1 **TITLE**

2 **Full title:** Whole genome sequencing of pharmacogenetic drug response in racially
3 and ethnically diverse children with asthma

4 **Short title:** Pharmacogenetic drug response in ethnically diverse children with
5 asthma

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ABSTRACT

Asthma is the most common chronic disease of children, with significant racial/ethnic differences in prevalence, morbidity, mortality and therapeutic response. Albuterol, a bronchodilator medication, is the first-line therapy for asthma treatment worldwide. We performed the largest whole genome sequencing (WGS) pharmacogenetics study to date using data from 1,441 minority children with asthma who had extremely high or low bronchodilator drug response (BDR). We identified population-specific and shared pharmacogenetic 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 pathways (*ADAMTS3* and *COX18*). Functional analyses centered on *NFKB1* revealed potential regulatory function of our BDR-associated SNPs in bronchial smooth muscle cells. Specifically, these variants are in linkage disequilibrium with SNPs in a functionally active enhancer, and are also expression quantitative trait loci (eQTL) for a neighboring gene, *SLC39A8*. Given the lack of other asthma study populations with WGS data on minority children, replication of our rare variant associations is infeasible. We attempted to replicate our common variant findings in five independent studies with GWAS data. The age-specific associations previously found in asthma and asthma-related traits suggest that the over-representation of adults in our replication populations may have contributed to our lack of statistical replication, despite the functional relevance of the *NFKB1* variants demonstrated by our

112 functional assays. Our study expands the understanding of pharmacogenetic
113 analyses in racially/ethnically diverse populations and advances the foundation for
114 precision medicine in at-risk and understudied minority populations.

115 **AUTHOR SUMMARY**

116 Asthma is the most common chronic disease among children. Albuterol, a
117 bronchodilator medication, is the first-line therapy for asthma treatment
118 worldwide. In the U.S., asthma prevalence is the highest among Puerto Ricans,
119 intermediate among African Americans and lowest in Whites and Mexicans. Asthma
120 disparities extend to mortality, which is four- to five-fold higher in Puerto Ricans
121 and African Americans compared to Mexicans [1]. Puerto Ricans and African
122 Americans, the populations with the highest asthma prevalence and death rate, also
123 have the lowest albuterol bronchodilator drug response (BDR). We conducted the
124 largest pharmacogenetic study using whole genome sequencing data from 1,441
125 minority children with asthma who had extremely high or low albuterol
126 bronchodilator drug response. We identified population-specific and shared
127 pharmacogenetic variants associated with BDR. Our findings help inform the
128 direction of future development of asthma medications and our study advances the
129 foundation of precision medicine for at-risk, yet understudied, racially/ethnically
130 diverse populations.

131 **INTRODUCTION**

132 Asthma is a chronic inflammatory disorder of the airways characterized by
133 recurrent respiratory symptoms and reversible airway obstruction. Asthma affects
134 5% of the world population [2] and is the most common chronic disease among
135 children [3, 4]. In the United States (U.S.), asthma is the most racially disparate
136 health condition among common diseases [5, 6]. Specifically, U.S. asthma prevalence
137 is highest among Puerto Ricans (36.5%), intermediate among African Americans
138 (13.0%) and European Americans (12.1%), and lowest among Mexican Americans
139 (7.5%) [7]. These disparities also extend to asthma mortality, which is four- to five-
140 fold higher in Puerto Ricans and African Americans compared to Whites and
141 Mexican Americans [1].

142 Current asthma guidelines recommend inhaled β_2-agonists (e.g., albuterol) for
143 treatment of acute asthma symptoms. Albuterol is a short-acting β_2-adrenergic
144 receptor (β_2AR) agonist, and it produces bronchodilation by causing rapid smooth
145 muscle relaxation in the airways. Albuterol is the most commonly prescribed asthma
146 medication in the world and is the mainstay of acute asthma management across all
147 ethnic groups [8, 9]. Among low income and minority populations in the U.S.,
148 albuterol is often the only medication used for asthma regardless of asthma severity
149 [10, 11]. Response to albuterol is quantified based on bronchodilator drug response
150 (BDR) using spirometry. We and others have demonstrated that there is significant
151 variability in BDR among individuals and between populations [12, 13]. Specifically,
152 the populations with the highest asthma prevalence and mortality also have the

153 lowest drug response to albuterol: Puerto Rican and African American children have
154 significantly lower BDR than Whites and Mexican American children [13, 14]. This
155 variation in drug response across racial/ethnic groups may contribute to the
156 observed disparities in asthma morbidity and mortality [15-19].

157 BDR is a complex trait, influenced by environmental and genetic factors, with
158 heritability estimates ranging from 47% to 92% [20-22]. Genome-wide association
159 studies (GWAS) have identified several common single nucleotide polymorphisms
160 (SNPs) associated with BDR in populations of European descent [23-25]. To date,
161 only one GWAS of BDR has been conducted among African Americans [26]. While
162 that study identified a novel BDR-associated locus, it did not replicate known
163 associations discovered in populations of European descent, suggesting that BDR
164 may be determined in part by population-specific variants. Our previous study of
165 genetic predictors of BDR in Latino populations identified a significant contribution
166 of population-specific rare variants to BDR [27].

167 GWAS were designed to identify common variants associated with disease
168 through the use of genotyping arrays that relied on linkage disequilibrium to
169 tag/represent variants not explicitly genotyped on the array itself. Early GWAS
170 arrays were optimized for performance in populations of European origin and
171 lacked the ability to capture race-/ethnic-specific genetic variation due to
172 differences in linkage disequilibrium (LD) across racially/ethnically diverse
173 populations [28]. Recent generations of arrays have attempted to tailor genotyping
174 panels for major HapMap populations (Affymetrix Axiom® World Arrays [29]), or to

175 include population-specific and trans-ethnic tag SNPs to statistically infer genotypes
176 not directly captured in diverse populations (Illumina Infinium® Multi-Ethnic
177 Genotyping Array [30]). However, imputation accuracy decreases significantly with
178 variant frequency [31, 32], making it difficult to use genotyping arrays to study rare
179 and/or population-specific variants.

180 The most striking weakness of GWAS is the inability to adequately capture rare
181 variation. Whole exome sequencing (WES) and other forms of targeted sequencing
182 were developed to address the inability of genotyping arrays to capture rare
183 variation. WES only allows for the capture of common and rare variants within
184 coding and flanking regions. Studies have shown that a large number of variants
185 associated with complex disease lie within non-coding regions of the genome
186 (reviewed in Zhang and Lupski, 2015 [33]). Additionally, the target capture
187 procedures result in uneven sequence coverage, limiting the reliability of SNP
188 calling for loci close to the boundary of targeted regions. WES also has limited usage
189 for the detection of structural variation, which depends heavily on uniform coverage
190 across the genome.

191 Whole genome sequencing (WGS) is the ideal technology for identifying disease-
192 causing variants that are rare and/or population-specific. Unlike GWAS genotyping
193 arrays or targeted sequencing technologies, WGS allows the detection of common
194 and rare variants in coding and non-coding regions. WGS is the only technology
195 capable of a truly comprehensive and agnostic evaluation of genetic sequence
196 variation in the context of complex disease. The persistent lack of large-scale genetic

197 studies conducted in populations of non-European descent further exacerbates
198 racial/ethnic disparities in clinical and biomedical research [34-36]. The application
199 of WGS to the evaluation of genetic factors within a racially/ethnically diverse study
200 population is a necessary step toward eliminating health disparities in BDR and
201 other complex phenotypes.

202 In this study, we performed WGS on 1,441 minority children with asthma from
203 the tails of the BDR distribution (**S1 Fig**). Our study included high and low drug
204 responders from three ethnic groups: Puerto Ricans (PR) (n=483), Mexicans (MX)
205 (n=483), and African Americans (AF) (n=475). An overview of the subject selection
206 process and main analyses performed in this study is presented in **Fig 1**. We
207 identified multiple BDR-associated common and rare variants that are population-
208 specific or shared among populations. This study is part of the National Heart, Lung,
209 and Blood Institute's Trans-Omics for Precision Medicine Whole Genome
210 Sequencing (TOPMed) program and represents the largest WGS study thus far to
211 investigate genetic variants important for bronchodilator drug response in racially
212 and ethnically diverse children with asthma.

213

214 **RESULTS**

215 **Descriptive characteristics of study subjects**

216 Descriptive characteristics for all study subjects (N=1,441, including 483 Puerto
217 Ricans, 483 Mexicans and 475 African Americans) are summarized in **Table 1**.
218 Covariates and demographic variables were assessed for significant differences
219 between high and low drug responders for each racial/ethnic group. Significant
220 differences were found for age (Mexicans, $p<0.001$), baseline lung function (pre-
221 FEV_1 % predicted, $p<0.001$), total Immunoglobulin E (tIgE, $p < 0.001$), and atopy.
222 Baseline lung function (pre-FEV_1 % predicted) was defined as the percentage of
223 observed FEV_1 relative to the expected population average FEV_1 estimated using the
224 Hankinson lung function prediction equations [37].

225 **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***	**High BDR**	**Low BDR**	***P***	**High BDR**	**Low BDR**	***P***
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 Age, yr (IQR)		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	76	67	0.32	100	96	0.85	82	83	0.85
	Non-Obese	163	177		143	144		151	159	
Pre-FEV_1 % Predicted, N	< 80%	149	56	<0.001	43	7	<0.001	47	6	<0.001
	≥ 80%	90	188		200	233		186	236	
Median ΔFEV_1, % (IQR)		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 tIgE, mL (IQR)		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

226
227 **BDR**: bronchodilator drug response. IQR: interquartile range. **Pre-FEV_1 % predicted**: percentage of measured FEV_1 relative to predicted FEV_1
228 estimated by the Hankinson lung function prediction equations prior to administration to albuterol. **ΔFEV_1**: a quantitative measure of BDR,
229 measured as the percent change in baseline FEV_1 after administration of albuterol. High and low drug responders were chosen from the
230 extremes of the BDR (ΔFEV_1) distribution. **tIgE**: measure of total Immunoglobulin E from serum in milliliters. **Atopy**: tIgE measurement greater
231 than or equal to 100.

232 We estimated genetic ancestry for all participants (see **Methods**) and found that
233 the major ancestry proportions in Puerto Ricans, Mexicans and African Americans
234 are European, Native American and African ancestries, respectively (**Table 1**, **S2**
235 **Fig**). Analysis of genetic substructure of the three admixed populations by principal
236 component analysis (PCA) demonstrated that the three populations displayed the
237 characteristic spectrum of ancestry found in admixed populations (**S3 Fig**).

238 **Variant summary statistics**

239 Genetic variant summary statistics revealed that the average number of variants by
240 population corresponded to the proportion of African ancestry: the most variants
241 were found among African Americans, followed by Puerto Ricans and Mexicans (**Fig**
242 **2a**, **Table 2**).

243 **Table 2.** Summary statistics of variants

	Puerto Ricans	Mexicans	African Americans
Range in variants, per individual (mean)			
All	3.7M – 4.4 M (4.0 M)	3.5M – 4.1M (3.7M)	4.0M – 4.5M (4.3M)
SNPs	3.4M – 4.0 M (3.6M)	3.2M - 3.7M (3.4M)	3.6M – 4.1M (4.0M)
Indels	284,067 – 344,493 (305,618)	272,635 – 321,778 (289,096)	311,997 – 354,646 (339,570)
Others*	21,233 – 29,177 (24,728)	19,703 – 26,772 (23,190)	22,014 – 30,993 (27,911)
No. of biallelic SNPs, union**	29.2M	28.1M	36.3M
By allele frequency of data			
Rare (< 1%)	18,169,292 (62%)	20,029,291 (71%)	22,847,938 (63%)
Common (≥ 1%)	11,007,125 (38%)	8,092,157 (29%)	13,459,004 (37%)
Population-specific***	6,680,909	9,687,651	14,114,142
Singleton	4,616,383 (69%)	7,983,950 (82%)	10,574,879 (75%)
< 5%	6,676,286 (> 99%)	9,675,271 (> 99%)	14,096,844 (> 99%)
≥ 5%	4,623 (< 1%)	12,380 (< 1%)	17,298 (< 1%)
By novelty (dbSNP150)			
Known	27,034,179 (93%)	25,911,801 (92%)	33,540,387 (92%)
Novel	2,142,238 (7%)	2,209,647 (8%)	2,766,555 (8%)
By protein impact****			
Coding	284,269 (1%)	289,055 (1%)	362,823 (1%)
Nonsynonymous	157,541	164,312	203,029
Stopgain / stoploss	3,194	3,531	4,158
Splicing	2,111	2,214	2,620
Other coding	121,423	118,998	153,016
Noncoding	28,833,461 (99%)	27,769,434 (99%)	35,878,723 (99%)
Not annotated^	58,687 (< 1%)	62,959 (< 1%)	65,396 (< 1%)

244 * Includes multi-nucleotide polymorphism (MNPs), complex, symbolic and mixed variants as
245 defined by GATK VariantEval.
246 ** Biallelic SNPs with less than 10% genotype missingness per population were included.
247 *** Biallelic SNPs that are present in only one of the three studied populations.
248 **** Other coding variants include those annotated as exonic and synonymous in ANNOVAR.
249 ^ Not annotated: biallelic SNPs not included in the annotation pipeline because they are not
250 present in TOPMed freeze 2 and 3 data releases.

251 The majority of observed variants (>90%) were SNPs. The union of biallelic SNPs
252 from all individuals in each population varied from 28.1M among Mexicans, 29.2M
253 among Puerto Ricans to 36.3M among African Americans. Approximately 65% of
254 biallelic SNPs were rare (non-reference allele frequency < 1%, **Fig 2b**, **Table 2**).
255 Biallelic SNPs that were population-specific (i.e., SNPs found in only one population)
256 accounted for 23% (6.68M / 29.2M in Puerto Ricans) to 39% (14.1M / 36.3M in
257 African Americans) of the biallelic SNPs observed in each population. Over 99% of
258 the population-specific SNPs had a non-reference allele frequency less than 5% and
259 the majority of these population-specific SNPs (69% to 82%) were also singletons
260 (**Fig 2c**, **Table 2**). Based on dbSNP build 150, an average of 8% of biallelic SNPs
261 were novel (**Fig 2d**, **Table 2**).

262 In all three populations, 99% of the biallelic SNPs were observed in noncoding
263 regions. Based on the Combined Annotation Dependent Depletion (CADD) score
264 [38], which estimates the deleteriousness of a variant, over 99% of highly
265 deleterious biallelic SNPs (CADD score ≥ 25) were observed in coding regions,
266 regardless of ethnicity (**Table 3**). This may be due to the relatively limited
267 availability of functional annotations as positive training data for CADD to estimate
268 deleteriousness in non-coding regions [39]. The percentage of singletons in these
269 highly deleterious biallelic SNPs varied from 51% (Puerto Ricans) to 70%
270 (Mexicans).

