

Abbreviations used

β_2 AR:	β_2 -Adrenergic receptor
BDR:	Bronchodilator response
GALA I:	Genetics of Asthma in Latino Americans
GALA II:	Genes-Environments & Admixture in Latino Americans
GWAS:	Genome-wide association study
IGF:	Insulin-like growth factor
LD:	Linkage disequilibrium
MAF:	Minor allele frequency
QC:	Quality control
SABA:	Short-acting β_2 -adrenergic receptor agonist
SLC:	Solute carrier
SNP:	Single nucleotide polymorphism

(β_2 AR). Response to SABAs is often measured based on the bronchodilator response (BDR), which is defined as the percentage change in FEV₁ after administration of a SABA. There is wide interindividual variability in the BDR, and not every patient responds to treatment.^{2,3} This variability can be caused by several factors, including asthma severity, drug-drug interactions, environmental exposures, age, and genetic factors.²

Five candidate genes⁴⁻¹⁰ and 1 gene from a genome-wide association study (GWAS)¹¹ have been reported as having variations associated with BDR. However, altogether, these results provide limited information about the genetic contributions to variation in BDR. The most commonly studied candidate gene is adrenoceptor β_2 , surface (*ADRB2*), which encodes the direct target of SABAs (β_2 AR). However, evidence for the association of variation in *ADRB2* and BDR is inconsistent, which might be in part due to variation in the single nucleotide polymorphisms (SNPs) studied, varying clinical end points/study conditions, and differences in the assumed genetic model.¹² Four other candidate genes have been studied to a lesser degree, including corticotropin releasing hormone receptor 2 (*CRHR2*), adenylate cyclase 9 (*ADCY9*), arginase 1 (*ARG1*), and thyroid hormone receptor, beta (*THRB*).⁷⁻¹⁰ Lastly, a recent GWAS of BDR in patients of European ancestry identified spermatogenesis associated, serine-rich 2-like (*SPATS2L*) as a novel candidate gene for regulating β_2 AR levels.¹¹ However, there are likely additional genes that contribute to differences in BDR that remain to be identified, notably in non-European populations.

BDR has been shown to vary among 4 ethnic groups in the United States. Despite having more severe asthma, Puerto Ricans have lower BDR compared with African Americans or Mexicans,^{13,14} and African Americans have lower BDR compared with patients of European ancestry.¹⁵ Although these differences might be due to environmental factors, they could also be due to varying frequencies of genetic variants affecting BDR between populations. Specifically, these differences might reside in the ancestral populations of admixed subjects, including Latinos with varying degrees of African, European, and Native American ancestry.^{5,13,14} Therefore admixture mapping, which tests for a correlation between BDR and local genetic ancestry (rather than genotype at an individual SNP), is a complementary strategy to a traditional GWAS that might identify novel genetic loci associated with BDR.

In the current study we performed a genome-wide association and admixture mapping study for BDR in 1782 Latinos with asthma from across the United States and Puerto Rico and attempted to replicate our findings in an additional 266 Mexicans and 265 Puerto Ricans with asthma.

METHODS**Study subjects**

Genes-Environments & Admixture in Latino Americans study. Patients with asthma in the discovery phase were recruited from the Genes-Environments & Admixture in Latino Americans (GALA II) study, which began in 2006 and is an ongoing clinical study of children between the ages of 8 and 21 years both with and without asthma. A total of 4045 subjects, including 1976 asthmatic patients, were recruited from urban study centers across the mainland United States and Puerto Rico through June 2011 (see Table E1 in this article's Online Repository at www.jacionline.org). All participants who met the criteria for enrollment (see Table E2 in this article's Online Repository at www.jacionline.org) completed in-person questionnaires related to their medical, asthma, allergic, social, environmental, and demographic histories. In addition, all participants provided blood for genetic analysis and underwent spirometry. Each participant or parent was also required to identify all 4 grandparents as Latino. Local institutional review boards approved the studies, and all subjects and legal guardians provided written informed assent/consent.

BDR was calculated as the percentage change in FEV₁ after 2 doses of albuterol (post-FEV₁) compared with baseline values before administration of albuterol (pre-FEV₁). Specifically, FEV₁ was measured before administering albuterol and again after 2 doses of albuterol, with a 15-minute waiting period after each dose. A total of 6 (if <16 years of age) to 8 (if ≥ 16 years of age) puffs of albuterol were administered. A total of 408 patients from the Centro de Neumología Pediátrica in Puerto Rico were recruited based on having a BDR of at least 8%; of these, 121 patients were recruited based on having a BDR of at least 12%.

Self-reported ethnicity was divided into categories of Puerto Rican, Mexican, mixed Latino, and other Latino. Puerto Rican and Mexican ethnicities were available as selections on the questionnaire, whereas mixed Latinos were defined as any subject who identified with more than 1 Latino ethnic group. Other Latinos were defined as those who chose only 1 Latino ethnic group from among Spanish/Hispanic/Latino, Cuban, Dominican, El Salvadorian, Guatemalan, Nicaraguan, Honduran, Colombian, Brazilian, and Argentinian. We performed our analyses in all of the GALA II participants and separately in the subsets of self-reported Puerto Ricans and Mexicans.

Genetics of Asthma in Latino Americans study.

Patients with asthma in the replication phase were participants in the Genetics of Asthma in Latino Americans (GALA I) study.¹³ Briefly, GALA I is a study of subjects with asthma (probands) age 8 to 40 years and their biological parents recruited from schools, clinics, and hospitals across 4 sites: the San Francisco Bay Area, New York City, Puerto Rico, and Mexico City. All probands who met the criteria for enrollment (see Table E2) completed in-person questionnaires related to their medical, asthma, allergic, and demographic histories. One of these criteria was to have self-reported ethnicity of either Mexican or Puerto Rican in all 4 of the proband's biological grandparents. All participants with asthma provided blood for genetic analysis and underwent spirometry before and after administration of albuterol. The Institutional Review Board at the University of California San Francisco and all recruitment sites approved the study. BDR was measured in a similar fashion as in GALA II but with a lower dosage of albuterol. Specifically, post-FEV₁ values were measured after only a single dose of albuterol (compared with 2 doses in GALA II). Two (if <16 years of age) to 4 (if ≥ 16 years of age) total puffs of albuterol were administered (compared with 4 [if <16 years of age] and 6 [if ≥ 16 years of age] in GALA II).

Genotyping and quality control

Genotyping of GALA II subjects was performed with the Axiom LAT1 array (World Array 4; Affymetrix, Santa Clara, Calif), which contained 817,810 SNPs before quality control (QC). This array was specifically designed for Latino populations and was shown to capture 80% of all variants with frequencies of greater than 1% with an r^2 value of 0.8 in the MXL population (Mexicans in Los Angeles, Calif) and with an r^2 value of 0.7 in the PUR population (Puerto Ricans in Puerto Rico) from the 1000 Genomes interim June 2011 release.¹⁶ We removed SNPs with less than 95% call rates and failed platform-specific SNP quality criteria ($n = 63,328$), those that

deviated from Hardy-Weinberg equilibrium within their respective populations (Puerto Rican, Mexican, and other Latino; $P < 10^{-6}$, $n = 1,845$), and those not present in the Phase II HapMap (to create a consensus dataset to estimate local genetic ancestry, $n = 179,092$). The total number of SNPs included in our analysis was 568,037. Subjects were filtered based on 97% call rates, discrepancy between genetic sex and reported sex, cryptic relatedness (identity by descent > 0.3), and standard Affymetrix Axiom metrics. We also removed 136 subjects with missing BDR data and 3 subjects who were outliers for BDR (BDR of > 80 or < -50). After QC, 1,782 asthmatic subjects were included in this study.

Genotyping and estimates of genetic ancestry for GALA I have been described previously.¹⁷ Briefly, GALA I subjects were genotyped with the Affymetrix 6.0 GeneChip, and after QC filtering on markers and subjects, genotypes were available for 729,685 markers in 529 children with asthma (253 Mexican and 276 Puerto Rican subjects).

Genetic ancestry estimation

We used genotypes from 3 populations to represent the ancestral haplotypes of Latinos for estimating local genetic ancestry: HapMap European (CEU), HapMap African (YRI), and 95 Native Americans. First, we combined the CEU and YRI genotypes across Phase II, Illumina Omni, and Affymetrix Axiom platforms for maximum coverage. In addition, we genotyped 95 Native American samples on the Axiom LAT1 array. Global ancestry was estimated by using ADMIXTURE¹⁸ unsupervised and assuming 3 ancestral populations (see Fig E1 in this article's Online Repository at www.jacionline.org). Local ancestry was estimated by using Local Ancestry in admixed Populations using LD (LAMP-LD) under a 3-population model, assuming 20 generations of admixture^{19,20} after phasing the ancestral haplotypes with BEAGLE.²¹

Replication

We attempted replication of significantly associated SNPs using *in silico* genotype imputation, followed by direct genotyping of 2 SNPs with a TaqMan SNP genotyping assay (Applied Biosystems, Carlsbad, Calif; rs1281748 and rs1281743). For *in silico* replication, we first phased the data using the program SHAPE-IT,²² accounting for relationships within trios. We then imputed 100-kb regions around each SNP with a 20-kb buffer on each side using the program IMPUTE2¹⁶ separately in the GALA I Mexicans and Puerto Ricans. Reference haplotypes for imputation were from the Phase I version 3 of the 1000 Genomes Project.²³ Finally, we removed SNPs that had an information score of less than 0.5 and used the gene dosage output for all analyses to account for uncertainty in imputation.

