

Genome-wide ancestry association testing identifies a common European variant on 6q14.1 as a risk factor for asthma in African American subjects

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

Objective: We sought to identify asthma-associated loci in African American subjects.

Methods: We compared local African and European ancestry estimated from dense single nucleotide polymorphism genotype data in African American adults with asthma and nonasthmatic control subjects. 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 chromosome 6q. Allelic tests for association within this region identified a single nucleotide polymorphism (rs1361549) on 6q14.1 that was associated with asthma exclusively in African American subjects with local European admixture (odds ratio, 2.2). The risk allele is common in Europe (42% in the HapMap population of Utah residents with Northern and Western European ancestry from the Centre d'Etude du Polymorphisme Humain collection) but absent in West Africa (0% in the HapMap population of Yorubans in Ibadan, Nigeria), suggesting the allele is present in African American subjects because of recent European admixture. We replicated our findings in Puerto Rican subjects and similarly found that the signal of association is largely specific to subjects who are heterozygous for African and non-African ancestry at 6q14.1. However, we found no evidence for association in European American or Puerto Rican subjects 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. (J Allergy Clin Immunol 2012;■■■■:■■■-■■■.)

Key words: Asthma, population structure, genome-wide association study, admixture mapping, ancestry association testing, admixed populations, African American, Puerto Rican

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 American subjects have higher rates of asthma and steroid dependency and a more limited response to treatment compared with European American subjects,¹ which might not be attributable to differences in access to health care alone.^{2,3} Although there is a strong environmental component, recent estimates of the heritability of asthma are approximately 75%,^{2,3} suggesting there are important genetic contributions to asthma susceptibility. As of 2006, polymorphisms in more than 100 genes had been associated with asthma and asthma-related phenotypes, with more than 40 genes showing replication in at least 1 independent population.⁴ More recently, numerous genome-wide association studies (GWASs) have identified additional candidate genes and genetic loci that contribute to asthma susceptibility.^{5–11} Although the majority of GWASs have focused largely on populations of European origin,^{6,7,9–11} candidate loci have also been identified through GWASs in African American,⁸ Puerto Rican,¹² and Mexican⁵ subjects.

One of the challenges of performing genetic association studies in African American subjects is avoiding confounding resulting from complex population structure. Because of differences in demographic histories, populations of African origin exhibit higher levels of genetic variation, reduced linkage disequilibrium (LD), and proportionally more rare variation compared with European populations.¹³ 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 American subjects, who have approximately 20% European ancestry on average.¹⁴ 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 control subjects is important to avoid confounding because of the population structure in genetic association studies. However, it can also be used to perform admixture mapping to identify novel disease susceptibility loci.¹⁵

Admixture mapping in African American subjects has proved to be a valuable tool for identifying novel risk alleles for diseases such as multiple sclerosis,¹⁶ prostate cancer,¹⁷ obesity,¹⁸ and hypertension.¹⁹ In theory admixture mapping is applicable to genetic studies of admixed populations for any disease in which risk alleles vary in frequency between 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 Rican subjects,¹² indicating that 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 (eg, Zhu et al²⁰) and by increasing genomic coverage through long-range tagging of single nucleotide polymorphisms (SNPs) with local ancestry

Abbreviations used

CEU:	Utah residents with Northern and Western European ancestry from the Centre d'Etude du Polymorphisme Humain collection
CSGA:	Collaborative Study on the Genetics of Asthma
GALA:	Genetics of Asthma in Latino Americans
GWAS:	Genome-wide association study
LAMP:	Local Ancestry in Admixed Populations
LD:	Linkage disequilibrium
PC1:	First principal component
SARP:	Severe Asthma Research Program
SNP:	Single nucleotide polymorphism
YRI:	Yoruba in Ibadan, Nigeria

(ancestry LD). Although admixture mapping has historically used a set of ancestry informative markers, methods are available to identify local ancestry by using dense SNP genotype data,^{21,22} enabling genome-wide ancestry association testing at a higher resolution and accuracy than more traditional admixture mapping.

To identify genetic loci that contribute to asthma susceptibility in African American subjects, we performed genome-wide ancestry association testing using estimates of local ancestry inferred from approximately 1 million genotyped SNPs in 355 African American adults with asthma and 444 African American adult control subjects. 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 Rican subjects. The risk allele is common in the HapMap population of Utah residents with Northern and Western European ancestry from the Centre d'Etude du Polymorphisme Humain collection (CEU) but absent in the HapMap population of Yorubans in Ibadan, Nigeria (YRI), suggesting the allele is present in African American subjects because of 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 nonasthmatic control subjects 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, the University of Maryland, and Wake Forest University from (1) families ascertained through affected sib pairs, (2) trios of affected children and their parents, (3) adults and children with severe persistent asthma, and (4) nonasthmatic control subjects (>18 years of age). The multicenter SARP study sample includes patients with mild-to-severe asthma and control subjects. In both studies subjects were carefully characterized by using similar procedures.^{23–25} In addition to having a physician's diagnosis of asthma, all patients with asthma 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 control subjects were older than 17 years at the time of recruitment, we limited our study to those subjects to reduce phenotypic heterogeneity. Among these subjects, the mean age at

