

Genome-wide association study and admixture mapping identify different asthma-associated loci in Latinos: The Genes-environments & Admixture in Latino Americans study

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Background: Asthma is a complex disease with both genetic and environmental causes. Genome-wide association studies of asthma have mostly involved European populations, and replication of positive associations has been inconsistent.

Objective: We sought to identify asthma-associated genes in a large Latino population with genome-wide association analysis and admixture mapping.

Methods: Latino children with asthma (n = 1893) and healthy control subjects (n = 1881) were recruited from 5 sites in the United States: Puerto Rico, New York, Chicago, Houston, and the San Francisco Bay Area. Subjects were genotyped on an Affymetrix World Array IV chip. We performed genome-wide association and admixture mapping to identify asthma-associated loci.

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Results: We identified a significant association between ancestry and asthma at 6p21 (lowest P value: rs2523924, $P < 5 \times 10^{-6}$). This association replicates in a meta-analysis of the EVE Asthma Consortium ($P = .01$). Fine mapping of the region in this study and the EVE Asthma Consortium suggests an association between *PSORS1C1* and asthma. We confirmed the strong allelic association between SNPs in the 17q21 region and asthma in Latinos (*IKZF3*, lowest P value: rs90792, odds ratio, 0.67; 95% CI, 0.61-0.75; $P = 6 \times 10^{-13}$) and replicated associations in several genes that had previously been associated with asthma in genome-wide association studies.

Conclusions: Admixture mapping and genome-wide association are complementary techniques that provide evidence for multiple asthma-associated loci in Latinos. Admixture mapping identifies a novel locus on 6p21 that replicates in a meta-analysis of several Latino populations, whereas genome-wide association confirms the previously identified locus on 17q21. (J Allergy Clin Immunol 2013;■■■:■■■-■■■.)

Key words: Asthma, Latinos, admixture mapping, genome-wide association study, local ancestry, 17q21, 6p21

Asthma is the most common chronic disease among children. In the United States childhood asthma prevalence is highest among Puerto Ricans (18.4%), followed by blacks (14.6%), whites (8.2%), and Mexicans (4.8%).^{1,2} The discrepancy in asthma burden, as well as the paucity of studies of asthma in Latinos, has led the American Academy of Pediatrics to identify asthma among Latinos as an urgent priority for further research.³

Genetic variation between populations might account for some of these differences. Estimates of the heritability of asthma based on twin studies range from between 75%⁴ and 92%.⁵ However, few genetic loci show consistent associations across studies.⁶⁻⁸ Of the 48 genes reported to be associated with asthma in genome-wide studies,⁹ only 5 have been identified in more than 1 genome-wide association study (GWAS).¹⁰⁻¹⁶ This might be due to differences in study designs, differences in environment between study populations, and heterogeneity in asthma phenotypes.^{17,18} Our previous work has found that few genes associated with asthma replicate in Latino populations. Most that do replicate are consistent across Latino ethnic groups; however, 5 genes replicated in either Mexicans or Puerto Ricans (but not both) and showed significant heterogeneity in their association with asthma.⁶ These observed differences in genetic variation might in part explain the discrepancy in asthma prevalence between the 2 groups.¹⁹

Most GWASs of asthma have been conducted in European and European American populations.²⁰ The recent identification of *PYHINI*, a gene associated with asthma in subjects of African descent but not in Latinos or European Americans,¹⁰ highlights the importance of studying diverse populations in genetic association analyses. Moreover, studies in non-European populations might help uncover some of the “missing heritability” in complex diseases and could provide insights into racial/ethnic disparities in asthma prevalence and severity.

Admixture mapping is a technique that can help identify asthma-associated loci in populations of mixed ancestry (eg, African Americans and Latinos).²¹ In these admixed populations it is possible to use dense genotyping to estimate ancestry at a locus-specific (local) level.^{22,23} If the allele frequency of risk variants is higher in one ancestral population than in others, there will be a correlation between ancestry at that locus and disease.

Comparing local ancestry in subjects with disease with that seen in control subjects will show a deviation from the expected distribution of ancestry. Like GWASs, admixture mapping provides an unbiased method to screen for disease-associated loci. However, because admixture is a relatively recent phenomenon, ancestry blocks are significantly larger than haplotype blocks.^{24,25} Therefore admixture mapping offers increased coverage of genetic variation and a lower multiple testing burden than GWAS, although this also means that admixture mapping peaks cover an area in the hundreds of kilobases, which makes narrowing down the causal variant more difficult. Admixture mapping has successfully identified disease-associated loci in breast²⁶ and prostate²⁷ cancer, renal disease,^{28,29} and white blood cell counts,^{30,31} among others. Our own prior admixture mapping study demonstrated that admixture mapping preferentially harbored asthma-associated genes,³² whereas an admixture mapping study in African Americans and Puerto Ricans identified a locus on 6q14.1 that harbored a risk allele for asthma exclusively in subjects with local European admixture.³³

To identify genetic risk factors for asthma, we used genotype data from the Genes-environments & Admixture in Latino Americans (GALA II) study. GALA II is an ongoing, multicenter, case-control study to identify novel clinical and genetic risk factors associated with asthma and related phenotypes in Latino populations at 5 sites in the United States: Puerto Rico, New York, Chicago, Houston, and the San Francisco Bay Area, which includes genome-wide genotype data on 3774 participants. We hypothesize that by using a large population of Latino participants, we would identify novel loci for asthma.

METHODS

Recruitment and genotyping

Institutional review boards at the University of California, San Francisco, and recruitment sites approved the study, and all participants/parents provided appropriate written assent/consent. Latino children were enrolled as a part of the ongoing GALA II case-control study. From July 2006 through June 2011, when genotyping began, a total of 4045 children (1976 participants with asthma and 2065 healthy control subjects) were recruited from 5 centers (Chicago, Bronx, Houston, San Francisco Bay Area, and Puerto Rico) through a combination of community and clinic-based recruitment. Participants were eligible if they were 8 to 21 years of age, self-identified as Latino, and had 4 Latino grandparents. Asthma cases were defined as participants with a history of physician-diagnosed asthma and the presence of 2 or more symptoms of coughing, wheezing, or shortness of breath in the 2 years preceding enrollment. Participants were excluded if they reported any of the following: (1) 10 or more pack years of smoking; (2) any smoking within 1 year of recruitment date; (3) history of lung diseases other than asthma (cases) or chronic illness (cases and control subjects); or (4) pregnancy in the third trimester. Details of recruitment are described elsewhere³⁴ and in the **Methods** section in this article's Online Repository at www.jacionline.org.

