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Whole-Genome Sequencing of Pharmacogenetic Drug Response in Racially Diverse Children with Asthma

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Abstract

Rationale: Albuterol, a bronchodilator medication, is the first-line therapy for asthma worldwide. There are significant racial/ethnic differences in albuterol drug response.

Objectives: To identify genetic variants important for bronchodilator drug response (BDR) in racially diverse children.

Methods: We performed the first whole-genome sequencing pharmacogenetics study from 1,441 children with asthma from the tails of the BDR distribution to identify genetic association with BDR.

Measurements and Main Results: We identified population-specific and shared genetic variants associated with BDR, including genome-wide significant ($P < 3.53 \times 10^{-7}$) and suggestive ($P < 7.06 \times 10^{-6}$) loci near genes previously associated with lung capacity (*DNAH5*), immunity (*NFKB1* and *PLCB1*), and β-adrenergic signaling (*ADAMTS3* and *COX18*). Functional analyses of the BDR-associated SNP in *NFKB1* revealed potential regulatory function in bronchial smooth muscle cells. The SNP is also an expression quantitative trait locus for a neighboring gene, *SLC39A8*. The lack of other asthma study populations with BDR and whole-genome sequencing data on minority children makes it impossible to perform replication of our rare variant associations. Minority underrepresentation also poses significant challenges to identify age-matched and population-matched cohorts of sufficient sample size for replication of our common variant findings.

Conclusions: The lack of minority data, despite a collaboration of eight universities and 13 individual laboratories, highlights the urgent need for a dedicated national effort to prioritize diversity in research. Our study expands the understanding of pharmacogenetic analyses in racially/ethnically diverse populations and advances the foundation for precision medicine in at-risk and understudied minority populations.

Keywords: albuterol; asthma; minority; *NFKB1*; Latinos

Asthma is a chronic inflammatory disorder characterized by recurrent respiratory symptoms and reversible airway obstruction. Asthma is the most common chronic childhood disease (1). In the United States, asthma prevalence is highest among Puerto Ricans (36.5%), intermediate among African Americans (13.0%) and European Americans (12.1%), and lowest among Mexican Americans (7.5%) (2). Asthma mortality is fourfold to fivefold higher in Puerto Ricans and African Americans compared with white persons and Mexican Americans (3).

Inhaled β_2-agonists (e.g., albuterol) are the preferred treatment for acute asthma symptoms. Albuterol produces bronchodilation by causing rapid smooth muscle relaxation in the airways. Albuterol is the most commonly prescribed asthma medication worldwide (4). Among low-income and minority populations, albuterol is often the only medication used for asthma regardless of asthma severity (5, 6).

Spirometry is used to quantify bronchodilator drug response (BDR), which varies significantly among individuals and between populations (7). Specifically, the populations with the highest asthma prevalence and mortality also have the lowest BDR: Puerto Rican and African American children have significantly lower BDR than white and Mexican American children (7, 8). This racial/ethnic variation in BDR may contribute to the observed disparities in asthma morbidity and mortality (9, 10).

BDR is a complex trait, influenced by social, environmental, and genetic factors, with heritability estimates ranging from 47% to 92% (11–13). Genome-wide association studies (GWASs) have identified several common SNPs associated with BDR in populations of European descent (14–16). Only one GWAS of BDR has been conducted among African Americans (17). Although that study identified a novel BDR-associated locus, it did not replicate associations discovered in populations of European descent, suggesting that BDR may be partly determined by population-specific variants. Our genetic investigation of BDR among Latinos identified a

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At a Glance Commentary

Scientific Knowledge on the Subject: Asthma is the most common chronic disease among children. Albuterol, a bronchodilator medication, is the first-line therapy for asthma treatment worldwide. In the United States, asthma prevalence is the highest among Puerto Ricans, intermediate among African Americans and white persons, and lowest in Mexicans. Asthma mortality is fourfold to fivefold higher in Puerto Ricans and African Americans compared with Mexicans. Puerto Ricans and African Americans, the populations with the highest asthma prevalence and mortality, also have the lowest albuterol bronchodilator drug response.

What This Study Adds to the Field: We conducted the largest pharmacogenetic study using whole-genome sequencing data from 1,441 minority children with asthma who had extremely high or low albuterol bronchodilator drug response. We identified population-specific and shared pharmacogenetic variants associated with bronchodilator drug response. We prioritized variants in *NFKB1* with multiple levels of existing biologic evidence and demonstrated their potential regulatory functions using chromatin immunoprecipitation sequencing, RNA sequencing, and luciferase enhancer assays. Our study reveals the challenges and importance of increasing diversity in research. Our findings help inform the direction of future development of asthma medications and advance the foundation of precision medicine for at-risk, yet understudied, racially/ethnically diverse populations.

significant contribution of population-specific rare variants (18).

Whole-genome sequencing (WGS) can identify rare and/or population-specific disease-causing genetic variants. WGS allows the detection of common and rare variants in coding and noncoding regions in a truly comprehensive and agnostic evaluation in the context of complex disease.

We performed WGS on 1,441 minority children with asthma from the tails of the BDR distribution (*see* Figure E1 in the online supplement). We examined high and low drug responders from three ethnic groups: Puerto Ricans, Mexicans, and African Americans. Subject selection and the main analyses are summarized in Figure 1. We identified BDR-associated common and rare variants that are population-specific or shared among populations. This study represents a highly collaborative effort and is part of the NHLBI TOPMed (Trans-Omics for Precision Medicine Whole Genome Sequencing) program. Our study represents the first WGS study to investigate genetic variants of BDR in racially diverse children with asthma. Some of the results of this study have been previously reported in the form of conference abstracts (19–22).

Methods

Study Cohorts and Sample Details

This study examined a subset of subjects with asthma from SAGE II (Study of African Americans, Asthma, Genes and Environments) (23) and GALA II (Genes-Environments and Admixture in Latino Americans) studies (18).

A total of 1,441 individuals from three ethnic groups (483 Puerto Ricans, 483 Mexicans, and 475 African Americans) representing the tails of the BDR distribution were selected for WGS (Figure 1). Sequencing quality control metrics are summarized in Table E1. Subject selection and filtering processes are described in the online supplement. Descriptive data of study participants are summarized in Table 1. Detailed descriptions of BDR assessment and analysis are described in the online supplement.

