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A Genome-Wide Association Study on African-Ancestry Populations For Asthma

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

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Abstract

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—To test the hypothesis that some genes may contribute to the profound disparities in asthma.

Methods—We performed a genome-wide association study in two independent populations of African ancestry (935 African American asthma cases and controls from the Baltimore-Washington, D.C. area, and 929 African Caribbean asthmatics and their family members from Barbados) to identify single-nucleotide polymorphisms (SNPs) associated with asthma.

Results—Meta-analysis combining these two African-ancestry populations yielded three SNPs with a combined *P*-value $<10^{-5}$ in genes of potential biological relevance to asthma and allergic disease: rs10515807, mapping to alpha-1B-adrenergic receptor (*ADRA1B*) gene on chromosome 5q33 (3.57×10^{-6}); rs6052761, mapping to prion-related protein (*PRNP*) on chromosome 20pter-p12 ($2.27\times10^{-}6$); and rs1435879, mapping to dipeptidyl peptidase 10 (*DPP10*) on chromosome 2q12.3-q14.2. The generalizability of these findings was tested in family and case-control panels of UK and German origin, respectively, but none of the associations observed in the African groups were replicated in these European studies.

Conclusions—Evidence for association was also examined in four additional case-control studies of African Americans; however, none of the SNPs implicated in the discovery population were replicated. 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.

Keywords

Asthma; GWAS; *ADRA1B*; *PRNP*; *DPP10*; African ancestry; ethnicity; polymorphism; genetic association

INTRODUCTION

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 rise [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 two recent genome-wide

association studies (GWAS) [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, two independent populations of African descent ascertained through physician's diagnosed asthma, have been recruited by a consortium entitled *Genomic Research on Asthma in the African Diaspora* (GRAAD). These populations have been genotyped using the Illumina HumanHap650Y BeadChip containing 655,352 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 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 two studies.

METHODS

Sample description

We analyzed 498 asthma cases and 500 non-asthmatic controls from the Baltimore-Washington, D.C. metropolitan area who self-reported as African American ethnicity. These subjects comprised the consortium for '*Genomic Research on Asthma in the African Diaspora*' (GRAAD) and represent eight separate, NIH-funded studies of asthma in pediatric and adult African American populations, plus one 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 controls with some potential for developing asthma. Informed consent was obtained from each study participant, and the study protocol was approved by the institutional review board 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 the American Thoracic Society [15] or International Study of Asthma and Allergy in Childhood (ISAAC) [16] was administered by a clinical coordinator. All controls (except 50, see below) were likewise administered a standardized questionnaire and were determined to be negative for a history of asthma. Asthma status on 50 controls 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 a total of 1,028 individuals 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 documented history of physician-diagnosed asthma (past or current), plus a history of wheezing without an upper respiratory infection (URI) for two out of four hallmark symptoms (wheezing with a URI, cough without a URI, shortness of breath, and tightness in the chest). All subjects gave verbal and written consent as approved by the Johns Hopkins Institutional Review Board (IRB) and the Barbados Ministry of Health.

European Ancestry Replication Samples—In addition we also used data from an earlier genome wide association study (GWAS) for childhood asthma in Caucasian samples described elsewhere [13]. Briefly, this study involved family and case-control panels

comprising 994 patients with childhood onset asthma and 1,243 non-asthmatics. 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 non-asthmatic aged-matched Caucasian UK controls (UK-C) were also studied. The case-control panel consisted of 728 asthmatic children from the MAGICS study and 694 matched non-asthmatic children recruited by the ISAAC study. All cases in both family and case-control panels had physician-diagnosed asthma.

African American Replication Samples

CHOP: 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 (CHOP) between 2006 and 2008. Cases included 1,456 patients with physician-diagnosed persistent asthma. Controls 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. Mean age of cases was 7.5±5.7 SD years and 57% were male; mean age of controls was 6.7±5.2 SD years and 49% male.