Participants were genotyped at 818,154 single nucleotide polymorphisms (SNPs) on the Affymetrix Axiom World Array IV.³⁵ Details of individual and SNP quality control procedures are described in the **Methods** section in this article's Online Repository.

Ancestry was estimated by using the program ADMIXTURE, with a 3-population model.³⁶ Details of the ancestral populations are described in the **Methods** section in this article's Online Repository. Local ancestry at all positions across the genome was estimated by using the program LAMPLD,²² assuming 3 ancestral populations.

Statistical methods

We tested the associations between each SNP and asthma status using logistic regression, adjusting for potential confounders, including global and

Abbreviations used

GALA II: Genes-environments & Admixture in Latino Americans
 GWAS: Genome-wide association study
 OR: Odds ratio
 SNP: Single nucleotide polymorphism

local ancestry (for details, see the [Methods](#) section in this article's Online Repository), by using a threshold for significance of 5×10^{-8} . Admixture mapping was performed by using a likelihood ratio test to compare a full model, including 2 local ancestry estimates, and potential confounders to a restricted model excluding the local ancestry estimates. Adjustment for multiple comparisons was made by using a permutation procedure (for details, see the [Methods](#) section in this article's Online Repository). Based on permutation testing, we used an adjusted significance threshold α^* value of 9×10^{-6} , corresponding to 5278 effective independent comparisons. Analyses were performed with R software (version 2.14.1).³⁷

We searched for allelic associations within any admixture mapping peaks. A peak was defined as a contiguous region with admixture mapping P values within $2 \log_{10}$ orders of the most significant P value. We examined the P values for significance, adjusting for multiple comparisons using both a Bonferroni correction for the number of SNPs within the peak and the effective number of comparisons accounting for linkage disequilibrium between SNPs measured through spectral decomposition.^{38,39} The former correction resulted in a significance threshold α^* value of 8.3×10^{-5} for 601 SNPs; however, accounting for the effective number of independent markers (approximately 174 SNPs) within this region resulted in a less conservative significance threshold α^* value of 2.9×10^{-4} .

Replication of admixture mapping results

We examined ancestry associations in each of the studies containing Latino participants in the EVE Asthma Consortium.¹⁰ Details of the individual studies and estimation of ancestry are described elsewhere^{32,40-42} and in the [Methods](#) section in this article's Online Repository.

Admixture mapping of the region of interest was performed by using logistic regression for case-control studies and the transmission disequilibrium test for trio-based studies. A meta-analysis of the 5 studies was performed using a fixed-effects model in PLINK.⁴³ A 2-sided significance level of .05 was chosen.

Replication of prior GWAS and admixture mapping results

Using the NHGRI Catalog of Published GWASs,⁹ we identified significant association(s) in prior GWASs for asthma (see [Table E1](#) in this article's Online Repository at www.jacionline.org). We then examined the association between the reported SNPs and asthma in the GALA II study, using a 2-sided significance level of .05. SNPs that were not genotyped were imputed with Impute2.^{44,45} For the imputation, we used haplotypes from all available populations in the 1000 Genomes Project phase I integrated variant set release v3 as a reference panel, and we filtered our results to include only those variants imputed with an information score cutoff of greater than 0.3.^{46,47}

We also investigated whether previously reported admixture mapping results in the setting of asthma replicated in the current study by examining the admixture mapping likelihood ratio test results underlying the peaks described in the 2 studies in Torgerson et al^{32,33} in Latinos and African Americans.

RESULTS

The characteristics of the GALA II study population are shown in [Table I](#). Nearly half of the subjects were Puerto Rican (47%), followed by Mexican subjects (31% to 35%) and Latinos of other ethnicities (15% to 18%). The percentage of male subjects was higher in cases than control subjects (55% vs 44%), and the cases

TABLE I. Characteristics of GALA II participants

	Cases	Control subjects	<i>P</i> value
No.	1893	1881	
Age (y)	12 (10-15)	13 (11-16)	<.001
Sex (male)	1046 (55%)	822 (44%)	<.001
Ethnicity			.001
Mexican	596 (31%)	661 (35%)	
Puerto Rican	894 (47%)	894 (48%)	
Mixed	62 (3%)	44 (2%)	
Other	341 (18%)	282 (15%)	
Ancestry			
African	15.3% (5% to 22%)	13.8% (4% to 20%)	<.001
Native American	30% (10% to 50%)	33% (10% to 55%)	<.001
European	55% (42% to 70%)	53% (39% to 70%)	.007
Total IgE (IU/mL)	232 (68-650)	80 (26-248)	<.001
History of atopy	1515 (80.0%)	606 (32.2%)	<.001
Lung function		n = 422	
FEV ₁	90.8 (81.3-100.6)	98.8 (89.8-107.1)	<.001
(% predicted)			
FEF ₂₅₋₇₅	82.4 (65.5-97.9)	100.6 (86.1-113.3)	<.001
(% predicted)			
FVC	95.1 (85.2-105.2)	98.1 (88.6-106.4)	.001
(% predicted)			
FEV ₁ /FVC ratio (%)	84.4 (80-90)	89 (85-93)	<.001
ΔFEV ₁ (% change)	10.3 (4.8-13.7)	—	—

For continuous variables, the median and interquartile range are displayed, and the differences between cases and control subjects were tested with a t test. For categorical variables, the number and proportion of subjects in each category are displayed, and a χ^2 test was used. History of atopy was defined as self-reported allergic rhinitis or eczema.

