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A Genome-wide Association and Admixture Mapping Study of Bronchodilator Drug Response in African Americans with Asthma

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Abstract

Short-acting β_2-adrenergic receptor agonists (SABAs) are the most commonly prescribed asthma medications worldwide. Response to SABAs is measured as bronchodilator drug response (BDR), which varies among racial/ethnic groups in the U.S[1, 2]. However, the genetic variation that contributes to BDR is largely undefined in African Americans with asthma[3]. To identify genetic

variants that may contribute to differences in BDR in African Americans with asthma, we performed a genome-wide association study (GWAS) of BDR in 949 African American children with asthma, genotyped with the Axiom World Array 4 (Affymetrix, Santa Clara, CA) followed by imputation using 1000 Genomes phase III genotypes. We used linear regression models adjusting for age, sex, body mass index (BMI) and genetic ancestry to test for an association between BDR and genotype at single nucleotide polymorphisms (SNPs). To increase power and distinguish between shared vs. population-specific associations with BDR in children with asthma, we performed a meta-analysis across 949 African Americans and 1,830 Latinos (Total=2,779). Lastly, we performed genome-wide admixture mapping to identify regions whereby local African or European ancestry is associated with BDR in African Americans. We identified a population-specific association with an intergenic SNP on chromosome 9q21 that was significantly associated with BDR (rs73650726, $p=7.69\times10^{-9}$). A trans-ethnic meta-analysis across African Americans and Latinos identified three additional SNPs within the intron of *PRKG1* that were significantly associated with BDR (rs7903366, rs7070958, and rs7081864, $p\leq5\times10^{-8}$). Our results failed to replicate in three additional populations of 416 Latinos and 1,615 African Americans. Our findings indicate that both population specific and shared genetic variation contributes to differences in BDR in minority children with asthma, and that the genetic underpinnings of BDR may differ between racial/ethnic groups.

INTRODUCTION

Albuterol, a short-acting β_2-adrenergic receptor agonist (SABA), is the most commonly prescribed asthma medication worldwide[4, 5]. SABAs cause rapid smooth muscle relaxation of the airways. Bronchodilator drug response (BDR) is a measure of a patient's clinical response to SABA treatment and is quantitatively assessed as a change in forced expiratory volume in one second (FEV_1) after administration of a SABA. BDR is a complex trait involving interactions among inflammatory cells[6], airway epithelium[7], smooth muscle cells[8], and the autonomic nervous system[9]. Variation in BDR is likely influenced by both population-specific and shared environmental and genetic factors[10–12]. In the United States (U.S.), BDR in children with asthma differs significantly between racial/ethnic groups[2, 10]. Specifically, African Americans have lower BDR compared to European populations even after controlling for asthma severity[13]. Compared to European Americans, African Americans suffer increased asthma morbidity and mortality[2, 11, 14] and decreased BDR likely contributes to these disparities in disease progression and outcomes. The extensive use of albuterol as a first-line therapy for asthma, coupled with the decreased drug response (BDR) and increased disease burden in African Americans underscores the importance of identifying genetic factors that influence BDR in African American children with asthma. Once identified, these factors may lead to the generation of novel therapies and targeted interventions that will serve to improve patient care and asthma outcomes in an over-burdened and under-studied population.

To date, knowledge of genetic variation that contributes to BDR in African Americans is limited to a single genome-wide association study (GWAS) in 328 individuals[3]. Previous GWAS and candidate gene studies performed in populations of predominantly European ancestry with asthma have identified several BDR candidate genes[12, 15–24]. A recent study in

Latinos with asthma replicated a number of these findings, and also identified novel population-specific associations with BDR[10]. Genetic effects identified in one population are not always generalizable across populations and several population-specific asthma-risk variants have been discovered in African-descent populations (e.g., African Americans and Latinos)[25–27]. Additionally, previous studies have shown that the varying degrees of African and European ancestry present in the African American population can be leveraged, through a technique known as admixture mapping, to identify the missing heritability of complex traits[28]. Admixture mapping is a genome-wide approach that uses the variable allele frequencies of multiple SNPs between different ancestral populations to test for an association between local ancestry and phenotype[28]. The likelihood of population-specific effects, the limited number and scale of prior studies of BDR performed in African Americans, and ability to perform admixture mapping analysis highlights the possibility of gaining novel information through evaluating the impact of common genetic factors on BDR in African American children with asthma.

