A genome-wide association study on African-ancestry populations for asthma

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Background: Asthma is a complex disease characterized by striking ethnic disparities not explained entirely by environmental, social, cultural, or economic factors. Of the limited genetic studies performed on populations of African descent, notable differences in susceptibility allele frequencies have been observed.

Objectives: We sought to test the hypothesis that some genes might contribute to the profound disparities in asthma.

Methods: We performed a genome-wide association study in 2 independent populations of African ancestry (935 African American asthmatic cases and control subjects from the Baltimore–Washington, DC, area and 929 African Caribbean asthmatic subjects and their family members from Barbados) to identify single-nucleotide polymorphisms (SNPs) associated with asthma.

Results: A meta-analysis combining these 2 African-ancestry populations yielded 3 SNPs with a combined *P* value of less than 10^{-5} in genes of potential biologic relevance to asthma and allergic disease: rs10515807, mapping to the α-1B-adrenergic receptor *(ADRA1B)* gene on chromosome 5q33 (3.57×10^{-6}); rs6052761, mapping to the prion-related protein *(PRNP)* gene on chromosome 20pter-p12 (2.27×10^{-6}); and rs1435879, mapping to the dipeptidyl peptidase 10 *(DPP10)* gene on chromosome 2q12.3-q14.2. The generalizability of these findings was tested in family and case-control panels of United Kingdom and German origin, respectively, but none of the associations observed in the African groups were replicated in these European studies. Evidence for association was also examined in 4 additional case-control studies of African Americans; however, none of the SNPs implicated in the discovery population were replicated.

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Conclusions: This study illustrates the complexity of identifying true associations for a complex and heterogeneous disease, such as asthma, in admixed populations, especially populations of African descent. (J Allergy Clin Immunol 2010;125:336-46.)

Key words: *Asthma, genome-wide association study, ADRA1B, PRNP, DPP10, African ancestry, ethnicity, polymorphism, genetic association*

Asthma is a complex disease characterized by intermittent inflammation of the airways. Morbidity and mortality rates are disproportionately high among ethnic minorities, including African Americans, and they continue to increase.[1] The striking ethnic disparities in asthma prevalence cannot be explained entirely by environmental, social, cultural, or economic factors. Nearly a dozen genome-wide linkage screens[2-12] and 2 recent genome-wide association studies (GWASs)[13,14] have confirmed a strong genetic component to asthma. It remains difficult, however, to identify specific causal genes and determine whether genetic control contributes to the observed ethnic disparities for this complex disease.

In this study 2 independent populations of African descent ascertained through physician-diagnosed asthma have been recruited by a consortium entitled Genomic Research on Asthma in the African Diaspora (GRAAD). These populations have been genotyped with the Illumina HumanHap650Y BeadChip containing 655,352 single nucleotide polymorphisms (SNPs) as part of a genome-wide search for genes controlling risk to asthma in ethnic minorities. The generalizability of findings from these populations of African descent was tested in European family and case-control panels of United Kingdom (UK) and German origin, respectively. Four samples of African Americans from independent case-control studies were also tested to replicate the top signals in these 2 studies.

Abbreviations used
ADRA1B: α-1B-adrenergic receptor
AIM: Ancestry informative marker
DPP10: Dipeptidyl peptidase 10
GNA13: G-α-13
GRAAD: Genomic Research on Asthma in the African Diaspora
GWAS: Genome-wide association study
IRB: Institutional review board
LD: Linkage disequilibrium
MAF: Minor allele frequency
PRNP: Prion-related protein
PrP: Prion protein
REACH: Reducing Emergency Asthma Care in Harlem
SNP: Single nucleotide polymorphism
UK: United Kingdom
URI: Upper respiratory tract infection

METHODS

Sample description

We analyzed 498 asthmatic cases and 500 nonasthmatic control subjects from the Baltimore–Washington, DC, metropolitan area who self-reported as being of African American ethnicity. These subjects comprised the GRAAD consortium and represent 8 separate, National Institutes of Health–funded studies of asthma in pediatric and adult African American populations, plus 1 study on healthy African Americans. Because asthma is often characterized by onset during childhood, there was a deliberate decision to favor adults in the control group to minimize including control subjects with some potential for development of asthma. Informed consent was obtained from each study participant, and the study protocol was approved by the institutional review board (IRB) at either the Johns Hopkins University or Howard University.

Among all cases, asthma was defined as both a reported history of asthma and a documented history of physician-diagnosed asthma (past or current). For each of the asthma studies, a standardized questionnaire based on either American Thoracic Society[15] or International Study of Asthma and Allergy in Childhood[16] questionnaires was administered by a clinical coordinator. All control subjects (except 50, see below) were likewise administered a standardized questionnaire and were determined to be negative for a history of asthma. Asthma status for 50 control subjects participating in a study of the genetics of human pigmentation[17] was not explicitly determined, although "known clinical disease" was among the exclusion criteria.

A replication population of 163 African Caribbean families ascertained through asthmatic probands from Barbados and containing 1,028 subjects was also included. Probands were recruited through referrals at local polyclinics or the Accident and Emergency Department at the Queen Elizabeth Hospital, as previously described, and their nuclear and extended family members were recruited.[18,19] Asthma was defined as both a reported history of asthma and a documented history of physician-diagnosed asthma (past or current) plus a history of wheezing without an upper respiratory tract infection (URI) for 2 of 4 hallmark symptoms (wheezing with a URI, cough without a URI, shortness of breath, and tightness in the chest). All subjects provided verbal and written consent, as approved by the Johns Hopkins IRB and the Barbados Ministry of Health.

European-ancestry replication samples. In addition, we also used data from an earlier GWAS for childhood asthma in ethnically white samples described elsewhere.[13] Briefly, this study involved family and case-control panels comprising 994 patients with childhood-onset asthma and 1,243 nonasthmatic subjects. The family panel consisted of 207 predominantly nuclear families ascertained through a proband with severe (step 3) childhood-onset asthma. These families contained 295 sib pairs, 11 half-sib pairs, and 3 singletons. An additional set of 437 nonasthmatic aged-matched white UK control subjects were also studied. The case-control panel consisted of 728 asthmatic children from the Multicenter Asthma Genetics in Children Study (MAGICS) and 694 matched nonasthmatic children recruited by the International Study of Asthma and Allergy in Childhood. All cases in both family and case-control panels had physician-diagnosed asthma.

African American replication samples. *Children's Hospital of Philadelphia.* For replication of findings in one of the studies with existing GWAS data, African American children were recruited at the Children's Hospital of Philadelphia between 2006 and 2008. Cases included 1,456 patients with physician-diagnosed persistent asthma. Control subjects included 1,973 subjects who were determined to have no history of asthma or reactive airway disease by questionnaire and who had never been prescribed asthma medications according to their medical records. The mean age of the cases was 7.5 ± 5.7 (SD) years, and 57% were boys; the mean age of the control subjects was 6.7 ± 5.2 (SD) years, and 49% were boys.

The Howard University Family Study. GWAS data from the National Human Genome Center at the Howard University Asthma Cohort is comprised of 200 self-identified African American asthmatic cases and 200 ethnically matched control subjects ascertained from a database of participants recruited by the genetic epidemiology group directed by Dr Charles Rotimi for the Howard University Family Study and the Admixture Mapping for Hypertension in African Americans, a follow-up to the Howard University Family Study conducted by Adeyemo and coworkers in this group. These 2 projects contain an extensive epidemiologic database on more than 1,750 participants randomly recruited from 6 of the 8 total Council Wards in Washington, DC. The asthma cohort from this resource was included in analyses reported herein. Characteristics of the study participants were obtained by using questionnaires, anthropometry, and measurements of blood pressure and

related physiologic intermediates. The mean age is 50.5 ± 8.8 years in cases and 53.0 ± 6.7 years in control subjects. In the case group 48.2% had a family history of asthma compared with 13.5% in the control subjects. Study protocols were previously approved by the Howard University IRB, and informed consent was obtained from each participant.