HUFS: GWAS data from the National Human Genome Center (NHGC) at Howard University Asthma Cohort is comprised of 200 self-identified African American asthma cases and 200 ethnically matched controls ascertained from a database of participants recruited by the genetic epidemiology group directed by Dr. Charles Rotimi for the "The Howard University Family Study (HUFS)" and the "Admixture mapping for hypertension in African Americans", a follow-up to the HUFS conducted by Adeyemo and co-workers in this group. These two projects contain an extensive epidemiological database on >1750 participants randomly recruited from 6 of the 8 total Council Wards in Washington, D.C. The asthma cohort from this resource was included in analyses reported herein. Characteristics of the study participants were obtained by 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 controls. In the case group, 48.2% had a family history of asthma compared to 13.5% in the controls. Study protocols were previously approved by the Howard University Institutional Review Board and informed consent was obtained from each participant.

SAGE: An additional 264 asthma cases and 186 non-asthmatic controls participating in the Study of African Americans, Asthma, Genes & Environments (SAGE) comprising asthma cases and controls 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 biological parents and all grandparents were of African American ethnicity. Asthma was defined according to a modified version of the 1987 American Thoracic Society (ATS) - 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 four SNPs were performed using Assay-on-Demand or Assay-by-Design pre-validated assays (Applied Biosystems, Foster City, CA) according to the manufacturer's instructions. Adjustments for population stratification was performed as previously described [22]. Local Institutional Review Boards (IRBs) and clinics approved the study and age-appropriate written consent was obtained from all study participants.

BASS / REACH: A total of 387 African Americans, including 208 asthma cases and 179 non-asthmatic controls, donated a blood sample 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 following a visit to the Harlem Hospital Emergency Department (ED) for an asthma exacerbation (cases) or for a non-allergic condition (controls). Ethnicity was self-reported, and asthma was defined based on an evaluation by a pulmonary physician within a median of 24 days following the ED visit. In the Baltimore Asthma Severity Study (BASS), the study population included a community-based convenience sample of 539 African American Baltimore City residents, including 203 physician-diagnosed asthma cases and 336 controls. The participants in both REACH and BASS responded to a standardized, interviewer-administered questionnaire that includes a modified version of the 1987 American Thoracic Society (ATS) - Division of Lung Disease Epidemiology Questionnaire to collect information on asthma and allergy symptoms. In addition to questionnaire data, participants in both cohorts gave 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 Institutional Review Boards (IRBs) and clinics approved both studies.

Genotyping

Genotypes were generated by the Johns Hopkins University SNP Center at the Center for Inherited Disease Research (CIDR) 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 on Version 1 arrays and 61 Barbados samples typed on Version 3 arrays. Allele cluster definitions for each SNP were determined using 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 below 95% (N=3,316). These SNPs were re-clustered 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 using the combined intensity data from 96 study samples and HapMap controls genotyped together, plus 120 HapMap samples genotyped at Illumina 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 re-clustered 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 error, more than 1 Mendelian error in the HapMap control trios, between 2-5% missing data along with a minor allele frequency less than 5%, or less than 2% missing data and less than 1% minor allele frequency. 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 (IBS) estimates over all SNPs using PLINK [25], and further verified using 103 equally spaced highly polymorphic SNPs (MAF>45%) across the 22 autosomes using RELPAL [26]. PLINK [25] was also used to evaluate Mendelian inconsistencies in the family-based sample as well as marker-level quality control (QC) parameters (MAF, differential missing rates between cases and controls, Hardy Weinberg Equilibrium). The genetic structure of African American cases and controls was evaluated using unrelated individuals from the three “continental” ancestral populations in the HAPMAP (www.hapmap.org) using 416 SNPs identified as ancestry informative markers

(AIMs) selected for maximal difference between African and European populations. The STRUCTURE program (V2.2; http://pritch.bsd.uchicago.edu/software) was used to estimate membership in distinct sub-populations [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 controls using AIMs, ~1000 randomly selected independent SNPs, and ultimately on the complete array of autosomal markers to further test for possible confounding 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 using Generalized Estimating Equations (GEE) 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 using the MQLS method [31] (software implemented by LL and GRA: http://www.sph.umich.edu/csg/liang/MQLS/) under an additive model for each SNP. Under an additive model, this method compares allele frequencies between cases and controls while taking into account family relationships. Genotyped individuals with missing phenotype and phenotyped individuals 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 standard error was available from MQLS, we simply combined test statistics taking the direction of the effect (*i.e.*, 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 individual, genotypes at untyped SNPs can be summarized by taking (1) the most likely genotype according to the posterior probability of the three 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 needs full likelihood inference or cumbersome multiple imputations. HapMap CEU samples (based on phased haplotype release July, 2006) were used to impute untyped SNPs for the English and German samples. A combined panel of HapMap CEU, YRI and JPT+CHB (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 allele count and true allele count < 0.3 (imputation R-square) and focus 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 LL and GRA: http://www.sph.umich.edu/csg/liang/MQLS/) using imputed allele dosage. For the case-control (African American) sample, a two sample t-test was used to compare the allele frequency (dosage) between cases and controls.