271 **Table 3.** CADD score summary statistics of biallelic SNPs*.

Population	CADD score	No. of SNPs*	% Coding	% Singletons
Puerto Ricans	0-9	26,936,285	0.5%	29%
	10-19	2,027,392	4%	32%
	20-24	123,481	32%	37%
	≥ 25	30,572	>99%	51%
Mexicans	0-9	25,932,006	0.5%	44%
	10-19	1,967,096	4%	49%
	20-24	124,173	34%	55%
	≥ 25	35,214	>99%	70%
African Americans	0-9	33,468,245	0.5%	36%
	10-19	2,573,523	4%	40%
	20-24	158,967	32%	46%
	≥ 25	40,811	>99%	64%

272 *Biallelic SNPs with less than 10% genotype missingness per population were included.

273

274 **BDR association testing with common variants**

275 We performed genome-wide association testing of common variants with BDR
276 (dichotomized as high/low drug responders from the extremes of the BDR
277 distribution) for each population, adjusting by age, sex, body mass index (BMI)
278 categories, and the first ten principal components (PCs) (see **Methods** section
279 "Single locus BDR association testing on common variants" for rationale on
280 including these covariates). We then performed a trans-ethnic meta-analysis on
281 these results across all three populations. For all three populations, 94% of all
282 common variants tested are known variants annotated in dbSNP Build 150 (**S1**
283 **Table**).

284 A universal *p*-value threshold of 5×10^{-8} is often used to determine significance in
285 GWAS. This statistical threshold was calculated based on Bonferroni correction
286 under the assumption of 1,000,000 independent tests using patterns of linkage
287 disequilibrium based primarily on individuals of European descent and has been
288 shown to be non-generalizable for WGS studies or genetic studies of non-European
289 populations in general [40-42].The number of independent tests varies by LD
290 patterns, which in turn vary by race/ethnicity [41]. We calculated the effective
291 number of independent tests for each population and for our trans-ethnic meta-
292 analysis, and generated racially/ethnically adjusted genome-wide significance
293 thresholds for each population (see **Methods**). Population-specific genome-wide
294 significance thresholds after correcting for the number of effective tests (adjusted
295 genome-wide significance level) were 1.57×10^{-7} for Puerto Ricans, 2.42×10^{-7} for
296 Mexicans, and 9.59×10^{-8} for African Americans (see **Methods**). These numbers are
297 highly concordant with WGS significance thresholds derived from the African
298 American (ASW), Mexican (MXL), and Puerto Rican (PUR) 1000 Genomes
299 sequencing data [41]. The adjusted genome-wide significance level for our trans-
300 ethnic meta-analysis was 3.53×10^{-7}. Significance thresholds for discovery analyses
301 in genome-wide association studies can often produce false negative results [43-46].
302 To minimize Type II error, suggestive associations are often included in replication
303 and functional validation studies. We identified suggestive associations based on the
304 following widely used formula: 1/(effective number of tests) [43-46].
305 While no significant associations were identified from the population-specific
306 analyses (**S8 Fig**), our trans-ethnic meta-analysis identified ten unique loci

307 (represented by 27 SNPs) significantly ($p < 3.53 \times 10^{-7}$) or suggestively ($p < 7.06 \times$
308 10^{-6}) associated with BDR status (**Fig 3a, Table 4, S2 Table)**. We annotated all 27
309 SNPs by performing a thorough bioinformatics search in ENCODE, NHGRI-EBL
310 GWAS Catalog and PubMed databases. Their previously reported lung-related
311 phenotype associations and functional annotations are reported in **S4 Table** and **S9**
312 **Table**.

313 Two SNPs, rs17834628 and rs35661809, located on chromosome 5 were
314 significantly associated with BDR ($p = 1.18 \times 10^{-8}$ and 3.33×10^{-8}); additionally,
315 population-specific analyses show that the direction of effect for these two variants
316 is concordant across all three populations (**Fig 3b, S3 Table**). **Fig 3c** displays a
317 LocusZoom plot of rs17834628 with 400 kb flanking regions. Three of the 27
318 identified SNPs were located within genes. Specifically, two SNPs are located in the
319 third and fifth introns of *NFKB1* (rs28450894 and rs4648006), and a third SNP,
320 rs16995064, mapped to intron 7 of *PLCB1* **(Table 4)**. Among the *NFKB1* SNPs, the
321 low BDR-associated T allele of rs28450894 is found predominantly among African
322 populations (minor allele frequency [MAF] 8.8% – 28.7%), followed by European
323 populations (MAF 3.7% - 7.6%) and Puerto Ricans (MAF 6.2%), and is relatively
324 rare in Mexicans (MAF 1.5%) based on 1000 Genomes data (**S6 Fig**). Combined,
325 these 27 SNPs explain 23%, 16%, and 18% of the variation in BDR status in Puerto
326 Ricans, Mexicans, and African Americans, respectively **(S5 Table)**.

7 **Table 4.** Results from trans-ethnic BDR association tests for common variants.

Chr	Start	rsID	Effect allele	OR (95%CI)	*p*	Effect allele frequency			Nearest genes
						PR	MX	AA	
5	12978566	rs17834628	A	1.67 (1.29-2.16)	1.18E-08*	0.32	0.42	0.17	*LINC01194* (-173k), *MIR4454* (-311k), *CTNND2* (-1074k), *DNAH5* (712k)
5	12968341	rs35661809	G	1.59 (1.20-2.10)	3.33E-08*	0.34	0.43	0.24	*LINC01194* (-163k), *MIR4454* (-300k), *CTNND2* (-1064k), *DNAH5* (722k)
5	12975934	rs17237639	G	1.61 (1.30-2.00)	1.22E-07	0.31	0.43	0.16	*LINC01194* (-171k), *MIR4454* (-308k), *CTNND2* (-1072k), *DNAH5* (715k)
5	12975187	rs1017452	G	1.60 (1.31-1.96)	2.11E-07	0.31	0.43	0.16	*LINC01194* (-170k), *MIR4454* (-307k), *CTNND2* (-1071k), *DNAH5* (715k)
5	12975322	rs1017454	A	1.60 (1.31-1.96)	2.11E-07	0.31	0.43	0.16	*LINC01194* (-170k), *MIR4454* (-307k), *CTNND2* (-1071k), *DNAH5* (715k)
5	12975265	rs1017453	C	1.56 (1.25-1.95)	6.40E-07	0.31	0.42	0.16	*LINC01194* (-170k), *MIR4454* (-307k), *CTNND2* (-1071k), *DNAH5* (715k)
5	12972636	rs17237443	C	1.59 (1.28-1.97)	9.85E-07	0.29	0.42	0.11	*LINC01194* (-170k), *MIR4454* (-307k), *CTNND2* (-1071k), *DNAH5* (715k)
1	209324294	rs10746419	T	1.29 (0.75-2.25)	1.19E-06	0.49	0.54	0.53	*MIR205HG* (278k), *MIR205* (281k), *CAMK1G* (433k), *LAMB3* (464k)
5	12961545	rs17833938	A	1.56 (1.28-1.91)	1.45E-06	0.30	0.42	0.12	*LINC01194* (-156k), *MIR4454* (-294k), *CTNND2* (-1057k), *DNAH5* (729k)
6	104240500	rs13437006	C	1.56 (1.21-2.02)	1.61E-06	0.22	0.24	0.32	*HACE1* (935k), *LINC00577* (1144k), *LIN28B* (1164k)
15	101230457	rs1565749	A	1.66 (1.18-2.32)	1.64E-06	0.18	0.15	0.18	*ASB7* (-39k), *LINS1* (-88k), *PRKXP1* (-131k)
5	12948369	rs34845041	T	1.56 (1.26-1.92)	1.77E-06	0.30	0.42	0.12	*LINC01194* (-143k), *MIR4454* (-280k), *CTNND2* (-1044k), *DNAH5* (742k)
5	12975108	rs1017451	T	1.55 (1.24-1.93)	1.96E-06	0.30	0.42	0.13	*LINC01194* (-170k), *MIR4454* (-307k), *CTNND2* (-1071k), *DNAH5* (715k)
5	12950432	rs62347395	G	1.55 (1.26-1.92)	2.02E-06	0.30	0.42	0.12	*LINC01194* (-145k), *MIR4454* (-282k), *CTNND2* (-1046k), *DNAH5* (740k)
15	101231049	rs57924834	A	1.59 (1.25-2.03)	2.04E-06	0.23	0.20	0.20	*ASB7* (-39k), *LINS1* (-89k), *PRKXP1* (-132k)
4	137382142	rs17048684	A	1.8 (1.06-3.05)	2.20E-06	0.11	0.14	0.18	*LINC00613* (-547k), *PCDH18* (1058k)
5	12959598	rs1438293	G	1.55 (1.24-1.93)	2.73E-06	0.29	0.42	0.11	*LINC01194* (-154k), *MIR4454* (-292k), *CTNND2* (-1055k), *DNAH5* (731k)
20	8635168	rs16995064	G	1.96 (1.12-3.43)	3.30E-06	0.12	0.13	0.05	***PLCB1* (intron 7)**, *PLCB4* (415k)
12	19821401	rs66544720	T	0.66 (0.55-0.78)	3.66E-06	0.33	0.37	0.16	*AEBP2* (-146k), *PLEKHA5* (-292k)
6	104235591	rs6926020	C	1.57 (1.25-1.97)	3.68E-06	0.19	0.23	0.27	*HACE1* (940k), *LINC00577* (1149k), *LIN28B* (1169k)
4	103453535	rs28450894	T	0.47 (0.34-0.64)	3.75E-06	0.06	0.03	0.12	*SLC39A8* (-187k), ***NFKB1* (intron 3)**, *MANBA* (99k)
4	103461559	rs4648006	T	0.47 (0.34-0.64)	3.75E-06	0.06	0.03	0.12	*SLC39A8* (-195k), ***NFKB1* (intron 5)**, *MANBA* (91k)
22	27826429	rs60163793	G	2.01 (1.20-3.38)	4.30E-06	0.04	0.14	0.15	*MN1* (318k), *PITPNB* (421k)
12	19824386	rs7313907	C	0.66 (0.55-0.79)	4.35E-06	0.33	0.37	0.16	*AEBP2* (-149k), *PLEKHA5* (-295k)
12	19820677	rs11044754	A	0.66 (0.55-0.79)	4.54E-06	0.33	0.37	0.16	*AEBP2* (-146k), *PLEKHA5* (-291k)
15	101233236	rs55638658	A	1.61 (1.13-2.30)	5.08E-06	0.18	0.15	0.18	*ASB7* (-41k), *LINS1* (-91k), *PRKXP1* (-134k)
6	54581204	rs13200833	A	0.66 (0.48-0.90)	5.15E-06	0.32	0.24	0.22	*TINAG* (-326k), *MLIP* (-450k), *FAM83B* (130k)

8 The top ten 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 trans-ethnic meta-analysis. **Chr and Start**:
9 chromosome locations of SNPs in GRCh37 coordinates. All significantly and suggestively associated common variants are presented above. **Nearest genes**: The four nearest transcripts from RefSeq
0 were identified and genes with multiple transcripts were reported once only with the distance to the nearest transcripts indicated in parentheses. Negative distances indicate upstream genes.
1 Genes that overlap with BDR-associated SNPs are bold. High drug responders were assigned as cases in analyses throughout this study.* p-values that achieve adjusted genome-wide significance
2 for trans-ethnic meta-analysis ($p < 3.53 \times 10^{-7}$).

333 It has been shown that functionally relevant variants do not always display the
334 lowest *p*-values in association studies [47]. To avoid false negative results, it is
335 strongly suggested that replication analyses for two-stage genome-wide studies [43-
336 46]. Therefore, we included all 27 SNPs in replication analyses we performed
337 separately and via meta-analysis in five independent populations (GALA I, SAGE I,
338 HPR, SAPPHIRE and CHOP) (**S6-S7 Table**). None of the 27 SNPs were significantly
339 associated with BDR status in our replication analyses **(S6-S7 Table)**. It is
340 important to note that our largest replication cohort (SAPPHIRE) did not include
341 children (median age = 30 and 28 for high and low BDR groups, respectively, **S6**
342 **Table**). All other replication populations included less than 500 individuals per
343 study **(S6 Table)**. It is well known that there are age-specific associations with
344 asthma and asthma-related phenotypes [48, 49]. It is unclear whether the same is
345 true for BDR. It is possible that the overrepresentation of adult patients in our
346 available replication populations may explain, in part, our lack of replication.

347 In addition to performing WGS association analyses to identify genetic variants
348 associated with variation in BDR, we also performed H3K27ac chromatin
349 immunoprecipitation sequencing analysis (ChIP-seq) experiments in primary
350 bronchial smooth muscle cells (BSMCs) to identify potential regulatory regions
351 marked by H3K27ac peaks. Albuterol's mechanism of action involves binding with
352 the β_2-adrenergic receptor in bronchial smooth muscle cells causing rapid onset of
353 airway tissue relaxation and bronchodilation. BSMCs are therefore considered one
354 of the most relevant cell types for molecular studies of BDR [50]. We observed two
355 H3K27ac ChIP-seq signals that overlapped with variants in moderate to high LD (R^2

356 = 0.47 to 0.82) with two *NFKB1* SNPs (rs28450894 and rs4648006) we identified
357 through trans-ethnic meta-analysis, implying that these variants may have
358 regulatory functions in BSMC (**S5 Fig a, S8 Table**).

359 The gold standard for identifying true signals in genetic association studies is to
360 use *p*-values from a primary and/or replication study to prioritize variants for
361 further investigation. The use of *p*-values as the sole metric for prioritization is
362 problematic for three reasons: (1) the *p*-value statistic is dependent on sample size
363 and effect magnitude, (2) *p*-values do not incorporate biological knowledge, and (3)
364 one cannot use *p*-values to distinguish between true association signals and noise of
365 the same magnitude [47, 51, 52]. Instead of relying solely on *p*-values, we applied
366 the Diverse Convergent Evidence (DiCE) [53] approach to prioritize each of the 27
367 BDR-associated SNPs from our trans-ethnic meta-analysis for inclusion in further
368 function analyses **(S15 Table, S4 Fig)**. After integrating information from our WGS
369 analysis, publicly available bioinformatics data, and ChIP-Seq experiments in BSMCs,
370 the *NFKB1* locus had the highest DiCE evidence score, indicating that this locus had
371 the strongest evidence of functional relevance to BDR variation **(S4 Fig)**. Therefore,
372 all further functional experiments were focused on variants within this locus.