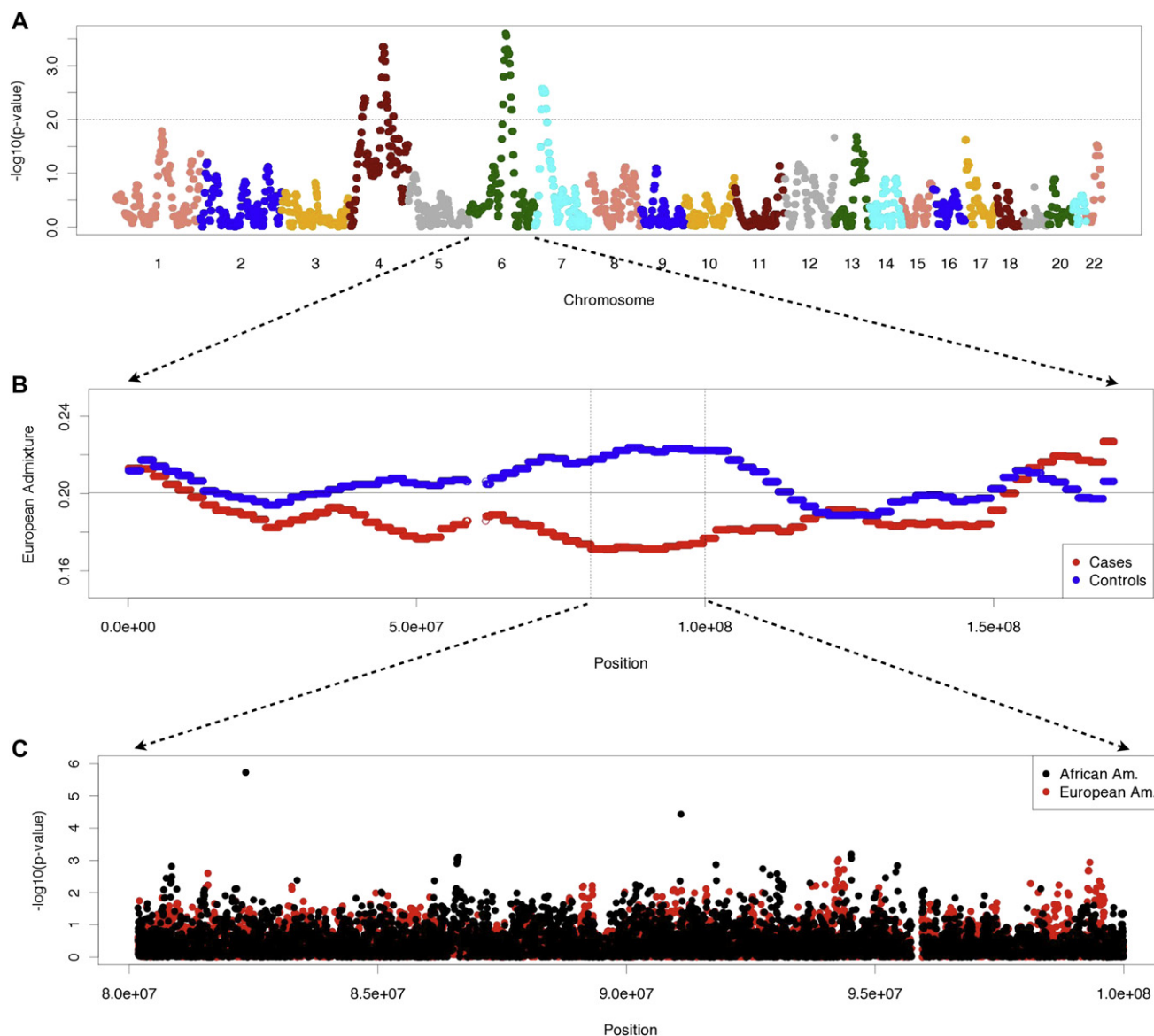


FIG 1. Results of genome-wide ancestry and allelic association testing in African American adults with asthma and healthy control subjects by using logistic regression. **A**, $-\log_{10}(P\text{ values})$ across the genome showing the location of 4 ancestry association peaks with P values of less than .01 (above the dashed line). **B**, Average local European ancestry in the cases (red) and control subjects (blue) on chromosome 6; vertical lines indicate the boundaries of the ancestry association peak with a P value of less than .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 American subjects (by using local ancestry as a covariate) and European American subjects in CSGA/SARP.

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 American subjects; however, we also present results from self-reported European American subjects who participated in the CSGA/SARP studies (complete results will be presented elsewhere).

Genotyping and quality control

Genotyping was performed on the Illumina 1Mv1 platform (Illumina, San Diego, Calif), with individual genotypes called by using clustering algorithms as implemented in the BeadStudio software by Illumina. After an initial round of clustering and genotype calling, 3 additional rounds of

clustering were performed, alternating between filtering of subjects and markers based on call rates of less than 95% and less than 90%, respectively. SNPs were then filtered for "Cluster_Sep" values of less than 0.3 (score of genotype cluster separation) and for "GenTrain" scores of less than 0.75. The resulting number of SNPs was 1,033,467 before additional quality control measures.