Study of African Americans, Asthma, Genes and Environments. An additional 264 asthmatic cases and 186 nonasthmatic control subjects participating in the Study of African Americans, Asthma, Genes, and Environments, comprising asthmatic cases and control subjects from community clinics within San Francisco and Oakland, California, were included in the replication studies. Ethnicity was self-reported, and subjects were only enrolled if both biologic parents and all grandparents were of African American ethnicity. Asthma was defined according to a modified version of the 1987 American Thoracic Society Division of Lung Disease Epidemiology Questionnaire to collect information on asthma and allergy symptoms[20] and included pulmonary function data collected in a standardized fashion.[21] Taqman genotyping assays of the 4 SNPs were performed by using Assay-on-Demand or Assay-by-Design prevalidated assays (Applied Biosystems, Foster City, Calif), according to the manufacturer's instructions. Adjustments for population stratification were performed as previously described.[22] Local IRBs and clinics approved the study, and age-appropriate written consent was obtained from all study participants.

Baltimore Asthma Severity Study/Reducing Emergency Asthma Care in Harlem. Three hundred eighty-seven African Americans, including 208 asthmatic cases and 179 nonasthmatic control subjects, donated a blood sample for genetic analysis in the context of the Reducing Emergency Asthma Care in Harlem (REACH) study.[23] This study population consisted of adult Harlem residents recruited after a visit to the Harlem Hospital Emergency Department for an asthma exacerbation (cases) or for a nonallergic condition (control subjects). Ethnicity was self-reported, and asthma was defined based on an evaluation by a pulmonary physician within a median of 24 days after the emergency department visit. In the Baltimore Asthma Severity Study the study population included a community-based convenience sample of 539 African American Baltimore City residents, including 203 physician-diagnosed asthmatic cases and 336 control subjects. The participants in both the REACH study and the Baltimore Asthma Severity Study responded to a standardized, interviewer-administered questionnaire that includes a modified version of the 1987 American Thoracic Society Division of Lung Disease Epidemiology Questionnaire to collect information on asthma and allergy symptoms. In addition to questionnaire data, participants in both cohorts provided written informed consent for venipuncture, skin testing, and spirometry. However, in the REACH study, because the asthmatic participants were enrolled within less than 6 weeks of a severe exacerbation requiring emergency care, pulmonary function data were obtained only on a subset of the asthmatic participants (n = 137). Local IRBs and clinics approved both studies.

Genotyping

Genotypes were generated by the Johns Hopkins University SNP Center at the Center for Inherited Disease Research for 665,352 polymorphic tagging SNPs using Illumina HumanHap650Y Versions 1 and 3 BeadChips and the Illumina Infinium II assay protocol.[24] Genotypes were released for 994 GRAAD samples, 948 Barbados samples typed on Version 1 arrays, and 61 Barbados samples typed on Version 3 arrays. Allele cluster definitions for each SNP were determined by using the Illumina BeadStudio Genotyping Module (Version 2.3.41) and the combined intensity data from the African American samples. For the African Caribbean (Version 1) sample set, SNP cluster definitions from the African American data release were used, except for SNPs with call rates of less than 95% (n = 3,316). These SNPs were reclustered by using the African Caribbean samples and BeadStudio Genotyping Module (Version 3.1.0.0). For the African Caribbean Version 3 sample set, allele cluster definitions were determined by using the combined intensity data from 96 study samples and HapMap controls genotyped together plus 120 HapMap samples genotyped at Illumina by using BeadStudio Genotyping Module (Version 3.1.0.0). Thirty replicates composed of 10 trios were included across array versions. All mitochondrial and Y chromosome SNPs were manually reviewed and reclustered as needed. Genotype calls were made when a genotype yielded a quality score (Gencall value) of 0.25 or higher. Genotypes were not released (n = 23,874) for SNPs with more than 5% missing data, 1 or more HapMap replicate errors, more than 1 Mendelian error in the HapMap control trios, between 2% and 5% missing data along with a minor allele frequency (MAF) of less than 5%, or less than 2% missing data and a less than 1% MAF. Four HapMap controls were placed in unique positions on each DNA plate, 1 per set of 3 columns processed together in the laboratory. Fifteen blind duplicate samples were included, and the overall reproducibility was 99.99%.

Statistical methods

Quality control. Relationships between individuals within each study were evaluated by calculating identity-by-state estimates over all SNPs with PLINK[25] and further verified by using 103 equally spaced, highly polymorphic SNPs (MAF >45%) across the 22 autosomes with RELPAL.[26] PLINK[25] was also used to evaluate Mendelian inconsistencies in the family-based sample, as well as marker-level quality control parameters (MAF, differential missing rates between cases and control subjects, and Hardy-Weinberg equilibrium). The genetic structure of African American cases and control subjects was evaluated by using unrelated individuals from the 3 "continental" ancestral populations in HapMap (www.hapmap.org), with 416 SNPs identified as ancestry informative markers (AIMs) selected for maximal difference between African and European populations. The STRUCTURE program (version 2.2; http://pritch.bsd.uchicago.edu/software) was used to estimate membership in distinct subpopulations.[27,28] STRUCTURE was similarly used to analyze these 416 AIMs on 298 founders from asthmatic families in the African Caribbean study. Principal component analysis was carried out on African American cases and control subjects by using AIMs, on approximately 1,000 randomly selected independent SNPs, and ultimately on the complete array of autosomal markers to further test for possible confounding by using the SMARTPCA package (http://rd.plos.org/david_reich_laboratory).[29]

Tests for association. The Cochran–Armitage trend test was used to test for association between individual SNPs and asthma among the African American group by using the generalized estimating equations method with an exchangeable covariance matrix to permit the 29 individuals identified as pairs of first-degree relatives to contribute.[30] Tests for association were performed in the African Caribbean families by using the MQLS method[31] (software implemented by Liming Liang and Goncalo Abecasis: http://www.sph.umich.edu/csg/liang/MQLS/) under an additive model for each SNP. This method compares allele frequencies between cases and control subjects while taking into account family relationships. Genotyped subjects with missing phenotypes and phenotyped subjects with no imputed genotypes were also included to increase power. A meta-analysis was then performed combining the single-SNP *P* values for all SNPs. Because no SE was available from MQLS, we simply combined test statistics by taking the direction of the effect (ie, the risk allele) into account. Under the null hypothesis of no association, both test statistics can be written as independent draws from a Normal(0,1), and thus their sum divided by the square root of 2 is itself a draw from a Normal(0,1). This allows for a simple and valid calculation of a combined meta-analysis *P* value.