373 ***Functional assays on the NFKB1 Locus***

374 Both H3K27ac ChIP-seq regions that overlapped with the BDR-associated *NFKB1*
375 locus were tested for enhancer activity using luciferase enhancer assays. The
376 sequences of these two *NFKB1* intronic regions were cloned into a pGL4.23
377 enhancer assay vector (Promega, Madison, WI, USA), which contains a minimal
378 promoter and a luciferase reporter gene. The pGL4.23 vector with the viral SV40

379 promoter was used as a positive control, and the pGL4.23 empty vector as a
380 negative control. All constructs were tested for their enhancer activity in BSMCs.
381 One enhancer, *NFKB1* Region 2, showed significantly increased enhancer activity
382 over empty vector (2.24-fold increase, $p = 8.70 \times 10^{-6}$, unpaired t-test; **S5 Fig b**).

383 Given the relevance of *NFKB1* in immune pathways and asthma, we also
384 performed RNA sequencing (RNA-seq) experiments to verify whether the identified
385 intronic *NFKB1* SNPs regulate gene expression of neighboring genes. Among genes
386 within 1Mb of rs28450894 meeting expression reliability cutoffs (see **Methods**), we
387 found that the low BDR-associated T allele of rs28450894 is significantly associated
388 with decreased expression of *SLC39A8* in blood (**S7 Fig**, $p = 0.0066$, FDR-adjusted p
389 $= 0.0856$, $\log_2(\beta) = -0.327$).

390 We observed that two known BDR candidate genes, *ADCY9* and *CRHR2*, which
391 achieved replication in a previous GWAS of BDR performed in the full GALA II
392 population but did not replicate in the current study (**S10 Table**) [27]. In the
393 previous study, GWAS array data, supplemented by imputation, were used to
394 evaluate genetic associations with BDR measured as a continuous trait. To
395 determine whether the discrepancy between findings was due to data type
396 (imputed array-based vs. WGS-based) or study design (continuous trait vs. extreme
397 phenotype), the common variant analysis in the current analysis was repeated
398 among the subset of samples that had array-based and WGS data (n = 1,414 out of
399 1,441). Based on the top 1,000 BDR-associated SNPs from the current common
400 variant analysis, there was high correlation between association p-values generated
401 from imputed array-based and WGS-based genotypes (Spearman correlation = 1.0),

402 suggesting that data type is not the cause of the observed discrepancy (**S9 Fig a**).
403 Nearly all SNPs with high imputation R^2 exhibited high genotype concordance
404 between array-based and WGS-based genotypes, confirming high imputation quality
405 for most common SNPs (≥ 99.7%). (**S9 Fig b and c**). We also performed linear
406 regression to analyze BDR (ΔFEV_1) as a continuous trait using imputed array-based
407 data. The most significantly associated SNP identified in the trans-ethnic meta-
408 analysis using extreme phenotype analysis displayed the same direction of effect as
409 analyzing BDR as a continuous trait (OR=1.67 in extreme phenotype analysis and
410 β=0.51 in continuous analysis). These observations indicate that the discrepancy
411 between findings may be due to differences in statistical power afforded by the
412 different study designs (continuous trait vs. extreme phenotype). For common
413 variant analyses, dichotomization of a continuous outcome results in a loss of
414 statistical power [54-57]. For example, a population of 2,000 individuals has 80%
415 power to identify moderate genetic associations (β=0.3) for common variants with
416 minor allele frequencies ≥ 0.05 when the outcome is continuous. If this population
417 were re-analyzed after dichotomizing the continuous outcome at the median
418 (cases=1,000, controls=1,000), power would be reduced to 62%. The opposite effect
419 is observed in rare variant analyses. The extreme phenotype study design is a
420 specific type of dichotomous outcome study design that has been shown to increase
421 power and the probability of identifying functional rare variants [56-58]. It should
422 also be noted that the previously published results were discovered in one
423 population (Puerto Ricans), whereas the results from our trans-ethnic meta-analysis

424 describe associations that are conserved across three populations (Puerto Ricans,
425 Mexicans, and African Americans).

426 **BDR association testing using common and rare variants**

427 We tested the combined effects of common and rare variants on BDR using SKAT-O
428 [59] to examine variants in 1kb sliding windows, which moved across the genome in
429 500bp increments. The same covariates used for common variant association
430 testing were applied.

431 After identifying the effective number of tests and adjusting for multiple
432 comparisons on each population separately (see **Methods**), we identified three
433 population-specific loci associated with BDR at genome-wide significance levels;
434 two were found in Mexicans on chromosome 1 and chromosome 11, and one in
435 African Americans on chromosome 19 (**Fig 4a-c, Table 5, S11 Table**).

436 **Table 5.** Results from association testing on combined effects of common and rare variants on BDR

Chr	Start	Stop	*p*	Population	nCommon	nRare	Nearest genes
1	114177000	114178000	4.40E-09	MX	2	1	***MAGI3* (intron 9)**, *PHTF1* (62k), *RSBN1* (126k)
11	27507000	27508000	6.59E-09	MX	2	3	*LOC105376671* (-3k), *LGR4* (-13k), *LIN7C* (8k)
19	10424000	10425000	3.12E-11	AA	1	2	*ZGLP1* (-4k), *ICAM5* (-17k), ***FDX1L* (intron 3)**, *RAVER1* (2k)
4	73478000	73479000	6.25E-08	Combined	10	23	*ADAMTS3* (-43k), *COX18* (441k)
8	97926000	97927000	1.32E-08	Combined	3	13	*SDC2* (-302k), ***CPQ* (intron 4)**, *LOC101927066* (37k), *TSPYL5* (359k)

437 **Chr, start and stop**: GRCh37 chromosome coordinates; **AA**: African Americans; **MX**: Mexicans; **Combined**: all individuals in all three populations. **nCommon**
438 and **nRare**: number of common and rare variants, respectively. **Nearest genes**: The four nearest transcripts from RefSeq were identified and genes with
439 multiple transcripts were reported once only with the distance to the nearest transcripts indicated in parentheses. Negative distances indicate upstream
440 genes. Genes that overlap with BDR-associated SNPs are bold.

441 We also performed association testing across all three populations in a single
442 analysis. Pooling subjects increased the sample size and thereby maximized the
443 power of the SKAT-O association test. To minimize any potential effect of
444 confounding by population substructure, association testing also included local
445 genetic ancestry, defined as the proportions of Native American and African
446 ancestries for the window under testing. Two loci on chromosomes 4 and 8 were
447 found to be genome-wide significant ($p < 1.53 \times 10^{-7}$) (**Fig 4d, Table 5**). A total of 60
448 variants were identified from all SKAT-O regions reported in **Table 5**. Six of the 60
449 variants were located within predicted regulatory regions (**S12 Table**). Specifically,
450 three variants located on chromosome 11 identified in Mexicans overlap with a
451 CTCF (transcriptional repressor) binding site and comprise a chromatin insulator
452 region. The five regions identified in our combined and population- specific SKAT-O
453 analyses independently explained 4% to 8% of the variation in BDR in their
454 respective populations (**Table 5, S5 Table**).

455 We examined alternative grouping strategies for rare variants, including
456 grouping by (1) genes from transcription start to end sites with or without 50kb
457 flanking regions, (2) transcription start site with 20kb flanking regions, and (3)
458 H3K27ac ChIP-seq peaks in airway epithelial cells and airway smooth muscle cells.
459 Association tests with these alternate grouping strategies identified no further
460 significant associations.

461 **DISCUSSION**

462 We identified population-specific and shared common and rare variants associated
463 with bronchodilator drug response in three ethnically diverse populations of
464 children with asthma. WGS, unlike GWAS genotyping arrays and targeted
465 sequencing, provides comprehensive detection of common and rare variants in
466 coding and non-coding regions. African Americans, Latinos, and other minorities
467 have been dramatically underrepresented in GWAS [34-36]. Combined, the 27
468 variants identified from our common variant analyses (**Table 4**) explained 23%,
469 16%, and 18% of the variation in BDR in Puerto Ricans, Mexicans, and African
470 Americans, respectively, after adjusting for clinical covariates (**S5 Table**). The five
471 SKAT-O regions identified in our combined and population-specific analyses
472 independently explained 4% to 8% of the BDR variation in their respective
473 populations (**Table 5, S5 Table**). Our study represents an important investment
474 from the NIH/NHLBI to include underrepresented populations in large whole
475 genome sequencing efforts and to improve racial/ethnic diversity in clinical and
476 biomedical research.

477 Our trans-ethnic common variants meta-analysis identified one locus on
478 chromosome 5 that was associated with BDR at a genome-wide significance level (p
479 $< 5 \times 10^{-8}$). The proximity of this BDR-associated locus to *DNAH5* and *LINC01194* is
480 of particular interest. A SNP in *DNAH5* has been associated with total lung capacity
481 in White subjects with chronic obstructive pulmonary disease [60]. In a separate
482 GWAS, the *DNAH5/LINC01194* locus was reported among Europeans to be
483 associated with levels of IgE [61, 62], a biomarker associated with asthma
484 endotypes. Baseline lung function (FEV_1) and total IgE levels are associated with

485 asthma severity and can predispose an individual to lower bronchodilator drug
486 responsiveness [13, 14, 63]. We found two *NFKB1* intronic variants on chromosome
487 4 associated with BDR at a suggestive significance level. The NFκB protein has a
488 known role in allergic response, and various studies have demonstrated that the
489 NFκB pathway is activated in patients with asthma, as reviewed by Edwards *et al.*
490 [64].

491 ChIP-seq and functional enhancer assays in BSMCs suggest these *NFKB1* intronic
492 variants may regulate expression of nearby genes. This was in fact supported by our
493 RNA-seq data, which showed that individuals with the low BDR-associated T allele
494 genotype displayed reduced expression of the neighboring gene *SLC39A8*, which has
495 previously been found to be responsive to cytokine treatment in airway epithelial
496 cells [65] and had reduced expression in mice with allergic airway inflammation
497 [66]. Recent studies have also shown that *SLC39A8* is unique among zinc
498 transporters in that upregulation of *SLC39A8* is sufficient to protect lung epithelium
499 against TNF-α-induced cytotoxicity [67]. Additionally, the higher frequency of the
500 low BDR-associated allele (T allele of rs28450894 in *NFKB1*) in African populations
501 suggests that the low BDR-associated allele tracks with African ancestry. This may
502 explain why admixed populations with higher proportions of African ancestry, i.e.,
503 African Americans and Puerto Ricans, have lower bronchodilator drug
504 responsiveness [14], and by extension may shed light on the higher asthma
505 morbidity and mortality in these populations.

506 Another intronic variant (chromosome 20, rs16995064, *PLCB1* intron 7) was
507 associated with BDR at a suggestive significance level. *PLCB1* is highly relevant, as

508 this gene has been reported to be differentially expressed in therapy-resistant
509 childhood asthma compared to controlled persistent asthma or age-matched
510 healthy control subjects in a Swedish cohort [68]. Functional studies also reported
511 that silencing *PLCB1* inhibited the effect of lipopolysaccharide-induced endothelial
512 cell inflammation through inhibiting expression of proinflammatory cytokines [69].
513 Further functional studies are necessary to establish the role of *NFKB1* and *PLCB1*
514 on BDR.

515 Apart from assessing the individual effect of common variants on BDR, we also
516 identified various combined effects of rare variants that were population-specific or
517 shared across populations. While some of the nearest genes are uncharacterized or
518 have no known functional relationship to BDR (*MAGI3*, *LOC105376671*, *LIN7C* and
519 *CPQ*), there appears to be functional relevance for the locus between *ADAMTS3* and
520 *COX18*. The *ADAMTS3* and *COX18* locus were associated with β-adrenergic responses
521 in cardiovascular-related traits in mice [70]. This locus was significantly associated
522 with cardiac atrial weight in mice treated with the β blocker atenolol; the
523 association also replicated in mice treated with the β agonist isoproterenol. These
524 findings suggest that SNPs found in this locus may modify β adrenergic signaling
525 pathways in BDR. In the present study, we also identified BDR association with rare
526 variants within the *CPQ* gene, which encodes a protein from the carboxypeptidase
527 family. Although no previous BDR association has been identified for *CPQ*, another
528 member of the carboxypeptidase family, carboxypeptidase A3 (CPA3), is known to
529 be expressed at higher levels in the airway epithelium among subjects with T_H2-

530 high asthma [71, 72]. Further studies are necessary to determine the role of *CPQ* in
531 BDR.

532 GWAS-based BDR-associated common variants in GALA II have previously been
533 reported [27]. However, these variants did not replicate in the current study, likely
534 due to different study designs between the previous and current investigations. The
535 previous BDR GWAS used an array-based genotyping panel to examine children
536 with asthma across the entire BDR spectrum, i.e., BDR was used as a continuous
537 variable. In contrast, the current study sequenced the entire genome to investigate
538 only the extremes of the BDR distribution (i.e., high and low drug responders). By
539 repeating our current analysis using a subset of individuals who had array and WGS
540 data, we confirmed that the major discrepancy between the two studies is due to
541 study design instead of differences in data type. The contrast in results between
542 GWAS and WGS due to differences in study design implies that varied study designs
543 are necessary for a comprehensive understanding of variants associated with
544 asthma-related phenotypes and drug response. Studying samples from the extreme
545 tails of drug response distribution has been recognized as one of the success factors
546 in the study design of pharmacogenomic GWAS [73]. Furthermore, it was recently
547 demonstrated that the power gain from studying extreme phenotypes is much
548 greater in analyses of rare variants compared to common variant studies [55]. Since
549 cost is often a limiting factor for WGS studies, choosing an extreme phenotypic study
550 design may be beneficial for the study of rare variants and the discovery of common
551 variant associations that may otherwise be missed when sampling across the entire
552 phenotypic spectrum.

553 We did not identify BDR-associated variants from β_2AR signaling pathways.
554 Instead, most of the BDR-associated genes identified in this study are related to lung
555 function and allergic response, including total IgE levels and cytokine production in
556 mast cells. This suggests that at least part of BDR may be due to the predisposition
557 or intrinsic state of airway smooth muscle cells. Genetic variation may determine
558 individuals' intrinsic expression levels of candidate genes, which in turn determine
559 whether their response to albuterol is beneficial.

560 A higher percentage of African ancestry often implies a higher degree of genetic
561 variation [74]. Although Puerto Ricans have higher proportions of African ancestry
562 than Mexicans (**Table 1**), they have fewer population-specific SNPs, an observation
563 that is consistent with findings from our contributions to the 1000 Genomes Project
564 [75] and our independent work. This is likely due to the fact that Puerto Ricans have
565 gone through recent population bottlenecks [76]. We demonstrated that Puerto
566 Ricans may be more genetically related than expected [76], suggesting that our
567 current relatedness filters may be too conservative for Puerto Ricans.