Data quality control was performed with PLINK,²⁶ R (<http://www.R-project.org>), and EIGENSTRAT.^{27,28} SNPs were filtered based on 95% call rates, Hardy-Weinberg equilibrium P values of greater than 10^{-6} , consistency in allele frequency from the HapMap population of African ancestry in the Southwest US ($P > 10^{-6}$, χ^2 test), and less than 5 heterozygous genotype calls in male subjects for X-linked markers. The total number of markers after quality control checks was 1,026,072. Subjects were filtered based on greater than

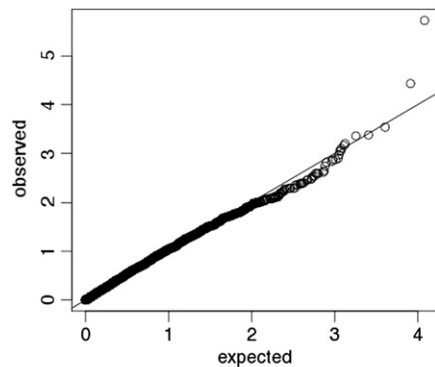


FIG 2. Comparison of the observed distribution of *P* values to a uniform distribution by using the function qqplot in R for SNPs within the admixture mapping peak on chr6q. Local ancestry was included as a covariate in tests of association.

95% call rates, consistency between genetic and reported sex (*F*-stat from X-linked markers between -0.2 and 0.3 for female subjects and between 0.8 and 1 for male subjects), consistency in self-reported ethnicity (no obvious clustering with the HapMap CEU population in a principal component analysis and 3 iterations of outlier removal on the first 3 principal components), and high or low heterozygosity (*F*-stat <0.5 and >-0.2). Samples were also flagged for unexpected pairwise relatedness (identity by descent, $>30\%$) or genetic identity (identity by state, $>90\%$). The total number of subjects remaining after quality control was 355 cases and 444 control subjects.

Population structure and ancestry association testing

Genotypes were oriented to the plus strand after the removal of 5634 A/T and C/G SNPs and merged with the 11 phase 3 HapMap populations by using PLINK to evaluate the extent of global European admixture. SNPs were filtered for LD based on r^2 values of greater than 0.5 , and a principal component analysis was conducted in EIGENSTRAT with no outlier iterations (outliers were removed in the quality control process). Global estimates of European admixture were estimated from the first principal component that separated the HapMap CEU and YRI populations (see Fig E1 in this article's Online Repository at www.jacionline.org). Global ancestry was compared between cases and control subjects by using logistic regression in R.

Segments of local European ancestry were estimated by using Local Ancestry in Admixed Populations (LAMP).²¹ Admixture in African American subjects 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 principal component 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 by using logistic regression in R, comparing the estimated number of African chromosomes in cases versus control subjects 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 the 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 control subjects. An ancestry association peak was defined as a window of unadjusted *P* values of less than $.01$.

Association testing

Tests of allelic associations were performed for SNPs within ancestry association peaks by 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 those explained by the local population structure. We also ran a stratified comparison to identify allelic associations specific to 1 ancestral background at a locus by separating cases and control subjects into 3 groups: (1) subjects with 2 chromosomes of European ancestry, (2) subjects with 1 chromosome of European ancestry and 1 chromosome of African ancestry, and (3) subjects with both chromosomes of African ancestry. The distribution of allelic association *P* values was compared to a uniform distribution by 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 by using IMPUTE2,^{30,31} with 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 control subjects 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 subjects of Mexican ancestry, we restricted our analysis to Puerto Rican subjects because they have a higher degree of African ancestry.³² Asthma was defined as a physician's diagnosis of mild-to-moderate asthma, having had 2 or more symptoms of asthma in the previous 2 years at the time of recruitment (wheezing, coughing, and/or shortness of breath), and current use of asthma medications. The mean age at enrollment was 14 years, the mean age of onset was 3.2 years, and the proportion of female subjects was 45%. Control subjects were recruited from the same locations and determined to have a negative history of asthma based on a standardized questionnaire.

Subjects were genotyped at greater than 900,000 SNPs by using the Affymetrix 6.0 GeneChip Array (Affymetrix, Santa Clara, Calif) and filtered by using standard quality control procedures including a greater than 95% call rate and consistency with Hardy-Weinberg equilibrium ($P > 10^{-5}$). Local ancestry was estimated with 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 by using ADMIXTURE.³³ Replication of ancestry and allelic association for the top associated SNP was performed by 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

After 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 nonasthmatic control subjects. Estimates of global African ancestry among self-reported African American subjects ranged from 30% to 100% African ancestry (mean, 81%) based on the first principal component with the HapMap CEU and YRI populations as a reference for European and African ancestry (see Fig E1). However, there was no significant difference in the distribution of global European admixture between cases and control subjects (logistic regression $P = .78$, see Fig E2 in this article's Online Repository at www.jacionline.org), suggesting that the proportion of European and African ancestry when pooled across the genome was balanced between cases and control subjects. Mean local ancestry as estimated by using LAMP was highly correlated with global estimates of ancestry based on the first principal component in EIGENSTRAT (see Fig E3 in this article's Online

TABLE I. Top signals of allelic association at SNPs within an ancestry association peak on 6q in African American subjects