WGS Data Generation, Processing, and Quality Control

Details regarding DNA processing, WGS, variant calling, data quality controls, and variant annotation are described in the online supplement.

Data Availability

TOPMed WGS data are available to download by submitting a data access request through dbGaP. The dbGaP study accession numbers for GALA II and SAGE II are phs000920.v1.p1 and phs000921.v1.p1, respectively.

BDR Association Testing and Replication

Single and multivariant testing for BDR association were performed. Logistic regression analysis (high vs. low responder status) was controlled for age, sex, body mass index category, and the first 10 principal components. We conducted population-specific analyses and also performed a transethnic meta-analysis using METASOFT (24). Multivariant analysis was also performed on individuals combined across all three populations, including local genetic ancestries as additional covariates. The contribution of individual common and rare variants to multivariant association significance was evaluated by excluding common or rare variants in reduced and drop-one analyses. Variation in BDR explained by identified associations was calculated using McFadden pseudo R^2 (25). Detailed descriptions of these analyses are described in the online supplement.

We were unable to identify another age- and population-matched asthma cohort of sufficient size to replicate our drug response findings. Replication was nevertheless attempted in smaller cohorts and is described in detail in the online supplement.

Variant Prioritization and Functional Validation

To prioritize BDR-associated variants for further evaluation, an H3K27ac chromatin immunoprecipitation sequencing (ChIP-seq) assay was performed to identify variants overlapping with regulatory regions in primary bronchial smooth muscle cells (BSMCs). The Diverse Convergent Evidence approach (26) was then used to score BDR-associated variants using multiple levels of observational, bioinformatic, and laboratory evidence. Prioritized variants were further validated by luciferase enhancer assays to verify enhancer activity and by RNA sequencing to identify potential expression quantitative trait locus function. Detailed descriptions of these procedures are described in the online supplement.

Results

Descriptive Characteristics of Study Subjects

Descriptive characteristics for all study subjects ($n = 1,441$) are summarized in Table 1. Covariates and demographic variables were assessed for significant

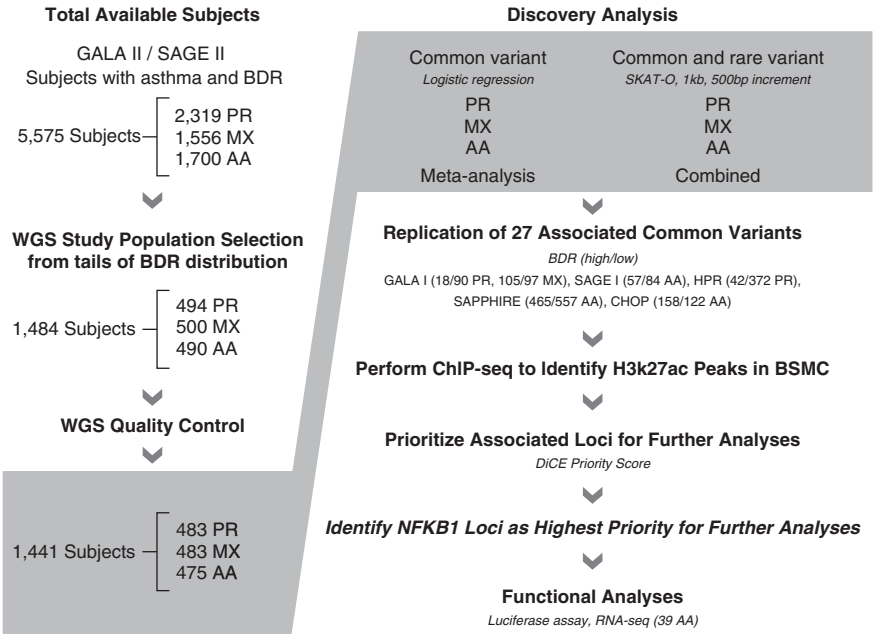

Figure 1. An overview of the main analyses performed in the current study. More detailed descriptions of the discovery and replication cohort demographics and analyses performed for common and rare variant analysis can be found in the METHODS section and the METHODS section in the online supplement. AA = African American; BDR = bronchodilator drug response; BSMC = bronchial smooth muscle cell; ChIP-seq = chromatin immunoprecipitation sequencing; CHOP = Children's Hospital of Philadelphia; DiCE = Diverse Convergent Evidence; GALA I = Genetics of Asthma in Latino Americans; GALA II = Genes-Environments and Admixture in Latino Americans; HPR = Hartford–Puerto Rico cohort; MX = Mexican; PR = Puerto Rican; SAGE = Study of African Americans, Asthma, Genes and Environments; SAPPHIRE = Study of Asthma Phenotypes and Pharmacogenomic Interactions by Race-Ethnicity; WGS = whole-genome sequencing.

differences between high and low drug responders for each racial/ethnic group. Significant differences were found for age (Mexicans, $P < 0.001$), baseline lung function (pre-FEV_1% predicted, $P < 0.001$), total IgE ($P < 0.001$), and atopy. Pre-FEV_1% predicted was defined as the percentage of observed FEV_1 relative to the expected population average FEV_1 based on the Hankinson lung function prediction equations (27). Results and descriptions of genetic ancestry and genetic substructure are shown in Table 1, supplemental text E1, and Figures E2 and E3.

BDR Association Testing with Common Variants

Detailed descriptions of variant summary statistics are described in supplemental text E2, Figure E4, and Tables E2 and E3. Study design is described in Figure E5. All subsequent analyses in this study were performed only with biallelic SNPs. Throughout this study, low drug responders were assigned as the reference (i.e., control) group, and high drug responders were classified as cases. We performed genome-wide association testing of common variants with BDR (dichotomized as high/low drug responders) for each population, adjusting by age, sex, body mass index category, and the first 10 principal components. We then performed a transethnic meta-analysis across all three populations.

The commonly used GWAS P value threshold of 5×10^{-8} is derived from a Bonferroni correction under the assumption of 1,000,000 independent tests, based primarily on patterns of linkage disequilibrium (LD) from individuals of European descent. This threshold has been shown to be nongeneralizable for genetic studies among populations of non-European descent (28, 29). We therefore empirically calculated the effective number of independent tests for each population and for our transethnic meta-analysis. The resulting genome-wide significance thresholds were 1.57×10^{-7} for Puerto Ricans, 2.42×10^{-7} for Mexicans, 9.59×10^{-8} for African Americans, and 3.53×10^{-7} for the transethnic meta-analysis. These numbers are highly concordant with WGS significance thresholds derived from the 1,000 Genomes sequencing data (28). Significance thresholds for discovery analyses in GWASs can often produce false-negative results. To minimize type II error, suggestive associations are often included in replication and functional validation studies. We identified suggestive associations based on the following widely used formula: 1/(effective number of tests) (30).