568 Including admixed populations in whole genome sequencing studies has
569 important scientific implications. First, it allows for discovery of genetic variation of
570 multiple ancestral populations in a single study. Second, it is extremely useful to
571 study admixed populations with ancestries that are currently underrepresented in
572 existing genetic repositories. For example, the widely popular PCSK9 inhibitors used
573 to treat hypercholesterolemia were discovered by studying the genetics of African
574 Americans but the biology and final drug development have benefited all patients
575 regardless of race/ethnicity [77]. Finally, studying admixed populations such as

576 Mexicans will enhance the understanding of genetic variation in Native American
577 ancestry, an area that is currently lacking in all major sequencing efforts.

578 Although an extensive effort was made to replicate the top BDR-associated
579 variants, we were unable to replicate our results because few studies of non-
580 European populations exist, as we and others have documented [34-36, 78]. Our
581 efforts to perform replication of rare BDR-associated variants were further hindered
582 by the lack of studies with whole genome sequencing data. These challenges
583 highlight the need to include more racially/ethnically diverse populations in all
584 clinical and biomedical research.

585 In an era of precision medicine, addressing questions about the impact of genetic
586 factors on therapeutic drug response in globally diverse populations is essential for
587 making precision medicine socially and scientifically precise [5]. This study
588 advances our understanding of genetic analysis in admixed populations and may
589 play an important role in advancing the foundation of precision medicine for
590 understudied and racially and ethnically diverse populations.

591 METHODS

592 Data availability

593 TOPMed whole genome sequencing data are available to download by submitting a
594 data access request through dbGaP. The dbGaP study accession numbers for GALA II
595 and SAGE II are phs000920.v1.p1 and phs000921.v1.p1. WGS and array genotype
596 data for each study are available through dbGaP under the same accession numbers.

597 **Study cohorts and sample details**

598 This study examined a subset of subjects with asthma from the Study of African
599 Americans, Asthma, Genes & Environments (SAGE II) [49, 79-81] and the Genes-
600 Environments & Admixture in Latino Americans (GALA II) study [27]. SAGE II
601 recruited African American subjects from the San Francisco Bay area. GALA II
602 recruited Latino subjects from Puerto Rico and the mainland United States (Bronx,
603 NY; Chicago, IL; Houston, TX; San Francisco Bay Area, CA). Ethnicity of the subjects
604 was self-reported and all four of the participant's biological grandparents must have
605 reported the same ethnicity.

606 A total of 1,484 individuals from three ethnic groups (494 Puerto Ricans, 500
607 Mexicans and 490 African Americans), representing the extremes of the
608 bronchodilator response (BDR, see below) distribution were selected for whole
609 genome sequencing. Genomic DNA was extracted and purified from whole blood
610 using Wizard® Genomic DNA Purification Kits (Promega, Madison, WI, USA).

611 **Bronchodilator response measurements**

612 Spirometry was performed and BDR (i.e., ΔFEV_1, defined as the relative change in
613 FEV_1) was calculated as previously described [27]. In brief, BDR was calculated
614 according to American Thoracic Society/European Respiratory Society guidelines
615 [82] as the percent change in FEV_1 after 2 doses of albuterol: that is, BDR = (post-
616 FEV_1 – pre-FEV_1) / pre-FEV_1.

617 High and low drug responders were selected from the extremes of BDR
618 distribution from GALA II and SAGE II (**Fig 1**). **S1 Fig** highlighted the BDR

619 distribution of the 1,441 individuals who passed WGS data quality control (see
620 "WGS data processing and quality control"). The ΔFEV_1 cutoffs for high and low
621 responders are as follows: high responders ($\Delta FEV_1 > 16.29$ for Puerto Ricans, > 8.55
622 for Mexicans and > 11.81 for African Americans); low responders ($\Delta FEV_1 < 7.23$ for
623 Puerto Ricans, < 6.05 for Mexicans and < 5.53 for African Americans).

624 **Analysis on descriptive data of study subjects**

625 Dichotomous variables were tested for association with BDR using Fisher's exact
626 test. Continuous variables were tested for normality using the Shapiro-Wilk test.
627 Normally and non-normally distributed continuous variables were tested using
628 Student's t-test and the Wilcoxon rank-sum test, respectively.

629 **Sample quality control and whole genome sequencing**

630 DNA samples were quantified by fluorescence using the Quant-iT PicoGreen dsDNA
631 assay (ThermoFisher Scientific, Waltham, MA, USA) on a Spectramax fluorometer
632 (Molecular Devices, Sunnyvale, CA, USA). Sample integrity was ascertained using the
633 Fragment Analyzer™ (Advanced Analytical Technologies, Inc., Ankeny, IA, USA).
634 Samples passing QC were genotyped using the HumanCoreExome-24 array
635 (Illumina®, San Diego, CA, USA). Genotyping results were analyzed using
636 VerifyIDintensity [83] to flag sample contamination. Sequencing libraries were
637 constructed using the TruSeq PCR-free DNA HT Library Preparation Kit (Illumina®,
638 San Diego, CA, USA) with 500ng DNA input. Briefly, genomic DNA was sheared using
639 a Covaris sonicator (Covaris, Woburn, MA, USA), followed by end-repair and bead-
640 based size selection of fragmented molecules. Selected fragments were then A-tailed

641 and sequence adaptors were ligated onto the fragments, followed by a final bead
642 purification of the libraries. Final libraries were reviewed for size distribution using
643 Fragment Analyzer and quantified by qPCR (Kapa Biosystems, Wilmington, MA,
644 USA). Libraries were sequenced on a HiSeq X system (Illumina®, San Diego, CA, USA)
645 with v2 chemistry, using a paired-end read length of 150 bp, to a minimum of 30x
646 mean genome coverage.

647 **WGS data processing and quality control**

648 Sequencing data were demultiplexed using bcl2fastq version 2.16.0.10 (Illumina®,
649 San Diego, CA, USA) and aligned to human reference hs37d5 with decoy sequences
650 using BWA-MEM v0.7.8 [84]. Data were further processed using the GATK best-
651 practices v3.2-2 pipeline [85]. Quality control procedures included marking of
652 duplicate reads using Picard tools v1.83 (http://picard.sourceforge.net),
653 realignment around indels, and base quality recalibration using 1000 Genomes
654 Phase 1 high confidence SNPs, HapMap v3.3, dbSNP v137, 1000 Genomes omni2.5,
655 1000 Genomes Phase 1 indels, and both Mills and 1000 Genomes gold standard
656 indels. Single-sample genotypes were called using GATK HaplotypeCaller followed
657 by joint genotyping of all subjects. The resulting multi-sample Variant Call Format
658 (VCF) file was used for variant quality score recalibration (VQSR). A 99.8% truth
659 sensitivity tranche level was used for SNPs and 99.0% for indel variants. SNP calls
660 were used to check for sample contamination using VerifyBAMId [83], and sample
661 identity was confirmed by requiring > 99.5% concordance with SNP array
662 (HumanCoreExome-24 array) genotypes.

663 As part of NIH's Trans-Omics for Precision Medicine (TOPMed) Program, BAM
664 files were submitted to the Informatics Resource Center (IRC) at the University of
665 Michigan. All 1,484 samples sequenced passed TOPMed's IRC quality control metrics
666 (mean genome coverage >30X; >95% of genome covered at >10X; and <3%
667 contamination).

668 VCF-level variants were filtered by GATK version 3.4.46 and VCFtools version
669 0.1.14 [86]. Variants were filtered according to the following procedures: (1)
670 remove variants that were not indicated as "PASS" in the VCF FILTER column, (2)
671 remove variants in low complexity regions [87] (downloaded from
672 https://github.com /lh3/varcmp/tree/master/scripts/LCR-hs37d5.bed.gz), and (3)
673 keep sample genotypes that have minimum read depths of 10 and genotype
674 qualities of 20 (DP ≥ 10 and GQ ≥ 20). The ratio of homozygous to heterozygous
675 variants (hom/het), ratio of transitions to transversions (Ti/Tv), and other variant
676 summary statistics were generated using GATK VariantEval. VCF files were
677 converted into PLINK format using PLINK 1.9 software [88] according to
678 recommended best practices [89]. Genotype consistency between WGS data and
679 previously published Axiom® Genome-Wide LAT 1 array (Affymetrix, Santa Clara,
680 CA) genotype data (dbGaP phs000920.v1.p1 and phs000921.v1.p1) was assessed
681 using VCFtools [86]. Individuals with percentage consistency three S.D. below the
682 mean (< 96.3%) were removed (N=7, **S10 Fig**). Cryptic relatedness was detected
683 using REAP [90]. Global ancestry and allele frequency used by REAP were estimated
684 using ADMIXTURE in supervised mode [91]. Related individuals (kinship coefficient
685 > 0.044, corresponding to a third degree relationship [92]) were excluded from

686 further analysis (N=36), yielding a final sample size of 1,441 for downstream
687 analysis. Downstream analyses were only performed on biallelic SNPs that passed
688 all quality filters mentioned above and had less than 10% of genotype missingness.
689 The 10% genotype missingness filter was applied per population instead of across
690 all three populations except for the rare variant analysis performed with all three
691 populations combined (see **Methods** section "Multi-variant analyses of combined
692 effects of rare variants on BDR").

693 **Principal component analysis**

694 Principal component analysis (PCA) was performed to control for hidden population
695 substructure using EIGENSTRAT's smartpca program [93]. After using PLINK 1.9 to
696 remove biallelic SNPs with low minor allele frequency (MAF ≤ 0.05) and in linkage
697 disequilibrium (R^2 > 0.5 in a 50-SNP window with a shift size of 5 SNPs), 710,256
698 variants remained for input into smartpca.

699 **Local ancestry estimation**

700 Reference genotypes for European and African ancestries were obtained from the
701 Axiom® Genotype Data Set [94]. SNPs with less than a 95% call rate were removed.
702 Since no Native American reference samples are available in the HapMap database,
703 reference genotypes for Native American ancestry were generated from 71 Native
704 American individuals previously genotyped on the Axiom® Genome-Wide LAT 1
705 array [27].

706 To call local ancestry tracts, we first created a subset of our WGS data
707 corresponding to sites found on the Axiom® Genome-Wide LAT 1 array, leaving

708 765,321 markers. Using PLINK 1.9, we merged these data with our European (CEU),
709 African (YRI), and Native American (NAM) reference panels, which overlapped at
710 434,145 markers. After filtering multi-allelic SNPs and SNPs with > 10% missing
711 data, we obtained a final merged dataset of 428,644 markers. We phased all samples
712 using SHAPEIT2 [95] and called local ancestry tracts jointly with RFMix [96] under a
713 three-way admixture model based on the African, European, and Native American
714 reference genotypes described above.

715 **Variant annotation**

716 TOPMed freeze 2 and 3 variants were annotated using the WGSA annotation
717 pipeline [97]. Annotated VCF files were downloaded from the TOPMed Data
718 Coordinating Center SFTP sites. dbSNP150 annotation was added separately by
719 using VCF file downloaded from NCBI dbSNP ftp site [98]. ENCODE (v4) annotations
720 were downloaded as BED files from the UCSC Table Browser (Feb.2009
721 [GRCh37/hg19] assembly). The conversion from GRCh37 to GRCh38 coordinates
722 was performed using liftOver from the UCSC Genome Browser Utilities [99].

723 **Single locus BDR association testing on common variants**

724 An additive logistic regression model was used to evaluate the association of
725 biallelic common variants (MAF > 1%) with BDR using PLINK 1.9 separately for
726 each population. Throughout this study, high drug responders were assigned as
727 cases. Logistic regression models included the covariates age, sex and body mass
728 index (BMI) categories to account for previously reported confounders of asthma
729 and BDR [100-107], and the first ten principal components (PCs) to correct for

730 population substructure in admixed populations. BMI and age- and sex-specific BMI
731 percentiles (BMI-pct) were calculated as previously described [49] and used for
732 assignment to BMI categories. For subjects aged 20 years and over, BMI categories
733 were defined as follows: underweight (BMI < 18), normal (18 ≤ BMI < 25),
734 overweight (25 ≤ BMI < 30) and obese (BMI ≥ 30). For subjects under 20 years of
735 age, BMI categories were defined as follows: underweight (BMI-pct < 5), normal (5 ≤
736 BMI-pct < 85), overweight (85 ≤ BMI-pct < 95) and obese (BMI-pct ≥ 95). Baseline
737 lung function (pre-FEV_1) has a significant impact on variation in BDR drug response.
738 Pre-FEV_1 was not included as a covariate in association analyses, as variation in pre-
739 FEV_1 was indirectly captured by several of the ten principal components already
740 included in association models (**S13 Table**). A correlation matrix showing the
741 relationship between pre-FEV_1, age, sex, BMI status, mean global ancestry, and the
742 top ten principal components is presented in **S13 Table**. The correlation matrix was
743 constructed using Spearman correlation coefficients and the accompanying
744 association tests for the significance of each correlation. Population-specific
745 genome-wide significance thresholds for the single locus analyses were calculated
746 based on genotypes using the autocorrelation-based *effectiveSize()* function in the R
747 package '*coda*' as published by Sobota *et al.* [41]. Population-specific genome-wide
748 significance thresholds after adjusting for the effective number of tests (adjusted
749 genome-wide significance) were 1.57×10^{-7} for Puerto Ricans, 2.42×10^{-7} for
750 Mexicans, and 9.59×10^{-8} for African Americans. Suggestive significance thresholds
751 were calculated as one divided by the effective number of tests [43]. Linkage
752 disequilibrium patterns (Genome build: hg19/1000 Genomes Nov 2014 AMR) of the

753 flanking regions of BDR-associated SNPs were visualized using LocusZoom [108].
754 Quantile-quantile (q-q) plots were generated using a uniform distribution as the
755 expected *p*-value distribution (**S11 Fig a-c**). The genomic inflation factor (λ_{GC}) was
756 calculated using the R package *'gap'*.

757 **Trans-ethnic meta-analysis of common variant effects on BDR**

758 A meta-analysis of the effects of common variants on BDR across the three
759 populations was performed using METASOFT [109]. We used the Han and Eskin
760 random effects model optimized for detecting associations under heterogeneous
761 genetic effects from different study conditions [109]. The number of effective tests
762 was estimated using the R package *'coda'* as described above, yielding an adjusted
763 genome-wide significance threshold of 3.53×10^{-7} and a suggestive significance
764 threshold of 7.06×10^{-6}. Allele frequency variation in the world population was
765 visualized using the Geography of Genetic Variants Browser (GGV) beta v0.92 [110].
766 The q-q plot and λ_{GC} were generated in the same way as described above (**S11 Fig d**,
767 see **Methods** section, "Single locus BDR association testing on common variants").