Genotype association testing

All statistical analyses were conducted with R software (version 2.14.1).²⁴ For each SNP, we used linear regression to test whether the number of minor alleles was associated with BDR after adjusting for ethnicity and local and global African and Native American ancestry. In addition, we performed the same analysis without adjusting for ethnicity separately in self-identified Puerto Ricans and Mexicans. Confidence bands on quantile-quantile plots were determined by using a $\beta(j, n-j+1)$ distribution for the j th order statistic, with n SNPs being tested.²⁵

For each genome-wide significant SNP ($P < 5 \times 10^{-8}$), we performed *in silico* fine mapping within 200 kb upstream and downstream using IMPUTE2¹⁶ and reference haplotypes from all available populations in the Phase I version 3 of the 1000 Genomes Project.²³ We also examined the dose-response relationship between the number of minor alleles and BDR, both before and after removing subjects with a BDR of greater than 60 who were at the upper end of the BDR distribution (but were not considered statistical outliers, which were removed during QC). SNPs that were genome-wide significant and met 3 criteria were validated by using Sanger sequencing and carried forward for replication in GALA I: (1) more than 5 copies of the minor allele, (2) a consistent dose-response relationship after removing subjects with BDRs of greater than 60, and (3) high QC measures and passed visual inspection of cluster plots. We also attempted replication

at an additional imputed SNP in GALA II identified as genome-wide significant through *in silico* fine mapping. Replication was performed with linear regression to test for an association between minor allele dosage and BDR separately in Mexicans and Puerto Ricans, adjusting for local and global African and Native American ancestry.

Admixture mapping

We also tested for an association between local ancestry (African, Native American, and European) and BDR in Mexicans and Puerto Ricans using linear regression and adjusting for global ancestry. Traditional thresholds for genome-wide significance using allelic associations are too stringent for ancestry associations because of increased ancestry linkage disequilibrium (LD) relative to SNP LD. Therefore we ran 100 random permutations of BDR maintaining the haplotype structure and the link between BDR and ethnicity to establish a threshold for a statistical significance of a P value of less than 1.6×10^{-4} for African ancestry and a P value of less than 1.0×10^{-4} for European and Native American ancestry. We attempted to replicate significant admixture mapping peaks in GALA I by testing for an association of BDR and local ancestry in the same manner as described above for GALA II.

Quantification of gene expression

The expression of *SLC22A15* was quantified in 12 human tissues by using real-time RT-PCR and visualized in normal human lung tissue by using immunohistochemistry available from the Human Protein Atlas (<http://www.proteinatlas.org/ENSG00000163393/normal/bronchus>). Total RNA of 13 human tissues (heart, adipose tissue, lung, placenta, total brain, small intestine, bladder, skeletal muscle, esophagus, peripheral blood monocyte, kidney, and liver) was purchased from Clontech (Mountain View, Calif). Each of these tissues was pooled from different subjects, as specified in the product insert from Clontech. Two micrograms of RNA was reverse transcribed to cDNA by using a High Capacity cDNA Reverse Transcription kit (Applied Biosystems) with the following thermal cycler conditions: 10 minutes at 22°C, 2 hours at 37°C, 5 minutes at 4°C, 10 minutes at 75°C, and 5 minutes at 4°C. Exonuclease I enzyme (10 U/mL) was added to each sample, and the following incubation conditions were used to remove excess primers: 1 hour at 37°C, 5 minutes at 4°C, and 10 minutes at 85°C to inactivate the exonuclease enzyme. Samples were then stored at -20°C . Real-time PCR was performed with Biotrove OpenArray technology (Life Technologies, Carlsbad, Calif). The primers that were used to detect *SLC22A15* transcripts were AACTCGCTTCTGCTGGGCGATGA (forward strand) and AGGCCGCAATCGATCCTGCAA (reverse strand). The housekeeping genes used in this experiment were human β -actin (*ACTB*), glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*), and β_2 -microglobulin (*B2M*); primers for these genes were predesigned by using BioTrove OpenArray technology. The mRNA expression level was normalized to the geometric mean of the housekeeping genes, and then the fold difference of *SLC22A15* transcription was determined by using the $\Delta\Delta$ cycle threshold method,²⁶ as described previously.²⁷ The fold expression in human liver tissue was normalized to 1 and used as a reference for relative quantification.

RESULTS

Allelic association tests

After QC, we tested for an association of BDR at a total of 568,037 SNPs in 1,782 asthmatic children in all Latino subjects regardless of self-reported ethnicity and also separately in subsets of 823 Puerto Ricans and 572 Mexicans. In all 3 analyses we observed an excess of small P values compared with those expected by chance (Fig 1 and see Fig E2 in this article's Online Repository at www.jacionline.org). However, this excess disappeared after removing SNPs with a minor allele frequency (MAF) of less than 5%, suggesting the excess of small P values is being driven by rare variation. We also observed an excess of small P values in variants in the proximity of solute carrier (SLC) genes

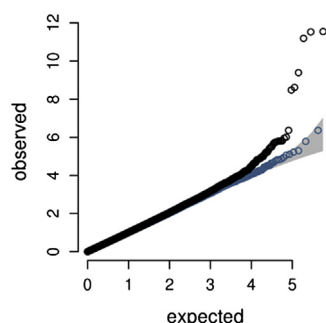


FIG 1. Quantile-quantile plot for genome-wide allelic associations with BDR. The expected distribution of P values is based on a uniform distribution. Black circles indicate all SNPs (inflation factor, $\lambda = 1.005$), whereas blue circles include only common SNPs with an MAF of 5% or greater ($\lambda = 1.004$). The shaded region represents the 95% concentration band. Quantile-quantile plots for the subset of Puerto Ricans and Mexicans are shown in Fig E2.

(see Fig E3 in this article's Online Repository at www.jacionline.org), which include membrane transport proteins involved in the transport of endogenous metabolites and xenobiotics.

Six SNPs were genome-wide significant in all subjects combined ($P < 5 \times 10^{-8}$; Fig 2 and see Fig E4 in this article's Online Repository at www.jacionline.org). Of these SNPs, 2 had minor alleles that were present in at least 5 subjects (rs8191725 and rs77441273, Table I), and both had a dose-response relationship, suggesting the minor allele confers an increase in BDR (Table I). This relationship remained consistent after removing subjects with BDRs of greater than 60, indicating the dose-response relationship is not being driven entirely by subjects with more extreme BDR. In the Puerto Rican and Mexican subsets 7 and 6 rare SNPs were genome-wide significant, respectively (see Fig E4). Three of these SNPs in Puerto Ricans and 2 in Mexicans had minor alleles present in more than 3 subjects (Table I). Only 1 of these variants was present in more than 5 subjects and taken forward for replication (rs77977790 in the Puerto Rican subset, Table I). Three of the 7 SNPs that were genome-wide significant in the Puerto Rican population were also significant in all of the GALA II participants; however, in all cases the association was driven by a single heterozygote with extreme BDR (BDR >60). *In silico* fine mapping identified 1 additional rare variant to be more strongly associated with BDR than a directly genotyped SNP (see Figs E5-E11 in this article's Online Repository at www.jacionline.org). Up to 7 carriers of the minor allele (when present) were validated for 6 of the variants listed in Table I by using Sanger sequencing. All genotypes were successfully validated with the exception of rs74973995 in a single subject who appeared homozygous. rs8191725 failed PCR design.

We found no significant associations at SNPs previously reported as being associated with BDR. However, 2 rare variants in the proximity of *ADCY9* and *CRHR2* were significantly associated with BDR in our study after adjusting for multiple testing based on the number of SNPs within 50 kb of each gene (Table II). No individual SNP was associated with BDR within 50 kb upstream or downstream of *SPATS2L*, a gene identified through a GWAS in patients of European ancestry.¹¹

We attempted to replicate 3 SNPs in GALA I that had been detected in more than 5 subjects in GALA II, including 2 SNPs identified in all of GALA II (rs8191725 and rs77441273) and 1 SNP identified in the Puerto Rican subset (rs77977790). We also

attempted replication of an additional rare variant in the proximity of rs77977790 (rs143010317) that was genome-wide significantly associated with BDR using *in silico* fine mapping in Puerto Ricans in GALA II ($\beta = -58$, $P = 3.2 \times 10^{-10}$, Fig E8). *In silico* replication through imputation in GALA I resulted in only 3 polymorphic SNPs with information scores of greater than 0.5. None were significantly associated with BDR ($P > .05$, see Table E3 in this article's Online Repository at www.jacionline.org); however, 2 of the SNPs showed an effect size in the same direction in Puerto Ricans (rs77441273: $\beta = 24.3$, $P = .38$; rs143010317: $\beta = -12.6$, $P = .24$). Attempting replication by means of direct genotyping of 3 SNPs in GALA I, we were unable to cluster any heterozygous genotypes for the minor alleles by using custom TaqMan SNP Genotyping Assays.

Admixture mapping

At each of the 568,037 loci, we tested for an association of BDR with local genetic ancestry in 1,782 asthmatic children in the subsets of 823 Puerto Rican and 572 Mexican children (Table III). In total, we identified 5 admixture mapping peaks that were significantly associated with BDR (permutation $P < .05$, Fig 3, Table II, and see Fig E12 in this article's Online Repository at www.jacionline.org). None of the admixture mapping peaks replicated in either Mexicans or Puerto Ricans in GALA I ($P > .05$).

To perform fine mapping, we tested for allelic associations with BDR for SNPs within each peak. Because we restricted our analyses to candidate regions, we applied a Bonferroni correction for the number of SNPs within each peak. Two rare SNPs in the intron and 3' untranslated region of *SLC22A15* were significantly associated with increased BDR in Mexicans under the peak at 1p13 (rs1281748 and rs1281743, $P = 8.8 \times 10^{-5}$ and MAF of 0.26% for both; Fig 4). Carrying 1 copy of the minor allele at these SNPs was associated with an increase of 18.8% in BDR. These allelic associations were specific to Mexicans in GALA II, despite the admixture mapping peak having been identified in Puerto Ricans. The SNPs were not significantly associated with BDR in the Puerto Rican subset of GALA II ($P = .28$ for both) or GALA I ($P = .28$ for rs1281748 and $P = .19$ for rs1281743). The associations similarly failed to replicate in GALA I Mexicans; however, only 1 and 2 subjects carried the minor allele at rs1281748 and rs1281743, respectively (Table IV).