SNP (major/minor)	Closest gene (distance)	MAF (cases, control subjects)	P value, local ancestry	P value, allelic, no correction	P value, allelic, global correction	P value, allelic, local correction	P value, allelic, European Americans (frequency: cases, control subjects)
rs1361549 (G/A)	FAM46A (−171661)	0.093, 0.069	.00079	.0472	.0527	1.87×10^{-6}	.428 (0.42, 0.40)
rs6926330 (G/A)	BACH2 (−32265)	0.013, 0.052	.00049	1.13×10^{-4}	1.14×10^{-4}	3.70×10^{-5}	.851 (0.0015, 0.0018)
rs576231 (A/G)	PRDM13 (−75107)	0.028, 0.079	.0090	3.19×10^{-5}	1.67×10^{-5}	4.38×10^{-4}	.15 (0.25, 0.28)
rs9363108 (C/T)	EPHA7 (−332718)	0.25, 0.31	.00062	.013	.014	6.32×10^{-4}	.52 (0.0066, 0.0089)
rs1414614 (T/C)	EPHA7 (−331225)	0.38, 0.46	.00062	1.65×10^{-3}	1.83×10^{-3}	6.93×10^{-4}	.40 (0.33, 0.31)

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 American subjects are shown in the last column, and the top signals of allelic association at SNPs using only a global correction for population structure are shown in Table E1.

TABLE II. Allele frequencies and estimated odds ratios for rs1361549 in African American, European American, and Puerto Rican cases and control subjects

Sample	Frequency in cases (N)	Frequency in control subjects (N)	Odds ratio (95% CI)
HapMap YRI population	—	0 (120)	—
African American subjects (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 American subjects	0.42 (678)	0.40 (561)	1.1 (0.9-1.3)
Puerto Rican subjects (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 population	—	0.42 (120)	—

Comparisons for African American and Puerto Rican cases and control subjects are shown for subjects regardless of local ancestry (all), subjects homozygous for African ancestry (African ancestry), subjects heterozygous for African/European or African/Native American ancestry (African/European ancestry, African/non-African ancestry), and subjects homozygous for non-African ancestry (Native American or European ancestry).

Repository at www.jacionline.org). Patterns of local European ancestry were variable both within and between subjects, as expected under a model of more recent European admixture (see Fig E4 in this article's Online Repository at www.jacionline.org).

We identified 4 ancestry association peaks suggestive of differences in local ancestry between African American cases and control subjects ($P < .01$; Fig 1, A). Two of the peaks were on chromosome 4 (4p and 4q) in the direction of increased European ancestry in cases ($P = 4.0 \times 10^{-3}$ and $P = 4.4 \times 10^{-4}$, respectively; Fig 1, B), and 2 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; Fig 1, C). The ancestry association peak on 6q reached genome-wide significance based on 100 permutations across approximately 1,000,000 SNPs, (permutation $P < .01$).

We then tested for allelic associations with asthma for 9266 SNPs within the ancestry association peak on 6q while adjusting for local ancestry (Fig 1, D). The most significant SNP, rs1361549, had an association *P* value that passed Bonferroni correction for 9266 tests at 6q14.1 ($P = 1.87 \times 10^{-6}$, $\alpha_{9,266} = 5.4 \times 10^{-6}$, Fig 2). Notably, rs1361549 is only weakly associated with asthma unless local ancestry is included as a covariate in the logistic regression model ($P = .047$

with no covariates and $P = .053$ with global ancestry from the first principal component (PC1) as a covariate, Table I). 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 control subjects with and without local European admixture indicated that the signal of association is exclusive to African American subjects with at least 1 chromosome of European ancestry at rs1361549 (Table II). The frequency of the minor allele in African American cases heterozygous for European ancestry at rs1361549 was 27% compared with only 14% in control subjects heterozygous for European ancestry (odds ratio, 2.2; 95% CI, 1.4-3.5). Although the number of subjects is small, the frequency of the risk allele was 63% in 8 African American cases homozygous for European ancestry compared with 27% in 22 African American control subjects homozygous for European ancestry (odds ratio, 4.3; 95% CI, 1.2-18). In subjects homozygous for local African ancestry, rs1361549 was monomorphic in both cases and control subjects. Stratified tests of allelic association based on local ancestry (see the Methods section) 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 (Fig 1, D, and see Table E1 in this article's Online Repository at www.jacionline.org); however, we replicated both the ancestry association ($P = 5.3 \times 10^{-3}$) and allelic association ($P = .025$) with asthma at rs1361549 in Puerto Rican subjects in GALA (Table III), which was attributable to subjects who were heterozygous for African and either Native American or European ancestry (non-African ancestry, Table II). Although we attempted to replicate the signal of allelic association in African American subjects in the Genomic Research on Asthma in the African Diaspora study and in families from Barbados,⁸ 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 7124 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 ($P = 2.7 \times 10^{-6}$, Fig 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 population) and shows similar patterns of allele frequencies as rs1361549

TABLE III. Replication of ancestry and allelic association with asthma at rs1361549 in Puerto Rican cases and control subjects included in the GALA study