Although no significant associations were identified from the population-specific analyses (*see* Figure E6), our transethnic meta-analysis identified 10 unique loci (represented by 27 SNPs) significantly ($P < 3.53 \times 10^{-7}$) or suggestively ($P < 7.06 \times 10^{-6}$) associated with BDR status (Figure 2A and Table 2; *see* Table E4). After LD pruning, these 27 SNPs explain 23%, 16%, and 18% of the variation in BDR status in Puerto Ricans, Mexicans, and African Americans, respectively (*see* Table E5). To demonstrate that the results of our regression models were robust, a *post hoc* analysis was performed on the 27 SNPs by including Native American and African local ancestries as covariates. The association results before and after adjusting for local ancestry remained consistent (*see* Text E3, Table E6). We annotated all 27 SNPs by performing a thorough bioinformatic search in ENCODE, the NHGRI-EBL GWAS Catalog, and PubMed. Their previously reported lung-related phenotype associations and functional annotations are reported in Tables E7 and E8.

Two SNPs located on chromosome 5 (rs17834628 and rs35661809) were significantly associated with BDR ($P = 1.18 \times 10^{-8}$ and 3.33×10^{-8}). The direction of effect for these two variants is concordant across all three populations (Figure 2B; *see* Table E9). Figure 2C displays a LocusZoom plot of rs17834628 with 400-kb flanking regions. Three of the 27 SNPs were located within genes. Specifically, two SNPs are located in the third and fifth introns of *NFKB1* (rs28450894 and rs4648006), and a third SNP, rs16995064, mapped to intron 7 of *PLCB1* (Table 2). Based on 1,000 Genomes data, the low BDR-associated T allele of *NFKB1* (rs28450894) is found predominantly among African populations (minor allele frequency [MAF], 8.8–28.7%), followed by

Table 1. Study Population Description ($N = 1,441$)

Descriptive Statistics	Puerto Ricans ($n = 483$)			Mexicans ($n = 483$)			African Americans ($n = 475$)		
	High BDR	Low BDR	*P* Value	High BDR	Low BDR	*P* Value	High BDR	Low BDR	*P* Value
Number of subjects	239	244	—	243	240	—	233	242	—
Percent male	53.6	53.3	1.0	60.1	52.1	0.08	55.4	47.9	0.12
Median (IQR) age, yr	11.6 (9.7–14.8)	12.2 (10.1–15.2)	0.18	11.7 (9.6–14.0)	13.3 (10.6–16.0)	<0.001	13.8 (11.0–16.8)	13.8 (10.9–17.1)	0.48
Mean global ancestry proportions									
AFR	0.24	0.22	0.44	0.05	0.05	0.37	0.79	0.79	0.80
EUR	0.63	0.64	0.27	0.37	0.36	0.84	0.19	0.20	0.70
NAM	0.13	0.13	0.93	0.58	0.59	0.90	0.02	0.02	0.90
BMI category, *n*									
Obese, nonobese	76, 163	67, 177	0.32	100, 143	96, 144	0.85	82, 151	83, 159	0.85
Pre-FEV_1% predicted, *n*									
<80%, ⩾80%	149, 90	56, 188	<0.001	43, 200	7, 233	<0.001	47, 186	6, 236	<0.001
Median (IQR) ΔFEV_1, %	21.2 (18.2–25.7)	5.0 (2.9–6.3)	—	12.7 (10.3–16.8)	3.6 (2.0–4.9)	—	15.5 (13.3–20.3)	3.3 (2.0–4.4)	—
Median (IQR) tIgE, ml	407.5 (126.8–952.8)	191.9 (50.5–542.2)	<0.001	247.5 (64.2–817.0)	105.7 (35.4–332.0)	<0.001	281.6 (97.3–552.4)	128.8 (36.6–351.3)	<0.001
Atopy, *n*	177	118	<0.001	155	117	<0.001	139	111	<0.001

Definition of abbreviations: AFR = African ancestry; BDR = bronchodilator drug response; BMI = body mass index; EUR = European ancestry; IQR = interquartile range; NAM = Native American ancestry; pre-FEV_1% predicted = percentage of measured FEV_1 relative to predicted FEV_1 estimated by the Hankinson lung function prediction equations before administration of albuterol; tIgE = measure of total IgE from serum.

Atopy was defined as tIgE measurement ⩾100 kU/L. ΔFEV_1 is a quantitative measure of BDR, measured as the percent change in baseline FEV_1 after administration of albuterol. High and low drug responders were chosen from the extremes of the BDR (ΔFEV_1) distribution.

