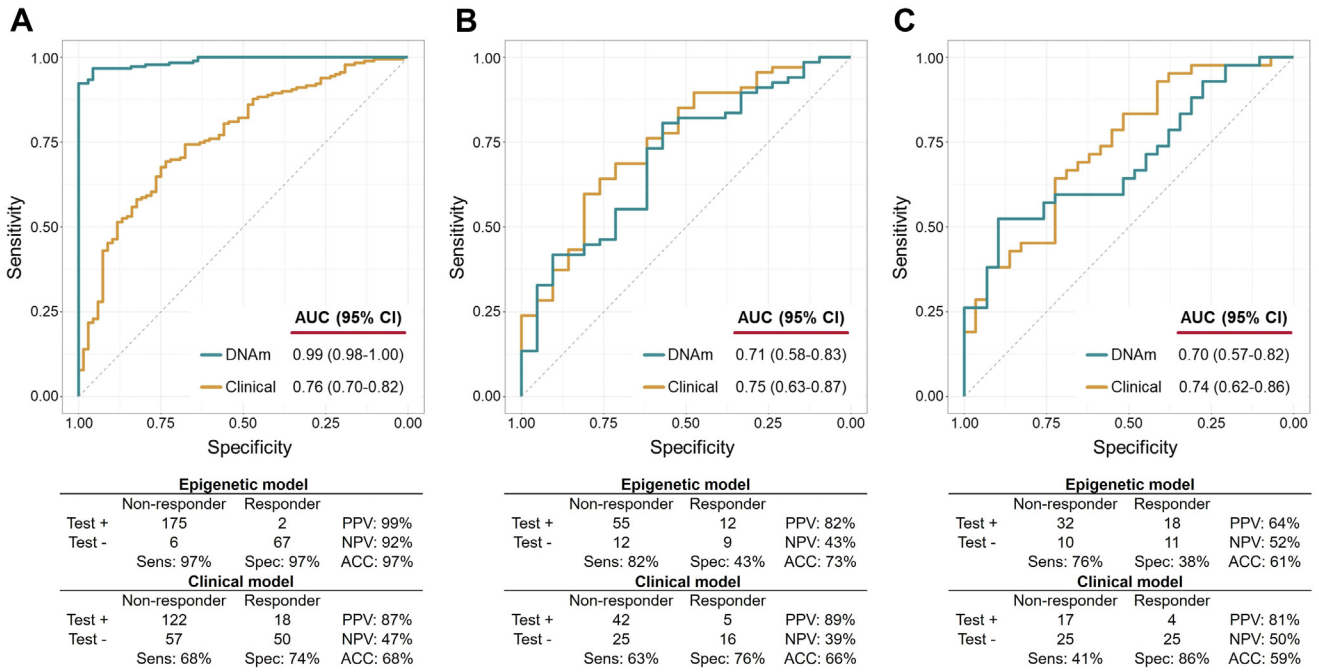

FIG 3. ROC curves and diagnostic test performance measures of epigenetic and clinical classification models of BDR in **(A)** training stage, **(B)** validation in African Americans, and **(C)** validation in Latinos. ROC curves of the epigenetic models are shown in *blue* (DNAm); clinical models, *yellow* (clinical). *ACC,* Accuracy; *NPV,* negative predictive value; *PPV,* positive predictive value; *ROC,* receiver operating characteristic; *Sens,* sensibility; *Spec,* specificity.

to improve the clinical prediction of BDR in minority populations with a high asthma burden.

The *FGL2* gene encodes for fibrinogen-like protein 2 (*FGL2*), a pan-expressed protein of the fibrinogen family that has an essential role during development.[19] *FGL2* can be secreted as a soluble protein by regulatory T-cells with a modulatory immune effect suppressing T_H1 and T_H17 cells, but not T_H2 cells, which are involved in allergic diseases.[20] Furthermore, *FGL2* over-expression in alveolar macrophages is associated with lung inflammation pathways in chronic obstructive pulmonary disease.[21] Here, we reported the association of the CpG cg08241295 and a DMR located on the *FGL2* promoter with BDR in African Americans. The CpG cg08241295 is located within the binding site of the transcription factor hypermethylated in cancer 1 (aka HIC1), which plays a role in the immune response by suppressing the regulatory T-cell activity.[22,23] Of note, it regulates gene expression through indirect and direct mechanisms of several genes, including *ADRB2,* the biological target of albuterol.[23] Moreover, we identified that DNAm at the CpG cg08241295 and the DMR located in *FGL2* was partially regulated by multiple independent meQTLs. The top-hit meQTL for the DMR on *FGL2* (rs3093273) was significantly associated with BDR, suggesting that both genetic and epigenetic variations may play a joint role in BDR.