FEF₂₅₋₇₅, Forced expiratory flow, midexpiratory phase; FVC, forced vital capacity.

had higher total IgE levels on average than the control subjects (234 vs 79 IU/mL). Differences in genomic ancestry between cases (11% African ancestry and 15% Native American ancestry) and control subjects (12% African ancestry and 15% Native American ancestry) were small but statistically significant. Individual-level ancestry proportions are shown in [Fig 1](#) and highlight the variability between and within Latino ethnicities.

Most of the cases have either severe (43%) or moderate persistent (28%) asthma based on medication/severity and symptoms/control classification.⁴⁸ The remaining cases are split between mild intermittent (17%) and mild persistent (12%) asthma.

Admixture mapping

We performed an admixture-mapping study of asthma in 3774 US children of Latino ethnicity ([Fig 2](#) and see [Fig E1](#) in this article's Online Repository at www.jacionline.org). Our most significant admixture mapping association was on chromosome 6p21 near the HLA region. It was centered on *MUC22*, where local ancestry was significantly associated with asthma ($P < 5 \times 10^{-6}$). The 0.05 genome-wide threshold established by means of permutation testing corresponds to a nominal P value of 9×10^{-6} . The permutation-corrected P value for the 6p21 peak was .02, adjusting for multiple testing. As expected with admixture mapping studies, the genome-wide significant region is broad, encompassing a genomic region of approximately 350 kb ([Fig 2, B](#)). Because of this, there is slight genomic inflation ($\lambda = 1.1$; see [Fig E1, A](#)). However, if chromosome 6 is excluded from the analysis, there is no genomic inflation in the remainder of the genome

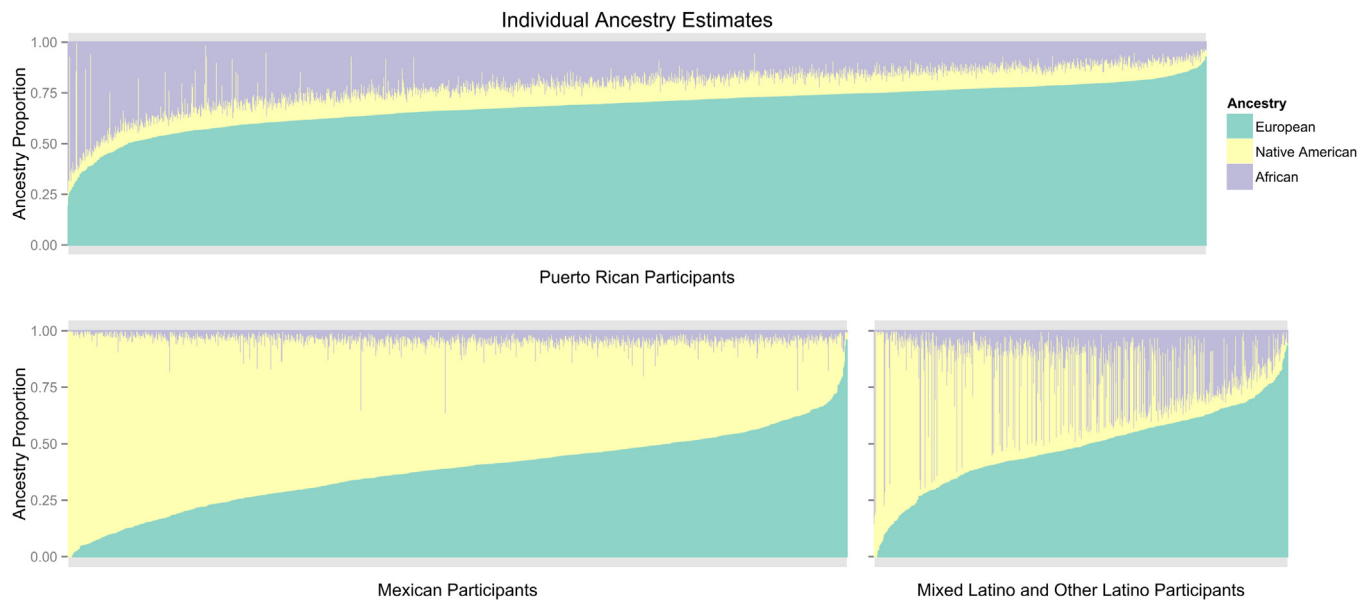


FIG 1. Proportions of individual Native American, European, and African ancestry in the GALA II participants. Each *bar* represents 1 subject. There is substantial variability in individual ancestry both within and between Latino ethnic subgroups.

($\lambda = 0.99$; see Fig E1, B). Within this genomic region, Native American ancestry is associated with lower odds of asthma (odds ratio [OR], 0.87; 95% CI, 0.82-0.93; $P = 1.7 \times 10^{-5}$).

We performed a meta-analysis of the association between Native American ancestry and asthma at this locus among 5 studies of Hispanic/Latino ethnicity from the EVE Asthma Consortium. Our initial results replicated in the same direction (OR, 0.88; 95% CI, 0.79-0.98; $P = .02$). A forest plot of the studies is shown in Fig 3.

We then searched for allelic associations within the peak, which we defined as the contiguous region with admixture mapping P values within 2 log orders of the most significant admixture association (Table E2 in this article's Online Repository at www.jacionline.org). This spanned a 354-kb region that included 13 genes and 1 open reading frame. We tested for allelic associations for the 601 SNPs identified within that region; the most significant association was between asthma and KG_6_31200283 (rs114235219), an SNP in *PSORS1C1* (OR, 0.61; $P = .002$). Given that we tested for 601 allelic associations within the peak, the association did not meet the threshold for significance after adjusting for multiple comparisons, either by using the within-peak Bonferroni correction ($\alpha^* = 8.3 \times 10^{-5}$) or adjusting for the effective number of comparisons accounting for linkage disequilibrium between SNPs ($\alpha^* = 2.9 \times 10^{-4}$; effective number of independent marker loci, 174).^{38,39}

We also tested for allelic associations within the peak in the EVE Asthma Consortium data.¹⁰ There were 399 SNPs within the region in the EVE Asthma Consortium data set. Unfortunately, rs114235219 was not analyzed in the EVE Asthma Consortium data set. The most significant allelic association within the peak was rs3132550, which was also within *PSORS1C1* ($P = 3 \times 10^{-4}$). There were 307 SNPs common to both the GALA II and EVE Asthma Consortium data sets under the admixture-mapping peak. Two of these SNPs were significant in both data sets. Both SNPs were in the *PSORS1C1* gene: rs3094663

(GALA II: OR, 0.85; 95% CI, 0.77-0.95; $P = .003$; EVE: $P = .01$) and rs3130564 (GALA II: OR, 1.30; 95% CI, 1.07-1.58; $P = .009$; EVE: $P = .02$). In both cases the direction of the association was consistent across the 2 studies (the T allele in rs2094663 and the C allele in rs3130564 were associated with increased asthma risk in both studies). A LocusZoom plot of this region is shown in Fig E2 in this article's Online Repository at www.jacionline.org.