Transcription of *SLC22A15* was detected in the lung and additional tissues by using RT-PCR (Fig 5, A), and was consistent with previous findings that *SLC22A15* (protein) is present in the lung.²⁸ Immunohistochemistry data from the Human Protein Atlas (<http://www.proteinatlas.org/ENSG00000163393/normal/bronchus>) confirmed our results and demonstrate that *SLC22A15* is expressed within the cytoplasm and membrane of bronchial epithelial cells (Fig 5, B).

DISCUSSION

We performed genome-wide tests for allelic and local ancestry associations with BDR in 1782 Latino children with asthma from across the United States and Puerto Rico and found 16 rare variants to be significantly associated with BDR in either Puerto Ricans, Mexicans, or all of the GALA II population combined (including Puerto Ricans, Mexicans, and other Latinos; $P < 5 \times 10^{-8}$). All of the associated variants had frequencies of 5% or less, and no common variants were significantly associated

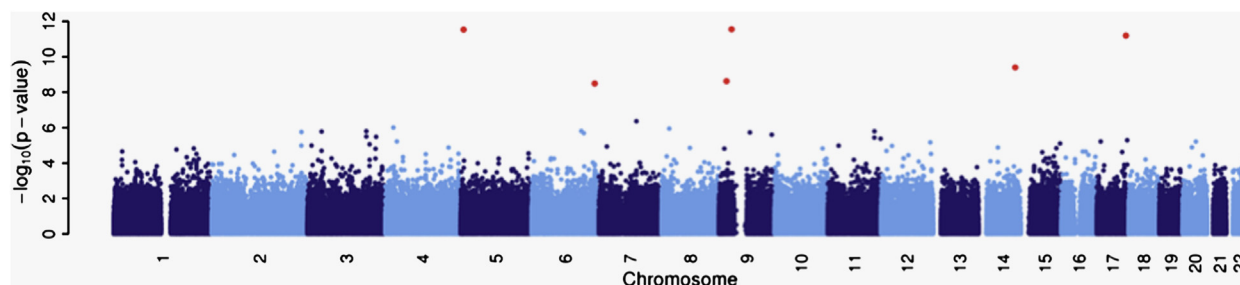


FIG 2. Manhattan plot of GWASs with BDR. Association testing for BDR was performed by using linear regression, including ethnicity and local and global African and Native American ancestry as covariates. SNPs meeting a genome-wide significance threshold of a P value of less than 5×10^{-8} are colored in red. Manhattan plots for the subset of Puerto Ricans and Mexicans are shown in Fig E4.

TABLE I. Top signals of allelic association with BDR

Population	SNP ID	Gene	Annotation	MAF	P value	β	Mean BDR by copies of minor allele (no. of subjects)		
							0	1	2
All	rs8191725*	<i>IGF2R</i>	Intronic	0.82%	3.3×10^{-9}	10.2	9.9 (1750)	20.4 (29)	—
	rs77441273*	<i>SLC24A4</i>	Missense	0.17%	4.0×10^{-10}	23.6	10 (1776)	33.6 (6)	—
Puerto Rican	rs77977790*	<i>PAPPA2</i>	Intronic	2.8%	4.6×10^{-10}	9.5	11.5 (776)	20.6 (46)	—
	rs77149876	<i>SPON1</i>	Intronic	0.18%	1.4×10^{-8}	32.5	11.9 (819)	44.1 (3)	—
	rs115501901	<i>NCOA3</i>	3'UTR	0.18%	4.2×10^{-8}	31.5	11.9 (819)	43.1 (3)	—
Mexican	rs116551936	Intergenic		0.26%	5.9×10^{-9}	27.0	7.8 (566)	36 (3)	—
	rs74973995	Intergenic		0.18%	1.3×10^{-8}	32.6	7.8 (569)	39.9 (2)	—

UTR, Untranslated region.

*SNPs we attempted to replicate in GALA I (see Table E3).

TABLE II. Top signals of allelic association within 50 kb of previously reported candidate genes

Gene	SNP ID	Position	P value	Coefficient	MAF	No. of SNPs
<i>ADRB2</i>	rs10476900	148162955	.040	1.32	10.3%	56
	<i>rs1042713</i>	148206440	.71	−0.37	44%	
	<i>rs1042714</i>	148206473	.76	−0.13	22%	
<i>ADCY9</i>	rs144315541	4191522	.0004*	−6.66	0.7%	81
	<i>rs2230739</i>	4033436	.33	0.98	27%	
<i>CRHR2</i>	rs73294475	30701596	.0011*	3.19	4.2%	39
	<i>rs7793837</i>	30726777	.98	−0.0096	39%	
<i>ARG1</i>	rs79631493	131951607	.077	2.00	2.6%	24
	<i>rs2781659</i>	131891820	.29	−1.06	45%	
<i>SPATS2L</i>	rs10203042	201354866	.0016	4.10	2.1%	71
	<i>rs295137</i>	201150040	.48	−0.71	38%	
	<i>rs295114</i>	201195602	.73	0.12	65%	
<i>THRB</i>	rs73038406	24573150	.010	−3.97	1.5%	179
	<i>rs892940</i>	24538838	.34	0.96	35%	

Candidate SNPs associated with BDR in prior studies are shown in italics.

*Statistically significant after Bonferroni correction for the total number of SNPs within 50 kb upstream and downstream of the transcription start and stop site of each gene.

with BDR after multiple testing correction. Although associations with rare variation are typically studied through the use of complete resequencing data, the Axiom LAT1 array used in our study was designed to have better coverage of rare variants compared with other commercial genotyping arrays and to have saturated coverage at 5000 genes previously identified by GWASs, including those for drug response.²⁹ Our use of this array might have allowed us to detect associations at rare variants not previously identified in genetic studies of BDR.

In addition, we replicated associations at 2 candidate genes for BDR (*ADCY9* and *CRHR2*) but at different variants than

originally reported; both of the top associations were with rare rather than common variants. We did not identify any associated SNPs in *SPATS2L*, looking at both the genome-wide level and a gene-based level. *SPATS2L* was identified as a top candidate in a recent GWAS for BDR in populations of European ancestry, in which top signals of allelic associations were observed at 2 common variants and replicated in independent studies (rs295137 and rs295114, MAF, >40%; neither were genome-wide significant).¹¹ One of the SNPs was genotyped in our study, and we imputed the other (rs295114; information score, 0.992). However, neither was associated with BDR in our study

TABLE III. Location of admixture mapping peaks

Population	Ancestry	<i>P</i> value	Coefficient*	Genome coordinates (hg19 [<i>P</i> < .01])	Cytoband
Puerto Rican	NAM/EUR	$9.4 \times 10^{-5}\ddagger$	-2.58/2.05	Chromosome 1: 116204807-117505312	1p13
Puerto Rican	NAM/EUR	$7.6 \times 10^{-6}\ddagger$	2.96/-2.56	Chromosome 2: 235202022-236278203	2q37
Puerto Rican	AFR	1.7×10^{-5}	-2.83	Chromosome 1: 157995576-158687163	1q23
Puerto Rican	AFR	1.5×10^{-5}	2.69	Chromosome 8: 5585682-6024650	8p23
Mexican	AFR	1.3×10^{-4}	3.88	Chromosome 14: 98812269-98932579	14q32

AFR, African; EUR, European; NAM, Native American.

*Coefficient is for the top of the admixture mapping peak.

†Minimum *P* value in Native American peak.

‡Minimum *P* value in European peak.

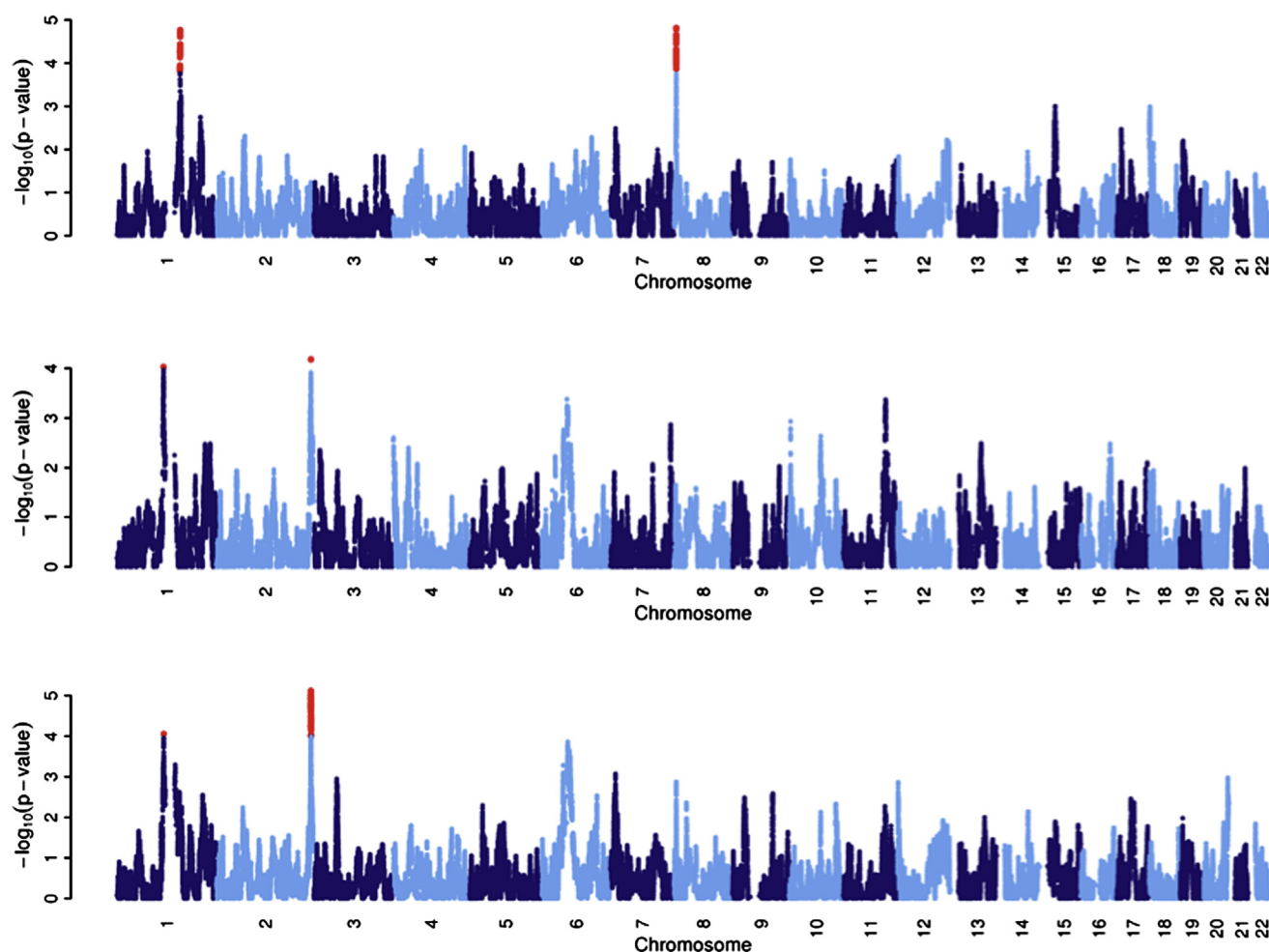


FIG 3. Admixture mapping in GALA II Puerto Ricans for African (A), Native American (B), and European (C) ancestry. Ancestry association testing for BDR was performed by using linear regression, including ethnicity and global African and global Native American ancestry as covariates. Peaks obtaining statistical significance based on permutations are indicated in red. Results for Mexicans are shown in Fig E12.