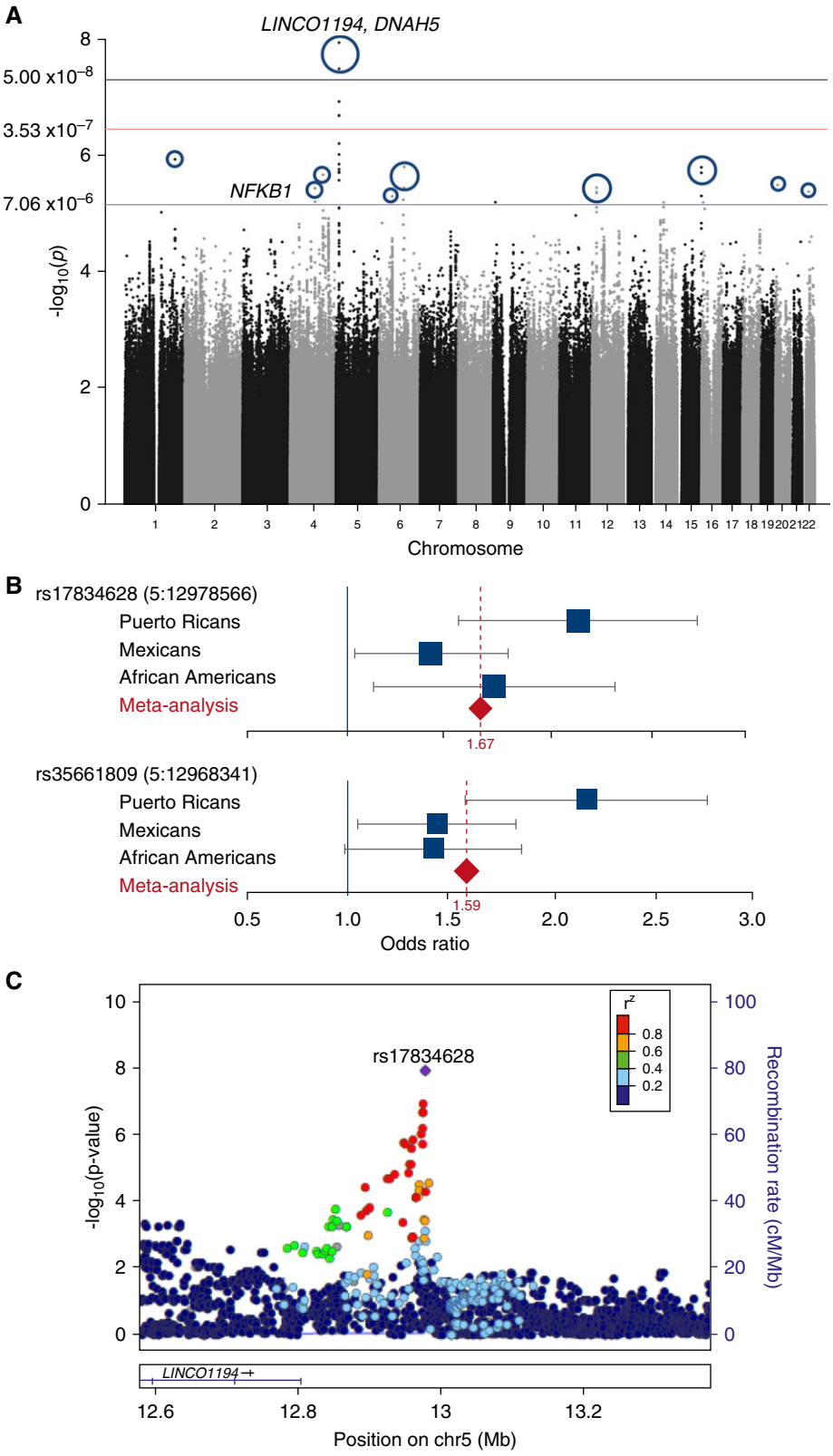

Figure 2. (*A*) Manhattan plot of the transethnic meta-analysis of single locus bronchodilator drug response association testing. Top 10 bronchodilator drug response–associated loci are circled. The black line indicates the universal genome-wide significance threshold (5.00×10^{-8}), the red line indicates the adjusted genome-wide significance threshold (3.53×10^{-7}), and the blue line indicates

European populations (MAF, 3.7–7.6%) and Puerto Ricans (MAF, 6.2%), and rare in Mexicans (MAF, 1.5%) (*see* Figure E7).

We were unable to identify age- and population-matched asthma cohorts with albuterol drug response data of sufficient sample size for replication. Nevertheless, we attempted to replicate all 27 SNPs in five independent populations (GALA I, SAGE I, HPR, SAPPHIRE, and CHOP) separately and by meta-analysis (*see* Tables E10 and E11). Although none of the 27 SNPs were significantly associated with BDR status in our replication analyses (*see* Table E12), it is important to note that age-specific associations with asthma and asthma-related phenotypes have been reported (31, 32), and that children were not included in our largest replication cohort (SAPPHIRE, median age >28) (*see* Table E10). All other replication populations were smaller than our discovery populations (ranging from 108 to 414 individuals per study), and some had an imbalance of cases and control subjects (*see* Table E10), which further diminished power for replication analyses.

Albuterol binds to β_2-adrenergic receptors in BSMC, causing rapid onset of airway tissue relaxation and bronchodilation. BSMCs are therefore considered one of the most relevant cell types for molecular studies of BDR (33). We performed H3K27ac ChIP-seq experiments in primary BSMCs to identify potential regulatory regions marked by H3K27ac peaks. We then overlapped the H3K27ac peaks with the 27 BDR-associated SNPs and SNPs that were in high LD with them ($R^2 \geq 0.8$ within 1 Mb in any of our study populations). Variants overlapping with H3K27ac peaks may imply regulatory functions in BSMC (*see* Table E8).

We applied the Diverse Convergent Evidence (26) approach to prioritize the 27 BDR-associated SNPs for inclusion in further functional analyses (*see* Table E12). After integrating information from our WGS analysis, publicly available bioinformatics data, and ChIP-seq experiments in BSMCs, the *NFKB1* locus had the highest Diverse Convergent Evidence score, indicating that *NFKB1* had the strongest evidence of functional relevance to BDR variation (*see* Figure E8). Therefore, all further functional experiments were focused on variants within this locus.

NFKB1 Functional Assays

Two H3K27ac ChIP-seq regions that overlapped with the BDR-associated *NFKB1* locus were tested for enhancer activity using luciferase enhancer assays in BSMCs (*see* Figure E9A and Table E13). One enhancer, NFKB1 Region 2, showed significantly increased enhancer activity over empty vector ($\log_2$ 2.24-fold increase, $P = 1.59 \times 10^{-5}$, unpaired Wilcoxon test) (*see* Figure E9B).

Given the relevance of *NFKB1* in immune pathways and asthma, we also performed RNA sequencing experiments in African American children with asthma to verify whether the identified intronic *NFKB1* SNPs regulate expression of neighboring genes. Among genes within 1 Mb of rs28450894 meeting expression reliability cutoffs, we found that the low BDR-associated T allele of rs28450894 is significantly associated with decreased expression of *SLC39A8* in blood (*see* Figure E10) [$P = 0.0066$, FDR-adjusted $P = 0.0856$, $\log_2(\beta) = -0.327$].