Logistic model	Primary/discovery sample, African American subjects, CSGA/SARP			Replication sample, Puerto Rican subjects, GALA		
	Regression coefficient	SE	P value	Regression coefficient	SE	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	.0472	0.01	0.036	.79
rs1361549 (global ancestry correction)	0.093	0.048	.0527	0.007	0.036	.85
rs1361549 (local ancestry correction)	0.28	0.058	1.9×10^{-6}	0.099	0.044	.025
rs1361549 (2 African chrom)	NA	NA	NA	NA	NA	NA
rs1361549 (1 African, 1 European/Native American)	0.25	0.059	2.5×10^{-5}	0.19	0.11	.073
rs1361549 (2 European/Native American)	0.35	0.12	5.4×10^{-3}	0.068	0.050	.18

The sign of the regression coefficient indicates the direction of the effect, which is consistent between the 2 studies. The regression coefficient for tests of association at rs1361549 in European American subjects in CSGA/SARP was 0.066 ($P = .43$). Also shown are stratified tests of association in African American subjects based on local ancestry. NA, Not applicable.

(frequency in the CEU population, 41%; frequency in the YRI population, 0%).

DISCUSSION

In this study we performed genome-wide ancestry association testing using genotypes from approximately 1 million SNPs in 344 African American adults with asthma and 444 nonasthmatic control subjects. Given the relatively low effect sizes of asthma-associated SNPs identified in previous GWASs,⁵⁻¹¹ our study was underpowered for performing a traditional GWAS. We therefore used genome-wide ancestry association testing to identify ancestry association peaks and then performed allelic tests of association within an associated region. This strategy significantly decreases the multiple-testing burden inherent in GWASs. 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 control subjects compared with 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 control subjects before including local ancestry as a covariate in the logistic model. The risk allele is common in Europe (frequency in the CEU population, 42%) and either rare or absent in West Africa (frequency in the YRI population, 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 compared with the control subjects 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 control subjects (because of increased European ancestry, for which the allele is more common). By including local ancestry in the model, this prior expectation was taken into consideration, leading to the identification of a signal of association with asthma at rs1361549 that would have otherwise gone unnoticed.

By comparing the frequency of rs1361549 between cases and control subjects after stratifying by local ancestry, we discovered that the frequency of the risk allele is only increased in African American 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 (see Table E2 in this article's Online Repository at www.jacionline.org). However, neither rs1361549 nor any of the nearby SNPs showed an association with asthma in European American subjects, 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 Rican subjects in the same direction (Table III) and similarly found that the frequency of the risk allele was predominantly increased in cases heterozygous for local African and non-African ancestry.

Although the number of subjects was small, rs1361549 also showed a signal of association with asthma in African American subjects homozygous for local European ancestry; however, we found no significant association in Puerto Rican subjects 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 can only be increased for admixed subjects 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 with control subjects in both African American and Puerto Rican subjects at this locus, we cannot rule out the possibility that there are additional untyped and untagged variants that increase the risk of asthma for subjects homozygous for African ancestry that are independent of the effect of rs1361549.

In silico fine mapping identified 1 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 approximately 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 monocytes,³⁴ suggesting a potential avenue for involvement in asthma susceptibility. In addition, there are 2 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 LD suggest the underlying causal variant or variants might be more proximal to the centromere (see Figs E5 and E6 in this

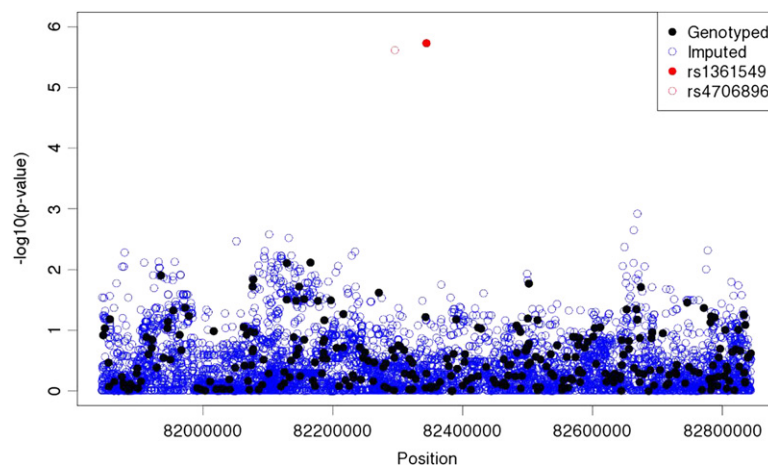


FIG 3. Tests of allelic association controlling for local ancestry within 500 Kb of rs1361549 in African American subjects in CSGA/SARP at both genotyped and imputed SNPs from the 1000 Genomes Project.

article's Online Repository at www.jacionline.org). Within this region, there are multiple candidate expression quantitative loci³⁵ in weak LD with rs1361549 ($r^2 > 0.3$) that show an association P value of less than .001 (see Table E1), including expression quantitative trait loci 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 have been found in patients with rheumatoid arthritis.³⁶ *CNR2* is a cannabinoid/G protein-coupled receptor that is also expressed in B cells and might function to mediate immunoglobulin class-switching from IgM to IgE.³⁷ 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 American and Puerto Rican subjects homozygous for African ancestry, which is consistent with our expectations based on the SNP being absent in the HapMap YRI population. Given our estimate that African American subjects in CSGA/SARP have approximately 19% European ancestry, if the variant were attributable solely to European admixture, we would expect the frequency of the minor allele to be approximately 8% given a frequency of 42% in the HapMap CEU population ($42\% \times 19\%$ European ancestry), which is similar to what we observe (7.8%). Therefore our results suggest that rs1361549 is present in African American subjects predominantly because of 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 might 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 because of 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 because signals were weakened in the combined subjects as a result of the majority of subjects having local African ancestry (and the SNPs not being associated in these subjects). 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 an SNP on 6q14.1 that increases the risk of asthma exclusively for subjects 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 stages, 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.