(Table II), nor did we find any association with local ancestry and BDR at *SPATS2L* ($P > .5$). The most significant association within 50 kb of *SPATS2L* was at a rare variant (rs10203042, $P = 1.6 \times 10^{-3}$, Table II). However, this variant lies within an intron of the neighboring gene *KCTD18* and was not statistically significant after correcting for 71 tests. There was little to no LD between rs10203042 and either of the GWAS-implicated SNPs (rs295137: $r^2 = 0.004$, $D' = 0.33$; rs295114: $r^2 = 0.003$, $D' = 0.29$). The difference between studies might be due to

differences in genetic risk factors between European and Latino populations or to differences in patterns of LD at *SPATS2L* (ie, differences in the coverage of variation between different genotyping arrays).

In addition to our allelic association results, we identified 5 admixture mapping peaks in Puerto Ricans and Mexicans in which local ancestry (European, Native American, or African ancestry) was significantly correlated with BDR. Given that rare variants are more likely than common variants to differ in

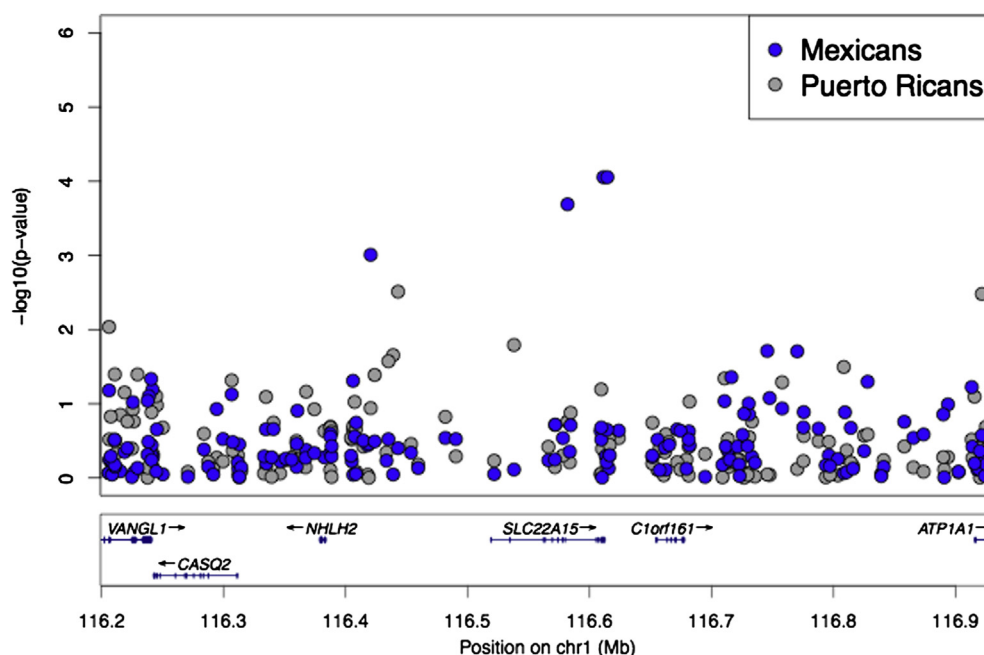


FIG 4. Top signals of allelic association under the admixture mapping peak at 1p13 in Mexicans. Plotted are the $-\log_{10}(P$ values) for tests of allelic association with BDR by using linear regression adjusting for ethnicity, local, and genomic African and Native American ancestry. Significant associations were observed at 2 rare variants in the intron and 3' untranslated region of *SLC22A15* in Mexicans after correcting for a reduced number of tests.

TABLE IV. Top signals of allelic association and replication under 1p13 admixture mapping peak

Study	rs1281748			rs1281743		
	P value	Coefficient	MAF	P value	Coefficient	MAF
GALA II Puerto Rican	.28	−2.65	1.04%	.28	−2.65	1.04%
GALA II Mexican	8.8×10^{-5}	18.8	0.26%	8.8×10^{-5}	18.8	0.26%
GALA I Puerto Rican	.28	5.9	1.80%	.19	7.7	1.50%
GALA I Mexican	.56	−5.25	0.39%	.47	−9.2	0.19%

frequency between the ancestral populations of modern Latinos,³⁰ the peaks we identified might be driven by differences in patterns of rare variation. In support of this, we identified 2 rare variants under one of the admixture mapping peaks (1p13) in which the minor allele was significantly associated with higher BDR after correcting for a reduced number of tests, whereas we found no significant associations at any common variants.

Five of our top associated variants fell directly within an annotated gene (Table I). All of these genes are plausible candidates for being associated with BDR and include an ion transporter (*SLC24A4*), a gene involved in transcriptional regulation (*NCOA3*), and a gene involved in vascular smooth muscle cell adhesion (*SPON1*). Two of the genes, *IGF2R* and *PAPPA2*, are especially noteworthy. *IGF2R* activates *TGFB2*³¹ and is a member of the TGF- β pathway, which is well studied in asthma susceptibility. *PAPPA2* cleaves insulin-like growth factor (IGF)-binding protein 5 and might be a local regulator of IGF bioavailability.³² IGF is known to be involved in airway inflammation and remodeling.³³

In addition to these genes identified through allelic associations, 2 rare variants identified under the admixture mapping peak at 1p13 are in *SLC22A15*, a member of the organic ion transporter family (SLC22), which mediates the uptake of pharmaceutical

drugs to different tissues used in the treatment of diseases, such as cancer and diabetes.³⁴ These 2 SNPs are in complete LD; however, one of the SNPs lies within a promoter region identified through ChIP-seq in the ENCODE studies.³⁵ Although we currently do not know the substrates of *SLC22A15*, using sequence-based profiling with additional bioinformatics analysis shows that *SLC22A15* is clustered close to *SLC22A7*, an organic anion transporter³⁶, which transports broad substrates of compounds, such as antiviral nucleosides analogs, and endogenous compounds, such as cyclic guanosine monophosphate.²⁷ Our expression studies and data from the protein atlas demonstrate that *SLC22A15* is expressed in the lung and bronchial epithelial cells; however, additional studies are required to identify whether rare variation in *SLC22A15* contributes to differential transcription.

We attempted to replicate our findings in an independent sample of Latino children with asthma (the GALA I study), which, to the best of our knowledge, is the only other large study of BDR in Latinos. Although 2 of the rare variants showed a consistent direction of effect in Puerto Ricans (rs77441273 and rs143010317), none of the rare variants were significantly associated with BDR in GALA I by using *in silico* approaches at a *P* value of less than .05. Imputation quality scores were

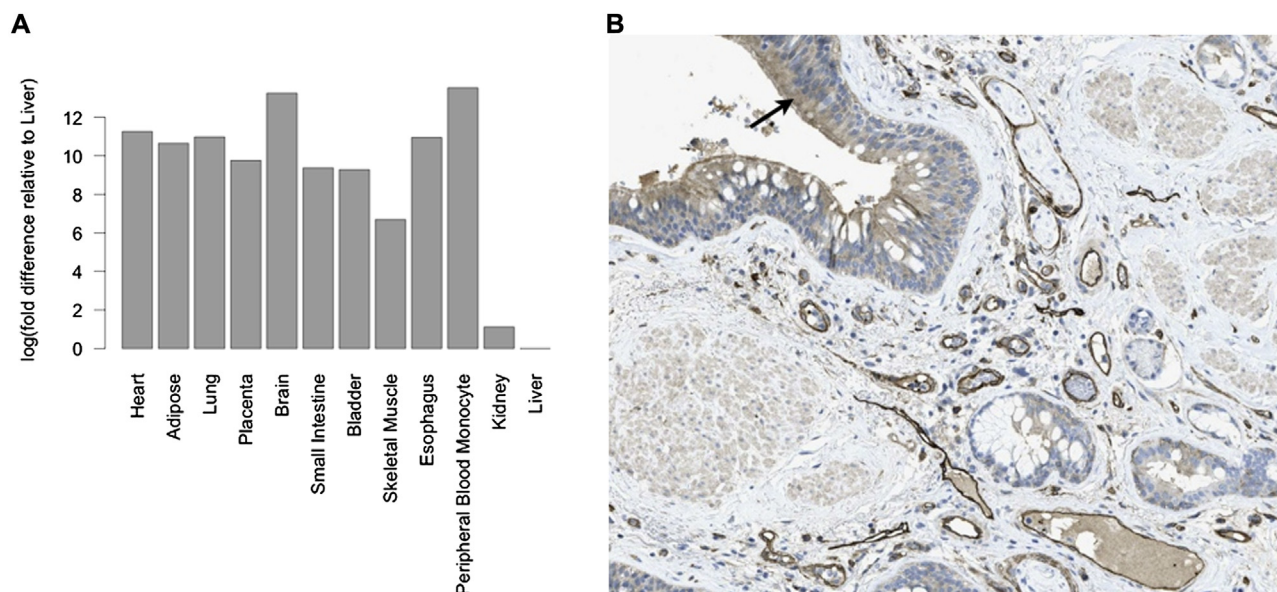


FIG 5. Expression of *SLC22A15* in the lung. **A**, Relative transcript abundance across human tissues assessed by using quantitative real-time RT-PCR; *SLC22A15* mRNA was detected at levels more than 50,000 times higher in the lung compared with the liver. **B**, Immunohistochemistry staining of lung tissue adapted from the Human Protein Atlas (<http://www.proteinatlas.org/ENSG00000163393/normal/bronchus>), showing the transcription of *SLC22A15* (brown) in the cytoplasm and membrane of bronchial epithelial cells (indicated by a black arrow).