Examining previously reported admixture mapping peaks,^{32,33} we found that the peak reported on cytoband 8q12 in both Puerto Ricans and Mexicans replicated in this study (likelihood ratio $P = .03$); Native American ancestry was associated with increased risk of asthma (OR, 1.08; 95% CI, 1.02-1.15; $P = .01$), as in Puerto Ricans in the original study. There was no association with African ancestry at this locus ($P = .8$). We were unable to replicate the association between ancestry and asthma in the admixture mapping peak in cytoband 6q15 found in Mexicans (likelihood ratio $P = .3$) or the association found in cytoband 6q14 in African Americans ($P = .2$). Of the 55 additional nonoverlapping loci identified by Torgerson et al^{32,33} as being enriched for asthma peaks, we found 13 additional loci that were nominally associated (likelihood ratio $P < .05$) with asthma in this study. The results are summarized in Table E3 in this article's Online Repository at www.jacionline.org.

Asthma GWAS

Results of our GWAS are summarized in Fig 4 and Table II. The genomic inflation factor (λ) for allelic association testing in this study is 1.03 (see Fig E3 in this article's Online Repository at www.jacionline.org). Thirty-one SNPs, all in the 17q21 region, were significantly associated with asthma at a genome-wide corrected significance level of less than 5×10^{-8} . A LocusZoom plot of the region is shown in Fig 4, B. The most significantly associated SNP (rs907092) is a synonymous coding mutation in the *IKZF3* gene, which lies within a region of broad linkage

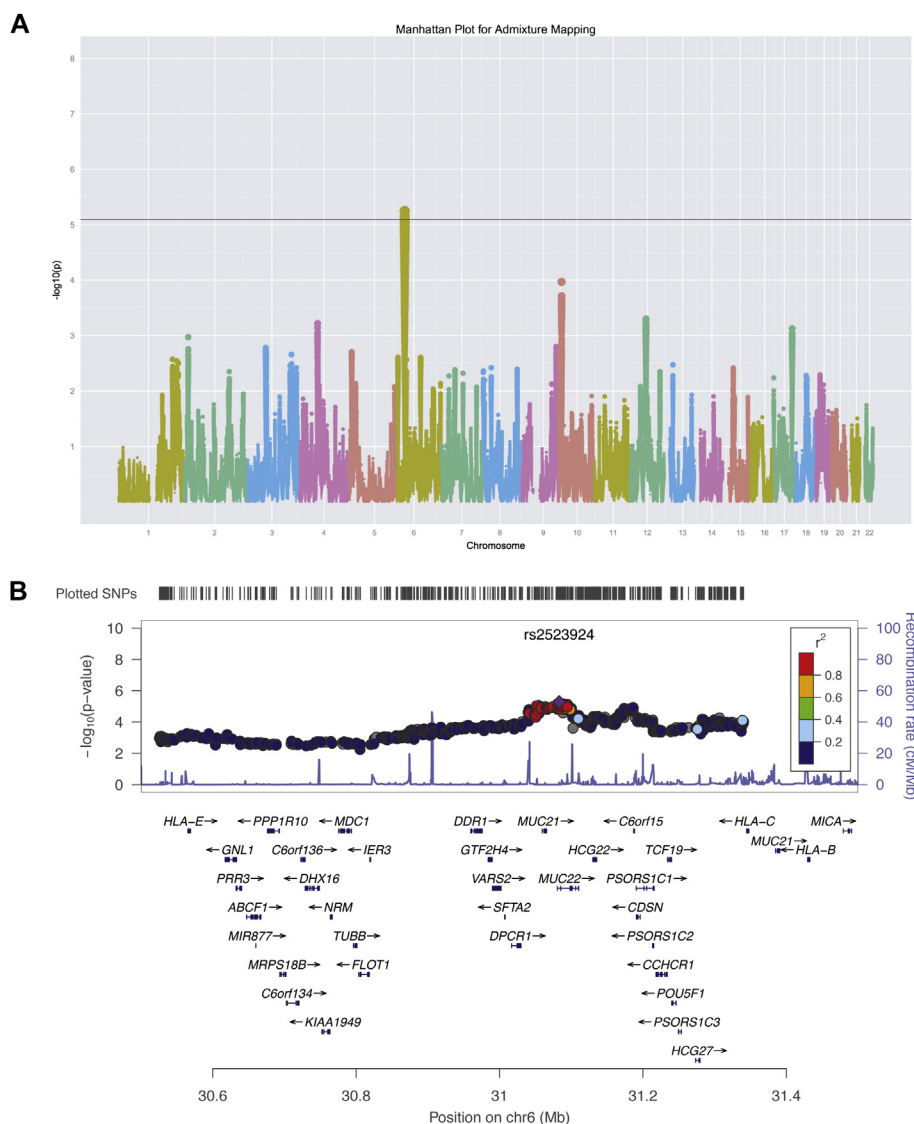


FIG 2. Admixture mapping results. **A**, Manhattan plot of the admixture mapping study showing a significant peak in chromosome 6. The red line represents the fifth percentile of the lowest P value in 10,000 permutations. **B**, LocusZoom plot of the admixture mapping peak, which is centered in the *MUC21* and *MUC22* gene cluster upstream of the *HLA-B* and *HLA-C* genes.

