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Case-control admixture mapping in Latino populations enriches for known asthma-associated genes

Dara G. Torgerson, PhD^a, Christopher R. Gignoux, MS^a, Joshua M. Galanter, MD^a, Katherine A. Drake, MS^a, Lindsey A. Roth, MA^a, Celeste Eng, BS^a, Scott Huntsman, MS^a, Raul Torres, BS^a, Pedro C. Avila, MD^b, Rocio Chapela, MD^c, Jean G. Ford, MD^d, José R. Rodríguez-Santana, MD^e, William Rodríguez-Cintrón, MD^f, Ryan D. Hernandez, PhD^g, and Esteban G. Burchard, MD, MPH^{a,g}

San Francisco, Calif, Chicago, Ill, Mexico City, Mexico, New York, NY, and Caguas and San Juan, Puerto Rico

^athe Department of Medicine, University of California San Francisco

^bthe Department of Medicine, Northwestern University, Chicago

^cINER, Mexico City

^dthe Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, New York

^eCentro de Neumología Pediátrica, Caguas

^fVA Medical Center, San Juan

^gthe Department of Bioengineering and Therapeutic Sciences, University of California San Francisco

Abstract

Background—Polymorphisms in more than 100 genes have been associated with asthma susceptibility, yet much of the heritability remains to be explained. Asthma disproportionately affects different racial and ethnic groups in the United States, suggesting that admixture mapping is a useful strategy to identify novel asthma-associated loci.

Objective—We sought to identify novel asthma-associated loci in Latino populations using case-control admixture mapping.

Methods—We performed genome-wide admixture mapping by comparing levels of local Native American, European, and African ancestry between children with asthma and nonasthmatic control subjects in Puerto Rican and Mexican populations. Within candidate peaks, we performed allelic tests of association, controlling for differences in local ancestry.

Results—Between the 2 populations, we identified a total of 62 admixture mapping peaks at a *P* value of less than 10^{-3} that were significantly enriched for previously identified asthma-associated genes (*P* = .0051). One of the peaks was statistically significant based on 100 permutations in the Mexican sample (6q15); however, it was not significant in Puerto Rican subjects. Another peak was identified at nominal significance in both populations (8q12); however, the association was observed with different ancestries.

Conclusion—Case-control admixture mapping is a promising strategy for identifying novel asthma-associated loci in Latino populations and implicates genetic variation at 6q15 and 8q12 regions with asthma susceptibility. This approach might be useful for identifying regions that contribute to both shared and population-specific differences in asthma susceptibility.

Keywords

Admixture mapping; genome-wide association study; asthma; Latino populations; population-specific risk factors

Asthma is a chronic respiratory disease that disproportionately affects different racial and ethnic groups in the United States. In the United States the highest incidence, morbidity, and mortality caused by asthma is found in Puerto Rican subjects, whereas the lowest is found in Mexican subjects. Both of these populations are largely considered Hispanic or Latino in studies of disease, despite their many differences in both social and genetic architecture. Discrepancies in the incidence of asthma between Puerto Rican and Mexican subjects might reside in differences in environmental exposures. However, estimates of the heritability of asthma are around 75%,^{1,2} suggesting there is also a strong genetic component to asthma susceptibility. More than 100 genes have been implicated in asthma susceptibility to date,³ including many identified through recent genome-wide association studies (GWASs), primarily in populations of European origin⁴⁻⁸ but also in African Americans⁹ and Latinos,^{10,11} and from meta-analyses including 3 ethnic groups.¹² Among previously identified asthma-associated genes, several appear to have differential effects in Mexican and Puerto Rican subjects,¹³ suggesting the possibility of population-specific genetic risk factors in different Latino populations.