generally low for the rare variants we attempted to replicate (0.19-0.88), and direct genotyping of 3 variants with TaqMan assays suggests they might be absent in GALA I. These findings are not surprising given the smaller sample size of the replication study ($n = 531$) and highlights the difficulty in replicating associations at individual rare variants. Apart from differences in statistical power, there are differences in the recruitment of patients between studies (see the Methods section), notably with respect to self-identified ethnicity, with subjects in GALA II having more diverse ethnic backgrounds. Therefore our results highlight the need for additional studies of BDR in larger samples of Latino populations to fully understand the contributions of individual rare variants to drug response.

In conclusion, we identified an enrichment of small P values in a GWAS for BDR that was driven by rare variants and implicated multiple individual rare variants as being significantly associated with BDR in Latino children with asthma. Additional studies involving complete resequencing and larger population samples are required to further understand the genetic contributions to differences in BDR. Future studies are also required to investigate the transport function of *SLC22A15* and its relationship to bronchodilation.

We thank the families and patients for their participation and the numerous health care providers and community clinics for their support and participation in GALA II. In particular, we thank the study coordinator, Sandra Salazar, and the recruiters who obtained the data: Duanny Alva, MD; Gaby Ayala-Rodriguez; Ulysses Burley; Lisa Caine; Elizabeth Castellanos; Jaime Colon; Denise DeJesus; Iliana Flexas; Blanca Lopez; Brenda Lopez, MD; Louis Martos; Vivian Medina; Juana Olivo; Mario Peralta; Esther Pomares, MD; Jihan Quraishi; Johanna Rodriguez; Shahdad Saeedi; Dean Soto; Ana Taveras; and Emmanuel Viera. We also thank Cheryl Winkler, PhD, and William Klitz, PhD, for providing data that were used to help estimate local ancestry.

Key messages

- A GWAS for BDR in Latino children with asthma suggests that rare variation contributes to a differential drug response, notably in SLC genes.
- Rare variants in 2 candidate genes (*ADCY9* and *CRHR2*) were associated with BDR; however, there was no association at the previously implicated common variants in either gene.
- Admixture mapping identified a novel candidate gene expressed in lung and bronchial epithelial cells (*SLC22A15*).

REFERENCES

1. Nelson HS. Beta-adrenergic bronchodilators. *N Engl J Med* 1995;333:499-506.
2. Palmer LJ, Silverman ES, Weiss ST, Drazen JM. Pharmacogenetics of asthma. *Am J Respir Crit Care Med* 2002;165:861-6.
3. Drazen JM, Silverman EK, Lee TH. Heterogeneity of therapeutic responses in asthma. *Br Med Bull* 2000;56:1054-70.
4. Martinez FD, Graves PE, Baldini M, Solomon S, Erickson R. Association between genetic polymorphisms of the beta2-adrenoceptor and response to albuterol in children with and without a history of wheezing. *J Clin Invest* 1997;100:3184-8.
5. Choudhry S, Ung N, Avila PC, Ziv E, Nazario S, Casal J, et al. Pharmacogenetic differences in response to albuterol between Puerto Ricans and Mexicans with asthma. *Am J Respir Crit Care Med* 2005;171:563-70.
6. Silverman EK, Kwiatkowski DJ, Sylvia JS, Lazarus R, Drazen JM, Lange C, et al. Family-based association analysis of beta2-adrenergic receptor polymorphisms in the childhood asthma management program. *J Allergy Clin Immunol* 2003;112:870-6.
7. Poon AH, Tantisira KG, Litonjua AA, Lazarus R, Xu J, Lasky-Su J, et al. Association of corticotropin-releasing hormone receptor-2 genetic variants with acute bronchodilator response in asthma. *Pharmacogenet Genomics* 2008;18:373-82.
8. Tantisira KG, Small KM, Litonjua AA, Weiss ST, Liggett SB. Molecular properties and pharmacogenetics of a polymorphism of adenylyl cyclase type 9 in asthma: interaction between beta-agonist and corticosteroid pathways. *Hum Mol Genet* 2005;14:1671-7.

9. Litonjua AA, Lasky-Su J, Schneider K, Tantisira KG, Lazarus R, Klanderman B, et al. ARG1 is a novel bronchodilator response gene: screening and replication in four asthma cohorts. *Am J Respir Crit Care Med* 2008;178:688-94.
10. Duan QL, Du R, Lasky-Su J, Klanderman BJ, Partch AB, Peters SP, et al. A polymorphism in the thyroid hormone receptor gene is associated with bronchodilator response in asthmatics. *Pharmacogenomics J* 2013;13:130-6.
11. Himes BE, Jiang X, Hu R, Wu AC, Lasky-Su JA, Klanderman BJ, et al. Genome-wide association analysis in asthma subjects identifies SPATS2L as a novel bronchodilator response gene. *PLoS Genet* 2012;8:e1002824.
12. Contopoulos-Ioannidis DG, Alexiou GA, Gouvias TC, Ioannidis JPA. An empirical evaluation of multifarious outcomes in pharmacogenetics: beta-2 adrenoceptor gene polymorphisms in asthma treatment. *Pharmacogenet Genomics* 2006;16:705-11.
13. Burchard EG, Avila PC, Nazario S, Casal J, Torres A, Rodriguez-Santana JR, et al. Lower bronchodilator responsiveness in Puerto Rican than in Mexican subjects with asthma. *Am J Respir Crit Care Med* 2004;169:386-92.
14. Naqvi M, Thyne S, Choudhry S, Tsai H-J, Navarro D, Castro RA, et al. Ethnic-specific differences in bronchodilator responsiveness among African Americans, Puerto Ricans, and Mexicans with asthma. *J Asthma* 2007;44:639-48.
15. Blake K, Madabushi R, Derendorf H, Lima J. Population pharmacodynamic model of bronchodilator response to inhaled albuterol in children and adults with asthma. *Chest* 2008;134:981-9.
16. Howie BN, Donnelly P, Marchini J. A flexible and accurate genotype imputation method for the next generation of genome-wide association studies. *PLoS Genet* 2009;5:e1000529.
17. Torgerson DG, Gignoux CR, Galanter JM, Drake KA, Roth LA, Eng C, et al. Case-control admixture mapping in Latino populations enriches for known asthma-associated genes. *J Allergy Clin Immunol* 2012;130:76-82.e12.
18. Alexander DH, Novembre J, Lange K. Fast model-based estimation of ancestry in unrelated individuals. *Genome Res* 2009;19:1655-64.
19. Sankararaman S, Sridhar S, Kimmel G, Halperin E. Estimating local ancestry in admixed populations. *Am J Hum Genet* 2008;82:290-303.
20. Baran Y, Pasaniuc B, Sankararaman S, Torgerson DG, Gignoux C, Eng C, et al. Fast and accurate inference of local ancestry in Latino populations. *Bioinformatics* 2012;28:1359-67.
21. Browning S. Multilocus association mapping using variable-length markov chains. *Am J Hum Genet* 2006;78:903-13.
22. Delaneau O, Marchini J, Zagury J-F. A linear complexity phasing method for thousands of genomes. *Nat Methods* 2012;9:179-81.
23. 1000 Genomes Project Consortium, Abecasis GR, Altshuler D, Auton A, Brooks LD, Durbin RM. A map of human genome variation from population-scale sequencing. *Nature* 2010;467:1061-73.
24. Team RDC. R: a language and environment for statistical computing. Vienna: R Foundation for Statistical Computing; 2010. Available at: <http://www.R-project.org>. Accessed March 31, 2012.
25. Casella G, Berger RL. Statistical inference. Pacific Grove (CA): Duxbury Press; 2002. p. 660.
26. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. *Methods* 2001;25:402-8.
27. Cropp CD, Komori T, Shima JE, Urban TJ, Yee SW, More SS, et al. Organic anion transporter 2 (SLC22A7) is a facilitative transporter of cGMP. *Mol Pharmacol* 2008;73:1151-8.
28. Eraly SA, Nigam SK. Novel human cDNAs homologous to Drosophila Orct and mammalian carnitine transporters. *Biochem Biophys Res Commun* 2002;297:1159-66.
29. Hoffmann TJ, Zhan Y, Kvale MN, Hesselson SE, Gollub J, Iribarren C, et al. Design and coverage of high throughput genotyping arrays optimized for individuals of East Asian, African American, and Latino race/ethnicity using imputation and a novel hybrid SNP selection algorithm. *Genomics* 2011;98:422-30.
30. Gravel S, Henn BM, Gutenkunst RN, Indap AR, Marth GT, Clark AG, et al. Demographic history and rare allele sharing among human populations. *Proc Natl Acad Sci U S A* 2011;108:11983-8.
31. Dennis PA, Rifkin DB. Cellular activation of latent transforming growth factor beta requires binding to the cation-independent mannose 6-phosphate/insulin-like growth factor type II receptor. *Proc Natl Acad Sci U S A* 1991;88:580-4.
32. Yan X, Baxter RC, Firth SM. Involvement of pregnancy-associated plasma protein-A2 in insulin-like growth factor (IGF) binding protein-5 proteolysis during pregnancy: a potential mechanism for increasing IGF bioavailability. *J Clin Endocrinol Metab* 2010;95:1412-20.
33. Yamashita N, Tashimo H, Ishida H, Matsuo Y, Arai H, Nagase H, et al. Role of insulin-like growth factor-I in allergen-induced airway inflammation and remodeling. *Cell Immunol* 2005;235:85-91.
34. Giacomini K, Sugiyama Y. In: Brunton L, Lazo JS, Parker RL, editors. Chapter 5: Membrane transporters and drug response. In: Goodman and Gilman's the pharmacological basis of therapeutics. 12th ed. New York: McGraw-Hill; 2006. pp. 41-70.
35. ENCODE Project Consortium. A user's guide to the encyclopedia of DNA elements (ENCODE). *PLoS Biol* 2011;9:e1001046.
36. Schlessinger A, Matsson P, Shima JE, Pieper U, Yee SW, Kelly L, et al. Comparison of human solute carriers. *Protein Sci* 2010;19:412-28.