Interestingly, all the epigenetic markers identified in African Americans that did not replicate in Latinos were shown to be regulated by genetic variation in meQTL analyses (cg08241295, and DMRs located in *CEBPD, ZNF205, CDKN1C, FGL2,* and *GUSB*), while the one that replicated (cg15341340, *DNASE2*) did not show any evidence of genetic regulation. Furthermore, we observed that the allele frequencies of the majority of top-hit meQTLs differed between African Americans and Latinos (eg, rs77056943 as a top-hit meQTL of cg08241295, Fig 2). This could be related to the absence of heterogeneity observed for cg08241295 among Latinos ($I^2 = 0.00$, Cochran Q $P = .604$), but the high heterogeneity across African Americans and Latinos ($I^2 = 85.97$, Cochran Q $P = 8.20 \times 10^{-4}$). Considering also that these individuals were recruited in different regions, we hypothesize that this heterogeneity across populations could be a consequence of differences in genetic backgrounds and environmental exposures, which have an essential role in DNAm patterns and their population-specific effect on BDR.

On the other hand, the CpG cg15341340 within *DNASE2* was genome-wide associated with BDR in African American and Latino children with asthma. A previous study showed that DNAm at this CpG is associated with long-term exposure to particulate matter ≤2.5 μm in diameter in elderly White subjects.[24] We observed the same association at a nominal level ($P = .021$; see Table E17 in the Online Repository available at www.jacionline.org) in a subset of African Americans with available data on early-life air pollution exposure during the first 3 years of life. Thus, we hypothesize that the shared effect of this CpG in BDR may be related to environmental exposure shared between populations. The *DNASE2* gene encodes for deoxyribonuclease II, a DNA nuclease widely expressed in mammalian tissues with optimum activity within lysosomes.[25] Genetic variation of *DNASE2* has been related to severe autoinflammatory syndromes mediated by interferons.[25] Besides, murine models have demonstrated that different types of DNases (eg, *DNASE2* and *DNASE1L2*) may jointly

participate in the same processes.[25] Considering that *DNASE1L3* has been previously associated with asthma exacerbations in Latinos and African Americans,[26] it is plausible that *DNASE2* may also play a role in asthma. Furthermore, inhaled DNAses have been suggested as a potential therapy in severe asthma to prevent airway epithelial cell damage due to extracellular neutrophils' DNA.[27] Additionally, cg15341340 was found to be associated with the gene expression of the catalytic center of the RNase enzyme *RNASEH2A* in whole blood of African Americans. Genetic variation near this gene has been previously associated with BDR in a genome-wide association study of African American adults with chronic obstructive pulmonary disease.[28]

We reported for the first time multiple DMRs associated with BDR. The most significant genomic region was annotated to *CEBPD,* which encodes for the transcription factor CCAAT/enhancer-binding protein δ (CEBPD). *CEBPD* is expressed in multiple tissues including the lungs, and its involvement in asthma is related to interleukin expression, air smooth muscle cell proliferation, and epithelial growth.[29] The expression of CEBP family members is regulated by glucocorticosteroids and β_2-agonist with disease-specific patterns.[30-32] Indeed, CEBP transcription factors are one of the mechanisms underlying the synergism between β_2-agonist and glucocorticosteroids.[33] *CEBPD* regulates the expression of the pro-inflammatory cytokines *IL5* and *IL6*.[27,34] IL5 participates in T_H2 inflammation and is also regulated by β_2-agonists and glucocorticosteroids,[27] whereas IL6 is a biomarker of asthma exacerbations and a potential therapeutic target in severe asthma.[34] Previous studies support the need for further investigation of the role of *CEBPD* in asthma treatment response.[31,32] Although the association of *CEBPD* with inhaled corticosteroid response has been reported,[35] our study is the first to relate *CEBPD* to BDR. In addition, we also identified a DMR located at *ZNF205* regulated by genetic variation and associated with *ZNF205* expression. *ZNF205* is a repressor of Mpv17-like protein (aka M-LPH), which confers protection from oxidative stress and mitochondrial apoptotic signaling under stress.[36] *ZNF205* is negatively regulated by forkhead box D3, aka FOXD3, and positively regulated by GA-binding protein, aka GABP.[36] GA-binding protein is a key regulator of T-cell homeostasis and immunity,[37] while forkhead box D3 downregulates IL-10 expression in B cells.[38] Genetic variation in other ZNF family members has been associated with BDR in asthma,[39] which together with our findings suggests that ZNF members may play a role in asthma treatment response.