Admixture mapping is different from a typical GWAS in that the variable compared between cases and control subjects is genetic ancestry at a given location in the genome (ie, local ancestry) rather than genotypes at single nucleotide polymorphisms (SNPs). The method can be applied to any disease whereby risk alleles are at varying frequencies in the ancestral populations of admixed subjects and in theory might have increased statistical power over traditional GWASs.¹⁴ Local ancestry is estimated by using information from multiple SNPs and can therefore capture more of the underlying genetic variation at a locus compared with analyzing tagging SNPs independently on commercial genotyping arrays. Local ancestry might also provide better coverage of rare variation because rare variants are more likely to differ in composition and frequency between populations with varying demographic histories.¹⁵ Admixture mapping with a selection of ancestry informative markers has successfully been used to identify novel genetic risk factors for several common diseases,¹⁶⁻¹⁹ including the 5q23 locus and asthma susceptibility in Puerto Rican subjects.¹⁰ However, methods have been developed to enable fine-scale estimation of local ancestry at individual SNPs,^{20,21} enabling ancestry association testing to identify candidate peaks at a higher resolution compared with using more traditional ancestry informative markers.

We performed case-control admixture mapping in 2 Latino populations to identify novel genetic loci that contribute to asthma susceptibility. We hypothesized that our approach would have increased power over more traditional SNP-based GWASs to obtain statistical significance in smaller sample sizes and that admixture mapping would identify regions enriched for asthma-associated genes.

METHODS

Study subjects

The Genetics of Asthma in Latino Americans (GALA) study²² includes subjects with asthma (probands) and their biological parents recruited from schools, clinics, and hospitals at 4 sites: San Francisco Bay Area, New York City, Puerto Rico, and Mexico City. In all health care centers medical records were reviewed to identify patients with physician-diagnosed mild or moderate-to-severe asthma based on medical billing records (International Classification of Diseases, ninth revision, codes). Patients were contacted to participate in the study if approved by their primary physician. Bilingual and bicultural physicians specialized in asthma were present at all interviews, and all forms and questionnaires for subjects were available in English and Spanish. On the basis of interviews and questionnaire data, subjects were included in the study if they were between the ages of 8 and 40 years with physician-diagnosed mild to moderate-to-severe asthma and had experienced 2 or more symptoms in the previous 2 years at the time of recruitment (including wheezing, coughing, and/or shortness of breath) and if both parents and 4 sets of grandparents self-identified as being of either Puerto Rican or Mexican ethnicity. We note that bronchial hyper-responsiveness was not used as the criterion for inclusion in the study because of the difficulty in assessing these parameters in children. The research was approved by the institutional review boards at each participating center.

Genotyping and quality control

Genotyping of GALA subjects was performed by using the Affymetrix 6.0 GeneChip Array (Affymetrix, Santa Clara, Calif) that contained more than 900,000 SNPs before quality control measures. Data quality control was performed with PLINK,²³ R (<http://www.R-project.org>), and EIGEN-STRAT.^{24,25} Markers were filtered based on 95% call rates, Hardy-Weinberg equilibrium *P* values of greater than 10^{-6} , and unambiguous mapping to the human reference genome. Subjects were filtered based on 95% call rates and consistency between genetic and reported sex (Fstat [a measure of homozygosity] from X-linked markers between -0.2 and 0.3 for female subjects and between 0.8 and 1 for male subjects). SNPs were filtered for linkage disequilibrium (LD) based on r^2 values of greater than 0.5 before performing a principal component analysis in EIGEN-STRAT.^{24,25} Three iterations of outlier removal were performed based on 3 principal components from a principal component analysis including unrelated subjects from 11 phase 3 HapMap populations. Subjects were also filtered based on high or low autosomal heterozygosity (Fstat <0.5 and >-0.2) and unexpected pairwise relatedness (identity by descent $>30\%$) or genetic identity (identity by state $>90\%$).