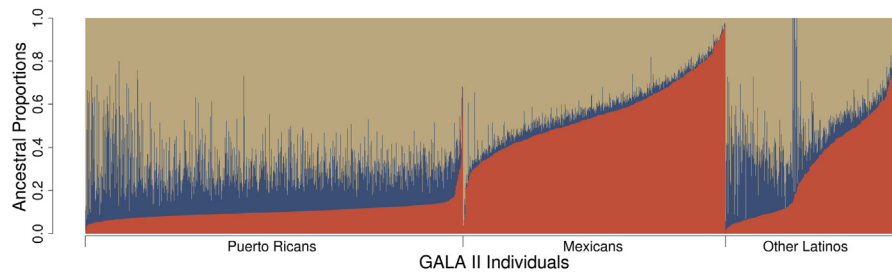


FIG E1. Admixture proportions for GALA II cases. Each *vertical bar* represents 1 subject. For each subject, the proportions of Native American (*red*), African (*blue*), and European (*tan*) ancestry are displayed.

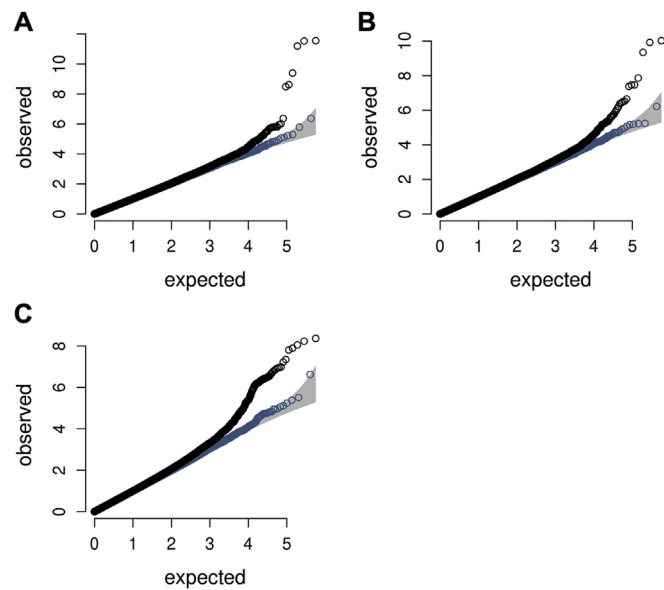


FIG E2. Quantile-quantile plots for genome-wide allelic associations with BDR in all of the GALA II population (inflation factor: $\lambda = 1.005$ for all SNPs and $\lambda = 1.004$ for common SNPs; **A**), GALA II Puerto Ricans ($\lambda = 0.98$ for all SNPs and $\lambda = 0.99$ for common SNPs; **B**), and GALA II Mexicans ($\lambda = 0.98$ for all SNPs and $\lambda = 0.996$ for common SNPs; **C**). The expected distribution of P values is based on a uniform distribution. *Black circles* indicate all SNPs, whereas *blue circles* include only common SNPs with an MAF of 5% or greater. The *shaded region* represents the 95% concentration band.

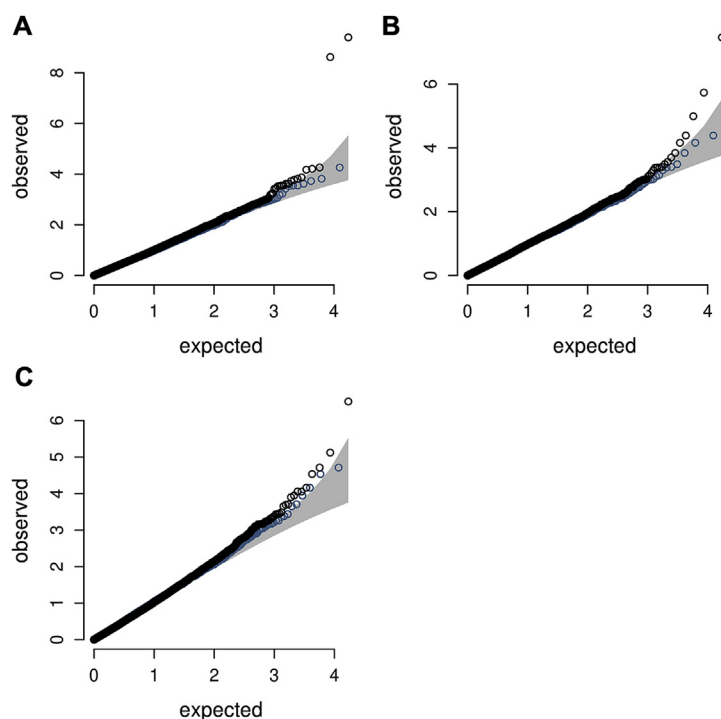


FIG E3. Quantile-quantile plots for genome-wide allelic associations with BDR for 17,295 SNPs in the proximity of 457 SLC genes in all of the GALA II population **(A)**, GALA II Puerto Ricans **(B)**, and GALA II Mexicans **(C)**. The expected distribution of *P* values is based on a uniform distribution. *Black circles* indicate all SNPs, whereas *blue circles* include only common SNPs with an MAF of 5% or greater. The *shaded region* represents the 95% concentration band.

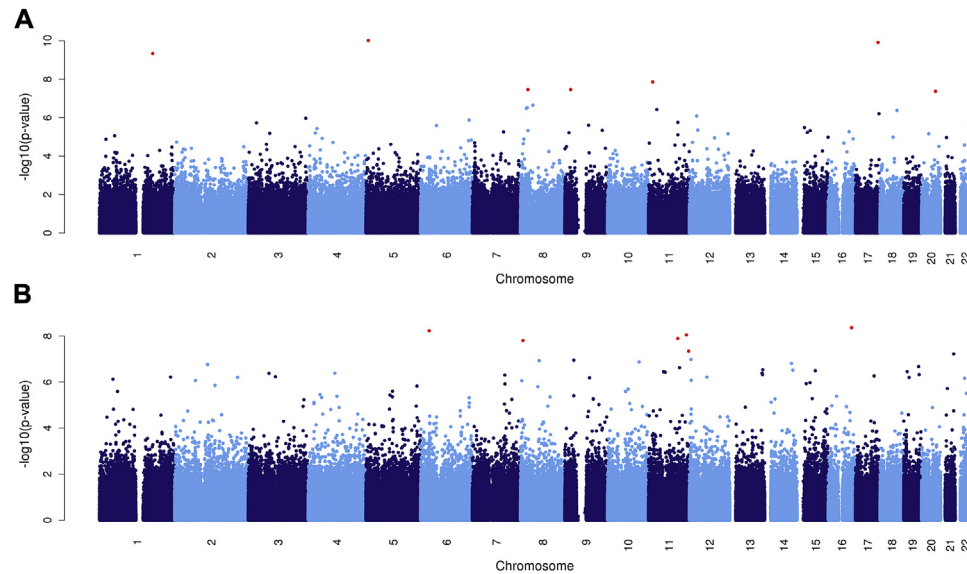


FIG E4. Manhattan plots of GWASs with BDR in Puerto Ricans (**A**) and Mexicans (**B**). Association testing for BDR was performed by using linear regression, including ethnicity and local and global African and Native American ancestry as covariates. SNPs meeting a genome-wide significance threshold of a P value of less than 5×10^{-8} are colored in *red*.

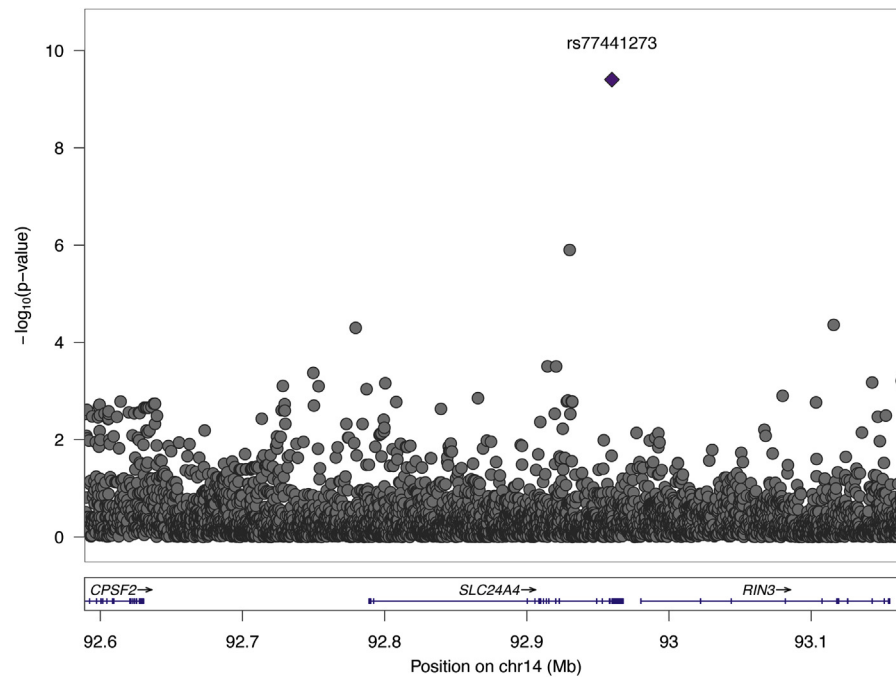


FIG E5. Results of *in silico* fine mapping around rs8191725 by using imputed genotypes from the Phase I Thousand Genomes Project in all GALA II asthma cases. Association testing for BDR was performed by using linear regression on genotype dosage, including ethnicity and local and global African and Native American ancestry as covariates.