To date, the accuracy of pharmacogenetic predictive models proposed for BDR in asthma even combined with clinical parameters is modest (range AUC: 0.60-0.75).[40,41] However, the panel that reported higher AUC has not been validated in independent populations, and minority and admixed populations that heavily rely on albuterol to treat asthma have been widely underrepresented in these studies (<6%). Previously, Forno et al[13] developed and validated in different populations a high-performance epigenetic classification model of atopy and atopic asthma, demonstrating the ability of epigenetics to predict asthma-related phenotypes. Here, we followed a similar approach and developed a methylation-based classification panel consisting of 70 CpGs that showed a good performance classifying BDR response, not only in African Americans but also in Latinos. This epigenetic panel showed similar discrimination of albuterol response as clinical variables closely related to BDR. We demonstrated that genetic variants associated with BDR, both previously reported in the bibliography or discovered in the study populations through a meta–genome-wide association study, do not allow the generation of classification panels with a significant discriminatory value. Our findings demonstrated that epigenetics is a promising field to discover biomarkers of asthma precision medicine that overcome genetics. Thus, we encourage further investigation of the clinical applicability of pharmacoepigenetics in respiratory diseases, especially when selecting the most appropriate treatment is crucial.

We acknowledge some strengths of this study. First, to our best, we have studied the largest epigenomic dataset of African American children with asthma. Second, we integrated different omic layers, which allowed us to provide a better understanding of the underlying molecular mechanisms. Third, we replicated our findings in other ethnically diverse populations including Mexican Americans and Puerto Ricans. However, we also acknowledge some limitations. First, the sample size is not too large for analyses that integrate different source data. Nonetheless, it is still higher than 50% of the EWAS conducted in the asthma field.[8-13] Second, we have assessed the DNAm in whole blood, and additional studies would be required to assess whether our findings have a role in other asthma-relevant tissues. Third, the use of microarray provided limited genomic coverage (3% of the whole genome). Whole-genome bisulfite sequencing solves this limitation, but its higher cost and technical variation than microarrays limit its application to association studies in large datasets.[42] Future studies that are based on targeted-sequencing approaches could provide a more comprehensive knowledge of DNAm in the regions we reported. Fourth, DNAm in whole blood cells was profiled at the same time when BDR was measured. Further studies are required to infer the causal association between these epigenetic markers and BDR. This is essential to assess the specific potential clinical applications of pharmacoepigenetics (eg, prevention or targeted therapy). Fifth, the classification model of BDR requires external validation in other independent cohorts with similar characteristics to ensure its validity and applicability to other populations.

In summary, we identified novel associations with BDR of 2 epigenetic markers at *FGL2* and *DNASE2* and multiple genomic regions in African Americans and Latinos. We found that DNAm at these loci was associated with genetic variation and/or gene expression. Finally, we developed epigenetic-based classification models of BDR in admixed populations and showed the potential clinical applicability of pharmacoepigenetics in precision medicine of respiratory diseases.

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Key messages

- **Multiple epigenetic markers were associated for the first time with BDR in minority and admixed children with asthma.**
- **The epigenetic markers associated with BDR were regulated by genetic variants and were associated with gene expression of nearby genes.**
- **Pharmacoepigenetics has potential clinical applicability to the classification of treatment response in pediatric asthma.**

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