Statistical analysis

Genomic levels of European, Native American, and African ancestry were evaluated separately for Puerto Rican and Mexican subjects by using the program ADMIXTURE,²⁶ assuming 3 ancestral populations ($K = 3$). Unrelated subjects from the phase 3 HapMap CEU (Utah residents with Northern and Western European ancestry from the CEPH collection [CEPH: Centre d'Etude du Polymorphisme Humain]) and YRI (Yoruba in Ibadan, Nigeria) populations and 88 Native American subjects courtesy of Mark Shriver (25 Aymaran, 24 Quechuan, 14 Nahuan, and 25 Mayan) were included as a reference. Local ancestry was estimated at each SNP independently for Mexican and Puerto Rican subjects by using the program LAMP²⁰ under a 3-population model, assuming 20 generations of admixture. Genomic proportions of European, Native American, and African ancestry were modeled based on the estimated proportions from the program ADMIXTURE²⁶ for each population. Windows were offset by a factor of 0.2 , the cutoff for linkage was set to 0.1 , and a constant recombination rate was set to 10^{-8} (bp)⁻¹. Ancestral allele frequencies at the

729,685 SNPs were estimated from the Hap-Map CEU population to represent European ancestry, the HapMap YRI population to represent African ancestry, and 88 Native American subjects (25 Aymaran, 24 Quechuan, 14 Nahuan, and 25 Mayan) to represent Native American ancestry.

Admixture mapping was performed at each SNP by using logistic regression to compare the proportion of local European, Native American, and African ancestry between cases and control subjects, including the respective global ancestry estimations from the program ADMIXTURE²⁶ as a covariate (asthma ~ local + global ancestry). Global ancestry is therefore the overall ancestry proportions across the genome. Candidate admixture mapping peaks were defined as having a peak association *P* value of less than 10^{-3} to a width at which the *P* value was less than .01. Statistical significance was evaluated by using 100 permutations for each population and ancestry by swapping the case-control labels to maintain the haplotype structure. We used permutations to correct for multiple testing over a traditional Bonferroni correction because of the high correlation between neighboring SNPs. To test for replication between the 2 populations, we extracted the smallest *P* value within each peak and performed a Bonferroni correction for 33 and 29 tests in Mexican and Puerto Rican subjects, respectively (the number of candidate peaks identified in each population).

A Fisher exact test was used to test for an enrichment of known genes within admixture mapping peaks that showed at least 1 positive association with asthma, as compiled from the Genetic Association Database.³ Known genes were defined as those included in RefSeq build 36,²⁷ and their positions in the human genome (build hg18) were downloaded from the UCSC genome browser.^{28,29} Allelic association testing within admixture mapping peaks was performed by using logistic regression, including 2 of the 3 local ancestries as covariates to correct for population structure. Genotypes were imputed from the 1000 Genomes Project (pilot study) by using the program Impute2³⁰ and using the European, African, and Asian haplotypes as a reference.

RESULTS

After quality control, the total number of SNPs included in the study was 729,685, and the total number of subjects was 529 children with asthma (253 Mexican and 276 Puerto Rican subjects) and 347 control subjects (158 Mexican and 189 Puerto Rican subjects). Global proportions of Native American, European, and African ancestry as estimated by using ADMIXTURE were 0.13, 0.67, and 0.20, respectively, in Puerto Rican subjects, and 0.51, 0.45, and 0.05, respectively, in Mexican subjects (Fig 1).

Comparisons of local ancestry between cases and control subjects identified 62 admixture mapping peaks with a difference in local ancestry at a *P* value of less than 10^{-3} (Fig 2 and see Figs E1-E3 and Table E1 in this article's Online Repository at www.jacionline.org). Extending the peak out to a *P* value of less than .01 resulted in an average peak width of 721 Kb, ranging in length from 79 Kb (14q23) to 5 Mb (4p12-4q12). A total of 29 peaks were identified in Puerto Rican subjects and 33 in Mexican subjects (see Table E1). On the basis of permutations, we observed an excess of small *P* values for the comparisons of local African ancestry between Mexican cases and control subjects genome wide (Fig 3 and see Fig E3). African ancestry was significantly lower in Mexican asthmatic patients compared with that seen in control subjects at 6q15 ($P = 4.6 \times 10^{-6}$; odds ratio, 0.56; Fig 4) and was statistically significant based on 100 permutations (permutation $P < .01$). One of the peaks overlapped between the 2 populations (8q12.1) in the direction of decreased African ancestry in Mexican cases compared with control subjects and increased Native American ancestry in Puerto Rican cases compared with control subjects. None of the peaks replicated

in the same direction for any ancestry across the 2 populations after a multiple testing correction (see Table E1).