Imputation. We imputed genotypes for all polymorphic HapMap SNPs by using a hidden Markov model programmed in MACH[32] (http://www.sph.umich.edu/csg/abecasis/MACH/). This method combines genotypes from the study samples with the HapMap samples and identifies shared stretches of haplotypes. For each subject, genotypes at untyped SNPs can be summarized by taking (1) the most likely genotype according to the posterior probability of the 3 possible genotypes at that marker and (2) allele dosage, the expected number of copies of the reference allele (a fractional value between 0 and 2). We used the imputed allele dosage for association analysis. Using the imputed allele dosage is a good balance between computation efficiency and fully taking into account the uncertainty of imputed genotypes, which requires full likelihood inference or cumbersome multiple imputations. HapMap CEU samples (based on phased haplotypes release July,

TABLE I. Clinical characteristics of the GRAAD population

	African American		
	Total	**Cases**	**Control subjects**
No. of subjects	935*	464	471
Male subjects, no. (%)	406 (43.4)	211 (45.5)	195 (41.4)
Age (y), mean (SD)	29.55 (18.10)	23.78 (17.85)	35.23 (16.51)
Total IgE (95% CI)†	213.7 (191.5-238.4)	315.6 (270.4-368.3)	143.3 (123.8-165.8)
Atopy, no. (%)	641 (75.2)	369 (85.2)	272 (64.9)

	African Caribbean		
	Total	**Founders**	**Asthmatic subjects**
No. of subjects	929*	299	355
Male subjects, no. (%)	454 (48.9)	145 (48.5)	175 (49.3)
Age (y), mean (SD)	30.63 (17.06)	47.25 (11.54)	20.78 (12.84)
Total IgE (95% CI)†	433.5 (385.6-487.3)	271.4 (218.6-337.0)	948.7 (815.2-1104)
Atopy, no. (%)	404 (71.4)	79 (46.2)	187 (71.4)

*Reflects the final genotyped dataset after all quality control steps.
†Geometric mean of serum total IgE level (in nanograms per milliliter).

2006) were used to impute untyped SNPs for the English and German samples. A combined panel of HapMap CEU, YRI, and JPT + CHB samples (phased haplotypes release July 2006) was used to impute untyped SNPs for both the African American and African Caribbean samples. We evaluated the imputations by masking 2% randomly picked genotypes and compared the imputed genotype with the experimentally obtained genotype. The genotype-mismatch error rate is 6.6%, and the allele-mismatch error rate is 3.4%. This indicated high quality of imputation. In the analysis we removed all SNPs with estimated correlation between imputed and true allele counts of less than 0.3 (imputation R^2) and focused only on high-quality imputed SNPs.

For the family-based datasets (African Caribbean and European), association tests were performed with the MQLS method[31] (software implemented by L. L. and G. R. A.: http://www.sph.umich.edu/csg/liang/MQLS/) using imputed allele dosage. For the case-control (African American) sample, a 2-sample *t* test was used to compare the allele frequency (dosage) between cases and control subjects.

RESULTS

Admixture analysis revealed ancestry misclassification for 7 of the African American subjects, and 18 subjects from an ethnically mixed family from Barbados were also excluded from subsequent analysis. Additionally, samples were dropped based on quality control analysis of familial relationships (n = 53) and Mendelian inconsistencies (n = 13). Fourteen samples in the African American group and 1 in the African Caribbean group revealed sex discrepancies compared with clinical records. Among all African American cases and control subjects combined, 27 individuals were dropped because identity-by-state estimates suggested duplicated samples. Twenty-nine pairs of subjects had an estimated identity-by-state value of 0.50, suggesting they were first-degree relatives, but they were retained for analyses, resulting in a total of 464 asthmatic cases and 471 nonasthmatic control subjects (Table I). Among the families from Barbados, 26 pairs of duplicated samples were identified, and 13 subjects had greater than 1% of available markers showing Mendelian inconsistencies, suggesting a biologic relationship different from the reported family structure. These individuals were dropped, resulting in 929 subjects from 163 pedigrees in the final family sample from Barbados (Table I).

A total of 644,709 SNPs were released by the Center for Inherited Disease Research for the African American data and 641,488 for the African Caribbean data. Only monomorphic SNPs were dropped before analysis (n = 206 in the African Americans and n = 598 in the African Caribbean subjects). All remaining SNPs were analyzed, but some were flagged for various quality control measures, including deviations from Hardy-Weinberg equilibrium at a *P* value of less than 10^{-6} (601 SNPs among African American cases, 354 SNPs among African American control subjects, and 111 SNPs among African Caribbean founders), an MAF of less than 1% (5,935 SNPs among African American cases, 6,692 SNPs among African American control subjects, and 13,336 SNPs among African Caribbean founders), differential missing rates between African American cases and control subjects (26 SNPs), and the presence of greater than 10 Mendelian inconsistencies in the African Caribbean families (10,975 SNPs). In total, 6,917 SNPs were flagged for 1 or more reasons in the African American data and 25,008 in the African Caribbean data.

We obtained a genomic control parameter, as described by Devlin and Roeder,[33] of 1.012 for the African American case-control group and 0.98 in the African Caribbean family group, indicating a very small degree of background stratification and minimal differences in admixture. This finding was further supported by the ancestry analyses. The estimated proportion of African ancestry was very similar for African American cases and control subjects (72.3% and 72.5%, respectively), suggesting little possibility of confounding in subsequent association tests (Fig 1, *A*). The admixture analysis among the 298 founders in the African Caribbean families revealed slightly higher African ancestry (77.4%; Fig 1, *B*). Principal component analysis of all autosomal markers revealed similar patterns, with virtually no difference between the African American case and control groups and a slightly higher proportion of African ancestry among founders from Barbados (data not shown). Although quantile-quantile plots of the $-\log_{10} P$ values appear to reveal deviations from the expected values in both populations (see Fig E1 in this article's Online Repository at www.jacionline.org), these are due to deviations for very low MAFs (<1%) and also, in the African American sample, for MAFs of less than 5%. This deviation

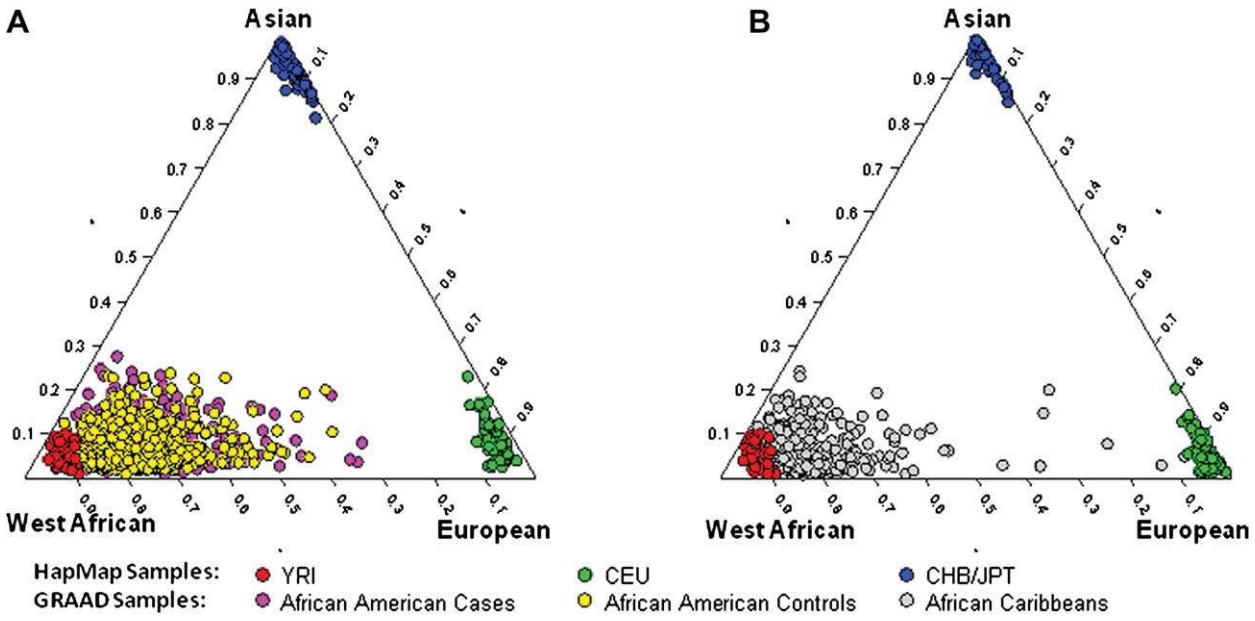

FIG 1. Triangle plots showing estimated admixture in 2 populations of African descent. Estimates were performed using 416 AIMs and data from the International HapMap Project on 60 YRI, 60 CEU, 90 CHB/JPT founders (see text for details). The figure depicts ancestry in 447 African American asthmatic cases and 459 nonasthmatic control subjects **(A)** and 298 African Caribbean founders **(B)**.