Comparison with Previous BDR Association

We observed that two known BDR candidate genes, *ADCY9* and *CRHR2*, which replicated in a previous BDR GWAS performed in the full GALA II population (18), did not replicate in the current study (*see* Table E14). In that study, imputed GWAS array data were used to evaluate genetic associations with BDR measured as a continuous trait. To determine whether the discrepancy between findings was caused by data type (imputed array-based vs. WGS-based) or study design (continuous trait vs. extreme phenotype), the common variant analysis in the current analysis was repeated among the subset of samples with array-based and WGS data ($n = 1{,}414$ out of 1,441). Based on the top 1,000 BDR-associated SNPs from the current common variant analysis, there was perfect correlation between association *P* values generated from imputed array-based and WGS-based genotypes (Spearman correlation, 1.0), suggesting that data type is not the cause of the observed discrepancy (*see* Figure E11A). Nearly all SNPs with high imputation R^2 exhibited high genotype concordance between array-based and WGS-based genotypes, confirming high imputation quality for most common SNPs (≥99.7%; *see* Figures E11B and E11C). We also performed linear regression to analyze BDR as a continuous trait (ΔFEV_1) using imputed array-based data. The most significantly associated SNP identified in the current extreme phenotype analysis displayed the same direction of effect as analyzing BDR as a continuous trait (odds ratio, 1.67 in extreme phenotype analysis; $\beta = 0.51$ in continuous analysis). These observations indicate that the discrepancy between findings may be caused by differences in statistical power afforded by the different study designs (continuous trait vs. extreme phenotype). For common variant analyses, dichotomization of a continuous outcome results in a loss of statistical power (34). The opposite effect is observed in rare variant analyses. The extreme phenotype study design has been shown to increase power and the probability of identifying functional rare variants (35, 36). Also noteworthy is that the previously published results were discovered in one population (Puerto Ricans), whereas the results from our transethnic meta-analysis describe associations that are conserved across three populations (Puerto Ricans, Mexicans, and African Americans).

BDR Association Testing Using Common and Rare Variants

We tested the combined effects of common and rare variants on BDR using SKAT-O (37) to examine variants in 1-kb sliding windows with 500-bp increments. The same covariates used for common variant association testing were applied.

After determining the effective number of tests and adjusting for multiple comparisons on each population separately, we identified three population-specific loci associated with BDR at genome-wide significance levels; two were found in Mexicans on chromosome 1 and chromosome 11, and one in African Americans on chromosome 19 (Figures 3A–3C and Table 3; *see* Table E15).

We also performed association testing across all three populations in a single analysis to maximize power. To minimize confounding by population substructure, association testing also included local genetic ancestry, defined as the proportions of Native American and African ancestries for the window under testing. Two loci on chromosomes 4 and 8 attained genome-wide significance ($P < 1.53 \times 10^{-7}$) (Figure 3D and Table 3). Sixty variants were identified from all SKAT-O regions reported in Table 3. Six of the 60 variants were located within predicted regulatory regions (*see* Table E16). Three variants on chromosome 11 identified in Mexicans overlap with a CTCF (transcriptional repressor) binding site and comprise a chromatin insulator region. The five regions identified in combined and population-specific SKAT-O analyses independently explained 4–8% of BDR variation in their respective populations (*see* Table E5).

To investigate whether common and rare variants both contributed to the BDR association *P* value, we performed reduced and drop-one SKAT analyses by excluding common or rare variants in the associated region one by one. Because excluding either all common or all rare variants would reduce the significance of the BDR association *P* value (*see* Table E17), the reduced (*see* Table E18) and drop-one (*see* Table E19) analyses indicated that both common and rare variants contribute to the significance of the BDR association.

Although we believe the sliding window method is the most appropriate approach for WGS data, we considered alternative grouping strategies for rare variants, including grouping by *1*) genes from transcription start sites to transcription end sites, with or without 50-kb flanking regions; *2*) transcription start sites with 20-kb flanking regions; and *3*) H3K27ac ChIP-seq peaks from airway epithelial cells and airway smooth muscle cells. Association tests

Figure 2. (Continued). the suggestive significance threshold (7.06×10^{-6}). (*B*) Forest plot of the two most significantly associated SNPs, rs17834628 and rs35661809. The R^2 between these two SNPs is 0.93 in Puerto Ricans, 0.96 in Mexicans, and 0.66 in African Americans. (*C*) The most significantly associated SNP (rs17834628) is plotted with 400-kb flanking regions on either side. Dot color shows each SNP linkage disequilibrium with rs17834628 based on the 1,000 Genomes November 2014 admixed American population. Multiple SNPs in high linkage disequilibrium ($R^2 > 0.8$, red) reached a suggestive significance level.