disequilibrium that also includes *ORMDL3*, *GSDMB*, and *ZPBP2*. The minor allele of the *IKZF3* SNP conferred protection against asthma with an OR of 0.67 (95% CI, 0.61-0.75; $P = 6 \times 10^{-13}$). Conditioning on rs907092 removes virtually all associations within the 17q21 region, with only rs12450091, a nonsynonymous coding mutation in *GSDMB*, maintaining nominal significance (OR, 1.23; 95% CI, 1.07-1.42; $P = .005$). Additionally, 6 regions had genotyped SNPs that showed a suggestive association with asthma at a P value of less than 5×10^{-6} (Table II). Examination of SNPs in each of these 6 regions in the EVE Asthma Consortium showed no significant associations with asthma in either the combined study ($P \geq .05$ for all SNPs) or in the subset of studies with subjects of Latino ethnicity ($P \geq .05$). Two additional regions showed suggestive associations with asthma at a P value of less than 10^{-6} by using data imputed with 1000 Genomes (Table II); neither of these was replicated in the EVE Asthma Consortium.

We also examined regions that had previously been associated with asthma in previously reported GWASs (see Table E1). We identified 20 studies reporting results of genome-wide associations in asthma, including 2 that were meta-analyses of other reported studies. Twelve studies reported 1 or more associations, although only 7 of these met strict genome-wide significance with P values of less than 5×10^{-8} . Thirty total SNPs were reported to be significant across the studies at genome-wide significance levels in at least 1 study. Twelve of these SNPs replicated in the current study (Fig 5^{10,49} and see Table E4 [boldface] in this article's Online Repository at www.jacionline.org), although in 4 cases the direction of the observed effect was in the opposite direction than that of the original study (denoted with a minus sign). In 5 cases the direction of the observed effect was in the same direction as that of the original study (denoted with an asterisk), and in 3 cases the original study did not report an OR.

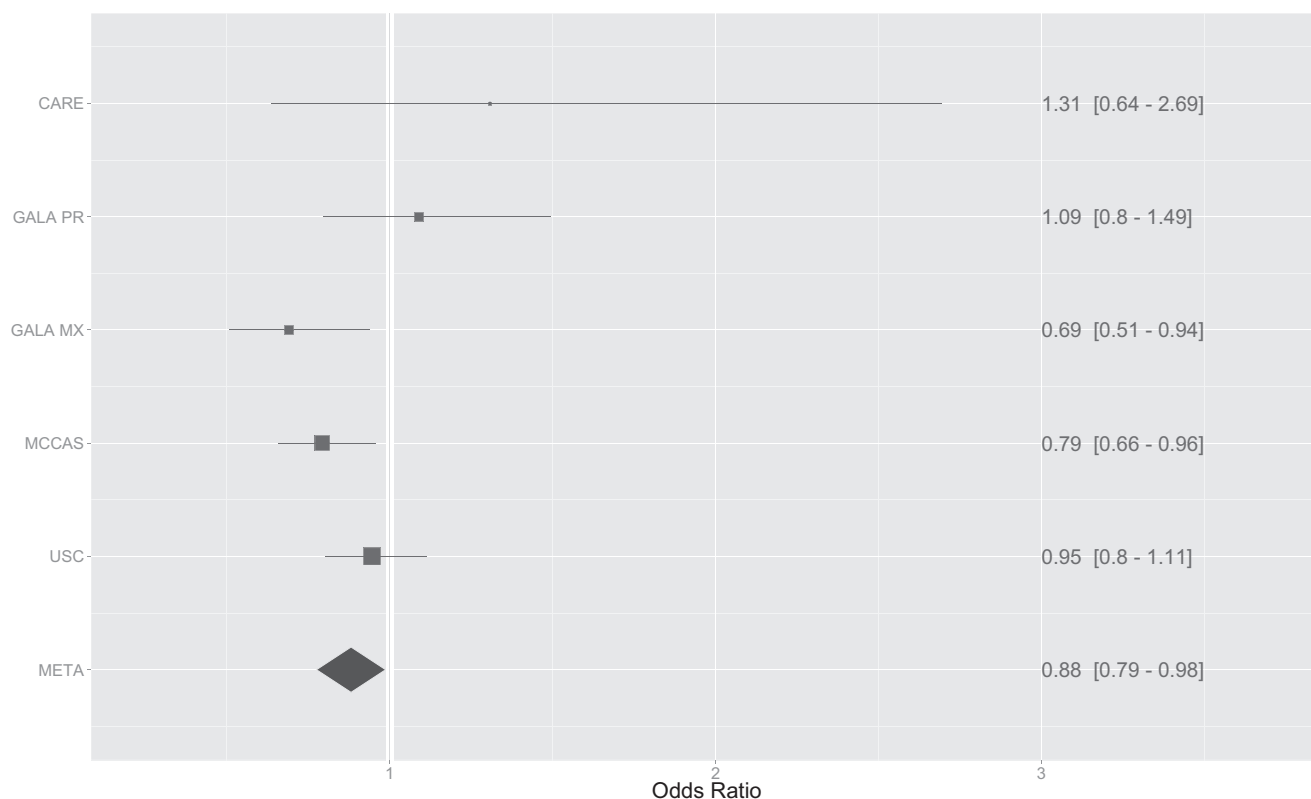


FIG 3. Forest plot of the meta-analysis of the effect of Native American ancestry on asthma in the Latino studies within the EVE Asthma Consortium. ORs and 95% CIs are listed next to each study.

DISCUSSION

We used the complementary techniques of admixture mapping and allelic genome-wide association analysis to identify novel asthma-associated loci and confirm prior genetic associations. In the admixture mapping study we found a genome-wide significant association between asthma and a peak at 6p21. This peak was centered on *MUC22* (likelihood ratio $P < 5 \times 10^{-6}$; permutation-adjusted $P = .02$). Native American ancestry in this genomic region was associated with decreased risk of asthma (OR, 0.87; 95% CI, 0.82-0.93; $P = 1.7 \times 10^{-5}$). This association was replicated in a meta-analysis of 5 studies of Latinos in the EVE Asthma Consortium, in which Native American ancestry was associated with a nearly identical protective effect (OR, 0.88; 95% CI, 0.79-0.98).