RESULTS

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

A total of 644,709 SNPs were released by the Center for Inherited Disease Research (CIDR) for the African American data and 641,488 in the African Caribbean data. Only monomorphic SNPs were dropped prior to analysis (N=206 in the African Americans and N=598 in the African Caribbeans). All remaining SNPs were analyzed, but some were flagged for various QC measures, including: deviations from Hardy Weinberg Equilibrium (HWE) at $P<10^{-6}$ (601 SNPs among African American cases, 354 SNPs among African American controls, and 111 SNPs among African Caribbean founders), minor allele frequency (MAF) <1% (5,935 SNPs among African American cases; 6,692 SNPs among African American controls; and 13,336 SNPs among African Caribbean founders), differential missing rates between African American cases and controls (26 SNPs) and presence of >10 Mendelian inconsistencies in the African Caribbean families (10,975 SNPs). In total, 6,917 SNPs were flagged for one or more reasons in the African American data and 25,008 in the African Caribbean data.

We obtained a genomic control parameter (λ_{GC}) 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 controls (72.3% and 72.5%, respectively), suggesting little possibility of confounding in subsequent association tests (Figure 1, Panel A). The admixture analysis among the 298 founders in the African Caribbean families revealed slightly higher African ancestry (77.4%; Figure 1, Panel 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). While quantile-quantile (qq) plots of the -log10 p-values appear to reveal deviations from the expected values in both populations (Figure E1, online repository), these are due to deviations for very low minor allele frequencies (< 1%), and also in the African American sample for minor allele frequencies less than 5%. This deviation is due to the approximation of the null distribution for the z-statistics derived from the generalized estimation equations, and for low minor allele frequencies the actual null distribution tends to be more discrete and somewhat different than the asymptotic standard normal distribution. As described below, 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 Figure 2. Three SNPs (rs13209883, rs10981955, rs16913596 in *RNGTT, ZNF618*, and *PRKG1*, respectively) met a pre-specified threshold for genome-wide significance ($<P\times10^{-8}$) in the African American case-control group (Figure 2, Panel A; note: for visual clarity, the Y-axis was truncated at $-\log_{10}$(P-Value) = 9 resulting in the exclusion of rs13209883, $P= 2.77 \times 10^{-11}$). However, all three of these SNPs had MAF <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 ($P=1.31 \times 10^{-8}$; Figure 2, Panel B), but the African American cases and controls 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 have been previously implicated in asthma.

To further test for possible concordant associations in these two study populations, we used a less stringent threshold of $P<0.01$ in both groups but required the same high risk allele showing apparent association in both groups, and a combined P-value $<10^{-5}$ from meta-analysis of these two independent populations. SNPs in four genes showed evidence of association with asthma in these two populations of African descent, and the combined strength of association ranged between 2.27×10^{-6} and 7.11×10^{-6} (Figure 2, Panel C; Table II): Dipeptidyl peptidase 10 (*DPP10*) on Chromosome 2q12.3-q14.2, Alpha-1B-adrenergic receptor (*ADRA1B*) on Chromosome 5q33, G-alpha-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 previously implicated in genome-wide linkage studies of multiplex asthmatic families [3, 7, 34, 35], and *DPP 10* was first identified by positional cloning [36]. One of these four SNPs (rs3972219 in *GNA13*) had a MAF <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% Confidence Interval (CI): 1.18–1.66); 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 two of these three genes in the African American data, *ADRA1B* and *PRNP*, was obtained by imputation. For *DPP10* however, none of the imputed SNPs around rs1435879 in *DPP10* were statistically significant (Figure 3, Panel C). The signal at rs6865665 in *ADRA1B* was supported by two imputed SNPs: rs11954917, located 483bp upstream ($P=0.0006$); and rs10077860, located 656bp downstream ($P=0.000041$) from the original signal (Figure 3, Panel A). The signal at rs6052761 in *PRNP* was supported by three imputed SNPs: rs10485513 and rs7270994, located 1415 and 1201 bp upstream respectively, ($P=0.0001$), and rs6037929, located 874bp downstream ($P=0.0041$) (Figure 3, Panel B). In the Barbados data, imputed SNPs did not lend further statistical support to peak signal of genotyped SNPs in any of these three genes (Figure E2, online repository).