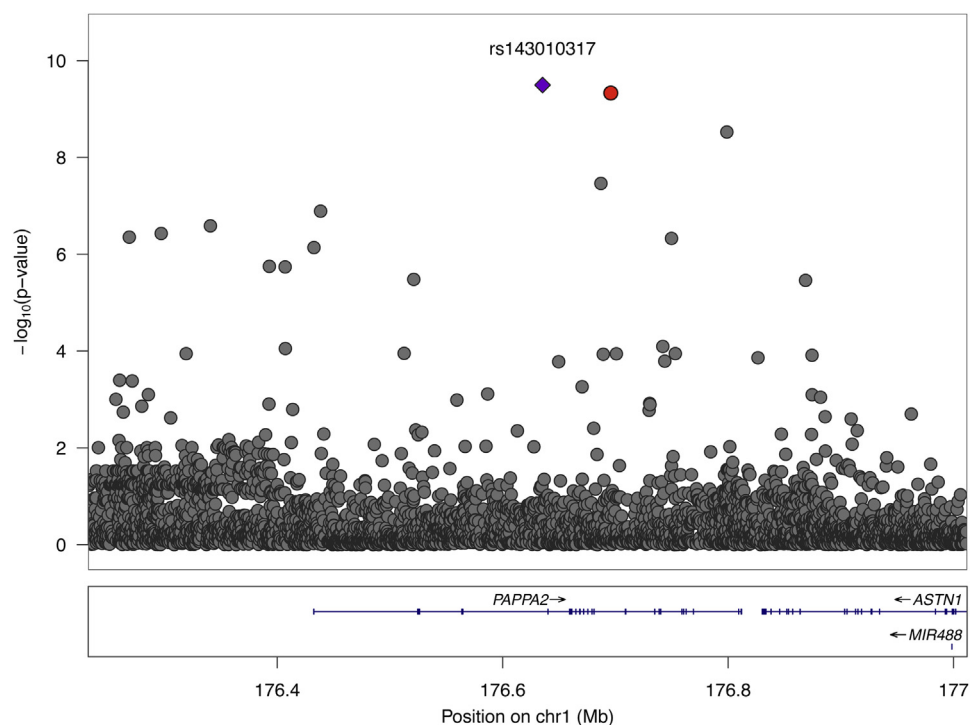


FIG E6. Results of *in silico* fine mapping around rs77441273 by using imputed genotypes from the Phase I Thousand Genomes Project in all GALA II asthma cases. Association testing for BDR was performed by using linear regression on genotype dosage, including ethnicity and local and global African and Native American ancestry as covariates.

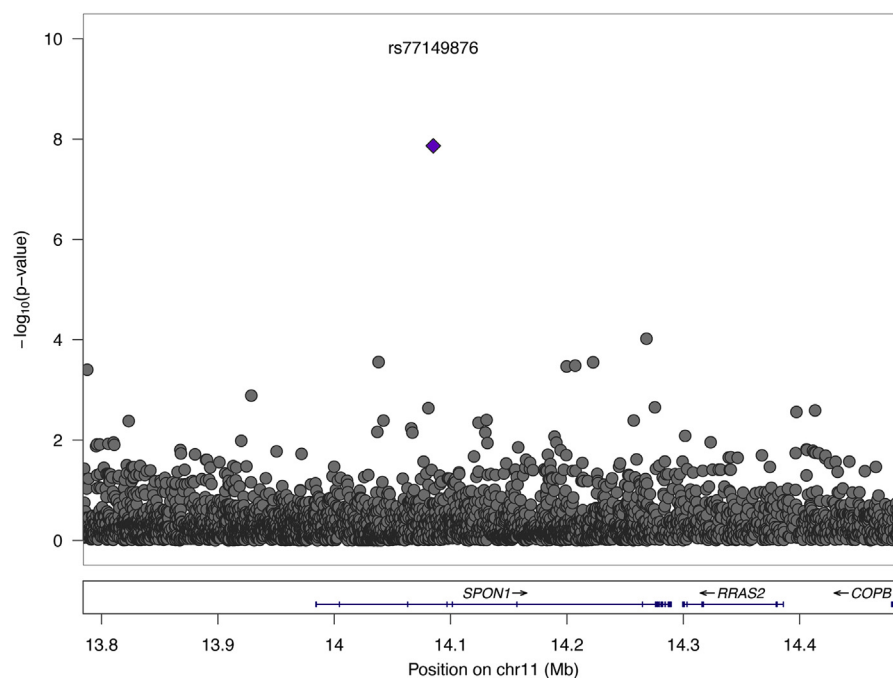


FIG E7. Results of *in silico* fine mapping around rs77977790 (red) by using imputed genotypes from the Phase I Thousand Genomes Project in Puerto Rican asthma cases. Association testing for BDR was performed by using linear regression on genotype dosage, including local and global African and Native American ancestry as covariates. A rare imputed variant (rs143010317, MAF = 0.20%) was slightly more significantly associated with BDR, but in the opposite direction ($\beta = -58$, $P = 3.2 \times 10^{-10}$), than the top genotyped SNP ($\beta = 9.5$, $P = 4.6 \times 10^{-10}$). rs143010317 did not replicate in GALA I Puerto Ricans by using imputed genotypes ($P = .24$); however, the effect was in a similar direction ($\beta = -12.6$).

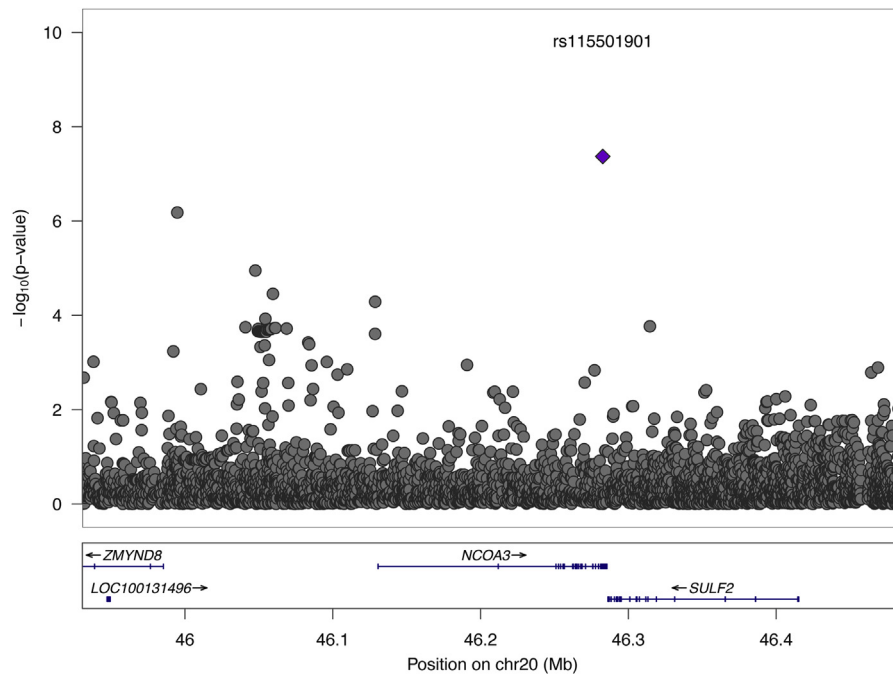


FIG E8. Results of *in silico* fine mapping around rs77149876 by using imputed genotypes from the Phase I Thousand Genomes Project in Puerto Rican asthma cases. Association testing for BDR was performed by using linear regression on genotype dosage, including local and global African and Native American ancestry as covariates.

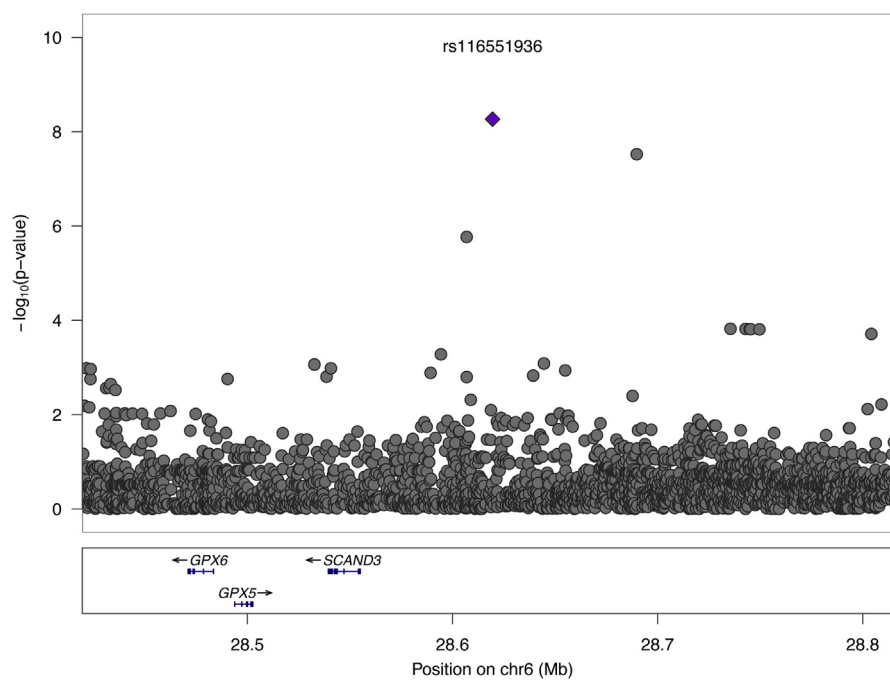


FIG E9. Results of *in silico* fine mapping around rs116551936 by using imputed genotypes from the Phase I Thousand Genomes Project in Puerto Rican asthma cases. Association testing for BDR was performed by using linear regression on genotype dosage, including local and global African and Native American ancestry as covariates.

