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Genome-wide Ancestry Association Testing Identifies a Common European Variant on 6q14.1 as a Risk Factor for Asthma in African Americans

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Abstract

Background—Genetic variants that contribute to asthma susceptibility may be present at varying frequencies in different populations, which is an important consideration and advantage for performing genetic association studies in admixed populations.

Objective—To identify asthma-associated loci in African Americans.

Methods—We compared local African and European ancestry estimated from dense single nucleotide polymorphism (SNP) genotype data in African American adults with asthma and non-asthmatic controls. Allelic tests of association were performed within the candidate regions identified, correcting for local European admixture.

Results—We identified a significant ancestry association peak on chromosomes 6q. Allelic tests for association within this region identified a SNP (rs1361549) on 6q14.1 that was associated with asthma exclusively in African Americans with local European admixture (OR=2.2). The risk allele is common in Europe (42% in the HapMap CEU) but absent in West Africa (0% in the HapMap YRI), suggesting the allele is present in African Americans due to recent European admixture. We replicated our findings in Puerto Ricans and similarly found that the signal of association is largely specific to individuals who are heterozygous for African and non-African ancestry at 6q14.1. However, we found no evidence for association in European Americans or in Puerto Ricans in the absence of local African ancestry, suggesting that the association with asthma at rs1361549 is due to an environmental or genetic interaction.

Conclusion—We identified a novel asthma-associated locus that is relevant to admixed populations with African ancestry, and highlight the importance of considering local ancestry in genetic association studies of admixed populations.

Keywords

asthma; population structure; genome-wide association study; admixture mapping; ancestry association testing; admixed populations; African Americans; Puerto Ricans

Introduction

Asthma is a chronic respiratory disease characterized by airway inflammation, airway obstruction, and episodic respiratory symptoms. Although it is a common disease shared across diverse human populations, African Americans have higher rates of asthma, steroid dependency, and a more limited response to treatment as compared to European Americans¹, which may not be attributable to differences in access to healthcare alone^{2,3}. Although there is a strong environmental component, recent estimates of the heritability of asthma are around 75%^{2,3}, suggesting there are important genetic contributions to asthma susceptibility. As of 2006, polymorphisms in over 100 genes had been associated with asthma and asthma-related phenotypes, with more than 40 genes showing replication in at least one independent population⁴. More recently, numerous genome-wide association studies (GWAS) have identified additional candidate genes and genetic loci that contribute to asthma susceptibility^{5–11}. While the majority of GWAS have focused largely on populations of European origin^{6,7,9–11}, candidate loci have also been identified through GWAS in African Americans⁸, Puerto Ricans¹² and Mexicans⁵.

One of the challenges of performing genetic association studies in African Americans is avoiding confounding resulting from complex population structure. Due to differences in demographic histories, populations of African origin exhibit higher levels of genetic variation, reduced linkage disequilibrium (LD), and proportionally more rare variation as compared to European populations¹³. The colonization of the Americas by Europeans, followed by the African slave trade, resulted in populations with “mixed” genetic ancestry in the Americas (admixed populations), including African Americans that have on average ~20% European ancestry¹⁴. Because European admixture is relatively recent, recombination has yet to break up the European and African haplotypes completely, resulting in long haplotypes specific to each ancestral background. Identifying regions whereby admixture varies between cases and controls is important to avoid confounding due to population structure in genetic association studies. However, it can also be used to perform admixture mapping to identify novel disease susceptibility loci¹⁵.

Admixture mapping in African Americans has proven to be a valuable tool for identifying novel risk alleles for diseases such as multiple sclerosis¹⁶, prostate cancer¹⁷, obesity¹⁸, and hypertension¹⁹. In theory, admixture mapping is applicable to genetic studies of admixed populations for any disease in which risk alleles vary in frequency between in the ancestral populations, such that detecting differences in ancestry can identify regions that contain variants associated with disease. Admixture mapping has previously implicated the 5q23 locus in asthma susceptibility in Puerto Ricans¹², indicating this method of analysis is relevant to genetic studies of asthma. A potential advantage of admixture mapping is to increase the power of genetic association studies, for example by identifying candidate regions to look for allelic associations to reduce the multiple-testing burden (e.g.²⁰), and by increasing genomic coverage through long-range tagging of single nucleotide polymorphisms (SNPs) with local ancestry (ancestry-LD). While admixture mapping has historically utilized a set of ancestral informative markers (AIMs), methods are available to identify local ancestry using dense SNP genotype data^{21, 22}, enabling genome-wide ancestry association testing at a higher resolution and accuracy over more traditional admixture mapping.

To identify genetic loci that contribute to asthma susceptibility in African Americans, we performed genome-wide ancestry association testing using estimates of local ancestry inferred from ~1 million genotyped SNPs in 355 African American adults with asthma and 444 African American adult controls. We performed allelic tests of association within an ancestry association peak on chromosome 6q while adjusting for local ancestry, and identified a polymorphism on 6q14.1 that is associated with asthma exclusively in African American adults with local European admixture. We replicated both the ancestry and allelic association in the same direction in an independent population of Puerto Ricans. The risk allele is common in the HapMap CEU but absent in the HapMap YRI, suggesting the allele is present in African Americans due to recent European admixture.