768 **Calculation of variation in BDR explained by common variants**

769 Total variation in BDR explained was estimated by calculating McFadden's pseudo
770 R^2 [111] in each population separately, after first pruning significantly and
771 suggestively associated variants (**Table 4**) for LD using the LD prune function in
772 PLINK 1.9 (R^2 cut-off: 0.6, window size = 50 SNPs, shift = 5 SNPs). Calculation of
773 pseudo R^2 was adjusted for age, sex, BMI category, and principal components 1-10.
774 McFadden's Pseudo R^2 is defined as:

$$R^2 = 1 - \frac{\ln L\ (M_{FULL})}{\ln L\ (M_{INTERCEPT})}$$

775 L: Estimated likelihood of model

776 M_{FULL}: Model with all predictors

777 $M_{INTERCEPT}$: Model with no predictors

778 **Multi-variant analyses of combined effects of common and rare variants on**
779 **BDR**

780 Combined effects of common and rare variants on BDR were analyzed using SKAT-O
781 [59]. Common and rare variants were collapsed into 1kb windows sliding across the
782 GRCh37 genome in steps of 500 base pairs. A total of 5.3 million windows were
783 analyzed and the R package *'coda'* was used to determine the number of effective
784 tests based on autocorrelation of the association *p*-value, as described above.
785 Adjusted genome-wide significance thresholds for Puerto Ricans (8.15×10^{-8}),
786 Mexicans (8.60×10^{-8}), African Americans (6.94×10^{-8}) and for all three populations
787 combined (1.53×10^{-7}) were used to identify windows of variants significantly
788 associated with BDR. The same covariates used for common variant association
789 testing were used for analyses of individual populations. For analyses of individuals
790 combined across all three populations, we avoided confounding from population
791 substructure by including local ancestry as additional covariates, defined as the
792 proportions of Native American and African ancestries for the window under
793 testing. The q-q plots and λ_{GC} were generated in the same way as described above

794 (**S12 Fig**, see **Methods** section, "Single locus BDR association testing on common
795 variants").

796 **Variation in BDR explained by associated SKAT-O regions**

797 Variation in BDR explained by SKAT-O regions was calculated as described above
798 for common variant analyses using McFadden's pseudo R^2 [111], but with one
799 addition: variants were weighted using a weighted kernel as described in SKAT-O
800 [112].

801 **Single locus BDR association and trans-ethnic meta-analysis of array data**

802 To address the discrepancy between our current common variant analysis results
803 with previously published BDR GWAS results [27], we used 1,414 of the 1,441
804 individuals who had both Axiom® Genome-Wide LAT 1 array (see **Methods** section,
805 "WGS data processing and QC") and WGS data available to rerun the single locus
806 BDR association testing and trans-ethnic meta-analysis. Array data were imputed to
807 the Haplotype Reference Consortium [113] (HRC release 1) panel using the
808 Michigan Imputation Server [114]. We used the top 1,000 BDR-associated SNPs to
809 examine the relationship between array-based and WGS-based association *p*-values,
810 genotype discordance, and imputation R^2. Correlation between the array-based and
811 WGS-based association *p*-values was determined by Spearman correlation. We also
812 performed single locus BDR association testing and trans-ethnic meta-analysis by
813 applying linear regression on 1,122 Puerto Ricans, 662 Mexicans and 1,105 African

814 Americans using BDR (ΔFEV_1) as a continuous trait. HRC imputed array-based data
815 and the same covariates as described above were used for the analysis.

816 **Replication of top BDR-associated common variants**

817 Replication cohorts included the Genetics of Asthma in Latino Americans Study
818 (GALA I) [13, 115], the Study of African Americans, Asthma, Genes & Environments
819 (SAGE I) [80], a case-control study of childhood asthma in Puerto Ricans (HPR)
820 [116], the Study of Asthma Phenotypes and Pharmacogenomic Interactions by Race-
821 Ethnicity (SAPPHIRE) [117] and a cohort from the Children's Hospital of
822 Philadelphia (CHOP) [118]. Descriptive statistics for the replication cohorts are
823 shown in **S6 Table**.

824 Logistic regression in replication analyses was performed using the same
825 population-specific extreme BDR cut-offs applied to the discovery analyses in the
826 current study (see **Methods** section, "Bronchodilator response measurements"). All
827 27 significantly and suggestively associated SNPs from the discovery analysis
828 (**Table 4**) were assessed in each replication study population. An additive genetic
829 model was assumed for each SNP tested. Association models were adjusted for sex,
830 age, BMI categories, and the first ten principal components as in the discovery
831 analysis. Meta-analysis across all replication studies was performed using
832 METASOFT as described above (**Methods**, "Trans-ethnic meta-analysis of common
833 variant effects on BDR").

834 The GALA I and SAGE I replication cohorts included 108 Puerto Ricans, 202
835 Mexicans and 141 African Americans with BDR measurements and complete data

836 for all covariates (age, sex, BMI categories and the first ten PCs). Genotype data were
837 imputed to the HRC panel using the Michigan Imputation Server [114]. Replication
838 in the HPR cohort involved 414 Puerto Rican subjects. Spirometry data were
839 collected as previously described [119]. Genome-wide genotyping was performed
840 using the Illumina HumanOmni2.5 BeadChip platform (Illumina Inc., San Diego, CA)
841 and processed as previously described [120]. Genotype data were phased with
842 SHAPE-IT [121] and imputation was performed with IMPUTE2 [122] using all
843 populations from 1000 Genomes Project Phase 3 as reference [75]. The SAPPHIRE
844 replication cohort consisted of 1,022 African Americans with asthma. Genome-wide
845 genotyping was performed using the Axiom® Genome-Wide AFR 1 array (Affymetrix
846 Inc., Santa Clara, CA) as previously described [26]. Genotype data were imputed to
847 the cosmopolitan 1000 Genomes Phase 1 version haplotypes using the Michigan
848 Imputation Server [114]. The CHOP replication cohort included 280 African
849 Americans. Genotyping was performed as described [118], and genotype data were
850 imputed to the HRC panel using the Sanger Imputation server [113].

851 **Identification of nearest genes for BDR-associated loci**

852 The four nearest transcripts to BDR-associated loci were identified by using the
853 "closest" command in BEDTools with the parameters "-d -k 4" and the RefSeq gene
854 annotations (Feb.2009 [GRCh37/hg19] assembly) downloaded in refFlat format
855 from the UCSC Table Browser [123]. Genes with multiple transcripts were reported
856 only once. When reporting the nearest gene, the "closest" command in BEDTools
857 with the parameters "-D a" was applied.

858 **Primary bronchial smooth muscle cell culture**

859 Cryopreserved primary human bronchial smooth muscle from two donors (from
860 Lonza catalog number CC-2576, lot number 0000212076 and from ATCC catalog
861 number PCS-130-011, lot number 62326179) was thawed and expanded in Lonza
862 Smooth Muscle Growth Media (SmGM; catalog number CC-3182) on T75 flasks (E&K
863 Scientific Products, catalog number 658175).

864 **H3K27ac ChIP-seq assay**

865 Upon reaching 80% confluency, BSMCs were serum-starved by replacing SmGM
866 with smooth muscle basal media (SmBM) for 24 hours. BSMCs were then grown in
867 SmBM containing 5% FBS for 4 hours, then fixed in 1% formaldehyde for 10 min
868 and quenched with 0.125 M glycine for 5 minutes. Cells were removed from the T75
869 flasks by scraping in cold PBS containing sodium butyrate (20 mM, Diagenode,
870 catalog number C12020010). Chromatin sheering was carried out using a Covaris S2
871 sonicator. Sheared chromatin was used for immunoprecipitation with antibodies
872 against active chromatin marks (H3K27ac; Abcam, ab4729) using the Diagenode
873 LowCell# ChIP kit (CAT#C01010072), following the manufacturer's protocol.
874 Libraries were prepared using the Rubicon DNA-Seq kit (CAT#R400406) following
875 the manufacturer's protocol and sequenced on an Illumina HiSeq 4000 using single-
876 end 50-bp reads to a sequencing depth of at least 25 million reads (submitted under
877 BioProject PRJNA369271). Uniquely mapping raw reads were aligned using Bowtie
878 [124] under default settings. Peak regions for each individual were called using

879 MACS2 [125, 126] and reproducible peaks identified using the ENCODE IDR pipeline
880 [127].

881 **Diverse Convergent Evidence approach for variant prioritization**

882 The Diverse Convergent Evidence (DiCE) approach is a logical, heuristic framework
883 for integrating multiple types of observational, bioinformatics, and laboratory
884 evidence to prioritize variants discovered from high throughput genetic studies for
885 further evaluation in functional experiments [53]. Results from the trans-ethnic
886 meta-analysis and from the replication analyses were considered observational
887 data. Laboratory evidence was provided by the identified peaks in our ChIP-seq
888 analyses performed in BSMCs. Informatic evidence was compiled using Ensembl,
889 PubMed, the NHGRI-EBI GWAS Catalog, and ENCODE to identify previously reported
890 associations with BDR or asthma-related phenotypes, and predicted biological
891 functions associated with assessed loci. After compiling the observational,
892 informatic and laboratory evidence for each suggestively or significantly associated
893 variant, DiCE constructs an evidence matrix to estimate the strength of the
894 information supporting each association. DiCE scores > 6 were considered strong
895 evidence that a given locus was involved in the pathophysiology of BDR, and
896 variants with the highest DiCE score, after meeting this criterion, were prioritized
897 for downstream functional analyses.

898 **Luciferase assays**

899 *NFKB1* candidate enhancer sequences were amplified from human genomic DNA
900 (Roche) using oligonucleotides designed in Primer3 with 18 and 20 bp overhangs
901 for forward and reverse primers, respectively (5′-GGCCTAACTGGCCGGTAC-3′ and
902 5′-CGCCGAGGCCAGATCTTGAT-3′), complementary to sequences flanking the KpnI
903 and EcoRV sites in the pGL4.23 Gate A vector (Promega, Madison, WI, USA) using
904 Phusion High-Fidelity PCR kit (NEB, catalog number M0531S). PCR primers were
905 designed around the edges of these ChIP-seq peaks and the most complete fragment
906 that was successfully amplified was used for luciferase assays. PCR products where
907 then cloned into the pGL4.23 vector using the Gibson Assembly method (NEB,
908 catalog number E2611S). Smooth muscle cells were plated at 50-70% confluency in
909 24-well cell culture plates (Falcon, catalog number 353047) and grown to 80%
910 confluency in SmGM. Transfections were carried out by combining
911 polyethyleneimine (PEI) with DNA vectors at a 1:1 ratio by weight in opti-MEM (Life
912 Technologies, catalog number 31985070). The transfection mixture consisted of
913 225 ng of enhancer assay vectors and 25 ng of pGL4.24 (Renilla transfection
914 efficiency control) with 250 ng of PEI in 50 μL of opti-MEM. After 15 minutes, 500
915 μL of SmBM was added to the transfection mixture and the combination added to
916 cell culture. Cells were incubated for 4 hours in SmBM plus the transfection mixture,
917 then media was replaced with SmGM for 24 hours. Cells were then washed with PBS
918 and enhancer assay cells were lysed with 100 μL of Passive Lysis Buffer (Promega,
919 Madison, WI, USA). Reporter activity was measured using the Dual-Luciferase
920 Reporter Assay System (Promega, Madison, WI, USA) and measured on the Glomax

921 96 well plate luminometer (Promega, Madison, WI, USA). The luciferase assay was
922 carried out in two separate experiments, with three independent replicates per
923 experiment (three wells of cells were transfected per construct per experiment).
924 Each well was then split into two technical replicates for luciferase activity
925 measurements with a luminometer.

926 **RNA extraction, library preparation and sequencing**

927 Among the African American subjects with WGS data in our study, 39 samples were
928 selected for RNA-seq based on BDR status and the number of copies of low-BDR
929 associated alleles at rs28450894. The number of samples in each category is shown
930 in **S14 Table**. Peripheral blood samples were collected into PAXgene Blood RNA
931 tubes (PreAnalytiX, Hombrechtikon, Switzerland). Total RNA was extracted from
932 PAXgene Blood RNA tubes using the MagMAX™ for Stabilized Blood Tubes RNA
933 Isolation Kit (CAT#4451894, Thermo Fisher Scientific, Waltham, MA, USA)
934 according to manufacturer's protocols. RNA integrity and yield were assessed using
935 an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA). Globin
936 depletion was performed using GLOBINclear™ kit (CAT#AM1980, Thermo Fisher
937 Scientific, Waltham, MA, USA). Library preparation and ribosomal depletion were
938 performed using KAPA Stranded RNA-seq Kit with RiboErase (CAT#KK8483, Kapa
939 Biosystems, Wilmington, MA, USA) according to the manufacturer's protocols. Each
940 sample was uniquely barcoded with NEXTflex™ DNA Barcodes (CAT#514104, Bioo
941 Scientific®, Austin, TX, USA). Barcoded libraries were pooled and sequenced on 4
942 lanes on a HiSeq 4000 sequencing system (Illumina®, San Diego, CA, USA) with a

943 paired-end read length of 100 bp at the University of California, San Francisco's
944 Center for Advanced Technology.

945 **RNA-seq data processing and analysis**

946 Raw sequencing reads were aligned to the human reference genome (hg19) using
947 STAR (v2.4.2a) [128]. Gene read counts were obtained from uniquely mapped reads
948 based on Ensembl annotation (v75) [129]. DESeq2 [130] was used to analyze read
949 counts for differential gene expression changes between genotypes, including an
950 interaction term with genotype and sex (genotype * sex). We used a linear model to
951 account for sex, age and library prep batch, and a custom model matrix to correct for
952 GC content difference between genes. After normalization for sequencing depth and
953 GC percentage, genes with fewer than an average of five normalized read counts per
954 sample and fewer than 20 samples with at least one read count were removed. This
955 filtering process kept 19,592 Ensembl genes for analysis. Fold change, raw and FDR-
956 adjusted *p*-value for the genotype term was reported. Genes were then further
957 filtered to analyze the locus surrounding rs28450894 for differential gene
958 expression by including all genes with a transcriptional start site within 1Mbp of
959 rs28450894. *P*-values were then corrected using the false discovery rate method to
960 account for the 13 genes in this locus. Significant level FDR-adjusted *p*-value is ≤ 0.1.

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1351 **FIGURE CAPTIONS**

1352 **Fig 1.** An overview of the main analyses performed in the current study. More
1353 detailed descriptions of the discovery and replication cohort demographics and
1354 analyses performed for common and rare variant analysis can be found in Methods.

1355

1356 **Fig 2.** (**a**) Number of variants per sample. The bin size is 0.025M variants. (**b**) Allele
1357 frequency of biallelic SNPs (relative to GRCh37). (**c**) Allele frequency of population-
1358 specific biallelic SNPs. (d) Novel biallelic SNPs based on dbSNP build150.