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Key messages

- Genome-wide comparisons of local ancestry in African American subjects 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 American and Puerto Rican subjects but not in European American subjects.
- A consideration of local genetic ancestry is essential when performing GWASs in admixed populations.

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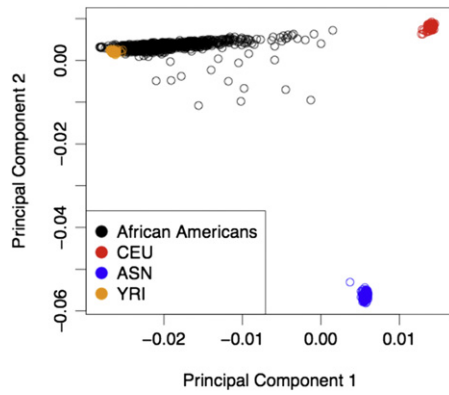


FIG E1. Principal component analysis of African American adults with asthma and healthy control subjects and the phase 3 HapMap CEU, YRI, and Asian (ASN) populations. Global estimates of European ancestry were extrapolated from PC1.

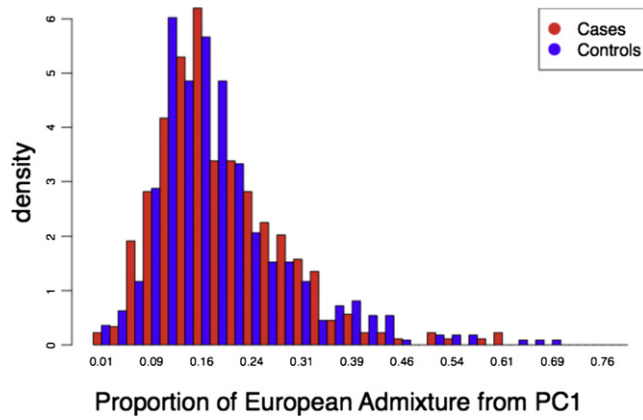


FIG E2. Distribution of global European admixture in African American adults with asthma and nonasthmatic control subjects, as estimated from PC1.

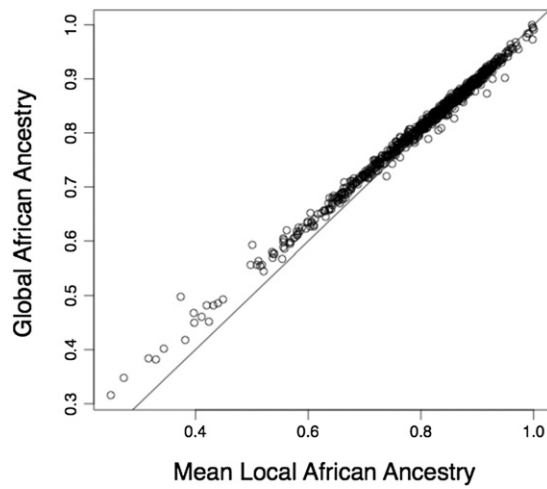


FIG E3. Correlation between mean local African ancestry calculated across the genome and estimates of global African ancestry from PC1.

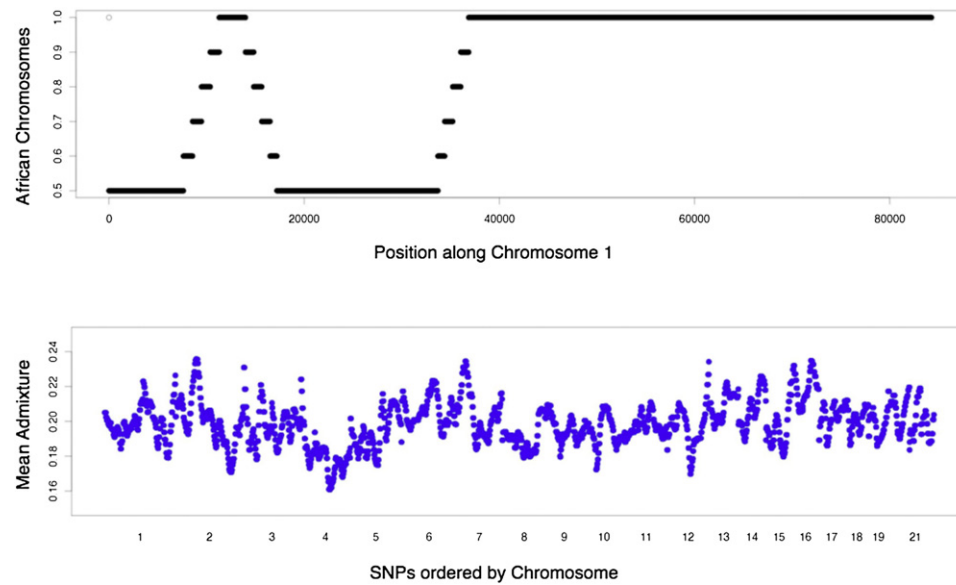


FIG E4. Estimates of local European ancestry in a single African American subject with asthma across chromosome 1 (*top*) and mean local European admixture across the genome in 444 nonasthmatic African American adults (*bottom*).