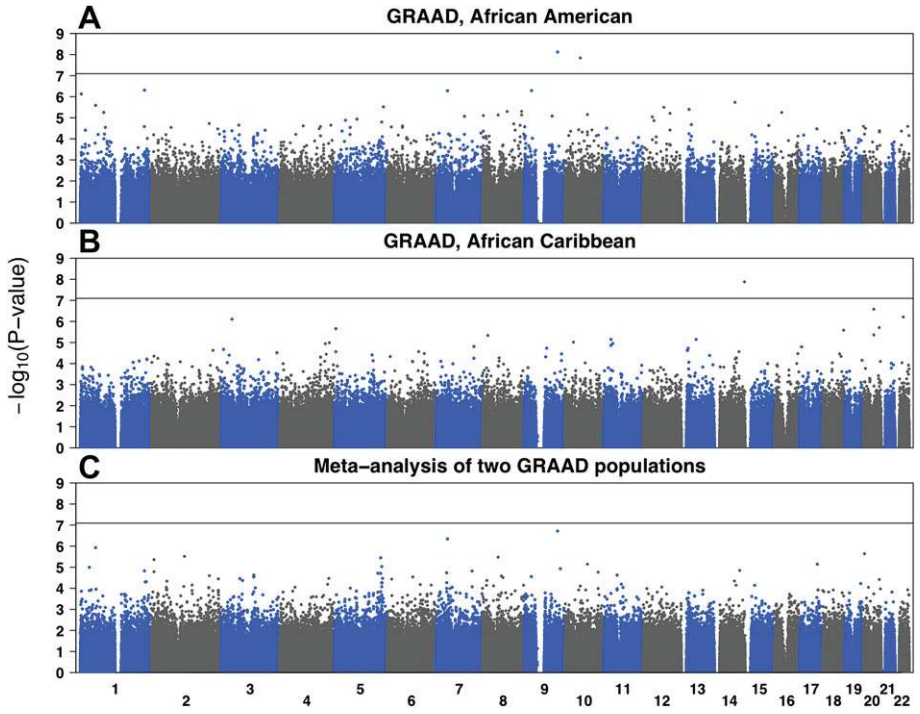

FIG 2. Genome-wide associations for asthma in 2 populations of African descent. **A**, African American asthmatic cases and control subjects. **B**, African Caribbean families. **C**, Meta-analysis of African American and African Caribbean GRAAD samples. Note that for visual clarity, the y-axis was truncated at a $-\log_{10}(P\text{value})$ of 9, resulting in the exclusion of a single data point (Fig 2, *A*: rs13209883, $P = 2.77 \times 10^{-11}$).

is due to the approximation of the null distribution for the *z* statistics derived from the generalized estimating equations, and for low MAFs, the actual null distribution tends to be more discrete and somewhat different than the asymptotic standard normal distribution. As described below, a low MAF was given much consideration in evaluating signals of association in these data.

Results of association tests between asthma status and individual SNPs across the entire genome are presented in Fig 2.

TABLE II. Associated SNPs with a combined *P* value of less than 10^{-5} in the African ancestry panels

					African American		African Caribbean		GRAAD combined
Marker	Chromosomal region	Genome position	Nearest gene	Risk allele	Risk allele frequency	*P* value	Risk allele frequency	*P* value	*P* value
rs1435879	2q12.3-q14.2	115,209,357	*DPP10*	A	0.9248	1.85×10^{-4}	0.9547	4.21×10^{-3}	3.05×10^{-6}
rs10515807	5q33	159,297,576	*ADRA1B*	T	0.0625	2.28×10^{-4}	0.0410	4.12×10^{-3}	3.57×10^{-6}
rs3972219	17q24.3	60,448,995	*GNA13*	G	0.0065	7.76×10^{-3}	0.0100	2.26×10^{-4}	7.11×10^{-6}
rs6052761	20pter-p12	4,605,017	*PRNP*	C	0.2828	5.96×10^{-5}	0.3339	7.54×10^{-3}	2.27×10^{-6}

Associated SNPs are those limited to some evidence for association ($P < .01$) in both African-ancestry panels and the same high-risk allele in both groups.

Three SNPs (rs13209883, rs10981955, rs16913596 in *RNGTT*, *ZNF618*, and *PRKG1*, respectively) met a prespecified threshold for genome-wide significance ($<P \times 10^{-8}$) in the African American case-control group (Fig 2, *A*; note that for visual clarity, the y-axis was truncated at a $-\log_{10}[P \text{ value}]$ of 9, resulting in the exclusion of rs13209883; $P = 2.77 \times 10^{-11}$). However, all 3 of these SNPs had MAFs of less than 1% in either the case or control group, as well as the Barbados founders. None of these SNPs showing significant association with asthma at this Bonferroni-adjusted threshold in the African American group showed evidence of association in the African Caribbean families. One marker (rs4264325 in *LOC400258*) was significantly associated in the African Caribbean group ($P = 1.31 \times 10^{-8}$; Fig 2, B), but the African American cases and control subjects showed no support for this SNP, and the MAF was low in both the Barbados founders (0.33%) and African Americans (0.65%). None of the genes in or near these significant markers has been previously implicated in asthma.

To further test for possible concordant associations in these 2 study populations, we used a less stringent threshold of a *P* value of less than .01 in both groups but required the same high-risk allele showing apparent association in both groups and a combined *P* value of less than 10^{-5} from meta-analysis of these 2 independent populations. SNPs in 4 genes showed evidence of association with asthma in these 2 populations of African descent, and the combined strength of association ranged between 2.27×10^{-6} and 7.11×10^{-6} (Fig 2, *C*, and Table II): dipeptidyl peptidase 10 *(DPP10)* on chromosome 2q12.3-q14.2, α-1B-adrenergic receptor *(ADRA1B)* on chromosome 5q33, G-α-13 *(GNA13)* on chromosome 17q24.3, and the prion-related protein *(PRNP)* on chromosome 20pter-p12. Two of these genes are in chromosomal regions 5q33 and 17q24.3, which were previously implicated in genome-wide linkage studies of multiplex asthmatic families,[3,7,34,35] and *DPP10* was first identified by means of positional cloning.[36] One of these 4 SNPs (rs3972219 in *GNA13*) had an MAF of less than 1% in both populations and was not included in further follow-up analyses. The estimated genotypic odds ratio under an additive model for the minor allele (T) at rs10515807 in *ADRA1B* was 1.40 (95% CI, 1.18–1.66), that for the minor allele (C) at rs6052761 in *PRNP* was 1.23 (95% CI, 1.07–1.41), and the minor allele (G) at rs1435879 in *DPP10* was protective (genotypic odds ratio, 0.65; 95% CI, 0.49–0.87).