1359

1360 **Fig 3. (a)** Manhattan plot of the trans-ethnic meta-analysis of single locus BDR
1361 association testing. Top ten BDR-associated loci are circled. Black line indicates
1362 universal genome-wide significance threshold (5.00×10^{-8}), red line indicates
1363 adjusted genome-wide significance threshold (3.53×10^{-7}), and blue line indicates
1364 suggestive significance threshold (7.06×10^{-6}). **(b)** Forest plot of the population-
1365 specific and joint effect of the two most significantly associated SNPs, rs17834628
1366 and rs35661809. The R^2 between these two SNPs is 0.93 in Puerto Ricans, 0.96 in
1367 Mexicans and 0.66 in African Americans. **(c)** The most significantly associated SNP
1368 (rs17834628) is plotted together with 400kb flanking regions on either side. Color
1369 of the dots shows the LD of each SNP with rs17834628 based on the 1000 Genomes
1370 Nov 2014 AMR population. Multiple SNPs in high LD ($R^2 > 0.8$, red) reached a
1371 suggestive significance level.

1372

1373 **Fig 4.** Manhattan plot of SKAT-O analysis of biallelic common and rare SNPs
1374 grouped by 1kb windows sliding across chromosome 1 to 22 in **(a)** Puerto Ricans
1375 **(b)** African Americans, **(c)** Mexicans, and **(d)** all populations combined. Bonferroni-
1376 corrected genome-wide and suggestive significance levels are marked by red and
1377 blue lines, respectively.
1378

1379 **SUPPORTING INFORMATION CAPTIONS**

1380 **S1 Fig.** Distribution of bronchodilator drug response (BDR) in Puerto Ricans,
1381 Mexican (GALA II) and African Americans (SAGE). The 1,441 subjects selected from
1382 the extreme of the BDR distribution for this study were highlighted.

1383 **S2 Fig.** Global ancestry composition for **(a)** Puerto Ricans, **(b)** Mexicans and **(c)**
1384 African Americans. Each individual is represented by a vertical line and the ancestry
1385 composition is colored based on the percentage composition of African (red),
1386 European (blue) and Native American (green) ancestries.

1387 **S3 Fig.** Plot of the first two principal components of variation based on WGS
1388 genotypes of 1,441 individuals.

1389 **S4 Fig.** Diverse Convergent Evidence (DiCE) prioritization of 27 common variants
1390 (**Table 4**). Y-axis indicates the points allotted per SNP for each form of evidence
1391 according to **S15 Table**: statistical evidence (grey), informatic evidence (blue), and
1392 experimental evidence (orange). Evidence-specific scores for each SNP are provided
1393 in the table. SNPs with a DiCE score ≥ 4 are labeled with the nearest gene.

1394 **S5 Fig.** Regions overlapped with SNPs in LD with BDR-associated SNPs show
1395 enhancer activity in BSMCs. **(a)** H3K27ac ChIP-seq peaks in BSMCs overlap with
1396 SNPs in LD with rs28450894 (marked red). The GRCh37 coordinates are
1397 chr4:103486504-103491377 for region 1 and chr4:103527184-103531814 for
1398 region 2. In addition to BSMCs, H3K27ac data is shown for Roadmap Epigenomic

1399 peripheral blood mononuclear cells (PBMSs) and lung tissue, as well as ENCODE
1400 project data for GM12878, H1 human embryonic stem cell (H1-ESC) line, human
1401 skeletal muscle cells and myoblasts (HSMM) and normal human epidermal
1402 keratinocytes (NHEK). **(b)** Luciferase assay results for regions tested for enhancer
1403 activity in BSMCs. *NFKB1* region 2 significantly increased the expression of
1404 luciferase over empty vector control (Fold change = 2.24, $p < 0.01$). A red line marks
1405 a fold change of one compared to the empty vector.

1406 **S6 Fig.** A GGV plot showing the allele frequency of rs2845894 for different
1407 populations based on 1000 Genomes Project. European populations include CEU
1408 (Utah residents [CEPH] with northern and western ancestry), FIN (Finnish in
1409 Finland), GBR (British in England and Scotland), TSI (Toscani in Italia) and IBS
1410 (Iberian population in Spain). American populations include MXL (Mexican ancestry
1411 from Los Angeles USA), PUR (Puerto Ricans from Puerto Rico), CLM (Colombians
1412 from Medellin, Colombia) and PEL (Peruvians from Lima, Peru). African populations
1413 include ASW (Americans of African ancestry in SW USA), ACB (African Caribbeans in
1414 Barbados), GWD (Gambian in western divisions in the Gambia), MSL (Mende in
1415 Sierra Leone), YRI (Yoruba in Ibadan, Nigeria), ESN (Esan in Nigeria) and LWK
1416 (Luhya in Webuye, Kenya).

1417 **S7 Fig.** Boxplot showing increasing number of copies of low BDR-associated T allele
1418 of rs28450894 is associated with decreased expression of *SLC39A8* in blood
1419 regardless of sex ($p = 0.0066$, FDR adjusted $p = 0.0856$, $\log_2(\beta) = -0.327$).

1420 **S8 Figure.** Manhattan plot of the single locus BDR association testing for **(a)** Puerto
1421 Ricans, **(b)** Mexicans and **(c)** African Americans. The horizontal lines are colored
1422 blue for the suggestive significance thresholds and red for the Bonferroni-adjusted
1423 genome-wide significance thresholds. Since no associations were close to the
1424 Bonferroni-adjusted genome-wide significance thresholds in Mexicans and African
1425 Americans, the red line is only marked in Puerto Ricans.

1426 **S9 Fig.** A comparison of the top 1000 BDR associations between the array-based
1427 and WGS-based genotype data. **(a)** A plot of association *p*-values of array-based and
1428 WGS-based data. The *p*-values from the two data types showed high correlation,
1429 especially for SNPs with more significant *p*-values. Genotype discordance of SAGE II
1430 **(b)** and GALA II **(c)** WGS SNPs. The corresponding imputation R^2 of the same SNPs
1431 in the HRC imputed array data is indicated by red ($R^2 \geq 0.8$) or blue ($R^2 < 0.8$).

1432 **S10 Fig.** Percentage genotype concordance between Axiom LAT1 array and WGS
1433 genotypes. Grey horizontal lines mark one, two and three standard deviations (S.D.)
1434 from the mean percentage genotype concordance.

1435 **S11 Fig.** Quantile-quantile (q-q) plots of the single locus BDR association for **(a)**
1436 Puerto Ricans, **(b)** Mexicans and **(c)** African Americans and **(d)** trans-ethnic meta-
1437 analysis. The genomic inflator factors (λ_{GC}) are shown on the q-q plots.

1438 **S12 Fig.** Quantile-quantile (q-q) plots of SKAT-O analysis of biallelic common and
1439 rare SNPs grouped by 1kb windows for **(a)** Puerto Ricans, **(b)** Mexicans and **(c)**

1440 African Americans and **(d)** all individuals in all three populations. The genomic
1441 inflator factors (λ_{GC}) are shown on the q-q plots.

1442 **S1 Table.** Novel common variants in discovery cohort by population.

1443 **S2 Table.** Chromosomal location of BDR-associated common variants.

1444 **S3 Table.** Common variant BDR association results by population.

1445 **S4 Table.** Lung-related phenotypes previously reported for BDR-associated
1446 common variants and their nearest genes.

1447 **S5 Table.** Variation in BDR Status explained by significant and suggestively
1448 associated variants identified by trans-ethnic meta-analysis and SKAT-O.

1449 **S6 Table.** Descriptive statistics for replication cohorts.

1450 **S7 Table.** Replication of common variant associations.

1451 **S8 Table.** Size of DNA inserts used in luciferase assay and key variants included
1452 within those sequences.

1453 **S9 Table.** Functional annotations for BDR-associated common variants in Table 4.

1454 **S10 Table.** Replication of previously reported BDR-associated SNPs in the current
1455 study by population and trans-ethnic meta-analysis.

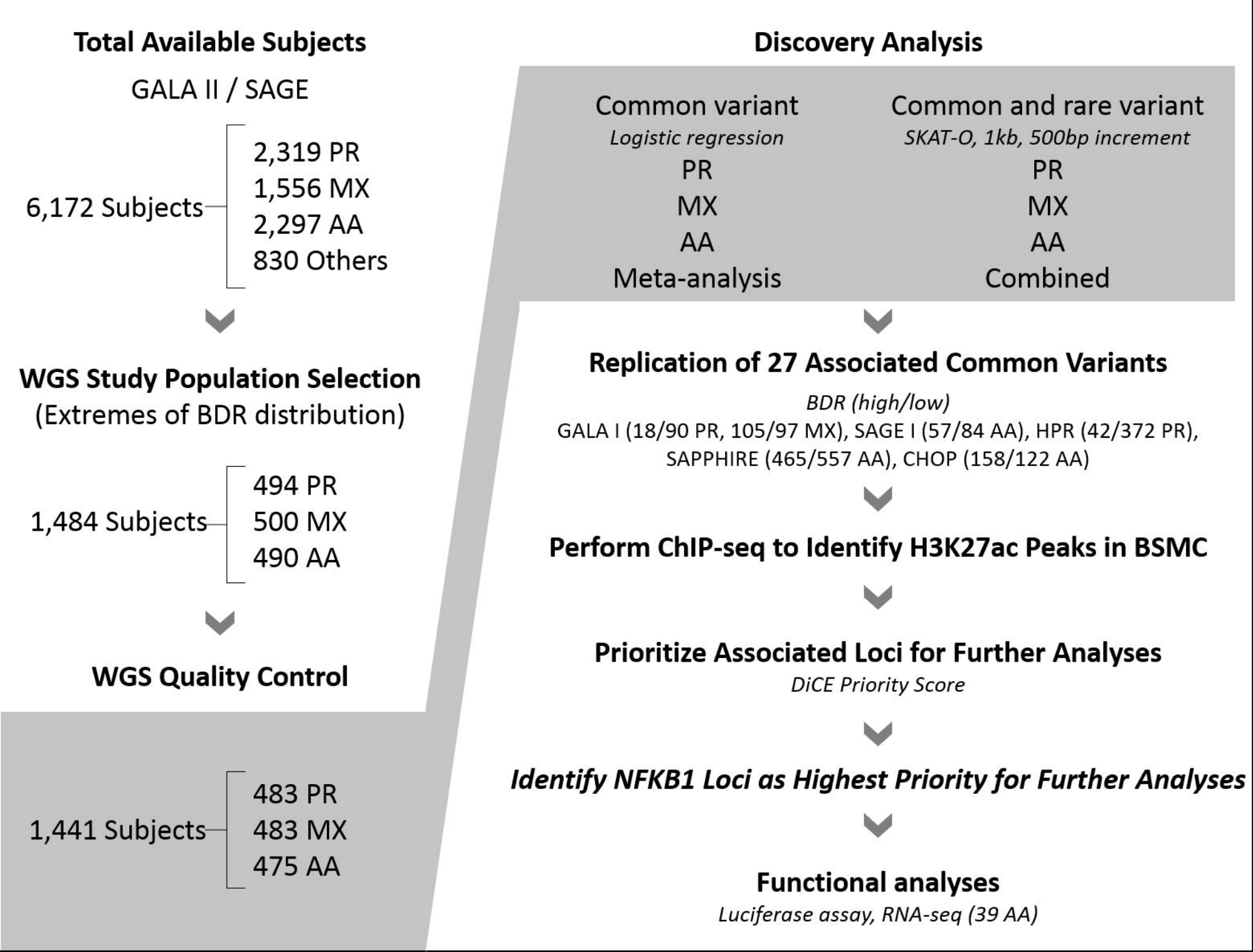
1456 **S11 Table.** Chromosomal location of identified SKAT-O regions by genome build.

1457 **S12 Table.** Functional annotations for variants within SKAT-O regions.

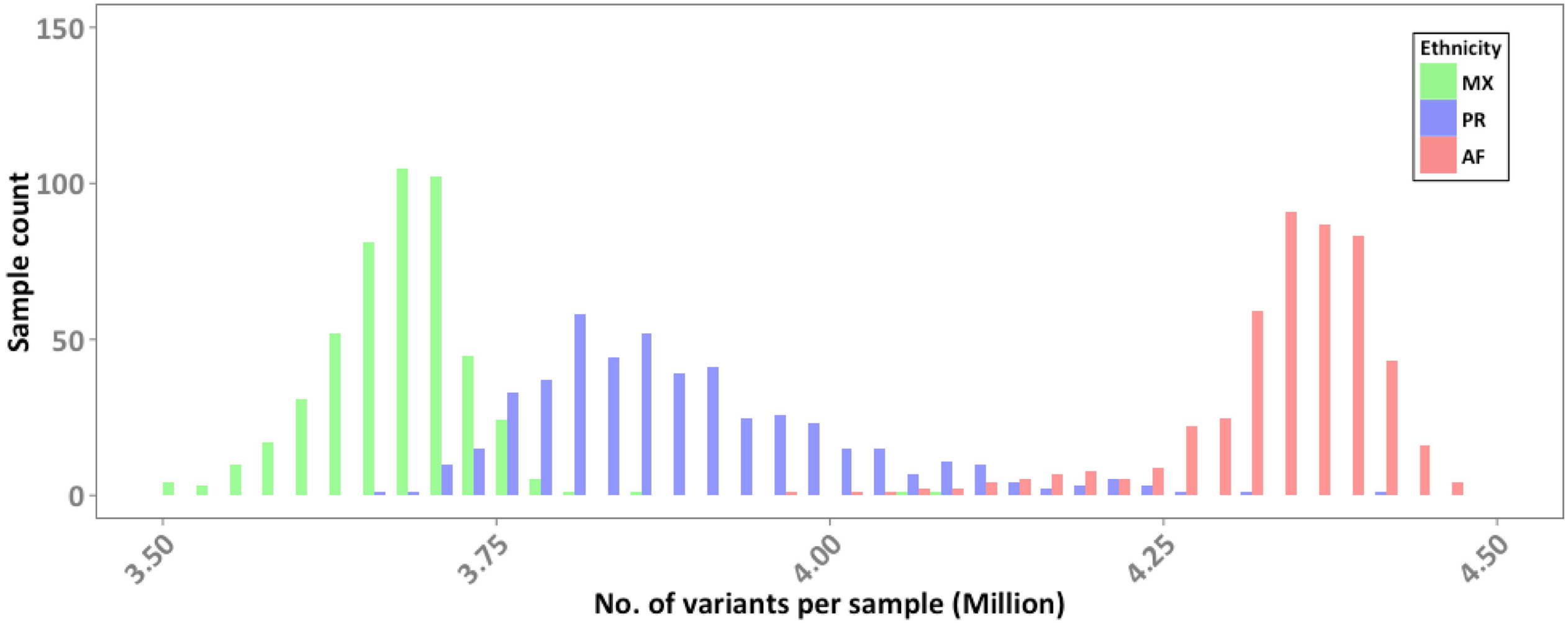
1458 **S13 Table.** Correlation between baseline lung function, top ten principle
1459 components, other covariates included in association analyses in the discovery
1460 study population (N =1,441).