We observed a significant enrichment of previously identified asthma-associated genes within the 62 admixture mapping peaks ($P = .0051$, Tables I and II). Six (9.6%) of the 62 admixture mapping peaks contained at least 1 gene that had been positively associated with asthma, as compiled from the Genetic Association Database.³ Moreover, 2 additional peaks were within 50 Kb of *CFTR* and *DPP10*, both of which have been previously associated with asthma.^{9,31,32} The enrichment is driven by both Mexican and Puerto Rican subjects independently, with 3 of the genes identified by means of admixture mapping in Mexican subjects ($P = .028$ for enrichment excluding Puerto Rican subjects) and 4 in Puerto Rican subjects ($P = .055$ for enrichment excluding Mexican subjects, see Table E2 in this article's Online Repository at www.jacionline.org). No individual SNP within the 62 admixture mapping peaks was significantly associated with asthma after multiple testing correction, including SNPs imputed from the 1000 Genomes Project. The top associated genotyped SNPs are shown in Tables E3 and E4 in this article's Online Repository at www.jacionline.org.

DISCUSSION

A major challenge in performing GWASs in Latino populations is lower coverage of the genetic variation present in non-European populations on commercial genotyping arrays. This leads to substantially reduced power to identify disease-associated genetic loci in non-European populations. Another challenge is that Latino populations have increased genetic variation and lower linkage compared with European populations, notably in populations with higher proportions of African ancestry. Furthermore, the majority of SNPs on commercial genotyping arrays was ascertained from European populations and might be a poor representation of the genetic variation present on ancestral Native American and African haplotypes present in Latino populations. To overcome these challenges, we applied genome-wide case-control admixture mapping in 2 Latino populations by performing tests of association with local Native American, European, and African ancestry compared with genotypes at individual SNPs. We identified 62 admixture mapping peaks with P values of less than 10^{-3} that were enriched for previously identified asthma-associated genes, demonstrating the ability of admixture mapping to identify novel genetic associations. Furthermore, the enrichment was observed in both Puerto Rican and Mexican subjects, although with different loci, highlighting the advantage of studying multiple and diverse Latino populations to maximize discovery in studies of complex disease. Although additional studies are required to identify and confirm the presence of novel asthma-associated genes within candidate peaks, our results confirm the utility of performing case-control admixture mapping in Latino populations in studies of complex disease.

Two of the admixture mapping peaks likely contain novel genes involved in asthma susceptibility, including 6q15 (a statistically significant peak) and 8q12 (a replicated peak across the 2 populations). African ancestry was protective for asthma in the Mexican sample at 6q15 and 8q12, and Native American ancestry was a risk factor for asthma in the Puerto Rican subjects at 8q12. The peak at 6q15 was statistically significant in Mexican subjects based on permutation tests; however, it was not associated with asthma in Puerto Rican subjects for any of the 3 ancestries compared. Failure to replicate genetic associations across populations are often attributed to differences in statistical power caused by varying patterns of LD between the associated SNP and underlying causal variants.¹² The same principal applies to admixture mapping, making it possible that the lack of replication at 6q15 in Puerto Rican subjects is due to lower LD between local African ancestry and protective alleles compared with the Mexican population. Alternatively, the 6q15 locus might contain

population-specific genetic risk factors, be subject to varying gene-environment or gene-gene interactions across populations, or might simply be a false-positive signal of association. Additional studies are required to distinguish between these possibilities.