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 ($P=0.04$), 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, one intronic SNP (rs1435879) towards the 3' end showed nominal significance ($P=0.0045$) and a cluster of multiple SNPs

0.6Mb away from the 3' UTR region of this gene were significantly associated with asthma (P = 0.01 – 0.001; Figure 4) in the European replication sample.

Four additional case-control studies on African American individuals (from Baltimore/New York City, Philadelphia, Washington, D.C., and San Francisco/Oakland, CA) were genotyped to test for SNP-by-SNP replication at these top three markers: rs1435879, rs10515807, and rs6052761. While the overall allele frequencies were comparable across datasets (Table E1, online repository), the differences in allele frequency between cases and controls seen in the discovery population of African descent were not seen in these additional four 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<0.05; Table E2, online repository).

DISCUSSION

In this paper we report the first genomewide association study for asthma focused on populations of African descent. Using two independent sets of samples, an African American case-control group from Baltimore-Washington, D.C. (N=935) and 163 African Caribbean families from Barbados (N=929), we have identified three genes as associated with asthma which each are biologically relevant to asthma pathology. However, these findings must be interpreted with caution due to 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 5Kb located 21Kb from the 5' end of the *ADRA1B* gene on Chromosome 5q33, which has been implicated in asthma studies previously [3], [7], [34], [35]. Upon 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 *IL12B* is 0.59 MB downstream from *ADRA1B*. However, none of the SNPs in these candidate genes were in LD with the *ADRA1B* SNP associated with asthma in this study. Evidence of association of *ADRA1B* was supported by several imputed SNPs (P-value range: 0.0001–0.0041) among the African American samples (Figure 3, Panel B). The estimated genotypic odds ratio for the minor allele (T) at rs10515807 under an additive model was 1.40 (95% Confidence Interval (CI): 1.18–1.66). ADRA1B is one of three alpha 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]. Alpha 1-adrenergic receptors are well known for their physiological responses to 'fight-or-flight' signaling and regulation of carbohydrate metabolism [38], but interestingly they have also been associated with pro-inflammatory responses [39]. Although no role for alpha-1-adrenergic receptors in asthma has yet been demonstrated, alpha-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].

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%–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-value range: 0.0001–0.0041) located within a small region (1.4Kb) upstream of marker rs6052761 which showed evidence among the African American (Figure 3, Panel B) and Barbados samples (Figure E2, Panel B, online repository). 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 non-neuronal tissues, including lung and lymphoid cells [42]. The biological 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 Foxp3+ regulatory T cells [46]) and most highly expressed on dendritic cells [47]. In a murine model, PrP(C) was shown to be up-regulated in T cells via a Stat6-dependent mechanism following treatment with IL-4 [48]. Marker rs6052761, a C to T substitution located 10.1Kb 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 non-synonymous marker (rs1435879; $P = 3.05\times10^{-6}$) towards the 5' end of a very large gene, *DPP10* (spanning ~1.4 Mb), on Chromosome 2q12.3-q14.2. The minor allele (G) at SNP rs1435879 was protective with an etimated genotypic odds ratio of 0.65 (95% CI: 0.49–0.87). DPP10 is a 796-amino-acid, multi-functional protein and is a member of a family of proteins in the S9B serine proteases subfamily [50]. Although structurally similar to dipeptidyl peptidase (DPP) IV, DPP10 shows nearly identical activity to DPPX 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 due to 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, QTL studies on murine models have linked airway hyper-responsiveness in mice to the mouse 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.* speculated 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 brain, spinal cord, pancreas, and adrenal glands [36]. 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.* may be of importance for asthma, especially in samples of African ancestry (Figure 4).