Methods

Study Subjects

This research was approved by the Institutional Review Boards at the University of Chicago and Wake Forest University. Adults and children with asthma and adult non-asthmatic controls were recruited as part of the Collaborative Study on the Genetics of Asthma (CSGA)²³ and the Severe Asthma Research Program (SARP)^{24, 25}. The CSGA study sample includes subjects recruited at the University of Chicago, University of Maryland, and Wake Forest University from a) families ascertained through affected sib pairs, b) trios of affected children and their parents, c) adults and children with severe persistent asthma, and d) non-asthmatic control subjects (over the age of 18 years). The multicenter SARP study

sample includes mild to severe asthmatics and controls. In both studies, subjects were carefully characterized using similar procedures^{23–25}. Besides having a physician's diagnosis of asthma, all asthma cases met objective criteria of bronchial hyperresponsiveness after a methacholine challenge, or reversibility to an inhaled bronchodilator. Subjects with a significant history of smoking were excluded.

Because 65% of the cases and 100% of the controls were over 17 years of age at time of recruitment, we limited our study to those subjects to reduce phenotypic heterogeneity. Among these subjects the mean age at enrollment was 36 years of age, the mean age of onset was 14 years of age, and 70% of the participants were female. All of the primary subjects included in the current study were self-reported African Americans, however we also present results from self-reported European Americans who participated in the CSGA/SARP studies (complete results will be presented elsewhere).

Genotyping and QC

Genotyping was performed on the Illumina 1Mv1 platform, with individual genotypes called using clustering algorithms as implemented in the BeadStudio software by Illumina. Following an initial round of clustering and genotype calling, 3 additional rounds of clustering was performed alternating between filtering of subjects and markers based on call rates < 95% and < 90%, respectively. SNPs were then filtered for "Cluster_Sep" values < 0.3 (score of genotype cluster separation), and for "GenTrain" scores < 0.75. The resulting number of SNPs was 1,033,467 prior to additional quality control measures.

Data quality control was performed using PLINK²⁶, R (<http://www.R-project.org>), and Eigenstrat^{27, 28}. SNPs were filtered based on 95% call rates, Hardy-Weinberg equilibrium p-values > 10⁻⁶, consistency in allele frequency from the HapMap ASW (chi-square p-value > 10⁻⁶), and < 5 heterozygous genotype calls in males for X-linked markers. The total number of markers following QC checks was 1,026,072. Subjects were filtered based on >95% call rates, consistency between genetic and reported sex (Fstat from X-linked markers between -0.2 and 0.3 for females, and between 0.8 and 1 for males), consistency in self-reported ethnicity (no obvious clustering with the HapMap CEU in a principal component analysis, 3 iterations of outlier removal on the first 3 principal components), and high or low heterozygosity (Fstat < 0.5 and > -0.2). Samples were also flagged for unexpected pairwise relatedness (IBD > 30%) or genetic identity (IBS > 90%). The total number of subjects remaining following QC was 355 cases and 444 controls.

Population Structure and Ancestry Association Testing

To evaluate the extent of global European admixture, genotypes were oriented to the plus strand following the removal of 5,634 A/T and C/G SNPs, and merged with the 11 phase 3 HapMap populations using PLINK. SNPs were filtered for linkage disequilibrium based on r² values > 0.5, and a principal component analysis was conducted in Eigenstrat with no outlier iterations (outliers were removed in the QC process). Global estimates of European admixture were estimated from the first principal component that separated the HapMap CEU and YRI populations (see Figure E1 in the Online Repository). Global ancestry was compared between cases and controls using logistic regression in R.

Segments of local European ancestry were estimated using LAMP - Local Ancestry in admixed Populations²¹. Admixture in African Americans was modeled under 7 generations of admixture with a 2-population model of 81% ancestry from Africa and 19% ancestry from Europe as estimated based on the first principal component in the PCA analysis described above. 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. Ancestry association testing

was performed using logistic regression in R by comparing the estimated number of African chromosomes in cases vs. controls at individual loci including global ancestry as a covariate (asthma ~ local ancestry + global ancestry). A total of 100 permutations were performed genome-wide by shuffling the case/control labels to maintain the haplotype structure of African and European segments. Statistical significance was evaluated based on the distribution of p-values expected at the genomic level under the null hypothesis of no significant differences in local ancestry between cases and controls. An ancestry association peak was defined as a window of unadjusted p-values < 0.01.