In this study, we performed a GWAS and admixture mapping study of bronchodilator drug response in 949 African American children with asthma from the Study of African Americans, Asthma, Genes & Environments (SAGE I and II)[29]. To increase power and distinguish between population-specific vs. shared associations, we also performed a trans-ethnic meta-analysis across our SAGE I and SAGE II participants and 1,830 Latinos from GALA II (Genes-environments and Admixture in Latino Americans) studies[26], respectively (total N=2,779). We further attempted replication of our population-specific and trans-ethnic meta-analysis results in 416 Latinos from the Genetics of Asthma in Latino Americans study (GALA I)[11, 30], 1,325 African Americans from the Study of Asthma Phenotypes and Pharmacogenomic Interactions by Race-Ethnicity (SAPPHIRE)[30, 31] and 290 African Americans from the Severe Asthma Research Program (SARP)[32, 33].

METHODS

Study subjects from the Study of African Americans, Asthma, Genes & Environments

The Study of African Americans, Asthma, Genes & Environments (SAGE) is an ongoing case-control study of asthma in children and adolescents recruited from the San Francisco Bay Area in California[29]. Subjects were eligible if they were 8–21 years of age and self-identified all four grandparents as African American. Exclusion criteria included: (1) 10 or more pack-years of smoking; (2) any smoking within 1 year of recruitment date; (3) pregnancy in the third trimester; or (4) history of one of the following conditions: sickle cell disease, cystic fibrosis, sarcoidosis, cerebral palsy, or history of heart or chest surgery. Asthma was defined by physician diagnosis, asthma medication use and reported symptoms of coughing, wheezing, or shortness of breath in the 2 years preceding enrollment. Detailed clinical measurements were recorded for each individual whom DNA was collected from. In addition, trained interviewers administered questionnaires to obtain baseline demographic data, as well as information on general health, asthma status, social, and environmental exposures. Pulmonary function testing was conducted with a KoKo® PFT Spirometer (nSpire Health Inc., Louisville, CO) according to American Thoracic Society recommendations[34], to obtain forced expiratory volume in one second (FEV_1) in addition to

other standard measurements of airway obstruction. Subjects with asthma were instructed to withhold their bronchodilator medications for at least 8 hours before testing. After completing baseline spirometry, subjects were given albuterol administered through a metered-dose inhaler (90 mcg/puff) with a spacer, and spirometry was repeated after 15 minutes to obtain post-bronchodilator measurements. The dose of albuterol was different in early stages of SAGE recruitment (2001–2005: SAGE I) than in more recent participants (2006-present: SAGE II). In SAGE I, post-bronchodilator FEV_1 values were measured after providing the participants 2 puffs of albuterol (180 μg) if they were younger than 16 years of age and 4 puffs of albuterol (360 μg) if they were 16 years of age or older. In SAGE II, two doses of albuterol were delivered. For the first dose, 4 puffs of albuterol (360 μg) were provided independently of the age of the participant. For the second dose, two puffs (180ug) for children < 16 years old were administered and 4 puffs for subjects older ≥ 16 years.

Body mass index (BMI) was calculated for each participant using weight and height measures and converted to a categorical scale of underweight, normal, overweight, and obese according to the Centers for Disease Control and Prevention. For participants under 20 years old, standardized sex- and age-specific growth charts were used to calculate BMI percentiles (http://www.cdc.gov/nccdphp/dnpao/growthcharts/resources/sas.htm) and categorize their BMI as: underweight (BMI percentile<5^{th}), normal (5^{th}≤BMI<85^{th}), overweight (85^{th}≤BMI<95^{th}), and obese (BMI≥95^{th}). For participants 20 years and older, BMI categories (http://www.cdc.gov/healthyweight/assessing/bmi/adult_bmi/index.html - interpretedAdults) were defined as: underweight (BMI<18.5), normal (18.5≤BMI<25), overweight (25≤BMI<30) and obese (BMI≥30). Further information about SAGE can be found in the Supplementary Text Supplementary Table 1.

Institutional review boards approved the study and all subjects/parents provided written assent/consent, respectively.