We initially undertook this GRAAD study assuming certain genes might contribute to the profound disparities in 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 three genes (*ADRA1B, PRNP* and *DPP10*). There was, however, significant association with SNPs towards the 3' end and in the 3' UTR 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 40-80%[57, 58], suggesting multiple genes are involved in disease etiology. 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 ~60% of SNPs in the human genome have MAF<5%, companies producing GWAS arrays are biased towards common tagging variants in support of the common-disease common-variant (CDCV) 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 upon Yoruban genomes (*i.e.*, YRI) to represent African Americans, particularly in light of the recent observations by Tishkoff and colleagues[62] demonstrating that, although ~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 four 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 two GRAAD populations. However, as highlighted recently by Li and Leal[63], it is not yet known if current statistical methods such as STRUCTURE or principal component analysis can adequately control for population substructure if rare variants are included. Although three of the four African American replicate samples were comprised of subjects from the same geographical region as the African American discovery sample (Baltimore, Washington, D.C., and Philadelphia), it is possible that slight differences in African and European admixture within the datasets precluded supporting findings. In the initial genomewide association study 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=0.016 in the case-control group with no evidence replication in the African Caribbean families). Examining both genotyped and imputed SNPs (N=2,702) in a 3Mb region (Chr17: 34Mb-37Mb) around *ORMDL3*, we only found minimal association signals in regions showing peak association signals in the European group (rs12150079, P=0.005 in the African Caribbean families, but no evidence in the GRAAD case-control group at P=0.89; data not shown). Further, two of the African American samples used for replication in the current study failed to 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 two 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 two GRAAD populations and three additional GWAS on asthma, including the European sample, CHOP sample, and GWAS data from non-Hispanic white families ascertained through childhood asthmatics aged 5-12 participating in the Childhood Asthma

Management Program (CAMP) [66]. Fifty six genes were selected for follow up in these three replicate populations meeting nominal significance criteria in both of our discovery populations with signals within 5kb (data not shown). It is notable that three genes appear to have a gene-based signal (qualified as a P-value <0.01) across the 5 ethnically diverse populations, including *DPP10*, the only gene identified by positional cloning for asthma as described above. While these analyses are purely exploratory and not formal, the findings suggest that the current standards requiring same SNP replication (for what are, afterall, 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 GWAS), illustrate the point that alternative approaches are warranted.

This is the first GWAS with a primary focus on independent populations of African descent which has highlighted key genes and regions that may be distinct from genes important in non-African populations. This study clearly illustrates the difficulty with replicating associations for complex and heterogeneous diseases (such as asthma) when the marker panel may provide imperfect coverage of common variants in admixed populations. Results of this study illustrate the complexity of identifying true associations for a complex and heterogeneous disease (such as asthma) in admixed populations, and emphasize the need to test for replication beyond a SNP-for-SNP level to fully evaluate fine mapping in follow-up strategies. Evidence of association between asthma and these three candidate genes (*ADRA1B, PRNP*, and *DPP10*) clearly warrant further studies to confirm 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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations used

GWAS	Genome wide association study
GRAAD	Genomic Research on Asthma in the African Diaspora
SNP	Single nucleotide polymorphism
MAF	Minor allele frequency
HWE	
ADRA1B	
PRNP	
DPP10	

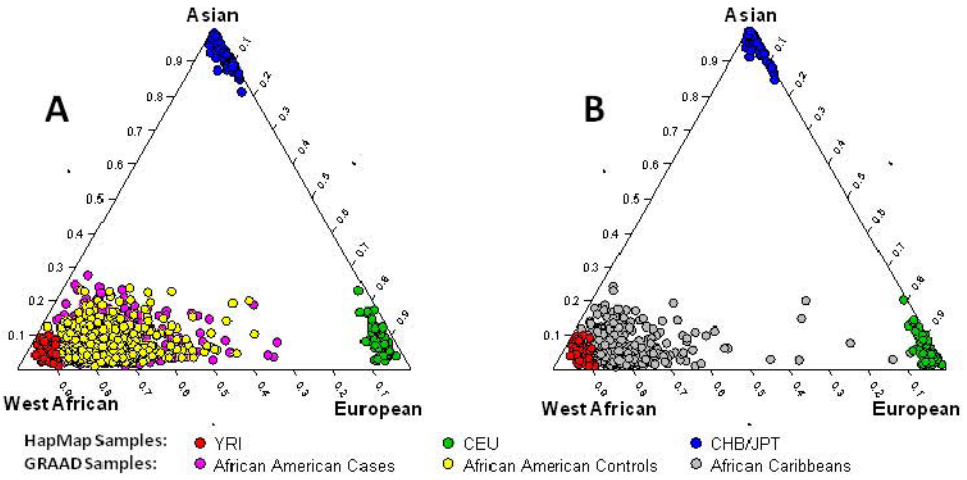

Figure 1. Triangle plots showing estimated admixture in two populations of African descent