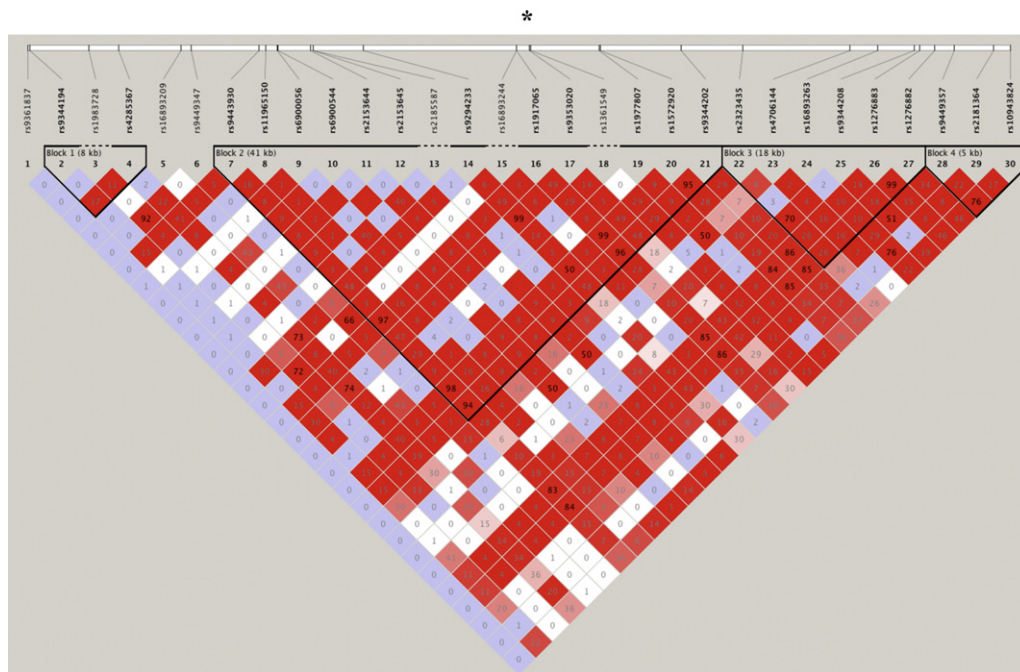


FIG E5. LD (r^2) between genotyped SNPs in African American subjects with local European ancestry within a 100-Kb region centered on rs1361549. *Location of rs1361549.

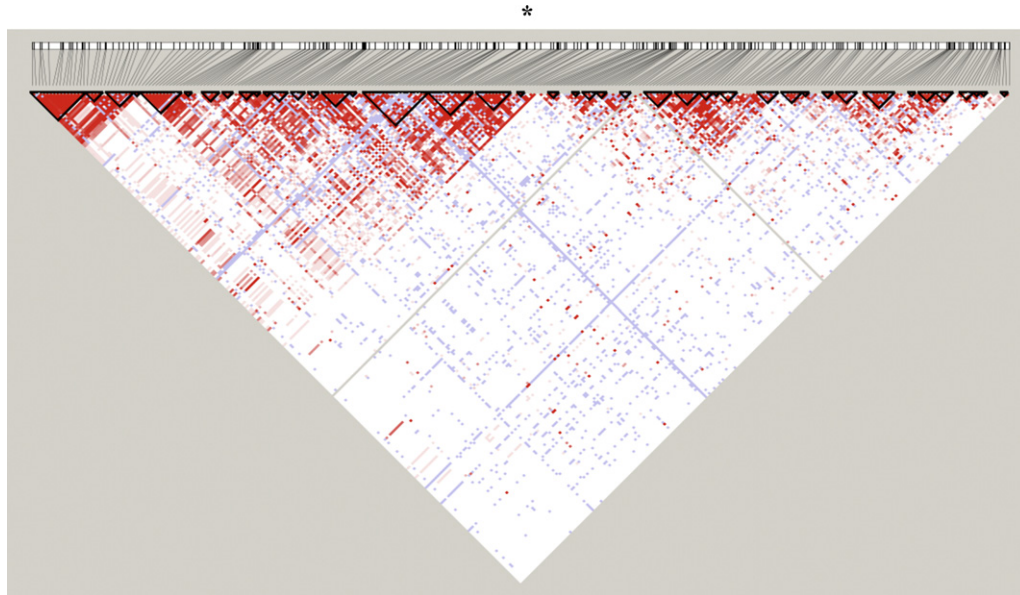


FIG E6. LD (r^2) between genotyped SNPs in African American subjects with local European ancestry at rs1361549 within a 1-Mb region centered on rs1361549. *Location of rs1361549.