Genotyping and quality control (SAGE)

A total of 1,821 samples (1,011 asthma cases and 810 controls) were genotyped with the Axiom® World Array 4 (Affymetrix, Santa Clara, CA) at ~800,000 SNPs. Quality control was performed by removing SNPs that failed manufacturer's quality control, had genotyping call rates below 95%, and/or had a deviation from Hardy-Weinberg equilibrium ($p<10^{-6}$) within controls. 772,135 genotyped SNPs passed quality control. Samples were filtered based on discrepancy between genetic sex and reported gender and cryptic relatedness (PI_HAT>0.3). We excluded 3 subjects who were outliers for BDR (BDR of >60, or <−10). After sample quality control we included 759 SAGE II and 190 SAGE I asthma cases, for a total of 949 individuals with both genome-wide SNP data and measurements of bronchodilator drug response in the current study (Table 1). Phasing of genotyped SNPs was performed using SHAPE-IT[35], and genotype imputation was performed using IMPUTE2[36, 37] using all populations from 1000 Genomes Project Phase III[38] as a reference. Following imputation, a total of 9,573,507 genotyped and imputed (info score >0.3) SNPs with a MAF>0.05 were analyzed for SAGE II and 9,605,653 were analyzed for SAGE I.

Study subjects from the Genes-environments & Admixture in Latino Americans study (GALA II)

A total of 1,830 Latino children with asthma genotyped with the Axiom LAT1 array (World Array 4, Affymetrix) were included in our analysis (Table 1). Asthma cases were defined in a similar manner as SAGE with detailed clinical measurements recorded for each individual whom DNA was collected from. Additionally, each individual underwent spirometry with BDR calculated as the percentage change in FEV_1 after 2 doses of albuterol (post-FEV_1) compared with baseline values before administration of albuterol (pre-FEV_1). Post-bronchodilator FEV_1 values were measured after providing the participants 2 doses of albuterol, with a 15-minute waiting period after each dose. A total of 6 (if <16 years of age) to 8 (if ≥16 years of age) puffs of albuterol were administered. A total of 408 patients from the Centro de Neumologia Pediatrica in Puerto Rico were recruited based on having a BDR of at least 8%; of these, 121 patients were recruited based on having a BDR of at least 12%. Further details about GALA II are described in the Supplementary Text, Supplementary Table 1 and in depth elsewhere[10]. Imputation procedures identical to those described above for SAGE I and SAGE II were implemented, resulting in a total of 7,498,942 genotyped and imputed (info score >0.3) SNPs with a MAF>0.05.

Study subjects from the Genetics of Asthma in Latino Americans study (GALA I)

Our replication phase included 247 Mexican and 169 Puerto Rican asthma cases genotyped with the Genome-Wide Human SNP Array 6.0 (Affymetrix). Subjects were included in the study if they were between the ages of 8–40 with physician diagnosed mild to moderate-severe asthma and had experienced two or more symptoms during the two years preceding time of recruitment (including wheezing, coughing and/or shortness of breath.)). BDR was measured in a similar way to GALA II, but with a lower dosage of albuterol. Specifically, post-FEV_1 values were measured after only a single dose of albuterol (compared with 2 doses in GALA II). Two (if <16 years of age) to 4 (if ≥16 years of age) total puffs of albuterol were administered (compared with 4 [if <16 years of age] and 6 [if ≥16 years of age] in GALA II). Further details of the study are described in the Supplementary Text, Supplementary Table 1 and elsewhere[11, 30].

Study subjects from the Study of Asthma Phenotypes and Pharmacogenomic Interactions by Race-Ethnicity (SAPPHIRE)

For additional replication, we included 1,325 Africans Americans with asthma from SAPPHIRE[3] genotyped with the Genome-Wide Human SNP Array 6.0 (Affymetrix). Subjects met the following criteria: age 12–56 years, had a diagnosis of asthma (based on both patient report and documentation in the medical record), did not have a prior diagnosis of chronic obstructive pulmonary disease or congestive heart failure (CHF), a baseline FEV_1 between 40–90% predicted, >12% baseline bronchodilator reversibility, no smoking in the preceding year or <10 pack-year smoking history total, no oral or inhaled corticosteroid use in the 4 weeks preceding screening, and not pregnant at the time of enrollment and not intending to get pregnant during the study period. Spirometry testing was performed using a KoKo® PFT Spirometer, (nSpire Health Inc., Louisville, CO) following 2005 ATS/ERS spirometry recommendations[34]. Patients with asthma who were using inhaled

bronchodilators were asked to withhold these medications for the 12 hours prior to spirometry tests. To assess BDR a 360 μg dose (i.e., 4 puffs) of inhaled albuterol sulfate hydrofluoroalkane (HFA) (GlaxoSmithKline, Research Triangle Park, NC) from a standard metered dose inhaler (MDI) using an AeroChamber Plus Flow-Vu® spacer (Monahan Medical Corp., Plattsburgh, NY) was administered to patients. Pulmonary function was reassessed 15 minutes after administering albuterol. BDR was measured as the change in forced expiratory volume at one second (FEV_1) between the baseline (pre-bronchodilator) measure and post-bronchodilator FEV_1. Estimates of local ancestry were obtained using RFMix[39].