The peak at 8q12 was associated with asthma in both Puerto Rican and Mexican subjects, suggesting this locus contains either shared genetic risk factors or shared risk loci, although with different risk alleles. The association was observed at 2 different ancestries, including African ancestry at 8q12 being protective for asthma in Mexican subjects and Native American ancestry being a risk factor for asthma in Puerto Rican subjects. However, this does not exclude the possibility of shared genetic risk factors between the populations because there might be shared risk alleles on both the European and Native American haplotypes in Mexican subjects that are driving the protective association with local African ancestry. The intersection of the 2 peaks is centered on the XK, Kell blood group complex subunit-related family, member 4 (*XKR4*) gene, which contains polymorphisms associated with attention deficit disorder³³ and response to 2 antipsychotic drugs.^{34,35} However, this peak also contains the v-yes-1 Yamaguchi sarcoma viral related oncogene homolog (*LYN*) gene that, when underexpressed in mice, results in severe persistent asthma,³⁶ making it an interesting candidate gene for follow-up studies of asthma susceptibility in human subjects.

One of the admixture mapping peaks (9q21.32-21.33) is flanking a locus previously identified as showing an under-transition of Native American ancestry in children with asthma from Mexico City (9q21.31).¹¹ However, in the current study we observed a deficit of Native American ancestry in Mexican control subjects compared with Mexican cases, which is opposite to what we would have expected based on the results from the study by Hancock et al.¹¹

No individual SNP within the 62 candidate peaks was significantly associated with asthma in either Mexican or Puerto Rican subjects when using standard GWAS techniques after correcting for ancestry and multiple tests, even within peaks containing known asthma-associated genes. However, because we observe a significant enrichment of asthma-associated genes within the peaks, it is likely we failed to identify any associated SNPs because of low statistical power. Causal variants might be common but have small effect sizes, might have large effect sizes but be rare in frequency, or might not be captured through LD on the Affymetrix 6.0 GeneChip (or attained through genotype imputation). Indeed, the majority of asthma-associated SNPs identified have relatively small effect sizes, with odds ratios ranging from 1.1 to 1.3.^{4-9,11,12} Given our sample size of Mexican and Puerto Rican subjects, we expect under an additive disease model to have at most 34% power to detect risk alleles at a *P* value of less than 10^{-5} (assuming a 50% minor allele frequency and a relative risk of 1.3). Thus we did not expect to identify even previously reported asthma-associated SNPs.

Furthermore, admixture mapping specifically targets genetic loci with risk alleles that differ in frequency between the ancestral populations. Given that the majority of genetic variation that differs between populations resides in rare variation,¹⁵ the candidate peaks we identified might be enriched for loci containing rare variation that contributes to differences in asthma susceptibility between populations. Rare causal alleles are not likely to be represented by the predominantly common SNPs on the Affymetrix 6.0 GeneChip and require an even larger sample size to reach statistical significance. Therefore additional studies involving the resequencing of candidate peaks are required to test for the presence of any associated variants within these regions.

Lastly, it is important to note that we identified a statistically significant admixture mapping peak despite a smaller number of cases and control subjects than is typically required for a

sufficiently powered GWAS based on allelic association testing. Admixture mapping in Latino populations might offer increased statistical power over traditional GWASs in smaller sample sizes by increasing coverage of the genome through admixture LD and reducing the number of statistical comparisons. Overall, our results demonstrate the utility of case-control admixture mapping for performing GWASs in Latino populations and implicate the 6q15 and 8q12 regions as being involved in asthma susceptibility. Furthermore, admixture mapping has the potential to identify both shared (8q12) and population-specific (6q15) genetic risk factors that contribute to asthma susceptibility.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations used

CEU	Utah residents with Northern and Western European ancestry from the CEPH collection (CEPH: Centre d'Etude du Polymorphisme Humain)
GALA	Genetics of Asthma in Latino Americans
GWAS	Genome-wide association study
LD	Linkage disequilibrium
SNP	Single nucleotide polymorphism
YRI	Yoruba in Ibadan, Nigeria

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Key messages

- Case-control admixture mapping identified 62 candidate peaks enriched for previously identified asthma-associated genes, highlighting the utility of this method to identify novel candidate regions in Latino populations.
- African ancestry was significantly associated with asthma in Mexican subjects at 6q15 ($P = 4.6 \times 10^{-6}$; odds ratio, 0.56) but not in Puerto Rican subjects.
- Ancestry at 8q12.1 was associated with asthma in both populations but with African and Native American ancestry in Mexican and Puerto Rican subjects, respectively.