Estimates were performed using 416 ancestry informative markers (AIMs) and data from the International Hapmap Project on 60 YRI, 60 CEU, 90 CHB/JPT founders. The figure depicts ancestry in **[A]** 447 African American asthma cases and 459 non-asthmatic controls and **[B]** 298 African Caribbean founders.

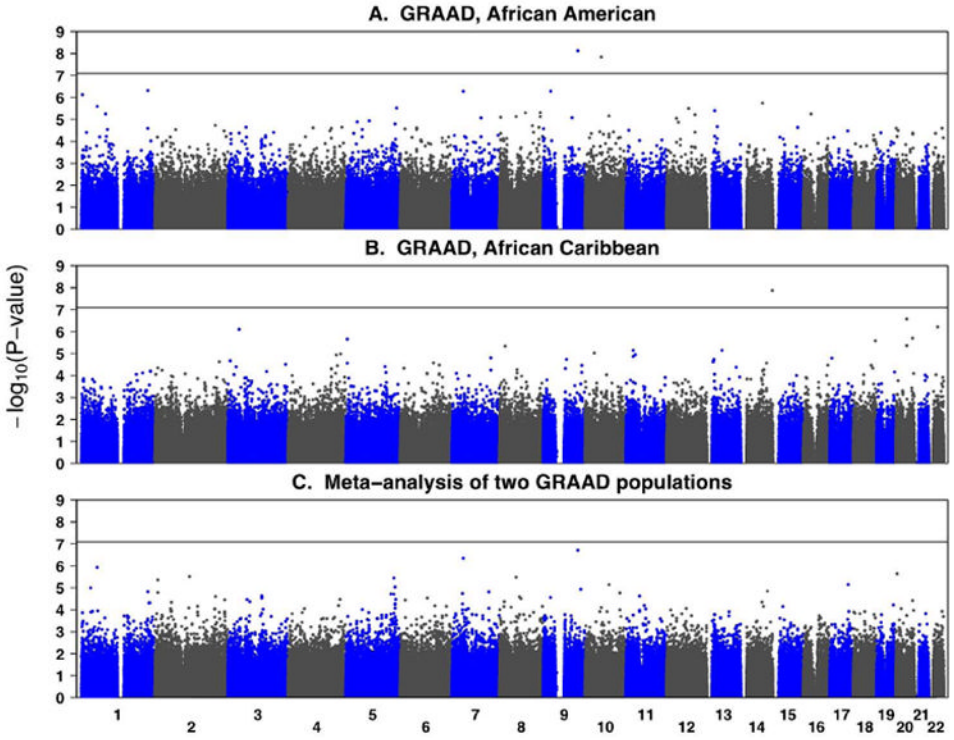

Figure 2. Genome-wide associations for asthma in two populations of African descent

[A] African American asthmatic cases and controls; **[B]** African Caribbean families; **[C]** Meta-analysis of African American and African Caribbean GRAAD samples. (Note: For visual clarity, the Y-axis was truncated at $-\log_{10}$(P-Value) = 9 resulting in the exclusion of a single data point; Panel A: rs13209883, P= 2.77×10^{-11}).