Association Testing

Tests of allelic associations were performed for SNPs within ancestry association peaks using logistic regression in R while controlling for local ancestry at each SNP (asthma ~ genotype + local ancestry). Using this approach we tested for allelic associations with asthma beyond that explained by local population structure. We also ran a stratified comparison to identify allelic associations specific to one ancestral background at a locus by separating cases and controls into three groups: 1) individuals with two chromosomes of European ancestry, 2) individuals with one chromosome of European ancestry and one chromosome of African ancestry, and 2) individuals with both chromosomes of African ancestry. The distribution of allelic association p-values was compared to a uniform distribution using the function qqplot in R. Tests of allelic association were performed in a similar manner for the European American samples for SNPs within the ancestry association peak identified in the African American sample. Imputation of genotypes from the 1000 Genomes Pilot Project²⁹ was performed using IMPUTE2^{30,31} using all of the European and African populations as a reference.

Replication Studies

The Genetics of Asthma in Latino Americans (GALA) Study included 277 asthma cases and 191 asthma controls recruited from schools, clinics and hospitals in New York City and Puerto Rico whose parents and 4 sets of grandparents self-identified as Puerto Rican. The research was approved by the Institutional Review Board at the University of California San Francisco. Although the GALA study also includes individuals of Mexican ancestry, we restricted our analysis to Puerto Rican individuals because they have a higher degree of African ancestry³². Asthma was defined as a physician diagnosis of mild to moderate asthma, having had two or more symptoms of asthma in the previous two years at time of recruitment (wheezing, coughing and/or shortness of breath), and current use of asthma medications. The mean age at enrollment was 14 years of age, the mean age of onset was 3.2 years of age, and the proportion of females was 45%. Controls were recruited from the same locations and determined to be negative for a history of asthma based on a standardized questionnaire.

Subjects were genotyped at > 900,000 SNPs using the Affymetrix 6.0 GeneChip Array and filtered using standard QC procedures including >95% call rate and consistency with Hardy-Weinberg equilibrium ($p > 10^{-5}$). Local ancestry was estimated using LAMP in a similar manner as for the CSGA/SARP subjects, but with 20 generations of admixture, and a 3-population model with genomic ancestral proportions of 16% African ancestry, 66% European ancestry, and 18% Native American ancestry as estimated using ADMIXTURE³³. Replication of ancestry and allelic association for the top associated SNP was performed using logistic regression in R, including global African ancestry as a covariate in tests of ancestry association, and local African and European ancestry as covariates in tests of allelic association (to account for 3 different local ancestries).

Results

Following subject and marker quality control, our study included a total of 1,026,072 genotyped SNPs in 355 African American adults with asthma, and 444 African American non-asthmatic controls. Estimates of global African ancestry among self-reported African Americans ranged from 30–100% African ancestry (mean=81%) based on the first principal component using the HapMap CEU and YRI individuals as a reference for European and African ancestry (see Figure E1 in the Online Repository). However, there was no significant difference in the distribution of global European admixture between cases and controls (logistic regression p -value=0.78, see Figure E2 in the Online Repository), suggesting that the proportion of European and African ancestry when pooled across the genome was balanced between cases and controls. Mean local ancestry as estimated using LAMP was highly correlated with global estimates of ancestry based on the first principal component in Eigenstrat (see Figure E3 in the Online Repository). Patterns of local European ancestry were variable both within and between individuals as expected under a model of more recent European admixture (see Figure E4 in the Online Repository).

We identified four ancestry association peaks suggestive of differences in local ancestry between African American cases and controls ($p < 0.01$) (Figure 1A). Two of the peaks were on chromosome 4 (4p and 4q) in the direction of increased European ancestry in cases ($p = 4.0 \times 10^{-3}$, $p = 4.4 \times 10^{-4}$, Figure 1B), and two of the peaks were on chromosomes 6 (6q) and 7 (7p) in the direction of increased African ancestry in cases ($p = 2.5 \times 10^{-4}$ and $p = 2.7 \times 10^{-3}$ respectively) (Figure 1C). The ancestry association peak on 6q reached genome-wide significance based on 100 permutations across ~1,000,000 SNPs, (permutation p -value < 0.01).