Table 2. Results from Transethnic BDR Association Tests for Common Variants

Chr	Start	rsID	Effect Allele	OR (95% CI)	*P* Value	Effect Allele Frequency			Nearest Genes
						PR	MX	AA	
5	12978566	rs17834628	A	1.67 (1.29–2.16)	1.18×10^{-8}*	0.32	0.42	0.17	*LINC01194* (−173 kb), *MIR4454* (−311 kb), *CTNND2* (−1,074kb), *DNAH5* (712 kb)
5	12968341	rs35661809	G	1.59 (1.20–2.10)	3.33×10^{-8}*	0.34	0.43	0.24	*LINC01194* (−163 kb), *MIR4454* (−300 kb), *CTNND2* (−1,064kb), *DNAH5* (722 kb)
5	12975934	rs17237639	G	1.61 (1.30–2.00)	1.22×10^{-7}	0.31	0.43	0.16	*LINC01194* (−171 kb), *MIR4454* (−308 kb), *CTNND2* (−1,072kb), *DNAH5* (715 kb)
5	12975187	rs1017452	G	1.60 (1.31–1.96)	2.11×10^{-7}	0.31	0.43	0.16	*LINC01194* (−170 kb), *MIR4454* (−307 kb), *CTNND2* (−1,071kb), *DNAH5* (715 kb)
5	12975322	rs1017454	A	1.60 (1.31–1.96)	2.11×10^{-7}	0.31	0.43	0.16	*LINC01194* (−170 kb), *MIR4454* (−307 kb), *CTNND2* (−1,071kb), *DNAH5* (715 kb)
5	12975265	rs1017453	C	1.56 (1.25–1.95)	6.40×10^{-7}	0.31	0.42	0.16	*LINC01194* (−170 kb), *MIR4454* (−307 kb), *CTNND2* (−1,071kb), *DNAH5* (715 kb)
5	12972636	rs17237443	C	1.59 (1.28–1.97)	9.85×10^{-7}	0.29	0.42	0.11	*LINC01194* (−170 kb), *MIR4454* (−307 kb), *CTNND2* (−1,071kb), *DNAH5* (715 kb)
1	209324294	rs10746419	T	1.29 (0.75–2.25)	1.19×10^{-6}	0.49	0.54	0.53	*MIR205HG* (278 kb), *MIR205* (281 kb), *CAMK1G* (433 kb), *LAMB3* (464 kb)
5	12961545	rs17833938	A	1.56 (1.28–1.91)	1.45×10^{-6}	0.30	0.42	0.12	*LINC01194* (−156 kb), *MIR4454* (−294 kb), *CTNND2* (−1,057kb), *DNAH5* (729 kb)
6	104240500	rs13437006	C	1.56 (1.21–2.02)	1.61×10^{-6}	0.22	0.24	0.32	*HACE1* (935 kb), *LINC00577* (1,144kb), *LIN28B* (1,164kb)
15	101230457	rs1565749	A	1.66 (1.18–2.32)	1.64×10^{-6}	0.18	0.15	0.18	*ASB7* (−39 kb), *LINS1* (−88 kb), *PRKXP1* (−131 kb)
5	12948369	rs34845041	T	1.56 (1.26–1.92)	1.77×10^{-6}	0.30	0.42	0.12	*LINC01194* (−143 kb), *MIR4454* (−280 kb), *CTNND2* (−1,044kb), *DNAH5* (742 kb)
5	12975108	rs1017451	T	1.55 (1.24–1.93)	1.96×10^{-6}	0.30	0.42	0.13	*LINC01194* (−170 kb), *MIR4454* (−307 kb), *CTNND2* (−1,071kb), *DNAH5* (715 kb)
5	12950432	rs62347395	G	1.55 (1.26–1.92)	2.02×10^{-6}	0.30	0.42	0.12	*LINC01194* (−145 kb), *MIR4454* (−282 kb), *CTNND2* (−1,046kb), *DNAH5* (740 kb)
15	101231049	rs57924834	A	1.59 (1.25–2.03)	2.04×10^{-6}	0.23	0.20	0.20	*ASB7* (−39 kb), *LINS1* (−89 kb), *PRKXP1* (−132 kb)
4	137382142	rs17048684	A	1.8 (1.06–3.05)	2.20×10^{-6}	0.11	0.14	0.18	*LINC00613* (−547 kb), *PCDH18* (1,058kb)
5	12959598	rs1438293	G	1.55 (1.24–1.93)	2.73×10^{-6}	0.29	0.42	0.11	*LINC01194* (−154 kb), *MIR4454* (−292 kb), *CTNND2* (−1,055kb), *DNAH5* (731 kb)
20	8635168	rs16995064	G	1.96 ($1.12–3._{43}$)	3.30×10^{-6}	0.12	0.13	0.05	***PLCB1* (intron 7)**, *PLCB4* (415 kb)
12	19821401	rs66544720	T	0.66 (0.55–0.78)	3.66×10^{-6}	0.33	0.37	0.16	*AEBP2* (−146 kb), *PLEKHA5* (−292 kb)
6	104235591	rs6926020	C	1.57 (1.25–1.97)	3.68×10^{-6}	0.19	0.23	0.27	*HACE1* (940 kb), *LINC00577* (1,149kb), *LIN28B* (1,169kb)
4	103453535	rs28450894	T	0.47 (0.34–0.64)	3.75×10^{-6}	0.06	0.03	0.12	*SLC39A8* (−187 kb), ***NFKB1* (intron 3)**, *MANBA* (99 kb)
4	103461559	rs4648006	T	0.47 (0.34–0.64)	3.75×10^{-6}	0.06	0.03	0.12	*SLC39A8* (−195 kb), ***NFKB1* (intron 5)**, *MANBA* (91 kb)
22	27826429	rs60163793	G	2.01 (1.20–3.38)	4.30×10^{-6}	0.04	0.14	0.15	*MN1* (318 kb), *PITPNB* (421 kb)
12	19824386	rs7313907	C	0.66 (0.55–0.79)	4.35×10^{-6}	0.33	0.37	0.16	*AEBP2* (−149 kb), *PLEKHA5* (−295 kb)
12	19820677	rs11044754	A	0.66 (0.55–0.79)	4.54×10^{-6}	0.33	0.37	0.16	*AEBP2* (−146 kb), *PLEKHA5* (−291 kb)
15	101233236	rs55638658	A	1.61 (1.13–2.30)	5.08×10^{-6}	0.18	0.15	0.18	*ASB7* (−41 kb), *LINS1* (−91 kb), *PRKXP1* (−134 kb)
6	54581204	rs13200833	A	0.66 (0.48–0.90)	5.15×10^{-6}	0.32	0.24	0.22	*TINAG* (−326 kb), *MLIP* (−450 kb), *FAM83B* (130 kb)

Definition of abbreviations: AA = African Americans; BDR = bronchodilator drug response; Chr = chromosome; CI = confidence interval; MX = Mexicans; OR = odds ratio; PR = Puerto Ricans.
The top 10 unique loci (represented by 27 SNPs) significantly ($P < 3.53 \times 10^{-7}$) or suggestively ($P < 7.06 \times 10^{-6}$) associated with BDR status in our transethnic meta-analysis.
Chr and Start: chromosome locations of SNPs in GRCh37 coordinates. All significantly and suggestively associated common variants are presented. Nearest genes: the four nearest transcripts from RefSeq were identified and genes with multiple transcripts were reported only once, with the distance to the nearest transcript indicated in parentheses. Negative distances indicate upstream genes. Genes overlapping with BDR-associated SNPs are bold. High drug responders were assigned as cases.
*P values that achieve adjusted genome-wide significance for transethnic meta-analysis ($P < 3.53 \times 10^{-7}$).