Study subjects from the Severe Asthma Research Program (SARP)

We included 290 African Americans with mild to severe asthma from SARP genotyped with the Illumina 1Mv1 platform[25]. SARP is a comprehensively characterized cohort with a range of asthma severities from mild to severe, but was enriched for severe disease defined by the American Thoracic Society (ATS) criteria for refractory asthma. Subjects met the definition of severe persistent asthma[32, 33, 40]. A physician's diagnosis of asthma was confirmed by evidence of methacholine bronchial hyperresponsiveness or bronchodilator reversibility and documented asthma symptoms. Baseline pre-bronchodilator spirometry was performed after withholding long and short-acting bronchodilators. Post-bronchodilator FEV_1 measurements were performed by increasing doses of albuterol of 200μg (two inhalations) up to a maximum dose of 800μg (eight inhalations).

Assessment of genetic ancestry

Genotypes from two populations were used to represent the ancestral haplotypes of African Americans for estimating local ancestry: HapMap European (CEU) and HapMap Africans (YRI). For Latinos, genotypes from 71 Native Americans were used as an additional ancestral population[41]. These 71 individuals included: 14 Zapotec, 2 Mixe, and 11 Mixtec from the southern State of Oaxaca[42] and 44 Nahua individuals from Central Mexico[43]. Global ancestry was estimated using ADMIXTURE[44] in a supervised analysis assuming two ancestral populations for African Americans and three ancestral populations for Latinos. Local ancestry was estimated using the program LAMP-LD[42] in the GALA and SAGE studies and with RFMix in SAPPHIRE[39].

Genotype association testing

All statistical analyses were conducted using R (version 2.15.3). For SAGE individuals, we used standard linear regression to test for an association between BDR and allele dosage at each individual SNP, adjusting for age, sex, BMI category, and both global and local African ancestry. A GWAS of BDR in GALA II has been previously published[10]. However, since this previous work did not include adjustment for BMI, we re-ran the GWAS using a new reference imputation panel and further adjusted by BMI in the present study[45]. For GALA II individuals, we adjusted for age, sex, BMI category, ethnicity, global Native American and African ancestry, and local ancestry. All analyses were performed using imputed genotypes from 1000 Genomes phase III. Using the fixed-effects model implemented in METAL[46], we performed a meta-analysis of common variants (MAF ≥ 5%) across African Americans (SAGE I and SAGE II) and Latinos (GALA II). We selected variants that were common

(MAF ≥ 5%) within each individual study and then took the intersection of SNPs for the meta-analysis.

Admixture mapping

We used local ancestry estimates generated across the genome to perform admixture mapping in African Americans. Linear regression models adjusted for age, sex, BMI category, and global African ancestry were used to identify significant associations between local ancestry estimates and BDR. The threshold for genome-wide significance was calculated using the empirical autoregression framework with the package *coda* in R to estimate the total number of ancestral blocks[47, 48]. The Bonferroni threshold was calculated as $\alpha=2.4\times10^{-4}$ based on 245 ancestral blocks. For African Americans, admixture mapping was performed separately in SAGE I and SAGE II and combined in a meta-analysis using METAL[46]. An admixture mapping study of BDR in GALA II has been previously published[10], but did not include adjustment for BMI. In the current study, we re-ran the admixture mapping study further adjusting by BMI[45] to be consistent with the SAGE I and SAGE II analyses. For GALA II Latinos, linear regression models adjusted for age, sex, ethnicity, BMI category, global Native American and African ancestry were used to identify significant associations between local ancestry estimates and BDR. We further combined the African ancestry results of SAGEI, SAGE II and GALA II in a meta-analysis using METAL[46].