We then tested for allelic associations with asthma for 9,266 SNPs within the ancestry association peak on 6q while adjusting for local ancestry (Figure 1D). The most significant SNP, rs1361549, had an association p -value that passed Bonferroni correction for 9,266 tests at 6q14.1 ($p = 1.87 \times 10^{-6}$, $\alpha_{9,266} = 5.4 \times 10^{-6}$, Figure 2). Notably, rs1361549 is only weakly associated with asthma unless local ancestry is included as a covariate in the logistic regression model ($p = 0.047$ with no covariates, $p = 0.053$ with global ancestry from PC1 as a covariate) (Table 1). Furthermore, when we correct for both local and global ancestry the association at rs1361549 is moderately increased ($p = 6.6 \times 10^{-7}$). Comparisons of allele frequencies at rs1361549 for cases and controls with and without local European admixture indicated that the signal of association is exclusive to African American individuals with at least one chromosome of European ancestry at rs1361549 (Table 2). The frequency of the minor allele in African American cases heterozygous for European ancestry at rs1361549 was 27%, as compared to only 14% in controls heterozygous for European ancestry (Odds Ratio=2.2, 95% confidence interval=1.4–3.5). Although the numbers of individuals is small, the frequency of the risk allele was 63% in 8 African American cases homozygous for European ancestry, as compared to 27% in 22 African American controls homozygous for European ancestry (Odds Ratio=4.3, 95% confidence interval=1.2–18). In individuals homozygous for local African ancestry, rs1361549 was monomorphic in both cases and controls. Stratified tests of allelic association based on local ancestry (see methods) for 287 SNPs within 500 Kb of rs1361549 identified additional SNPs that show similar, but weaker signals of association exclusively for African American subjects with local European ancestry that were not identified in the logistic model. None of the SNPs within 500 Kb of rs1361549 were significantly associated with asthma in the European American subjects (Figure 1D, and Table E1); however, we replicated both the ancestry association ($p = 5.3 \times 10^{-3}$) and allelic association ($p = 0.025$) with asthma at rs1361549 in Puerto Ricans in GALA (Table 3), which was attributable to individuals that were heterozygous for African and either Native American or European ancestry (non-African ancestry) (Table 2).

Although we attempted to replicate the signal of allelic association in African Americans in the Genomic Research on Asthma in the African Diaspora (GRAAD) study and in families from Barbados⁸, rs1361549 was not genotyped directly and imputed genotypes were of low quality (imputation $R^2=0.43$).

Lastly, we performed *in silico* fine mapping by imputing 7,124 SNPs from the 1000 Genomes Project within 500 Kb of rs1361549, and identified an additional variant (rs4706896) that shows a similarly strong signal of association when adjusting for local ancestry ($p=2.7\times 10^{-6}$) (Figure 3). The SNP is 49 Kb more proximal to the centromere than rs1361549, however it is in high LD ($r^2=0.97$ in the HapMap CEU) and shows similar patterns of allele frequencies as rs1361549 (frequency in the CEU = 41%, frequency in the YRI = 0%).

Discussion

In this study we performed genome-wide ancestry association testing using genotypes from ~ 1 million SNPs in 344 African American adults with asthma and 444 non-asthmatic controls. Given the relatively low effect sizes of asthma-associated SNPs identified in previous GWAS⁵⁻¹¹, our study was underpowered for performing a traditional GWAS. We therefore utilized genome-wide ancestry association testing to identify ancestry association peaks, and then performed allelic tests of association within the associated region. This strategy significantly lowers the multiple testing burden inherent in GWAS. We identified a significant ancestry association peak at 6q14.1 that is more likely to have underlying differences in patterns of allele frequencies between cases and controls as compared to a randomly selected region of the genome. By focusing on SNPs within this peak, we identified an intergenic SNP, rs1361549, with an association p-value that surpassed Bonferroni correction for a reduced number of tests after adjusting for local ancestry.

The signal of association with asthma at rs1361549 is highly dependent on correcting for local rather than global ancestry. We observed only a minor difference in the allele frequency between cases and controls prior to including local ancestry as a covariate in the logistic model. The risk allele is common in Europe (frequency in the CEU = 42%) and either rare or absent in West Africa (frequency in the YRI = 0%), highlighting the importance of adjusting for population structure in allelic tests of association at this SNP. Admixture association testing identified increased African ancestry in the cases as compared to the controls at 6q14.1, and therefore under the null hypothesis of no association the frequency of the minor allele is expected to be higher in the controls (due to increased European ancestry, where the allele is more common). By including local ancestry in the model this prior expectation was taken into consideration, leading to the identification a signal of association with asthma at rs1361549 that would have otherwise gone unnoticed.

By comparing the frequency of rs1361549 between cases and controls after stratifying by local ancestry, we discovered that the frequency of the risk allele is only increased in African Americans cases with local European admixture. Moreover, we found additional, but weaker signals of association at SNPs more proximal to the centromere that showed a similar pattern to rs1361549 (Table E2). However, neither rs1361549 nor any of the nearby SNPs showed an association with asthma in European Americans, suggesting that the underlying causal variant (or variants) only increases the risk of asthma when present on an African genetic background. In support of this, we replicated our signal of association at rs1361549 in Puerto Ricans in the same direction (Table 3), and similarly found that the frequency of the risk allele was predominantly increased in cases that were heterozygous for local African and non-African ancestry.

Although the number of individuals was small, rs1361549 also showed a signal of association with asthma in African American individuals that were homozygous for local European ancestry, however we found no significant association in Puerto Ricans homozygous for non-African ancestry (either Native American or European). Our findings suggesting the association at rs1361549 could be due to an environmental or genetic interaction with a variant (or variants) more common in Africa. Under the latter scenario, we hypothesize that the risk of asthma may only be increased for admixed individuals who carry both the minor allele at rs1361549 and an unobserved allele that is more common on African haplotypes. However, because we observed an increase in African ancestry in cases compared to controls in both African Americans and Puerto Ricans at this locus, we cannot rule out the possibility there are additional untyped and untagged variants that increase the risk of asthma for individuals that are homozygous for African ancestry that is independent of the effect of rs1361549.