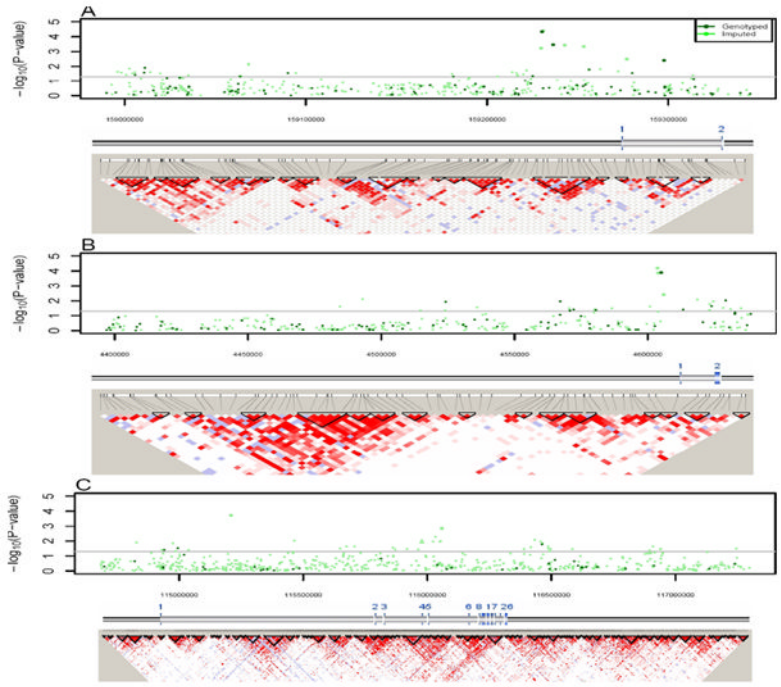

Figure 3. Evidence of association with asthma and linkage disequilibrium around *ADRA1B, PRNP,* and *DPP10*

Upper plots summarize association P-values for all genotyped and imputed SNPs in the African American case-control group for *ADRA1B* (Panel A), *PRNP* (Panel B), and *DPP10* (Panel C). Lower plots illustrate patterns of linkage disequilibrium (R^2) in these samples; red squares for strong LD, blue squares for non-significant LD, and white squares for little or no LD.

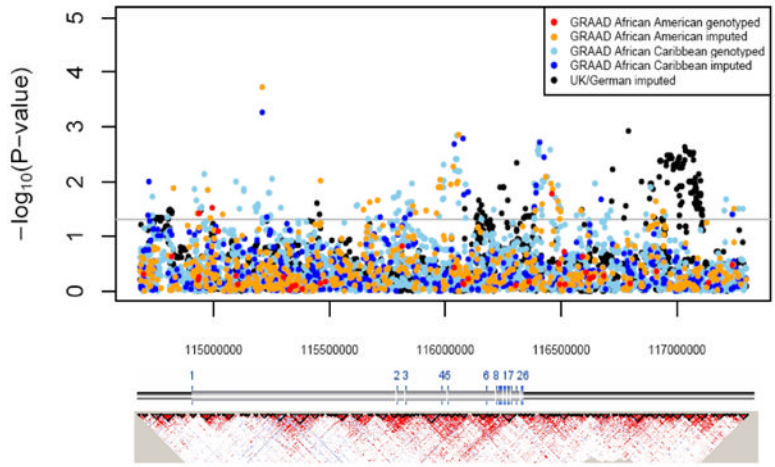

Figure 4. Evidence of association with asthma and linkage disequilibrium around *DPP10*

Upper plot summarizes association P-values of genotyped and imputed SNPs for the African American cases and controls, African Caribbean families, and the European GABRIEL replicate population. Lower plots illustrate patterns of linkage disequilibrium (R^2) in these samples; red squares for strong LD, blue squares for non-significant LD, and white squares for little or no LD.

Table I

Clinical characteristics of the GRAAD population.

Pane Panel A l A	African American		
	Total	**Cases**	**Controls**
N	935‡	464	471
Males; N (%)	406 (43.4%)	211 (45.5%)	195 (41.4%)
Age; 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; N (%)	641 (75.2%)	369 (85.2%)	272 (64.9%)
Panel B	**African Caribbean**		
	Total	**Founders**	**Asthmatics**
N	929‡	299	355
Males; N (%)	454 (48.9%)	145 (48.5%)	175 (49.3%)
Age; 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; N (%)	404 (71.4%)	79 (46.2%)	187 (71.4%)

†Geometric mean of serum total IgE (ng/ml).

‡Reflects the final genotyped dataset following all quality control steps.

Table II

Associated SNPs with combined $P < 10^{-5}$ in the African ancestry panels

Associated SNPs are those limited to some evidence for association ($P<0.01$) in both African ancestry panels and same high risk allele in both groups.

					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}