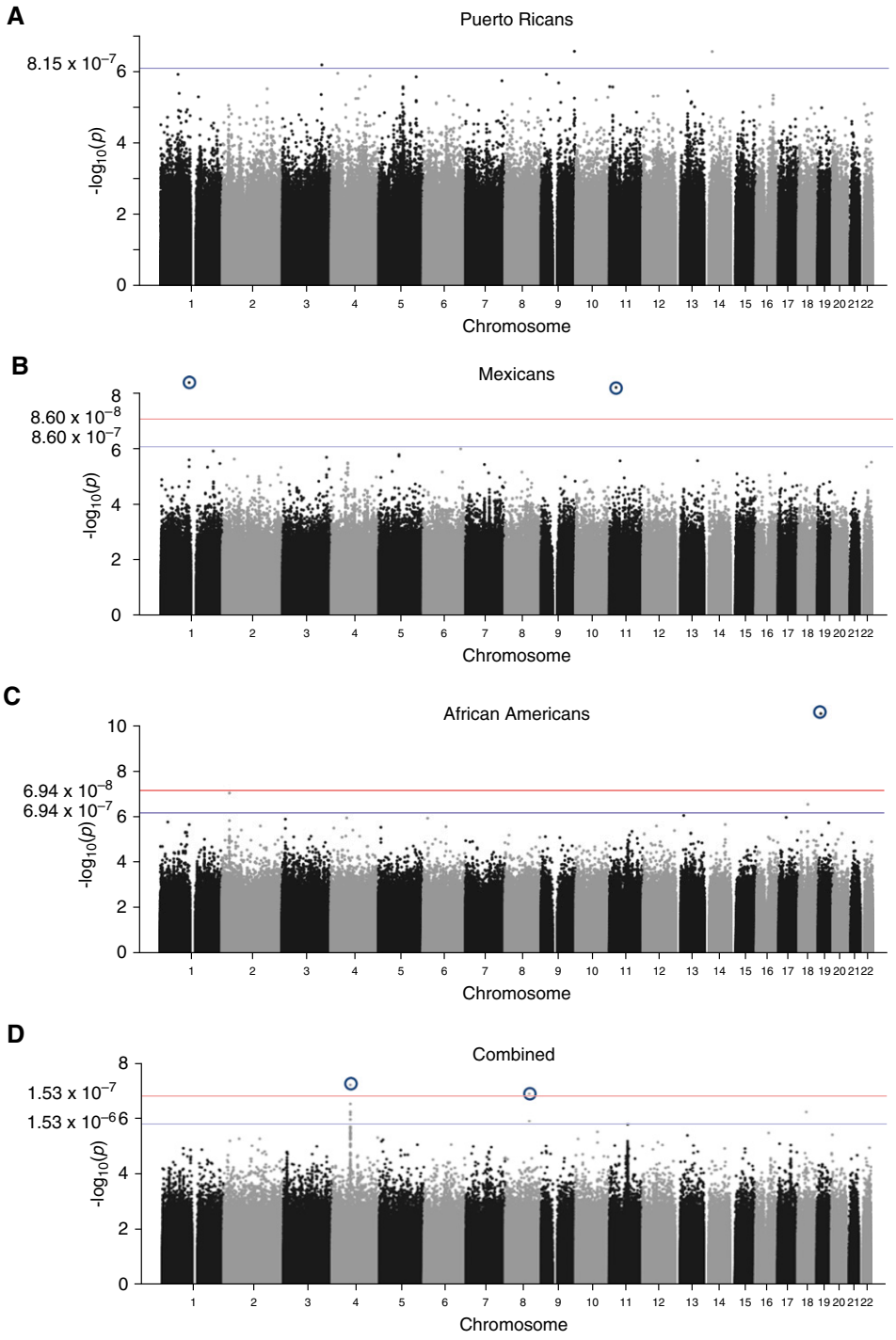

Figure 3. Manhattan plot of SKAT-O analysis of biallelic common and rare SNPs grouped by 1-kb windows sliding across chromosome 1–22 in (*A*) Puerto Ricans, (*B*) Mexicans, (*C*) African Americans, and (*D*) all populations combined. Bonferroni-corrected genome-wide and suggestive significance levels are marked by red and blue lines, respectively.

with these grouping strategies identified no further significant associations. Because these analyses were performed *post hoc*, multiple testing correction for testing alternative grouping strategies was not applied to the results of the sliding window approach.

Discussion

We identified population-specific and shared common and rare variants associated with BDR in three racially and ethnically diverse populations of children with asthma. WGS provides comprehensive detection of common and rare variants in coding and noncoding regions. Combined, the 27 variants (after LD pruning) identified from our common variant analyses (Table 2) explained 23%, 16%,

Table 3. Association Testing for Combined Effects of Common and Rare Variants on BDR

Chr	Start	Stop	*P* Value	Population	nCommon	nRare	Nearest Genes
1	114177000	114178000	4.40×10^{-9}	MX	2	1	***MAGI3* (intron 9)**, *PHTF1* (62 kb), *RSBN1* (126 kb)
11	27507000	27508000	6.59×10^{-9}	MX	2	3	*LOC105376671* (−3 kb), *LGR4* (−13 kb), *LIN7C* (8 kb)
19	10424000	10425000	3.12×10^{-11}	AA	1	2	*ZGLP1* (−4 kb), *ICAM5* (−17 kb), ***FDX1L* (intron 3)**, *RAVER1* (2 kb)
4	73478000	73479000	6.25×10^{-8}	Combined	10	23	*ADAMTS3* (−43 kb), *COX18* (441 kb)
8	97926000	97927000	1.32×10^{-7}	Combined	3	13	*SDC2* (−302 kb), ***CPQ* (intron 4)**, *LOC101927066* (37 kb), *TSPYL5* (359 kb)

Definition of abbreviations: AA = African Americans; BDR = bronchodilator drug response; Chr = chromosome; MX = Mexicans; nCommon = number of common variants; nRare = number of rare variants.
Chr, Start, and Stop: GRCh37 chromosome coordinates. Combined: African Americans, Mexicans, and Puerto Ricans. Nearest genes: the four nearest transcripts from RefSeq were identified, and genes with multiple transcripts were reported once only, with distance to nearest transcripts indicated in parentheses. Negative distances indicate upstream genes. Genes that overlap with BDR-associated SNPs are bold. Details of all variants within the windows are presented in Table E16.

and 18% of the variation in BDR in Puerto Ricans, Mexicans, and African Americans, respectively (*see* Table E5). The five SKAT-O regions identified in our combined and population-specific analyses independently explained 4–8% of BDR variation (Tables 3; *see* E5). Our study represents an important investment from the NIH/NHLBI to improve racial and ethnic diversity in clinical and biomedical research.