In silico fine-mapping identified one additional SNP in high LD with rs1361549 that showed a similar pattern of association (rs4706896). Both rs1361549 and rs4706896 lie within an intergenic region that is ~ 170 Kb downstream of the closest annotated gene, *FAM46A*. *FAM46A* codes for a protein of unknown function, however it is expressed in the lung and various immune-related cells including B-cells, natural killer cells, and monocytes³⁴ suggesting a potential avenue for involvement in asthma susceptibility. In addition, there are two reported transcripts within 36 Kb of rs1361549 in GenBank, including AF130064, a cDNA identified from fetal liver cells, and AK126547, a cDNA identified from mesenchymal cells. Patterns of linkage disequilibrium suggest the underlying causal variant(s) may be more proximal to the centromere (see Figures E5 and E6 in the Online Repository). Within this region there are multiple candidate eQTLs³⁵ in weak LD with rs1361549 ($r^2 > 0.3$) that show an association p-value < 0.001 (Table E1), including eQTLs for Fc receptor-like and mucin-like 1 (*FCRLA*), proline rich 15 (*PRR15*), and cannabinoid receptor 2 (macrophage) (*CNR2*). The function of *PRR15* is unknown, however *FCRLA* is involved in B-cell development, and increased levels of the FCRLA protein has been found in patients with rheumatoid arthritis³⁶. *CNR2* is a cannabinoid/G-protein coupled receptor that is also expressed in B-cells and may function to mediate immunoglobulin class switching from IgM to IgE³⁷. IgE production is known to play an important role in asthma; however, additional characterization of the genetic variation within this region is required to determine the underlying mechanism behind the association of rs1361549 with asthma susceptibility.

An important consideration is whether estimates of local European admixture around rs1361549 were accurately assigned. In support of this, we found that rs1361549 was monomorphic in both African Americans and Puerto Ricans that were homozygous for African ancestry, consistent with our expectations based on the SNP being absent in the HapMap YRI. Given our estimate that African Americans in CSGA/SARP have ~19% European ancestry, if the variant were attributable solely to European admixture we would expect the frequency of the minor allele to be around 8% given a frequency of 42% in the HapMap CEU ($42\% \times 19\%$ European ancestry), which is similar to what we observe (7.8%). Therefore, our results suggest that rs1361549 is present in African Americans predominantly due to recent European admixture, and that estimates of local ancestry at this locus are accurately distinguishing between African and European chromosomes.

From a methodological viewpoint, our results highlight the importance of considering local ancestry in genetic association studies of admixed populations. This was accomplished by including local ancestry as a covariate in allelic tests of association. However, it is foreseeable that in some situations a stratified analysis may be necessary to identify allelic associations that are unique to a specific genetic background. For example, we identified a

hidden signal of association at rs1361549 when using local ancestry as a covariate in a logistic model, in part due to the SNP being monomorphic on the African chromosomes that minimized the signal to noise ratio. Signals of association at nearby SNPs that were polymorphic on both African and European chromosomes were exclusively identified in a stratified analysis, as signals were weakened in the combined subjects due to the majority of individuals having local African ancestry (and the SNPs not being associated in these individuals). Although we did not address the issue of false positive associations in our current study, it is important to note that correcting for local ancestry is likely equally important for avoiding spurious signals of association.

In summary, we identified an ancestry association peak on 6q, and a SNP on 6q14.1 that increases the risk of asthma exclusively for individuals heterozygous for local African and non-African ancestry. Genetic associations that are dependent on local ancestry present unique challenges at both the discovery and replication stage, highlighting the need for additional large-scale genetic studies of asthma in admixed populations. Overall our results demonstrate the utility of ancestry association testing for identifying novel genetic variants that are associated with asthma, and the importance of considering local rather than global genetic ancestry in association studies involving admixed populations.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

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|-------------|---|
| GWAS | Genome-wide association study |
| SNP | Single nucleotide polymorphism |
| OR | Odds ratio |
| LD | Linkage disequilibrium |
| CSGA | Collaborative Study on the Genetics of Asthma |
| SARP | Severe Asthma Research Program |

| | |
|--------------|---|
| CEU | Utah residents with Northern and Western European ancestry from the CEPH collection |
| CEPH | Centre d'Etude du Polymorphisme Humain |
| YRI | Yoruba in Ibadan, Nigeria |
| GRAAD | Genomic Research on Asthma in the African Diaspora |
| GALA | Genetics of Asthma in Latino Americans |

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Key Messages

- Genome-wide comparisons of local ancestry in African Americans identified an admixture mapping peak on 6q that is more likely to contain variants associated with asthma.
- An allele at 6q14.1 that is common to European populations is associated with an increased risk of asthma in African Americans and Puerto Ricans, but not in European Americans.
- A consideration of local genetic ancestry is essential when performing genome-wide association studies in admixed populations.