Admixture mapping and genome-wide association analysis depend on tagging causal variants through local ancestry blocks and haplotype blocks, respectively. Because admixture mapping relies on relatively large ancestry blocks to identify disease-associated regions of the genome, there are fewer effective comparisons and therefore there is a lower statistical penalty for multiple comparisons. In this case permutation procedures lead to a significance threshold of 9×10^{-6} , suggesting that there are approximately 5000 to 6000 effective comparisons. In contrast, the traditional significance threshold for GWASs is 5×10^{-8} , which is equivalent to approximately 1,000,000 haplotype blocks. Although the reduced multiple comparison penalty associated with admixture mapping makes it an attractive technique for identifying regions associated with a disease, the drawback of the technique is that it results in a larger genomic region of interest, as in our study.

In the current study the region was narrowed down to a 350-kB region centered on a cluster of mucin-producing genes (ie, *MUC21*, *MUC22*, and *PBMUC2*; Fig 2) in the MHC region on chromosome 6. The genes of the mucin family are intriguing candidates for involvement in differential asthma susceptibility. These highly glycosylated macromolecules are broadly expressed in the airway epithelium, where they can be secreted or tethered to airway epithelial membranes and play a critical role in innate immune defense.⁵⁰ The overproduction of mucin has been associated with chronic airway disease, including asthma.⁵⁰ A genetic polymorphism in a promoter in *MUC5B* has also been strongly associated with idiopathic pulmonary fibrosis, suggesting a link between mucin genes and lung disease.⁵¹ *MUC22* itself was identified by searching for genes in the region associated with diffuse panbronchiolitis,⁵² which, like asthma, is an obstructive lung disease. The same study found that *MUC22* was expressed in the lung and in human bronchial epithelial cells differentiated at an air-liquid interface.

Although *MUC22* is an intriguing candidate for association with asthma, we cannot exclude other genes under the admixture mapping peak, including *HLA-C* and *HLA-B*. *HLA-B* has been evaluated as a candidate gene for asthma in 2 studies, with mixed results. In a study of Croatian children with atopic asthma, the HLA-B8 antigen was associated with asthma,⁵³ whereas an earlier study in Greek children with allergic asthma found no specific *HLA-B* alleles associated with asthma.⁵⁴

We attempted to narrow the region of interest by searching for allelic associations under the admixture-mapping peak. We found no allelic associations that met region-wide significance criteria ($\alpha^* = 8.3 \times 10^{-5}$, adjusting for 601 SNPs evaluated) in the

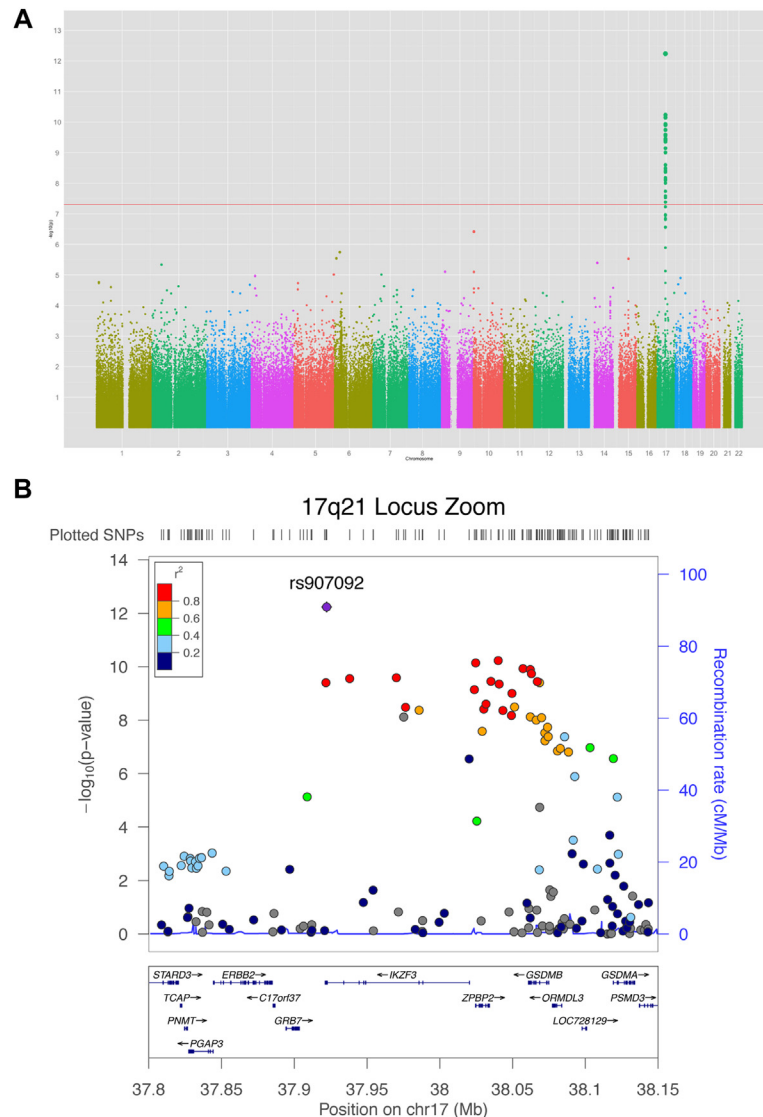


FIG 4. GWAS results. **A**, Manhattan plot of the GWAS showing a genome-wide significant peak at chromosome 17q21. **B**, LocusZoom plot of the 17q21 peak, which shows a broad area of linkage disequilibrium, with the most significant finding centered on the *IKZF3* gene.