Further support for 2 of these 3 genes in the African American data, *ADRA1B* and *PRNP*, was obtained by means of imputation. For *DPP10*, however, none of the imputed SNPs around rs1435879 in *DPP10* was statistically significant (Fig 3, *C*). The signal at rs6865665 in *ADRA1B* was supported by 2 imputed SNPs: rs11954917, which is located 483 bp upstream from the original signal ($P = .0006$), and rs10077860, which is located 656 bp downstream ($P = 0.000041$) from the original signal (Fig 3, *A*). The signal at rs6052761 in *PRNP* was supported by 3 imputed SNPs: rs10485513 and rs7270994, which are located 1415 and 1201 bp upstream, respectively ($P = .0001$), and rs6037929, which is located 874 bp downstream ($P = .0041$; Fig 3, *B*). In the Barbados data imputed SNPs did not lend further statistical support to peak signal of genotyped SNPs in any of these 3 genes (see Fig E2 in this article's Online Repository at www.jacionline.org).

To test the generalizability of these findings in other ethnic populations, we compared our results with GWAS data from a European study including both family and case-control panels of UK and German origin, respectively.[13] Because the European study genotyped a smaller number of markers (300,567 autosomal markers from the Illumina Sentrix HumanHap300 BeadChip), comparisons were made both with genotyped and imputed data. We observed nominal replication for the *ADRA1B* gene ($P = .04$) but no replication for *PRNP*. Although there was no replication for the *DPP10* markers in the region showing the strongest evidence for association in these GRAAD samples, 1 intronic SNP (rs1435879) toward the 3′ end showed nominal significance ($P = .0045$), and a cluster of multiple SNPs 0.6 Mb from the 3′ untranslated region of this gene were significantly associated with asthma ($P = .01$–$.001$, Fig 4) in the European replication sample.

Four additional case-control studies on African American subjects (from Baltimore/New York City; Philadelphia; Washington, DC; and San Francisco/Oakland, California) were genotyped to test for SNP-by-SNP replication at these top 3 markers: rs1435879, rs10515807, and rs6052761. Although the overall allele frequencies were comparable across datasets (see Table E1 in this article's Online Repository at www.jacionline.org), the differences in allele frequency between cases and control subjects seen in the discovery population of African descent were not seen in these additional 4 populations, nor were significant associations observed, with the exception of a trend for association between the *PRNP* SNP (rs6052761) in the dataset from Baltimore and New York City ($P < .05$, see Table E2 in this article's Online Repository at www.jacionline.org).

DISCUSSION

In this article we report the first GWAS for asthma focused on populations of African descent. Using 2 independent sets of samples, an African American case-control group from Baltimore–Washington, DC (n = 935), and 163 African Caribbean families from Barbados (n = 929), we have identified 3 genes as associated with asthma, each of which are biologically relevant to asthma pathology. However, these findings must be interpreted

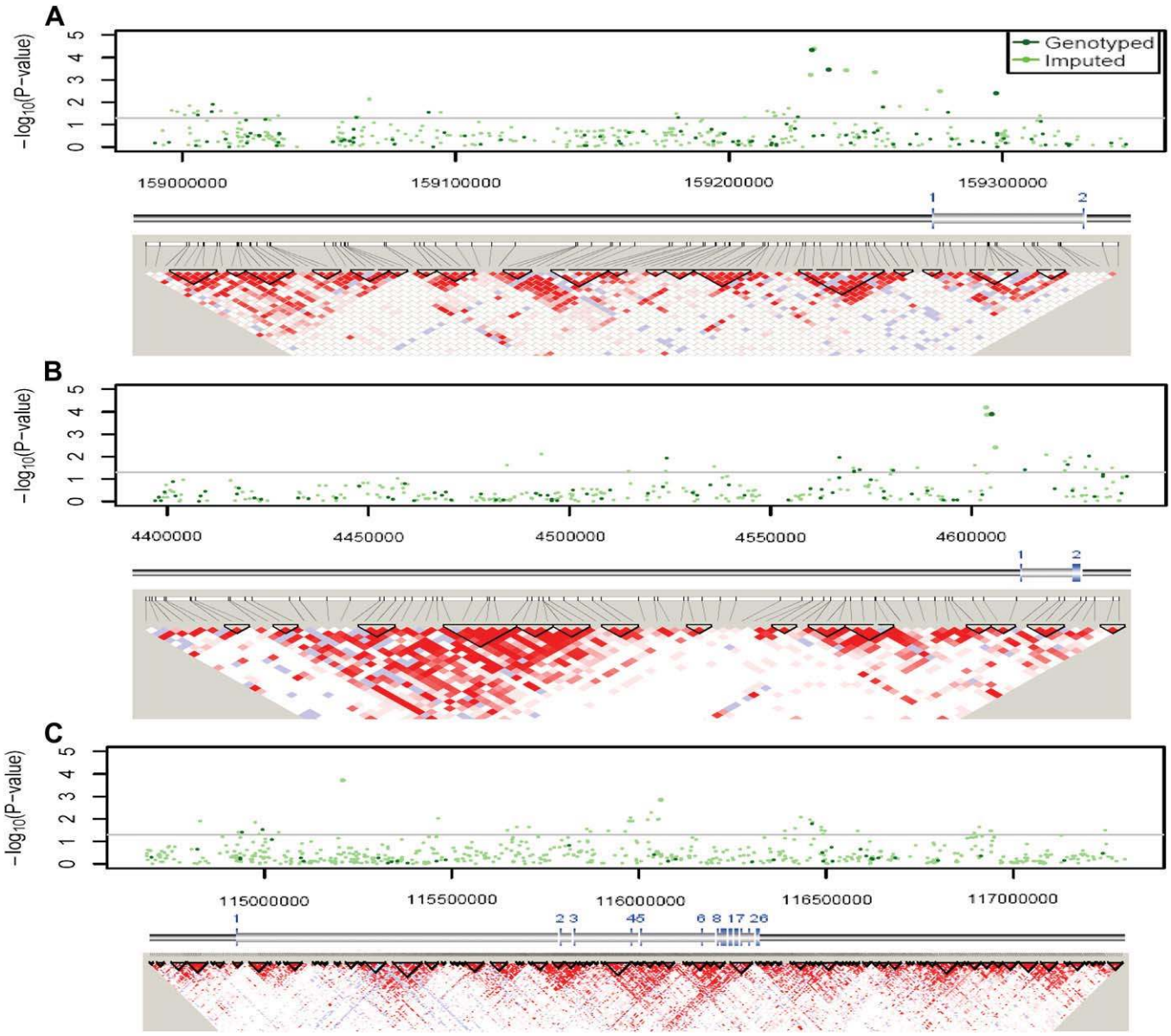

FIG 3. Evidence of association with asthma and LD around *ADRA1B*, *PRNP*, and *DPP10*. *Upper plots* summarize association *P* values for all genotyped and imputed SNPs in the African American case-control group for *ADRA1B* **(A)**, *PRNP* **(B)**, and *DPP10* **(C)**. *Lower plots* illustrate patterns of LD *(R^2)* in these samples: *red squares* for strong LD, *blue squares* for nonsignificant LD, and *white squares* for little or no LD.

with caution because of limitations of sample size and the underlying complexity and heterogeneity of this disease, as well as our inability to replicate findings at the SNP-for-SNP level.