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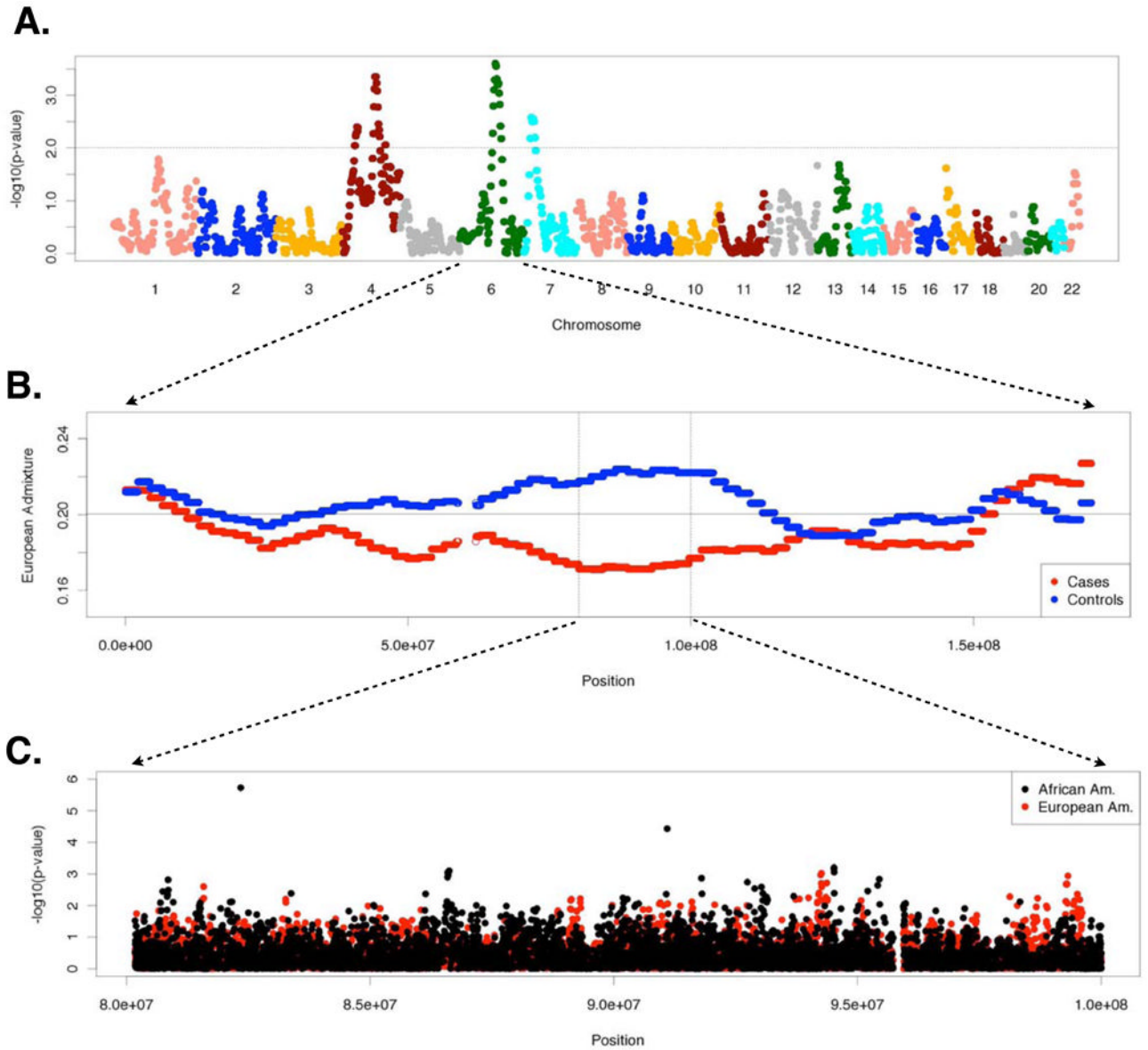


Figure 1.

Results of genome-wide ancestry and allelic association testing in African American adults with asthma and healthy controls using logistic regression. **A.** $-\log_{10}(\text{p-values})$ across the genome showing the location of four ancestry association peaks with $\text{p-values} < 0.01$ (above the dashed line). **B.** Average local European ancestry in the cases (red) and controls (blue) on chromosome 6; vertical lines indicate the boundaries of the ancestry association peak with $\text{p} < 0.01$, and the horizontal line indicates local European ancestry averaged across the genome. **C.** Tests of allelic association within the ancestry association peak on 6q in African Americans (using local ancestry as a covariate) and European Americans in CSGA/SARP.