TABLE II. Significant and suggestive allelic associations with asthma

SNP	Chromosome	Gene	MAF	OR	CI	P value
rs12328045	2	Intergenic	0.221	1.33	1.18-1.50	4.6×10^{-6}
rs4566922	6	Intergenic	0.440	0.79	0.72-0.87	2.9×10^{-6}
rs3804327	6	<i>DCDC2</i>	0.160	1.37	1.20-1.56	1.8×10^{-6}
rs7086533	10	Intergenic	0.117	1.51	1.29-1.77	3.8×10^{-7}
KG14-33639557	14	<i>NPAS3</i>	0.014	0.36	0.23-0.56	4.0×10^{-6}
rs12594238	15	<i>RAB11A</i>	0.169	0.73	0.64-0.83	3.0×10^{-6}
rs907092	17	<i>IKZF3</i>	0.304	0.67	0.61-0.75	5.7×10^{-13}

SNP	Chromosome	Gene	Info score	MAF	OR	CI	P value
rs148728975	1	<i>VPS13D</i>	0.890	0.032	0.47	0.35-0.63	7.0×10^{-7}
rs10211025	2	Intergenic	0.992	0.214	0.73	0.65-0.83	9.7×10^{-7}
rs3936215	9	<i>BNC2</i>	0.996	0.263	1.38	1.21-1.56	8.6×10^{-7}
rs11252191	10	Intergenic	0.962	0.121	1.54	1.31-1.80	1.3×10^{-7}
rs182722816	12	<i>BTBD11</i>	0.708	0.011	5.70	2.86-11.34	7.0×10^{-7}

Top, Genotyped SNPs; bottom, imputed SNPs.

MAF, Minor allele frequency.

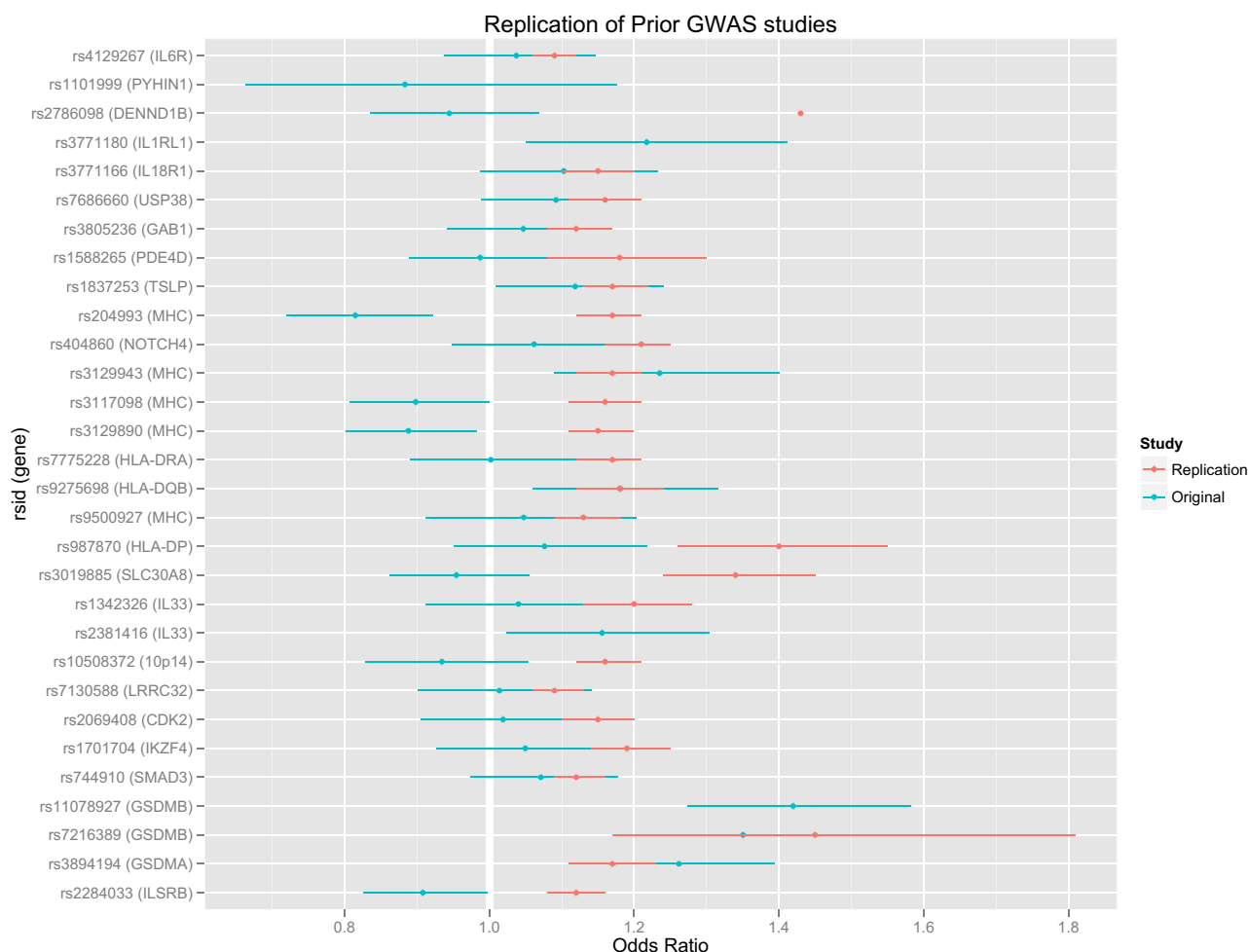


FIG 5. Replication of previous genome-wide findings in the GALA II study. When provided, the original study's effect estimate and CI are shown in *red*, whereas the effect estimate and CI in the present study are shown in *teal*. One study, including 3 SNPs reported only a summary *P* value.¹⁰ A second study, including 1 SNP, reported an OR but not a CI.⁴⁹ +, Replication was in the same direction as the original study; –, replication was in the opposite direction as the original study; ?, Direction of replication could not be assessed.