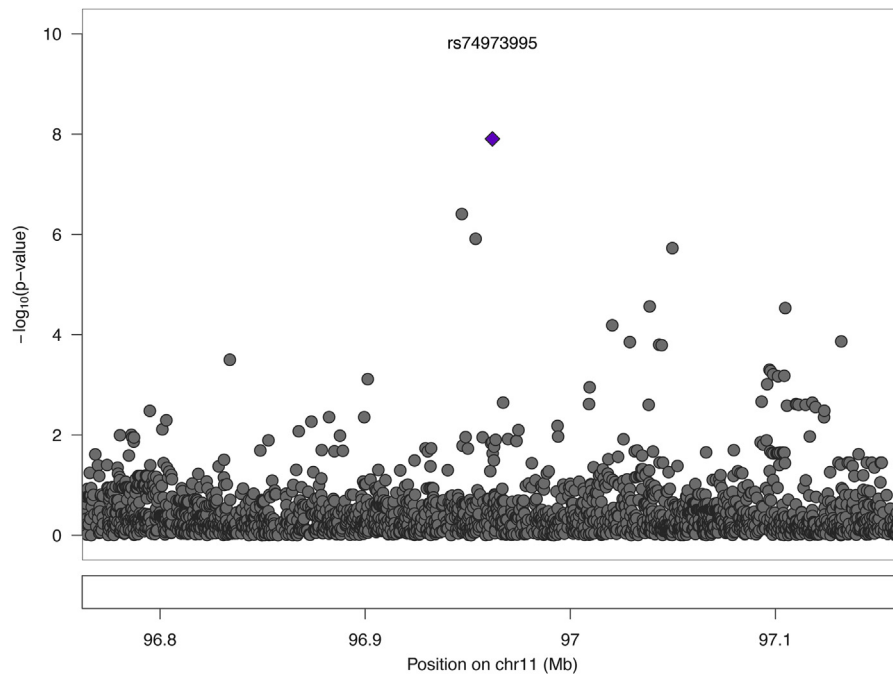


FIG E10. Results of *in silico* fine mapping around rs116551936 by using imputed genotypes from the Phase I Thousand Genomes Project in Mexicans asthma cases. Association testing for BDR was performed by using linear regression on genotype dosage, including local and global African and Native American ancestry as covariates.

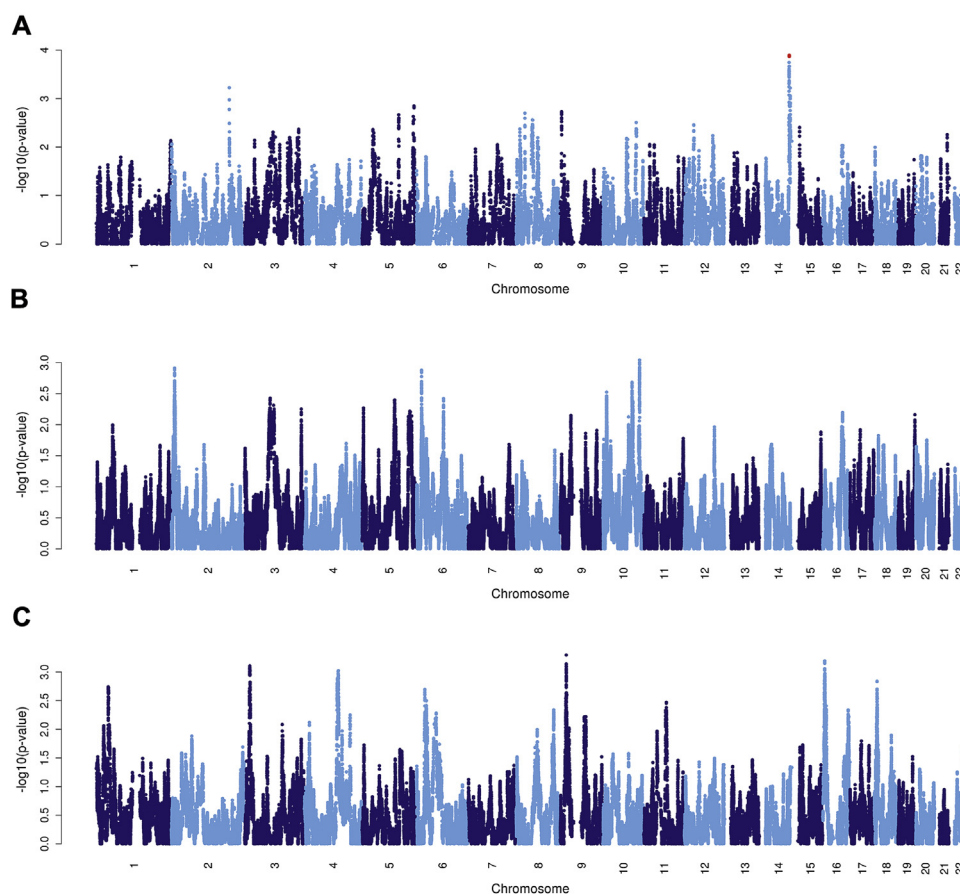


FIG E11. Results of *in silico* fine mapping around rs74973995 by using imputed genotypes from the Phase I Thousand Genomes Project in Mexican asthma cases. Association testing for BDR was performed by using linear regression on genotype dosage, including local and global African and Native American ancestry as covariates.

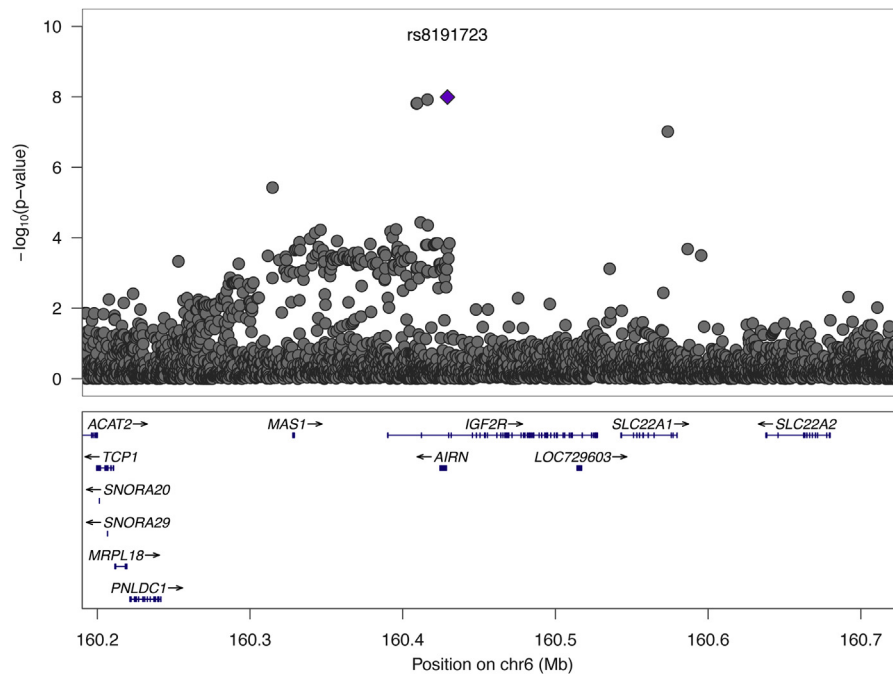


FIG E12. Genome-wide ancestry associations with BDR in GALA II Mexicans for African ancestry (A), Native American ancestry (B), and European ancestry (C). Y-axis, $-\log_{10}$ of the *P* value for the specified local ancestry in the linear regression of BDR on the specified local ancestry, corresponding global ancestry, and ethnicity. X-axis, Chromosome and position. Colors alternate for clarity. Significant loci are highlighted in red.

TABLE E1. Participating study centers and institutions in the GALA II study with the number of cases with asthma included in the current study

Study center	Institution	No. of cases
Chicago	Northwestern University	95
	Children's Memorial Hospital	206
Houston	Baylor College of Medicine Ben Taub Children's Center, Texas Children's Hospital	194
New York City	Jacobi Medical Center	285
Puerto Rico	Centro de Neumologia Pediatrica (San Juan)	408
	VA Medical Center (San Juan)	296
San Francisco Bay Area	Kaiser Permanente	
	Richmond Medical Center	5
	Oakland Medical Center	4
	Vallejo Medical Center	33
	Bay Area Pediatrics (Oakland)	3
	Children's Hospital (Oakland)	14
	La Clinica de la Raza (Oakland)	99
	San Francisco General Hospital	130
	Alta Vista (Oakland)	10

TABLE E2. Eligibility criteria for participation of asthmatic patients in GALA II versus GALA I

Criterion	GALA II	GALA I
Age (y)	8-21	8-40
Self-reported ethnicity of child and all 4 grandparents	Hispanic or Latino	Mexican or Puerto Rican
History of physician-diagnosed asthma	Yes	Yes
Symptoms of coughing, wheezing, or shortness of breath in the past 2 y	Yes	Yes
No respiratory tract infections for ≥ 6 wk (clinical stability)	Yes	Yes
No asthma exacerbations for ≥ 6 wk (clinical stability)	Yes	Yes
Less than 10 pack year smoking history and no smoking in the last year	Yes	Yes
If pregnant, less than third trimester	Yes	Yes
No history of other lung diseases or other chronic illnesses	Yes	Yes

TABLE E3. Results from *in silico* replication of four SNPs through imputation in GALA I

SNP	GALA I Puerto Ricans			GALA I Mexicans		
	Information score	P value	Coefficient	Information score	P value	Coefficient
rs8191725	0.57	.990	−0.4	0.88	.939	1.1
rs77441273	0.66	.376	24.3	.37	NA	NA
rs77977790	.28	NA	NA	.19	NA	NA
rs143010317	.59	.24	−12.6	.75	NA	NA

NA, Not applicable.