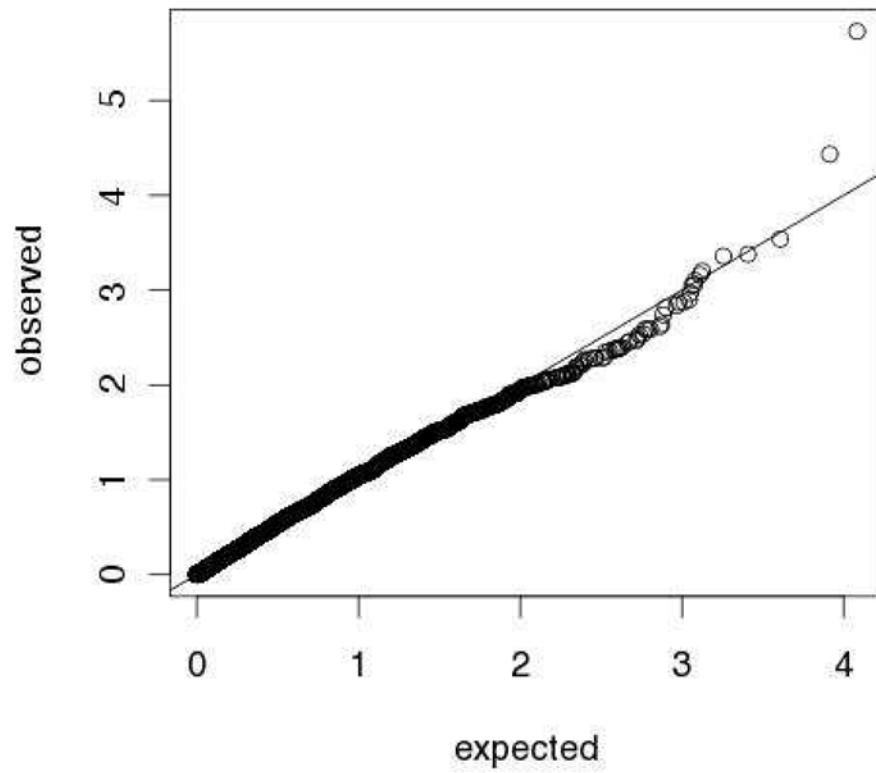


Figure 2. QQplot comparing the observed distribution of p-values to a uniform distribution using the function qqplot in R for SNPs within the admixture mapping peak on chr6q. Local ancestry was included as a covariate in tests of association.

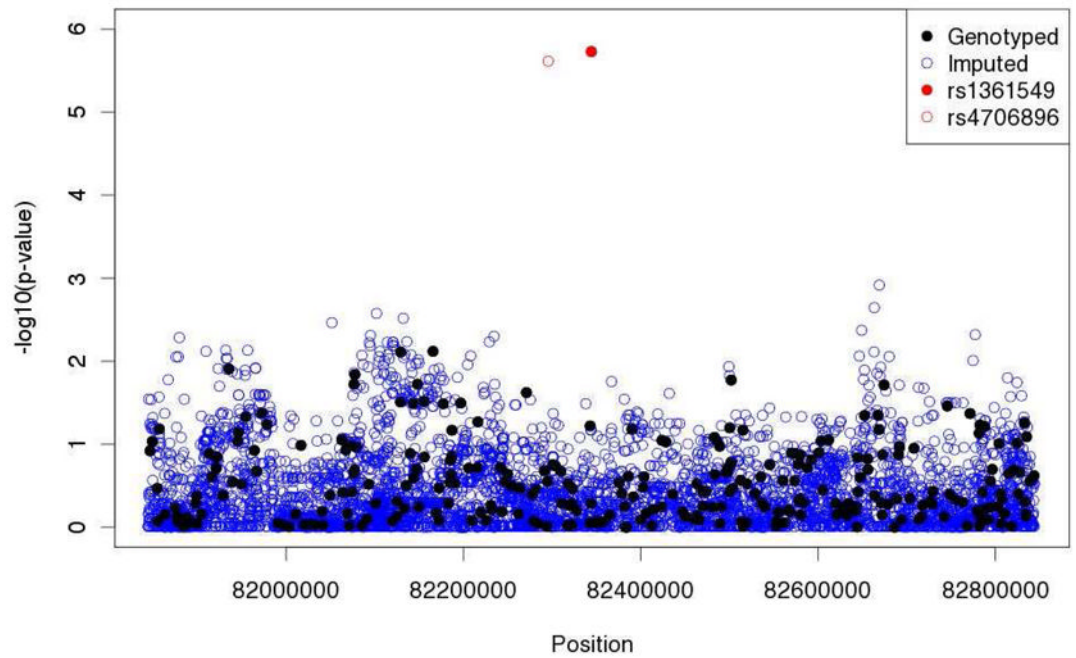


Figure 3. Tests of allelic association controlling for local ancestry within 500 Kb of rs1361549 in African Americans in CSGA/SARP at both genotyped and imputed SNPs from the 1000 Genomes Project.