GALA II study. However, the most significant allelic association within the region in GALA II (rs114235219, KG_6_31200283, $P = .002$) and the most significant SNP in the EVE Asthma Consortium study (rs1312550, $P = 3 \times 10^{-4}$) were both located on *PSORSIC1*, a psoriasis susceptibility candidate,⁵⁵ as were the 2 SNPs that achieved nominal significance in both studies (rs3094663 and rs130564). *PSORSIC1* was identified as a risk locus for systemic sclerosis,⁵⁶ Stevens-Johnson syndrome,^{57,58} Crohn disease,⁵⁹ chronic obstructive pulmonary disease biomarkers,⁶⁰ and Behçet disease⁶¹ in GWASs. A recent study showed that SNPs associated with both asthma and autoimmune diseases have opposite effects on immunopathogenesis.⁶²

Although the evidence suggests that variation within *PSORSIC1* is associated with asthma, it might be necessary to sequence the region to identify the causal variant or variants. The lack of a definitive allelic association within 6p21 might be due to one of several reasons. It is possible that the genotyping platform includes SNPs that poorly tag the causal variants. Although the World Array IV was specifically designed to maximize coverage in Latinos, its coverage of variation in Mexicans in Los Angeles from the 1000 Genomes Project is still imperfect,

especially for uncommon and rare variants. Approximately 80% of SNPs with a minor allele frequency of between 0.05 and 0.1 were tagged with an r^2 value of greater than 0.7, and for rarer alleles, coverage was less than 70%. The Eve Asthma Consortium study was genotyped on a variety of older platforms, which might not have captured variation in non-European populations. Furthermore, the MHC region is highly variable, which increases the probability that the admixture mapping signal was driven by untyped variation. Under these circumstances, admixture mapping has an advantage over allelic and imputed associations because the breadth of ancestry blocks allows for nearly complete coverage across the genome. As long as the allele frequency of the causal variant or variants differs between the ancestral populations, an admixture mapping association might be detectable, even if the untyped causal SNP is in poor linkage disequilibrium with genotyped SNPs. Admixture mapping can also find associations driven by rare variants unlikely to be identified through conventional means. Finally, the lack of allelic association could also be due to a number of variants with small effects clustered in the region, which would not be surprising in the MHC region given its highly variable nature.

We were also able to replicate the admixture mapping association between 8q12 and asthma, as well as a substantial minority (13/55, see Table E3) of the additional admixture mapping peaks previously reported as being enriched for asthma genes.³² This provides additional evidence that admixture mapping is able to uncover novel disease-associated regions.

Our genome-wide association analysis replicated the previously described association between the 17q21 region and asthma. Our reported OR of 0.67 (95% CI, 0.61-0.75) is consistent with the previously reported effect magnitudes at this site. Although our most statistically significant SNP was in *IKZF3* (approximately 50 kb upstream from reported associations in *ORMDL3* and *GSDMB*),¹⁵ there is a high degree of linkage disequilibrium in this region, and our statistically significant results extended across all 3 genes in the region. Moreover, functional work by Verlaan et al⁶³ has shown that genetic variants in this region are associated with domain-wide *cis*-regulatory effects on expression of multiple genes in the region, likely through their effect on chromatin states.

Our evaluation of the previous GWASs described in Table E1 found that nearly half of the associations with asthma nominally replicated in our study. This contrasts with our earlier evaluation of principally candidate gene studies, in which we found that only 17 genes contained SNPs that replicated in at least 1 Latino population of 124 genes that had been previously associated with asthma.⁶ This might be partially explained by the fact that this study, which included nearly 4000 subjects, was better powered to detect associations than our prior study, which included nearly 700 trios. However, it also seems likely that GWAS findings might reflect stronger effect sizes than candidate gene studies, might be less likely to reflect type I error, and might be more likely to confer universal risk across ethnic groups.

Our prior study also showed that for a small number of genes, there was an ethnicity-specific replication pattern with significant statistical heterogeneity between Mexicans and Puerto Ricans. In this study we found no genes with a statistically significant gene-ethnicity interaction. This suggests that associations with asthma uncovered through genome-wide studies are more universal than those from candidate gene studies, which might also explain why they are easier to replicate.

We do note that in 4 of 25 of the associations for which the original direction of effect was reported, the direction of replication was in the opposite direction. Such “flip-flop” associations have been previously reported in an asthma GWAS in which a protective minor allele in European subjects was associated with increased risk in African American participants.⁴⁹ Such a finding was ascribed to differences in the underlying genomic architecture between the 2 study populations, as could occur when the genotyped SNP was correlated through linkage disequilibrium or interaction with a causal variant, but the direction of the correlation varies between populations, resulting in an observed SNP that tags opposite alleles of the causal variant.^{64,65}

It should be noted that our case definition of asthma was based on physician’s diagnosis and self-report of symptoms. Although our cases, on average, had evidence of airway obstruction, with significantly lower FEV₁ and forced expiratory flow, midexpiratory phase values than control subjects (Table I), and many had significant response to albuterol, participants who did not have evidence of bronchodilator response were not required to undergo methacholine provocation. The lack of objective confirmation of the asthma diagnosis might have resulted in misclassification

of some participants. Such misclassification bias, which would be expected to be nondifferential with respect to the predictor, biases the results toward the null, understating our results. Nonetheless, our finding of a strong association at the 17q21 region with comparable, if not larger effect magnitudes than the original study suggests that this effect was likely to have been small.

In summary, our GWAS confirmed significant associations at the 17q21 region in Latinos, and our admixture mapping study identified a novel asthma-associated locus at 6p21 centered on the *MUC22* gene near the MHC region. Fine mapping in this region points to the involvement of the gene *PSORS1C1* in the pathogenesis of asthma. This confirms the advantages of performing admixture mapping studies in diverse populations to identify novel asthma-associated genes.⁶⁶ Follow-up of results from admixture mapping and GWASs with sequencing and functional studies in minority populations carried out in large multiethnic cohorts could help to relate the significance of such findings to disparities in asthma prevalence and severity.

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Key messages

- Admixture mapping of asthma in nearly 4000 Latinos identifies a novel asthma locus on 6p21 that replicates in a meta-analysis of several additional studies of Latinos.
- Fine mapping of this region suggests involvement of the *PSORS1C1* gene with asthma.
- Genome-wide association of asthma confirms the previously identified asthma locus on 17q21 and replicates several genes that had previously been associated with asthma in GWASs.

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