1461 **S14 Table.** Number of African American samples selected for RNA-Seq based on
1462 BDR status and number of copies of low BDR-associated allele.

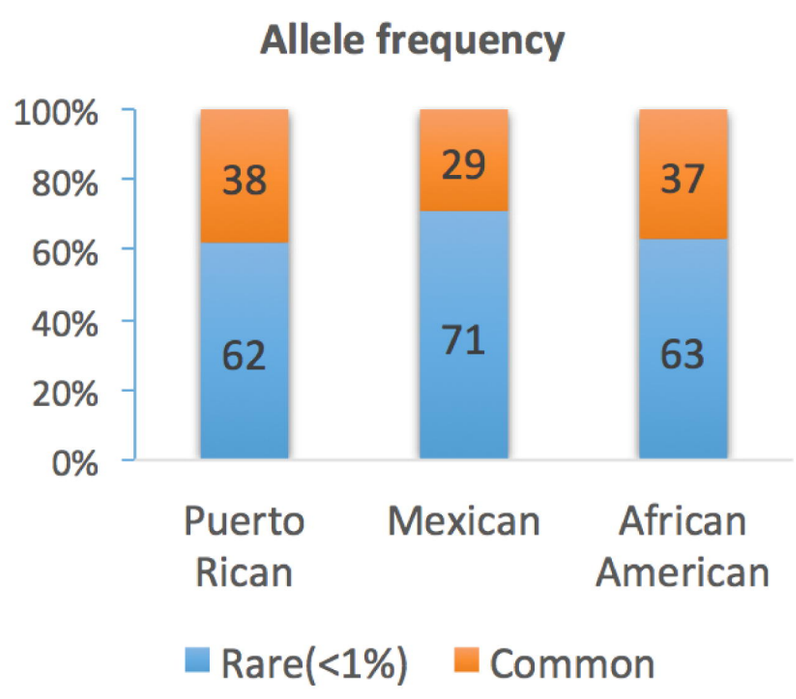
1463 **S15 Table.** Diverse Convergent Evidence (DiCE) approach scoring rubric.


Total Available Subjects
GALA II / SAGE
6,172 Subjects
2,319 PR
1,556 MX
2,297 AA
830 Others
WGS Study Population Selection
(Extremes of BDR distribution)
1,484 Subjects
494 PR
500 MX
490 AA
WGS Quality Control
1,441 Subjects
483 PR
483 MX
475 AA
Discovery Analysis
Common variant
Logistic regression
PR
MX
AA
Meta-analysis
Common and rare variant
SKAT-O, 1kb, 500bp increment
PR
MX
AA
Combined
Replication of 27 Associated Common Variants
BDR (high/low)
GALA I (18/90 PR, 105/97 MX), SAGE I (57/84 AA), HPR (42/372 PR),
SAPPHIRE (465/557 AA), CHOP (158/122 AA)
Perform ChIP-seq to Identify H3K27ac Peaks in BSMC
Prioritize Associated Loci for Further Analyses
DiCE Priority Score
Identify NFKB1 Loci as Highest Priority for Further Analyses
Functional analyses
Luciferase assay, RNA-seq (39 AA)

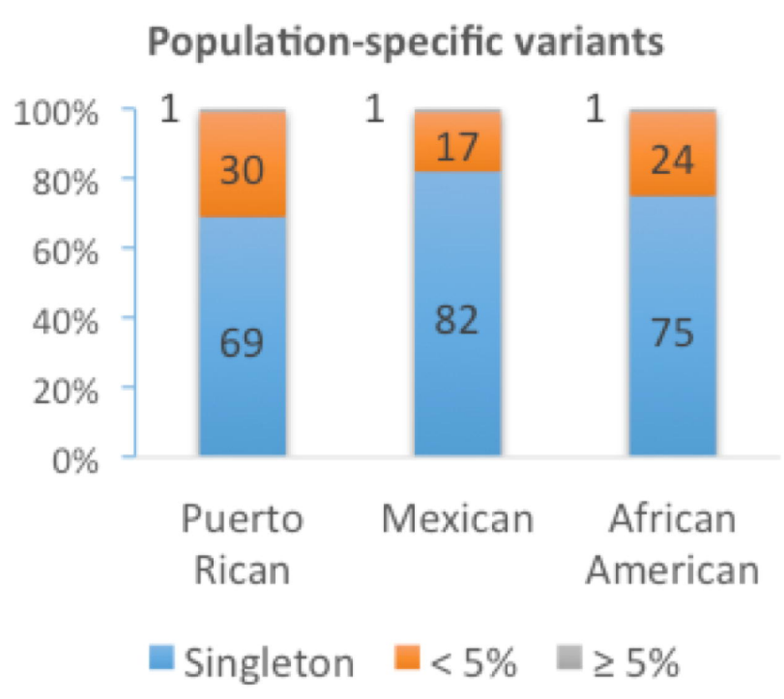
a


Ethnicity
MX
PR
AF
Sample count
150
100
50
0
3.50
3.75
4.00
4.25
4.50
No. of variants per sample (Million)

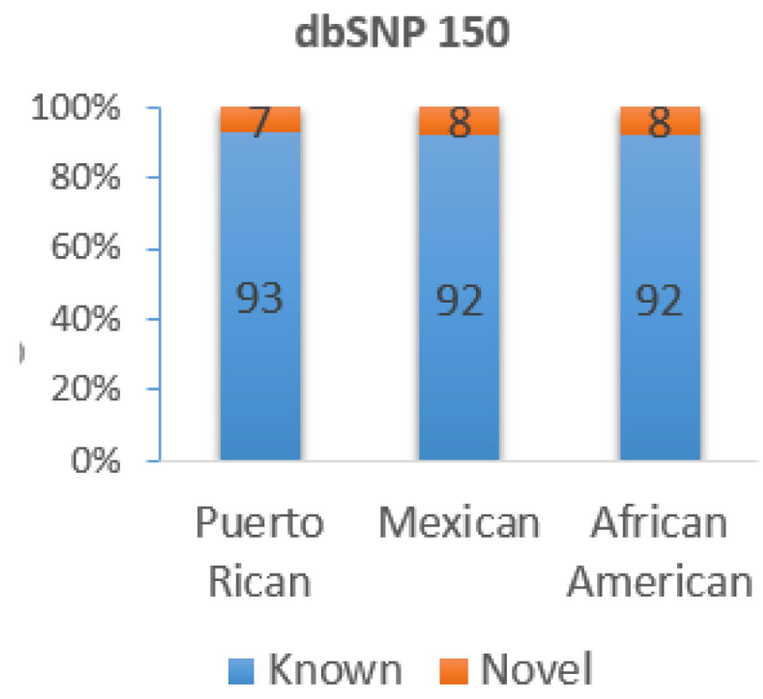
b


Allele frequency
100%
80%
60%
40%
20%
0%
38
29
37
62
71
63
Puerto Rican
Mexican
African American
Rare(<1%)
Common

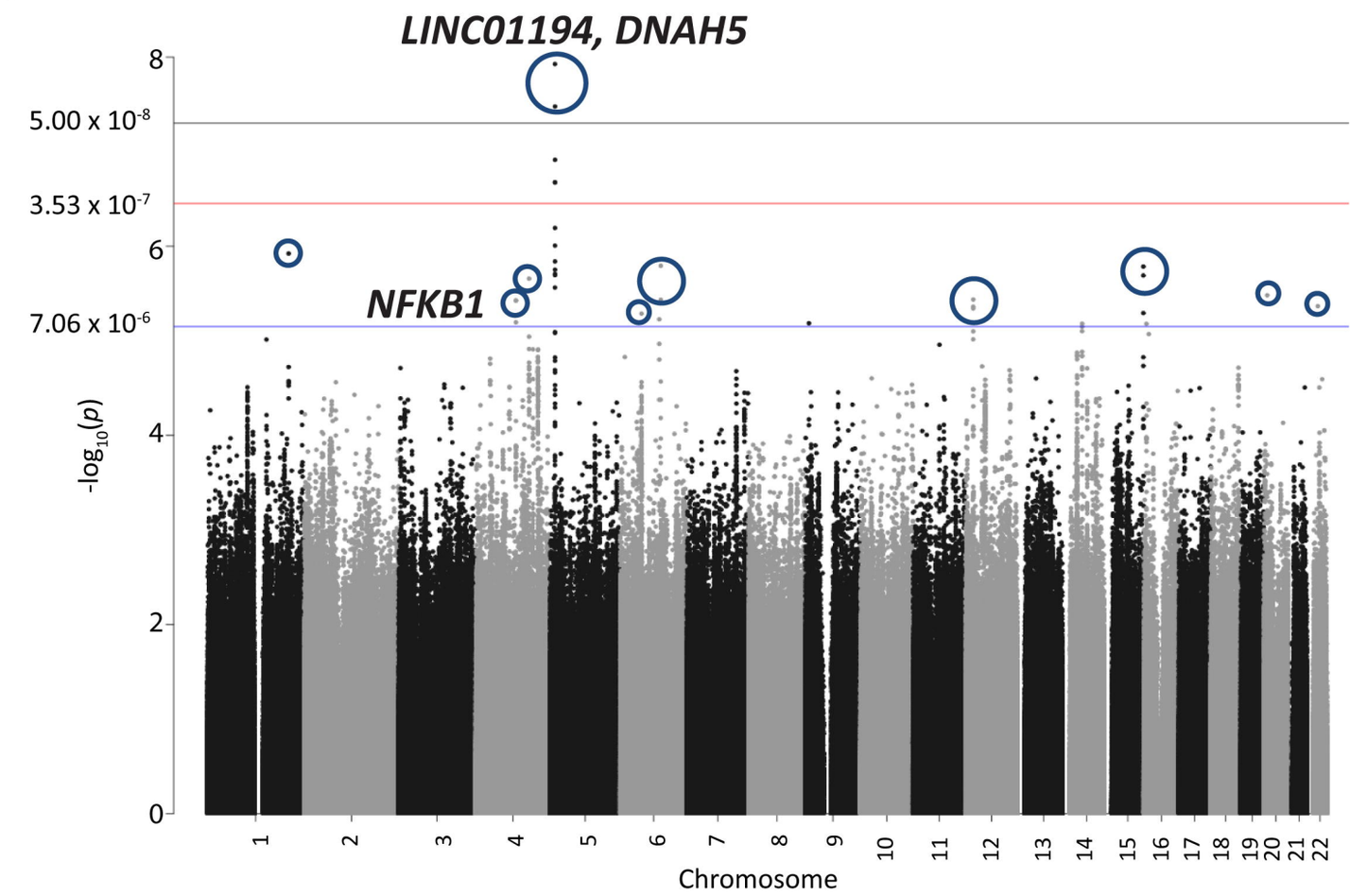
c


Population-specific variants
100%
80%
60%
40%
20%
0%
1
1
1
30
17
24
69
82
75
Puerto Rican
Mexican
African American
Singleton
< 5%
≥ 5%

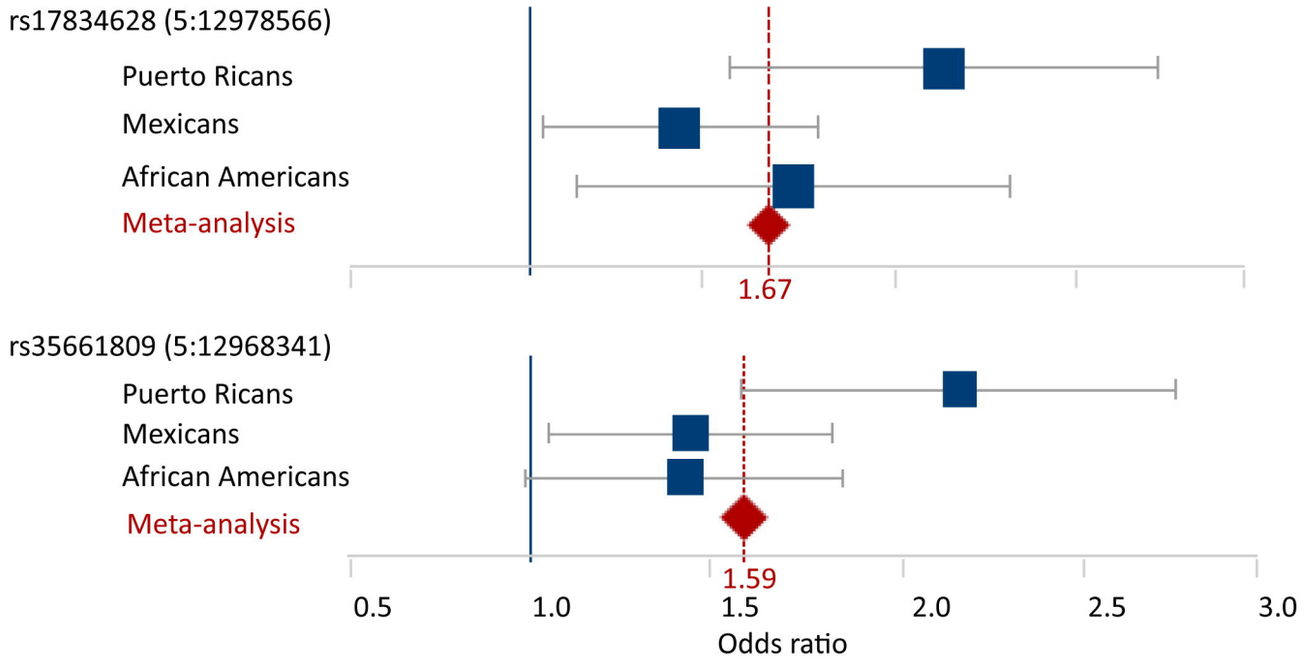
d


dbSNP 150
100%
80%
60%
40%
20%
0%
7
8
8
93
92
92
Puerto Rican
Mexican
African American
Known
Novel