Significant association ($P = 3.57 \times 10^{-6}$) was seen between asthma and the marker rs10515807 in an intronic linkage disequilibrium (LD) block spanning 5 Kb located 21 Kb from the 5′ end of the *ADRA1B* gene on chromosome 5q33, which has been implicated in asthma studies previously.[3,7,34,35] On examination for genes flanking *ADRA1B* for which there is previous evidence for association for asthma, we observed that the gene encoding *ADRB2* is 11 Mb upstream of *ADRA1B*, and interleukin 12B *(IL12B)* is 0.59 Mb downstream from *ADRA1B*. However, none of the SNPs in these candidate genes was in LD with the *ADRA1B* SNP associated with asthma in this study. Evidence of association of *ADRA1B* was supported by several imputed SNPs ($P = .0001$–$.0041$) among the African American samples (Fig 3, *B*). The estimated genotypic odds ratio for the minor allele (T) at rs10515807 under an additive model was 1.40 (95% CI, 1.18–1.66). *ADRA1B* is one of 3 α_1-adrenergic receptor subtypes in the G protein–coupled family of transmembrane receptors, and the protein product of this gene is expressed in the lung.[37] α_1-Adrenergic receptors are well known for their physiologic responses to fight-or-flight signaling and regulation of carbohydrate metabolism,[38] but interestingly, they have also been associated with proinflammatory responses.[39] Although no role for α_1-adrenergic receptors in asthma has yet been demonstrated, α_1-adrenergic receptor stimulation has been shown to increase the rate of DNA synthesis and to induce proliferation in various cell types, including vascular smooth muscle cells.[40]

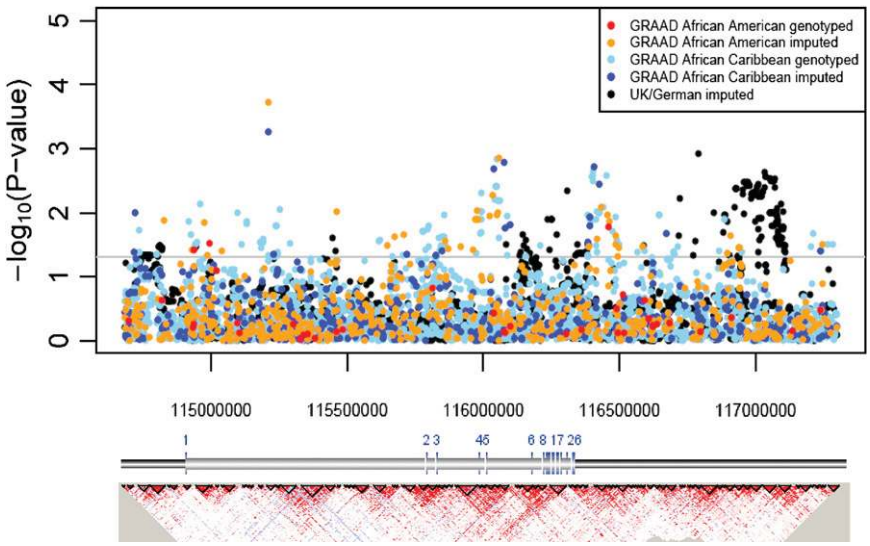

FIG 4. Evidence of association with asthma and LD around *DPP10*. The *upper plot* summarizes association *P* values of genotyped and imputed SNPs for the African American cases and control subjects, African Caribbean families, and the European GABRIEL replicate population. *Lower plots* illustrate patterns of LD (R^2) in these samples: *red squares* for strong LD, *blue squares* for nonsignificant LD, and *white squares* for little or no LD.

The second locus yielding significant evidence of association in the combined samples of African descent was the relatively common C allele of marker rs6052761 (MAF = 28% to 33%) in the *PRNP* gene on chromosome 20pter-p12. The estimated genotypic odds ratio for the minor C allele was 1.23 (95% CI, 1.07–1.41). Association between asthma and marker rs6052761 was modestly supported by several nearby imputed SNPs (P = .0001–.0041) located within a small region (1.4 Kb) upstream of marker rs6052761, which showed evidence among the African American (Fig 3, *B*) and Barbados samples (see Fig E2, *B*). The *PRNP* gene, encoding the prion protein (PrP), has mainly been implicated in various transmissible neurodegenerative spongiform encephalopathies, including Creutzfeldt-Jakob disease and Kuru.[41] The normal cellular isoform (PrP[C]) is, however, abundantly expressed in nonneuronal tissues, including lung and lymphoid cells.[42] The biologic role of PrP(C) is not fully understood, although it has been shown to be involved in immune cell activation,[43,44] signal transduction, cell adhesion, and antioxidant activity.[45] In lymphoid cells PrP(C) is detected on human T and B lymphocytes (preferentially expressed by $CD4^+$, $CD25^+$, and forkhead box protein 3–positive regulatory T cells[46]) and most highly expressed on dendritic cells.[47] In a murine model PrP(C) was shown to be upregulated in T cells through a signal transducer and activator of transcription 6–dependent mechanism after treatment with IL-4.[48] Marker rs6052761, a C-to-T substitution located 10.1 Kb upstream of the *PRNP* gene, is relatively close to regulatory regions previously identified as harboring variants associated with Creutzfeldt-Jakob disease.[49]

The third region of association was observed at an intronic nonsynonymous marker (rs1435879, $P = 3.05 \times 10^{-6}$) toward the 5′ end of a very large gene, *DPP10* (spanning approximately 1.4 Mb), on chromosome 2q12.3-q14.2. The minor allele (G) at SNP rs1435879 was protective, with an estimated genotypic odds ratio of 0.65 (95% CI, 0.49–0.87). DPP10 is a 796-amino-acid, multifunctional protein and is a member of a family of proteins in the S9B serine proteases subfamily.[50] Although structurally similar to dipeptidyl peptidase IV, DPP10 shows nearly identical activity to dipeptidyl peptidase X in that both proteins induce Kv4.2 protein trafficking from the endoplasmic reticulum to the cell surface.[51] DPP10 is moderately expressed in the trachea[36]; however, it is abundantly expressed in nodose and dorsal root ganglia, suggesting a possible role in controlling bronchial reactivity caused by alterations in the magnitude of the A-type K^+ current and subsequent changes in the excitability of cell membranes.[52] Importantly, it has been well established that perturbations and perversions of afferent nerve function contribute to manifestations associated with inflammatory airway disease.[53] Consistent with these findings, Quantitative Trait Locus (QTL) studies on murine models have linked airway hyperresponsiveness in mice to the murine homolog of human *DPP10*.[54,55] Very recently, DPP10 was found to be both expressed and regulated in the bronchial epithelium of the airways of rats with and without an allergic-like inflammation status.[56]

DPP10 was originally identified as a candidate gene for asthma through positional cloning, followed by extensive sequencing and association to additional SNPs in its first exon.[36] Because there were no known coding polymorphisms in this exon at the time of their study, Allen et al[36] speculated that the association might reflect alternative splicing between membrane-bound and other forms of the protein, a hypothesis supported by observations that *DPP10* was strongly expressed with multiple splice variants in the brain, spinal cord, pancreas, and adrenal glands. The *DPP10* gene is substantial in size, extending over 1 Mb of genomic DNA. Allen et al genotyped a limited number of SNPs, with a focus primarily around the initial few exons of the gene. The present study included both genotyped and imputed SNPs and provided much greater coverage within and surrounding the *DPP10* gene. Consequently, we were able to highlight other *DPP10* SNPs in addition to those reported by Allen et al that might be of importance for asthma, especially in samples of African ancestry (Fig 4).

We initially undertook this GRAAD study assuming certain genes might contribute to the profound disparities in the risk and severity of asthma morbidity and mortality between European-derived populations and those of African descent. In the European population used here for replication of findings from our samples of African ancestry, we also did not observe significant associations at SNPs in and around the markers providing the strongest evidence of association in these 3 genes (*ADRA1B*, *PRNP*, and *DPP10*). There was, however, significant association with SNPs toward the 3′ end and in the 3′ untranslated region of *DPP10* in the European sample (Figure 4).