TABLE E1. Top signals of allelic association at SNPs within an ancestry association peak on 6q in African American subjects correcting for global ancestry (global correction)

SNP	Position (hg18)	<i>P</i> value, local ancestry	<i>P</i> value, allelic, global correction	<i>P</i> value, allelic, local correction	Freq CEU	Freq YRI
rs10498989	95446907	6.30×10^{-4}	8.56×10^{-6}	1.46×10^{-3}	0.295	0.897
rs9345075	91799831	4.94×10^{-4}	1.61×10^{-5}	1.36×10^{-3}	0.902	0.367
rs9363229	95415689	6.30×10^{-4}	1.67×10^{-5}	2.29×10^{-3}	0.323	0.895
rs576231	100245282	9.18×10^{-4}	1.67×10^{-5}	4.38×10^{-4}	0.239	0.014
rs2207428	95209649	6.17×10^{-5}	4.20×10^{-5}	3.47×10^{-3}	0.332	0.003

None of the SNPs pass a Bonferroni correction for 9266 tests ($P < 5.4 \times 10^{-6}$). Included in the table are logistic regression *P* values from local ancestry association testing, and allelic association testing is corrected for local ancestry (local correction). Included are the reference allelic frequencies (*Freq*) in the HapMap CEU and YRI populations (release 28), indicating that all of the top SNPs are informative for European-African ancestry.

TABLE E2. SNPs with association *P* values of less than .01 among 257 genotyped SNPs within a 1-Mb region centered on rs1361549 in stratified tests for African American subjects with 1 or more chromosomes of local European ancestry (admixed) or 2 chromosomes of local African ancestry (African)

SNP	Position	<i>P</i> value, African	Coefficient, African	<i>P</i> value, admixed	Coefficient, admixed	<i>P</i> value, European American subjects	Distance from rs136154 and LD (<i>r</i> ²)	eQTL candidates* (gene, population, <i>P</i> value)
rs10455382	82077709	.97	0.0049	9.4×10^{-5}	−0.69	.33	−266695 (0.24)	PRR15, CEU, 3e-05 SPSB1, YRI, 4e-05 ASB2, YRI, 8e-05
rs12055792	82079376	.86	0.03	9.6×10^{-3}	−0.58	.51	−265028 (0.08)	IGF2BP3, CEU, 3e-05
rs4706889	82129155	.88	−0.02	6.4×10^{-4}	0.62	.28	−215249 (0.32)	PRR15, CEU, 4e-05
rs1015916	82129430	.38	−0.28	4.9×10^{-3}	−0.76	.69	−214974 (0.05)	IGF2BP3, CEU, 5e-05
rs9341883	82148380	.91	−0.015	4.8×10^{-4}	−0.62	.91	−196024 (0.24)	NA
rs2323252	82155910	.86	−0.024	3.5×10^{-4}	0.67	.23	−188494 (0.33)	PRR15, CEU, 2e-05
rs11756740	82165844	.48	−0.41	5.5×10^{-3}	−0.92	.30	−178560 (0.033)	IGF2BP3, CEU, 0.0001
rs7741650	82185636	.64	0.06	6.5×10^{-3}	−0.47	.91	−158768 (0.22)	CNR2, YRI, 2e-05 GMIP, YRI, 0.0001
rs1343232	82187051	.53	−0.09	3.5×10^{-3}	0.53	.30	−157353 (0.32)	FCRLA, YRI, 2e-06 CNR2, YRI, 2e-05 PRR15, CEU, 0.0001
rs6940202	82190504	.29	0.14	3.9×10^{-3}	−0.50	.83	−153900 (0.26)	CNR2, YRI, 2e-05 FCRLA, YRI, 9e-05 VIPR1, YRI, 0.0001
rs2120533	82206201	.38	−0.12	2.0×10^{-3}	0.56	.31	−138203 (0.30)	NA
rs17438648	82216223	.38	0.12	1.9×10^{-3}	−0.57	.30	−128181 (0.30)	FCRLA, YRI, 1e-05 CNR2, YRI, 6e-05 PRR15, CEU, 0.0001
rs1361549	82344404	NA	NA	1.1×10^{-5}	−1.1	.43	0 (1)	NA
rs2298053	82424008	1.4×10^{-3}	1.6	.32	−0.42	.58	79604 (0.012)	NA
rs12664410	82428442	1.1×10^{-3}	1.5	.21	−0.52	.58	84038 (0.013)	NA
rs1935420	82745959	.60	0.20	6.9×10^{-3}	1.4	.82	401555 (0.034)	FGL2, CEU, 6e-05 TP53INP1, CEU, 0.0001

Association *P* values are also shown for the CSGA/SARP European American subjects. SNPs are ordered based on position; *P* values of less than .01 in any one of the stratified tests are shaded; the strongest signal of association is with rs1361549 (in boldface). Estimates of LD with rs1361549 were calculated in African American subjects with local European ancestry by using Haploview (Figs E5 and E6).

eQTL, Expression quantitative trait locus; NA, not applicable.

*From www.scandb.org.^{E1}