FIG 1.

Global ancestry proportions of Mexican (**A**) and Puerto Rican (**B**) subjects in the GALA study estimated by using ADMIXTURE ($K = 3$); included are the HapMap CEU, YRI, and 88 Native American subjects kindly provided by Mark Shriver (25 Aymaran, 24 Quechuan, 14 Nahuatl, and 25 Mayan). Mexican subjects were, on average, 51% Native ancestry, 5% African ancestry, and 45% European ancestry, whereas Puerto Rican subjects were, on average, 13% Native ancestry, 20% African ancestry, and 67% European ancestry.

FIG 2.

Admixture mapping in GALA Puerto Rican and Mexican subjects. The Manhattan plot for comparisons of local African ancestry in Mexican cases and control subjects shows a statistically significant peak on 6q15 based on 1000 permutations. Candidate admixture mapping peaks were defined as those with association P values of less than 10^{-3} (*gray line*). The complete set of Manhattan plots for all ancestries/populations is shown in Figs E1 and E2.

FIG 3.

Admixture mapping quantile-quantile plot for genome-wide ancestry association testing of local African ancestry in Mexican cases and control subjects. Red *points* show the observed versus expected *P* values, and the *gray shaded area* represents the 95% quantile range of the permutations. Quantile-quantile plots for the other comparisons are shown in Fig E3.

FIG 4.

Admixture mapping peak on 6q15 in Mexican subjects. African ancestry (*left y-axis*) is significantly lower in cases (*red line*) compared with control subjects (*blue line*, $P = 1.3 \times 10^{-6}$) at 6q15. The $-\log_{10}(P \text{ values})$ for genotyped (*black circles*) and imputed SNPs from the 1000 Genomes Project (*gray circles*) are indicated on the *right y-axis*. The location of genes within the peaks are indicated with *green arrows* indicating the direction of transcription.

TABLE I

Admixture mapping peaks containing genes previously associated with asthma

Population	Band	Ancestry	Mean cases	Mean control subjects	P value	Associated genes
Mexican	2q14.1	European	0.63	0.89	6.2×10^{-4}	<i>DPP10</i> *
Mexican	4q22.1	African	3.3	1.9	5.2×10^{-4}	<i>FAM13A</i>
Mexican	5q32-q33.1	Native American	0.91	1.1	7.5×10^{-4}	<i>SPINK5 SCGB3A2</i>
Mexican	7q31.2-31.31	Native American	0.58	0.83	1.3×10^{-4}	<i>CFTR</i> *
Puerto Rican	4q13.1	African	1.4	0.89	6.8×10^{-4}	<i>MUC7</i>
Puerto Rican	5q31.2	Native American	1.3	1.9	6.9×10^{-4}	<i>EGR1</i>
Puerto Rican	5q33.3	Native American	1.2	0.7	6.5×10^{-4}	<i>IL12B</i>
Puerto Rican	7p11.2	Native American	0.87	1.4	5.0×10^{-4}	<i>EGFR</i>

Local ancestry was adjusted by genomic ancestry at the individual level before calculating the mean.

* Gene is within 50 Kb of a candidate peak.

TABLE II

Fisher exact test comparing the proportion of genes showing a previous positive association with asthma in at least 1 study in the Genetic Association Database for genes within and outside of admixture mapping peaks ($P = .0051$)

	No. of genes within peaks	No. of genes outside peaks
Asthma-associated genes	7	144
Other genes	299	21,716
Percentage	2.3%	0.66%

The number of unique genes was compiled from the National Center for Biotechnology Information RNA reference sequences collection (RefSeq). The number of genes within peaks does not include *CFTR* or *DPP10*, which were both within 50 Kb of a peak.