Numerous studies have demonstrated that asthma and its associated phenotypes, like other complex traits, have heritabilities in the range of 40% to 80%,[57,58] suggesting that multiple genes are involved in the disease's cause. The GWAS approach has been very productive in discovering genes controlling risk to complex diseases and phenotypes because it provides an unbiased and comprehensive approach.[59] However, the fact that GWAS has only identified a modest number of common variants of relatively modest effect supports the notion that numerous rare functional SNPs are major contributors to susceptibility to common diseases,[60] such as asthma. Although it is estimated that approximately 60% of SNPs in the human genome have MAFs of less than 5%, companies producing GWAS arrays are biased toward common tagging variants in support of the common-disease common-variant hypothesis, and consequently, there are relatively few rare SNPs in coding and promoter regions in their SNP genotyping panels.[61] Of greater concern in the context of the current study, it has been demonstrated that currently available commercial chips, including the panel used in the GRAAD discovery population, are inadequate in content for African-origin populations. These findings also underscore the shortcoming of relying only on Yoruban genomes (ie, YRI) to represent African Americans, particularly in light of the recent observations by Tishkoff et al[62] demonstrating that although approximately 71% of the African ancestry of African Americans can be attributed to West African populations, other African groups account for at least 8% of the African ancestry.

A possible explanation for the failure to observe SNP-for-SNP replication in the 4 independent African ancestry populations is subtle differences in admixture across each of the samples. In the discovery samples we detected minimal background stratification and minimal differences in admixture; principal component analysis of all autosomal markers revealed similar patterns between the 2 GRAAD populations. However, as highlighted recently by Li and Leal,[63] it is not yet known whether current statistical methods, such as STRUCTURE or principal component analysis, can adequately control for population substructure if rare variants are included. Although 3 of the 4 African American replicate samples were comprised of subjects from the same geographic region as the African American discovery sample (Baltimore; Washington, DC; and Philadelphia), it is possible that slight differences in African and European admixture within the datasets precluded supporting findings. In the initial GWAS by Moffatt et al[13] on the European sample used in the current study, the most significant association ($P < 10^{-12}$) was for markers near the gene encoding *ORMDL3* on chromosome 17q21. We closely examined these SNPs in both of our African ancestry groups and found little evidence for association with any genotyped SNPs in the *ORMDL3* gene and its flanking regions (rs9910635 had a nominal *P* value of .016 in the case-control group, with no evidence of replication in the African Caribbean families). Examining both genotyped and imputed SNPs (n = 2,702) in a 3-Mb region (Chr17: 34-37 Mb) around *ORMDL3*, we only found minimal association signals in regions showing peak association signals in the European group (rs12150079, $P = .005$ in the African Caribbean families but no evidence in the GRAAD case-control group at $P = .89$; data not shown). Furthermore, 2 of the African American samples used for replication in the current study did not support associations in the same *ORMDL3* SNPs.[64,65]

In the current study the only suggestion of replication for one of the genes *(DPP10)* was similar to the *ORMDL3* observations at the level of the gene rather than the SNP, with signals far apart in the 2 ethnic groups, supporting a strategy of gene versus SNP when examining replication across populations. To better evaluate this idea, we queried the level of significance at the gene level (minimum *P* value for all SNPs mapped to a gene) across the 2 GRAAD populations and 3 additional GWASs on asthma, including the European sample, the Children's Hospital of Philadelphia sample, and GWAS data from non-Hispanic white families ascertained through childhood asthmatic subjects aged 5 to 12 years participating in the Childhood Asthma Management Program.[66] Fifty-six genes were selected for follow-up in these 3 replicate populations meeting nominal significance criteria in both of our discovery populations with signals within 5 Kb (data not shown). It is notable that 3 genes appear to have a gene-based signal (qualified as $P < .01$) across the 5 ethnically diverse populations, including *DPP10*, the only gene identified by means of positional cloning for asthma, as described above. Although these analyses are purely exploratory and not formal, the findings suggest that the current standards requiring same SNP replication (for what are, after all, not causal variants but rather tagging SNPs in LD with an unknown disease-causing variant selected primarily from European genomes), combined with the stringent demand for levels of significance ($P < 10^{-8}$) to account for the considerable multiple comparisons (using statistical approaches not originally designed for GWASs), illustrate the point that alternative approaches are warranted.

This is the first GWAS with a primary focus on independent populations of African descent, and it has highlighted key genes and regions that might be distinct from genes important in non-African populations. This study clearly illustrates the difficulty with replicating associations for complex and heterogeneous diseases (eg, asthma) when the marker panel might provide imperfect coverage of common variants in admixed populations. The results of this study illustrate the complexity of identifying true associations for a complex and heterogeneous disease (eg, asthma) in admixed populations and emphasize the need to test for replication beyond an SNP-for-SNP level to fully evaluate fine mapping in follow-up strategies. Evidence of association between asthma and these 3 candidate genes (*ADRA1B*, *PRNP*, and *DPP10*) clearly warrants further studies to confirm the possible uniqueness of these associations to populations of African descent, with particular attention to fine mapping around these genes because of the difficulty in achieving SNP-for-SNP replication across studies in additional populations of African descent.

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Clinical implications: Identification of immune- and inflammatory-related polymorphisms uniquely controlling risk for asthma in African-ancestry populations might lead to a better understanding of the underlying disparities in this minority group.

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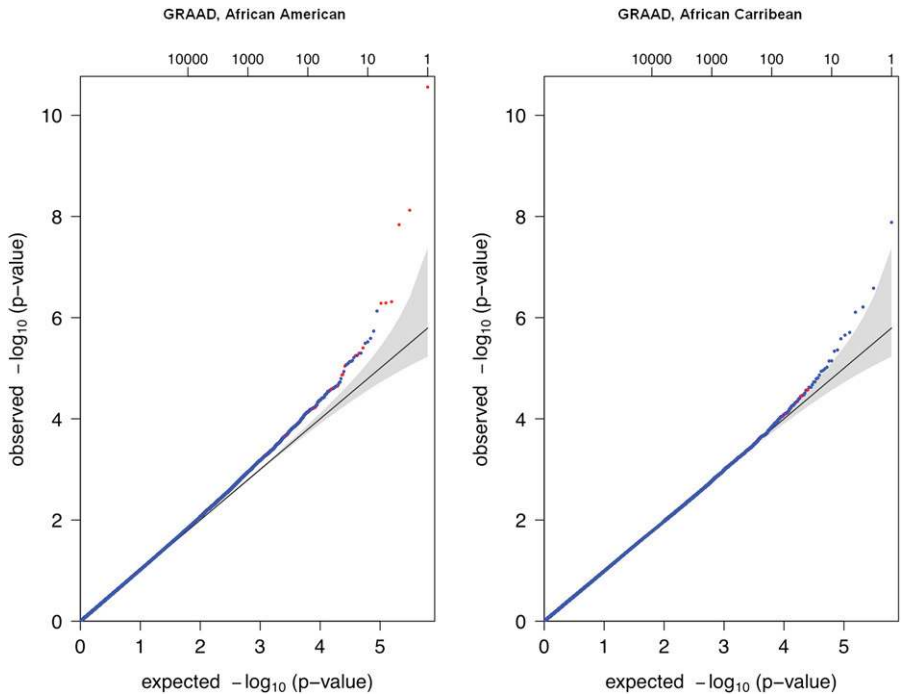