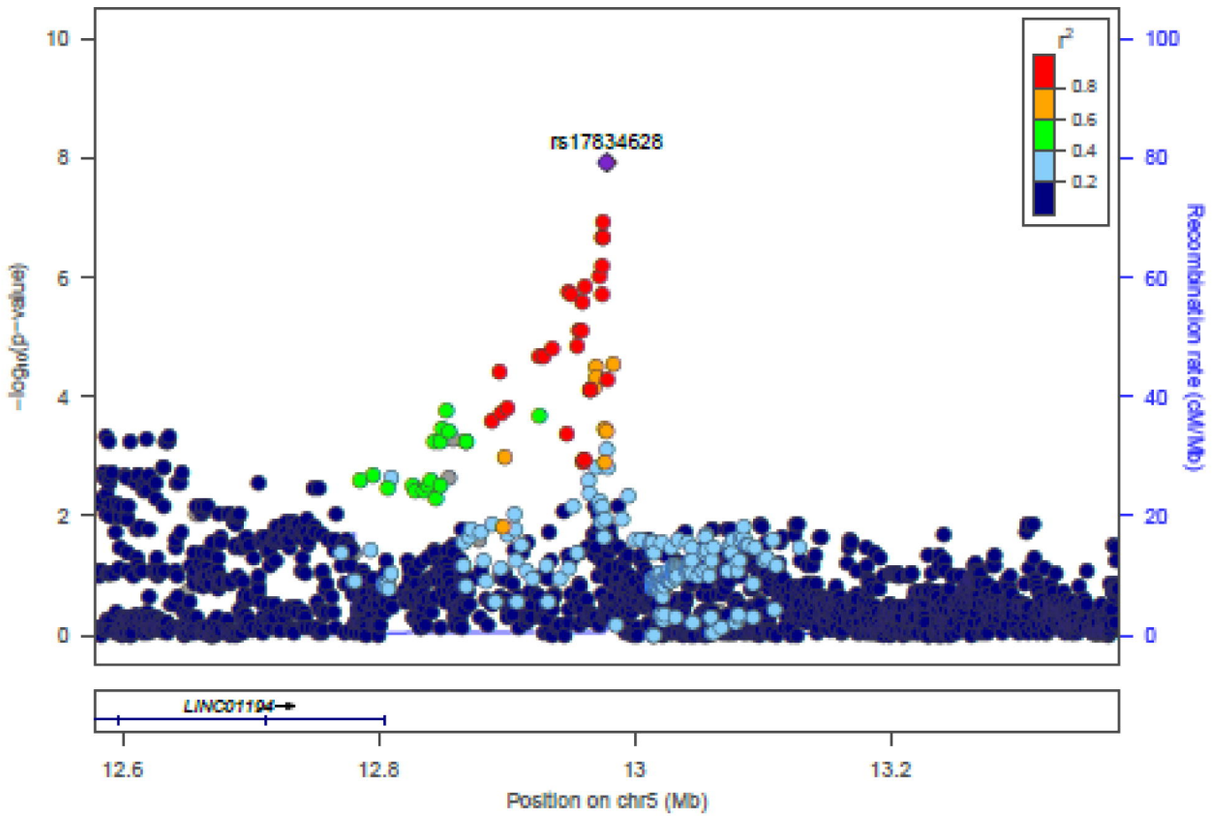
a


LINC01194, DNAH5
NFKB1
5.00 x 10-8
3.53 x 10-7
7.06 x 10-6
-log10(p)
Chromosome

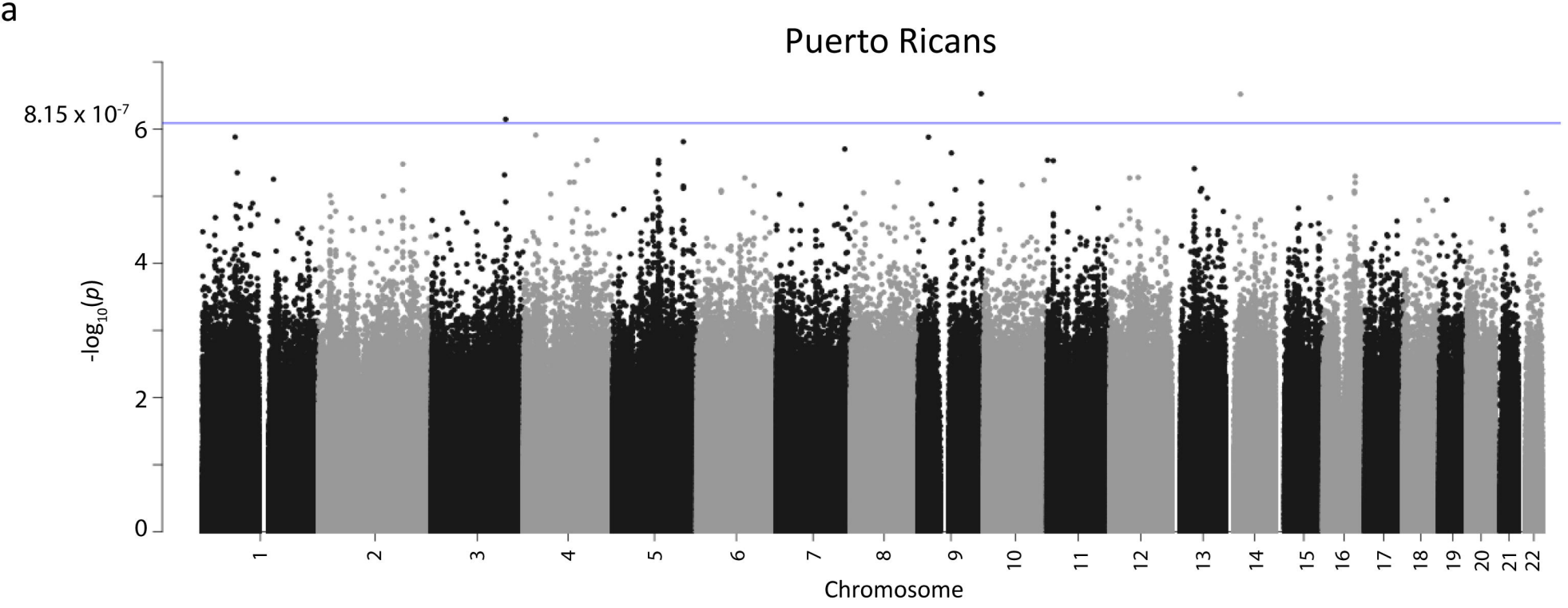
b


rs17834628 (5:12978566)
Puerto Ricans
Mexicans
African Americans
Meta-analysis
1.67
rs35661809 (5:12968341)
Puerto Ricans
Mexicans
African Americans
Meta-analysis
1.59
0.5
1.0
1.5
2.0
2.5
3.0
Odds ratio

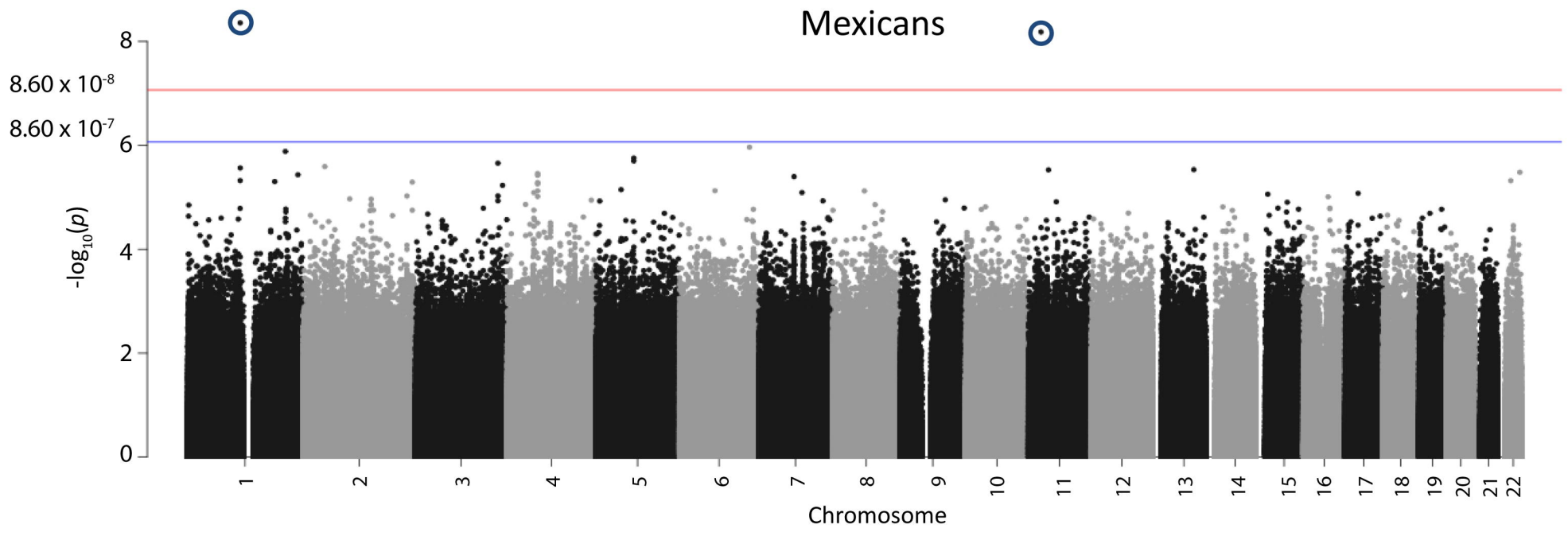
c


rs17834628
r2
0.8
0.6
0.4
0.2
-log10(p-value)
Recombination rate (cM/Mb)
LINC01194
12.6
12.8
13
13.2
Position on chr5 (Mb)

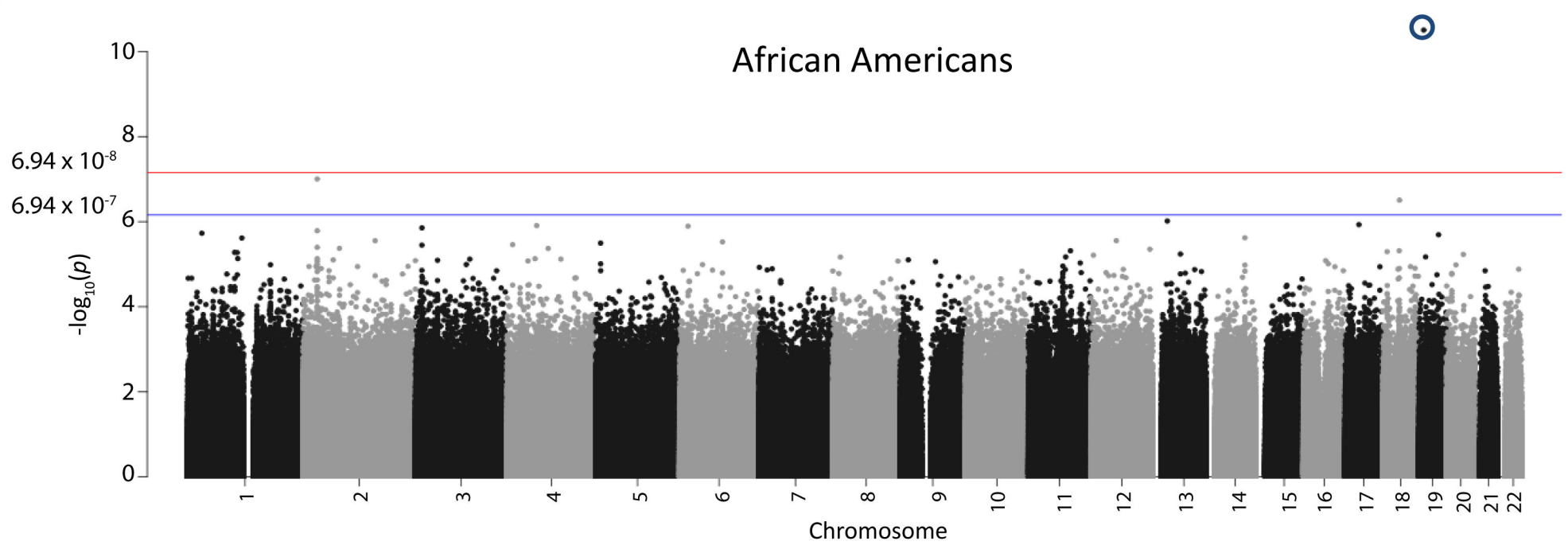
a


Puerto Ricans
8.15 x 10-7
-log10(p)
Chromosome

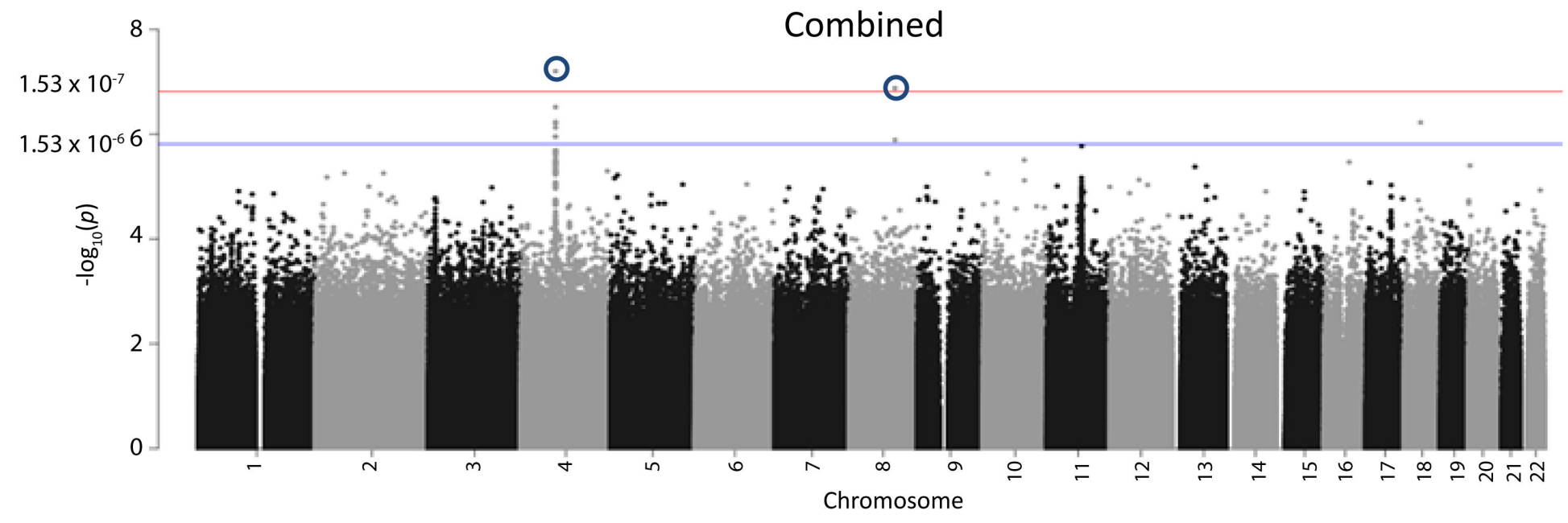
b


Mexicans
8.60 x 10-8
8.60 x 10-7
-log10(p)
Chromosome

c


African Americans
6.94 x 10-8
6.94 x 10-7
-log10(p)
Chromosome

d


Combined
1.53 x 10-7
1.53 x 10-6
-log10(p)
Chromosome