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Table 1

Top signals of allelic association at SNPs within an ancestry association peak on 6q in African Americans. Included in the table are logistic regression p-values from local ancestry association testing, and allelic association testing with no correction for ancestry (no correction), a correction for global ancestry based on PC1 (global correction), and a correction for local ancestry (local correction). Results of association testing in European Americans is in the last column, the top signals of allelic association at SNPs using only a global correction for population structure are in Table E1.

| SNP (major/minor) | Closest Gene (distance) | MAF (cases, controls) | p-value local ancestry | p-value allelic, no correction | p-value allelic, global correction | p-value allelic, local correction | p-value, allelic European Americans (freq cases, controls) |
|-------------------|-------------------------|-----------------------|------------------------|--------------------------------|------------------------------------|-----------------------------------|--|
| rs1361549 (G/A) | FAM46A (-171661) | 0.093, 0.069 | 0.00079 | 0.0472 | 0.0527 | 1.87×10^{-6} | 0.428 (0.42, 0.40) |
| rs6926330 (G/A) | BACH2 (-32265) | 0.013, 0.052 | 0.00049 | 1.13×10^{-4} | 1.14×10^{-4} | 3.70×10^{-5} | 0.851 (0.0015, 0.0018) |
| rs576231 (A/G) | PRDM13 (-75107) | 0.028, 0.079 | 0.0090 | 3.19×10^{-5} | 1.67×10^{-5} | 4.38×10^{-4} | 0.15 (0.25, 0.28) |
| rs9363108 (C/T) | EPHA7 (-332718) | 0.25, 0.31 | 0.00062 | 0.013 | 0.014 | 6.32×10^{-4} | 0.52 (0.0066, 0.0089) |
| rs1414614 (T/C) | EPHA7 (-331225) | 0.38, 0.46 | 0.00062 | 1.65×10^{-3} | 1.83×10^{-3} | 6.93×10^{-4} | 0.40 (0.33, 0.31) |

Table 2

Allele frequencies and estimated odds ratios for rs1361549 in African American, European American, and Puerto Rican cases and controls. Comparisons for African American and Puerto Rican cases and controls are shown for individuals regardless of local ancestry (all), individuals homozygous for African ancestry (African Ancestry), individuals heterozygous for African/European or African/Native American ancestry (African/European Ancestry, African/Non-African Ancestry), and individuals homozygous for non-African ancestry (Native American or European ancestry).

| Sample | Frequency in Cases (N) | Frequency in Controls (N) | Odds Ratio (95% CI) |
|------------------------------|------------------------|---------------------------|---------------------|
| HapMap YRI | - | 0 (120) | - |
| African Americans (all) | 0.09 (355) | 0.07 (444) | 1.4 (0.98–2.1) |
| African Ancestry | 0 (243) | 0 (257) | - |
| African/European Ancestry | 0.27 (104) | 0.14 (165) | 2.2 (1.4–3.5) |
| European/European Ancestry | 0.63 (8) | 0.27 (22) | 4.3 (1.2–18) |
| European Americans | 0.42 (678) | 0.40 (561) | 1.1 (0.9–1.3) |
| Puerto Ricans (all) | 0.31 (277) | 0.29 (191) | 1.1 (0.79–1.4) |
| African Ancestry | 0 (21) | 0 (3) | - |
| African/Non-African Ancestry | 0.20 (73) | 0.11 (47) | 2.1 (0.92–5.0) |
| Non-African Ancestry | 0.38 (183) | 0.36 (141) | 1.1 (0.78–1.5) |
| HapMap CEU | - | 0.42 (120) | - |

CI = 95% confidence interval.

Table 3

Replication of ancestry and allelic association with asthma at rs1361549 in Puerto Rican cases and controls included in the GALA study. The sign of the regression coefficient indicates the direction of the effect, which is consistent between the two studies. The regression coefficient for tests of association at rs1361549 in European Americans in the CSGA/SARP was 0.066 ($p=0.43$). Also shown are stratified tests of association in African Americans based on local ancestry.

| Logistic Model | Primary/Discovery Sample African Americans, CSGA/SARP: | | | Replication Sample Puerto Ricans, GALA: | | |
|---|---|----------------|----------------------|--|----------------|----------------------|
| | Regression Coefficient | Standard Error | p-value | Regression Coefficient | Standard Error | p-value |
| Local African Ancestry | 0.96 | 0.29 | 7.9×10^{-4} | 0.22 | 0.080 | 5.4×10^{-3} |
| rs1361549 (No Correction) | 0.094 | 0.047 | 0.0472 | 0.01 | 0.036 | 0.79 |
| rs1361549 (Global Ancestry Correction) | 0.093 | 0.048 | 0.0527 | 0.007 | 0.036 | 0.85 |
| rs1361549 (Local Ancestry Correction) | 0.28 | 0.058 | 1.9×10^{-6} | 0.099 | 0.044 | 0.025 |
| rs1361549 (2 African Chrom) | n/a | n/a | n/a | n/a | n/a | n/a |
| rs1361549 (1 African, 1 European/Native American) | 0.25 | 0.059 | 2.5×10^{-5} | 0.19 | 0.11 | 0.073 |
| rs1361549 (2 European/Native American) | 0.35 | 0.12 | 5.4×10^{-3} | 0.068 | 0.050 | 0.18 |