FIG E1. Expected quantiles versus observed quantiles for the $-\log_{10}$ *P* values in the GRAAD and Barbados populations. The *red dots* flag SNPs with MAF <1% and the *gray region* indicates the 95% confidence band, which was calculated by using the Stirling approximation.[E1]

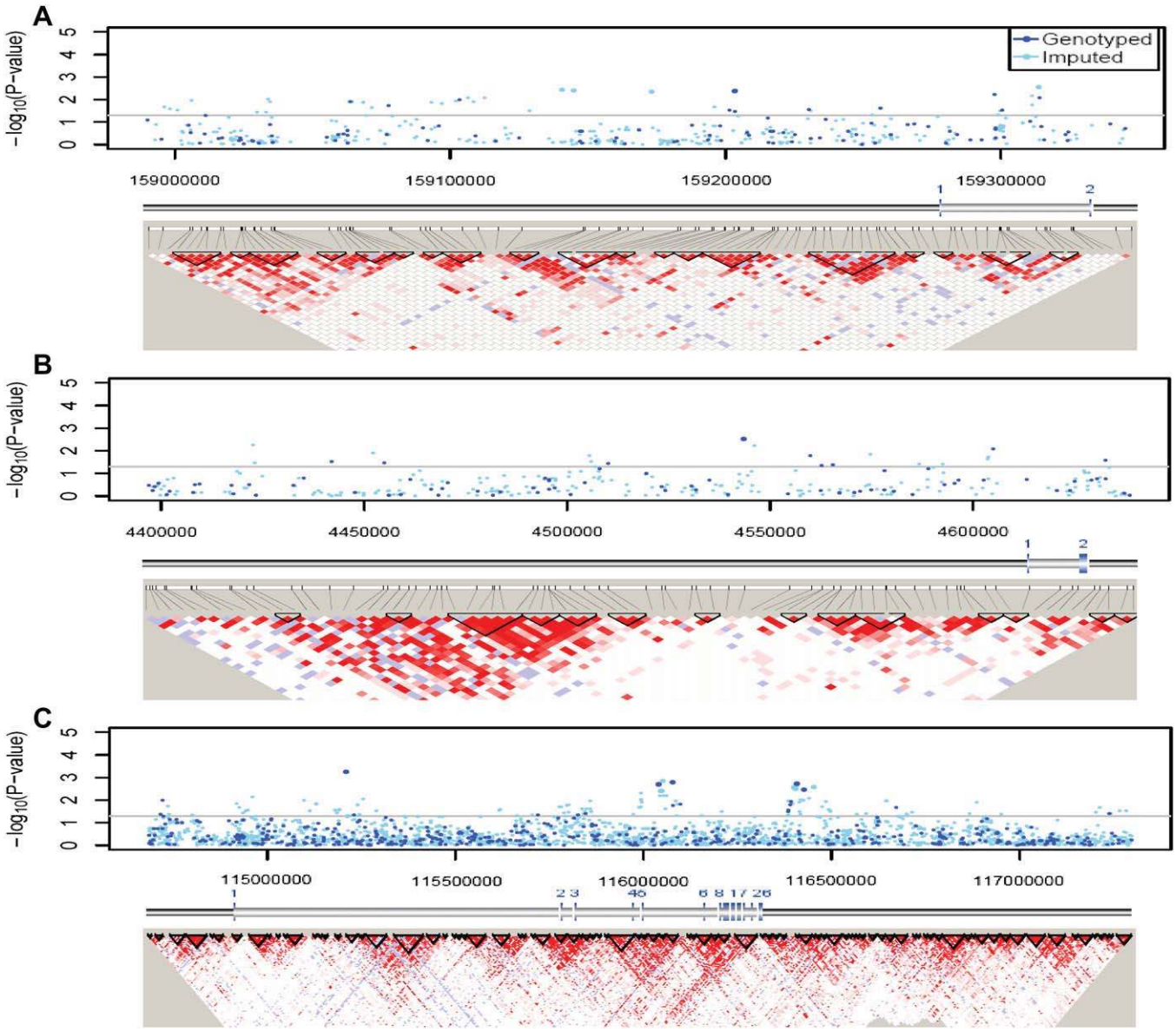

FIG E2. Evidence of association with asthma and LD around the genes *ADRA1B*, *PRNP*, and *DPP10*. *Upper plots* summarize all genotyped *(dark green circles)* or imputed *(light green circles)* SNPs with association *P* values (additive test) in the African Caribbean family-based group for *ADRA1B* **(A)**, *PRNP* **(B)**, and *DPP10* **(C)**. *Lower plots* in each panel illustrate patterns of LD *(R^2)* in the samples represented as *red squares* for strong LD, *blue squares* for nonsignificant LD, and *white squares* for little or no LD.

TABLE E1. Allele frequencies of 3 SNPs with a combined *P* value of less than 10^{-5} in the discovery populations comparing the African American GRAAD sample and 4 replicate African American populations

Study (cases/control subjects):			GRAAD (464/471)				CHOP (1,456/1,973)		HUFS (200/200)		SAGE (264/186)		BASS/REACH (411/515)	
Nearest gene	**Marker**	**Allele**	**Risk allele**	**Control**	**Case**	**Diff (%)**	**Control**	**Case**	**Control**	**Case**	**Control**	**Case**	**Control**	**Case**
DPP10	rs1435879	A	A	0.925	0.963	3.9	0.935	0.931	0.940	0.926	0.922	0.920	0.943	0.934
		G		0.075	0.037		0.065	0.069	0.060	0.075	0.078	0.080	0.057	0.066
ADRA1B	rs10515807	C		0.971	0.938		0.962	0.960	NA	NA	0.965	0.969	0.955	0.964
		T	T	0.029	0.063	3.3	0.038	0.040	NA	NA	0.035	0.031	0.045	0.036
PRNP	rs6052761	C	C	0.282	0.364	8.2	0.331	0.319	0.332	0.300	0.289	0.312	0.284	0.328
		T		0.718	0.636		0.669	0.682	0.668	0.700	0.711	0.688	0.716	0.672

CHOP, Children's Hospital of Philadelphia; *HUFS*, Howard University Family Study; *SAGE*, Study of African Americans, Asthma, Genes, and Environments; *BASS*, Baltimore Asthma Severity Study; *REACH*, Reducing Emergency Asthma Care in Harlem; *Diff*, difference in allele frequency between cases and control subjects; *NA*, not available.

TABLE E2. *P* values of tests for association of GRAAD top SNPs in replicate populations of African descent

					African American	African Caribbean	GRAAD total	CHOP	HUFS	SAGE	BASS/ REACH
Marker	Chromosomal region	Genome position	Nearest gene	Risk allele	*P* value (Armitage trend test)			*P* value	*P* value (adjusted for age and sex)	*P* value (Armitage trend test)	*P* value (Armitage trend test)
rs1435879	2q12.3-q14.2	115,209,357	*DPP10*	A	1.85×10^{-4}	4.21×10^{-3}	3.05×10^{-6}	.3261	.201	.0879	.417
rs10515807	5q33	159,297,576	*ADRA1B*	T	2.28×10^{-4}	4.12×10^{-3}	3.57×10^{-6}	.546	NA	.746	.388
rs6052761	20pter-p12	4,605,017	*PRNP*	C	5.96×10^{-5}	7.54×10^{-3}	2.27×10^{-6}	.1676	.316	.455	**.042**

CHOP, Children's Hospital of Philadelphia; *HUFS*, Howard University Family Study; *SAGE*, Study of African Americans, Asthma, Genes, and Environments; *BASS*, Baltimore Asthma Severity Study; *REACH*, Reducing Emergency Asthma Care in Harlem; *NA*, not available.

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