Our transethnic common variant meta-analysis identified one locus on chromosome 5 that was associated with BDR at a genome-wide significance level ($P < 5 \times 10^{-8}$). The proximity of this BDR-associated locus to *DNAH5* and *LINC01194* is of particular interest. An SNP in *DNAH5* has been associated with total lung capacity in white subjects with chronic obstructive pulmonary disease (38). In a separate GWAS, the *DNAH5*/*LINC01194* locus was reported among Europeans to be associated with levels of IgE (39, 40), a biomarker associated with asthma endotypes. Baseline lung function (FEV_1) and total IgE levels are associated with asthma severity and can predispose an individual to lower BDR (7, 8, 41). We found two *NFKB1* intronic variants on chromosome 4 that were suggestively associated with BDR. The nuclear factor-κB protein has a known role in allergic response, and various studies have demonstrated that the nuclear factor-κB pathway is activated in patients with asthma, as reviewed by Edwards and colleagues (42).

ChIP-seq and functional enhancer assays in BSMCs imply that the regions containing the NFKB1 intronic variants regulate expression of nearby genes, but do not directly suggest that the genetic variants themselves alter the expression of nearby genes. However, the latter was supported by our RNA sequencing data, which showed that individuals with the low BDR-associated T allele displayed reduced expression of the neighboring SLC39A8 gene. Additionally, SLC39A8 has been found to be responsive to cytokine treatment in airway epithelial cells (42) and had reduced expression in mice with allergic airway inflammation (43).

Studies have shown that up-regulation of *SLC39A8* is sufficient to protect the lung epithelium against tumor necrosis factor-α–induced cytotoxicity (44). Additionally, the higher frequency of the low BDR-associated allele (T allele of rs28450894 in *NFKB1*) in African populations suggests that the low BDR-associated allele tracks with African ancestry. This may explain why admixed populations with higher proportions of African ancestry (e.g., African Americans and Puerto Ricans) have lower BDR (8), and by extension may shed light on the higher asthma morbidity and mortality in these populations.

Another intronic variant (chromosome 20, rs16995064, *PLCB1* intron 7) suggestively associated with BDR is relevant to childhood asthma. *PLCB1* is differentially expressed among children with therapy-resistant asthma versus controlled persistent asthma or age-matched healthy control subjects (45). Silencing *PLCB1* inhibited the effect of lipopolysaccharide-induced endothelial cell inflammation by inhibiting expression of proinflammatory cytokines (46). Additional functional studies are necessary to establish the role of *NFKB1* and *PLCB1* on BDR.

We identified various combined effects of rare variants that were population-specific or shared across populations. Although some nearest genes show no known functional relationship to BDR (*MAGI3*, *LOC105376671*, *LIN7C*, and *CPQ*), the locus between *ADAMTS3* and *COX18* may be functionally relevant. The *ADAMTS3*/*COX18* locus was associated with β-adrenergic responses in murine cardiovascular-related traits (47). This locus was significantly associated with cardiac atrial weight in mice treated with the β-blocker atenolol and replicated under β-agonist isoproterenol treatment. These findings suggest that SNPs in this locus may modify β-adrenergic signaling pathways in BDR. In the present study, we also identified BDR association with rare variants within *CPQ*, which encodes a protein from the carboxypeptidase family. Although no previous BDR association has been identified for *CPQ*, another member of the carboxypeptidase family, carboxypeptidase A3, is expressed at higher levels in the airway epithelium among subjects with Th2-high asthma (48, 49). Further studies are necessary to determine the role of *CPQ* in BDR.

GWAS-based BDR-associated common variants in GALA II have previously been reported (18). However, those variants were not significantly associated with extreme BDR in the current study, likely because of differences in study design. The previous BDR GWAS used an array-based genotyping panel to examine children with asthma from the

entire BDR spectrum. In contrast, our current study sequenced the entire genome to investigate only the extremes of the BDR distribution. By repeating our current extreme phenotype analysis using a subset of individuals who had array and WGS data, we confirmed that the major discrepancy between the two studies was caused by study design instead of differences in data type. The contrast in results between GWAS and WGS caused by differences in study design implies that varied study designs are necessary for a comprehensive understanding of variants associated with asthma-related phenotypes and drug response. The extreme phenotype approach is recognized as one of the success factors in the study design of pharmacogenomic GWASs (50). Furthermore, the power gain from studying extreme phenotypes is much greater in analyses of rare variants compared with common variant studies (51). An extreme phenotype study design provides a cost-effective means for studying common and rare variant associations that may otherwise be missed when sampling across the entire phenotypic spectrum.

We did not identify BDR-associated variants from β_2-adrenergic receptors signaling pathways. Instead, most of the BDR-associated genes identified in this study are related to lung function and allergic response, including total IgE levels and cytokine production in mast cells. This suggests that BDR depends in part on the intrinsic state of airway smooth muscle cells. Genetic variation may determine individuals' intrinsic expression levels of candidate genes, which in turn determine whether their response to albuterol is beneficial.

Including admixed populations in WGS studies has important scientific implications. First, it facilitates discovery of genetic variation of multiple ancestral populations in a single study. Second, studying multiethnic admixed populations with ancestries that are underrepresented in existing genetic repositories can yield novel pharmacogenomic insights. For example, the widely popular PCSK9 inhibitors used to treat hypercholesterolemia were discovered by studying the genetics of African Americans but the drug development eventually benefited patients of all ethnicities (52). Finally, studying admixed populations, such as Mexicans, enhances the understanding of genetic variation in Native American ancestry, a population largely absent in major sequencing efforts.

We and others have documented the implications and challenges posed by the lack of non-European study populations in biomedical research (53–55). We made extensive efforts to test our top BDR-associated variants in other populations, but the unique characteristics of our discovery cohort (minority children with asthma who have BDR and WGS data) posed significant challenges for finding comparable replication cohorts. Testing ancestry-dependent pharmacogenetic variants without age- and ethnicity-matched replication populations poses an impossible statistical bar for publication. We therefore conducted additional analyses involving bioinformatics and experimental assays (56). These challenges highlight the need to include more racially/ethnically diverse populations in all clinical and biomedical research.

In an era of precision medicine, addressing questions about the impact of genetic factors on therapeutic drug response in globally diverse populations is essential for making precision medicine socially and scientifically precise (57). This study advances the understanding of genetic analysis in admixed populations and helps to lay the foundation of precision medicine for understudied and racially and ethnically diverse populations. ■

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