Replication in GALA I, SAPPHIRE, and SARP

We attempted replication of significant population-specific (SAGE I and SAGE II) and cosmopolitan (SAGE I, SAGE II, GALA II) associations with BDR in the GALA I, SAPPHIRE, and SARP studies. Replication in GALA I was performed using genotype imputation (i.e., *in silico* replication), followed by an examination at a locus-wide level for SNPs within +/− 50 kb. We imputed 100 kb regions around each SNP using the program IMPUTE2 for Mexican and Puerto Rican participants run separately using 1000 Genomes phase III haplotypes as a reference. Linear regression was used to test for an association between allele dosage and BDR separately in Mexicans and Puerto Ricans, adjusting for age, sex, BMI category, global and local ancestry. Replication in SAPPHIRE was performed using linear regression to test for an association between allele dosage and BDR in African Americans while adjusting for age, sex, BMI category, and global and local African ancestry. Replication in SARP was performed using linear regression to test for an association between allele dosage and BDR in African Americans while adjusting for age, sex, BMI, and global African ancestry. For GALA I and SAPPHIRE replication, statistical significance at the SNP level was evaluated at $p<0.05$, and at the locus-wide level was established using a conservative Bonferroni correction adjusting by the number of SNPs within +/− 50 kb of the original candidate SNP. For SARP replication, statistical significance was evaluated at $p<0.05$ at the SNP level only.

RESULTS

GWAS results

After filtering variants with a MAF ≥ 5% and with imputation quality score (info score) ≥ 0.3, we tested for an association of BDR at a total of 9,190,349 SNPs in 949 African Americans with asthma ($\lambda = 1.006$). We identified a single genome-wide significantly associated SNP within an intergenic region on chromosome 9 (rs73650726, imputation quality score=0.86) (Figures 1, 2, Supplementary Figure 1A, Table 2). At this variant, additional copies of the A1 allele (A), was associated with decreased drug response ($\beta=-3.8$, $p=7.69\times10^{-9}$) (Table 2 & Supplementary Figure 2, Table 2). The SNP rs73650726 is common in African Americans but rare in Latinos, with a minor allele frequency of 8% in both SAGE studies, but at a frequency of 1% in GALA II. This is consistent with allele frequencies observed in the 1000 Genomes Project, where the variant is common in African populations (8%), rare in Latino populations (1–2%), and absent in European and Asian populations (Figure 3)[49].

In order to increase power and identify associations shared between populations we performed a trans-ethnic meta-analysis across African American and Latino participants from SAGE I, SAGE II, and GALA II. Following quality control and filtering on variants common in each study (MAF ≥ 5%), we took the overlap between the three studies and performed a meta-analysis on 6,570,864 SNPs. We identified genome-wide significant associations at three SNPs located on chromosome 10 within the intron of *PRKG1*: rs7903366 ($\beta=1.23$, $p=3.94\times10^{-8}$), rs7070958 ($\beta=-1.24$, $p=4.09\times10^{-8}$), and rs7081864 ($\beta=1.23$, $p=4.94\times10^{-8}$) (imputation quality scores > 0.98, Figures 4 & 5, Table 2, Supplementary Figures 1B & 2, Table 2). All three SNPs are in linkage disequilibrium and are eQTLs for *PRKG1* in lung tissue from the Genotype-Tissue Expression (GTEx) database (Table 3)[50], with the minor allele associated with decreased expression.

Replication of African American population-specific (rs73650726) and shared (rs7903366, rs7070958, rs7081864) variants was attempted in three independent Latino (GALA I) and African American (SAPPHIRE and SARP) studies. The African American population-specific association between rs73650726 and BDR, identified in the SAGE studies, was in the same direction in GALA I Puerto Ricans ($\beta = -6.22$) and the SAPPHIRE cohort of African Americans ($\beta = -0.65$), but in the SARP African American cohort the association was in the opposite direction ($\beta = 6.12$, $p=0.04$) (Supplementary Table 3). In addition, none of the SNPs within 50 kb of the four original SNPs were significantly associated with BDR following Bonferroni correction (Supplementary Table 4). Lastly, we evaluated previously identified candidate SNPs from prior candidate gene and GWAS with BDR in patients with asthma. After accounting for fifteen comparisons, no SNPs met the statistical significance threshold ($p<3.33\times10^{-3}$) (Supplementary Table 5); only rs9551086 in *SPATA13* had a p-value below 0.05 ($p=0.02$).

Admixture mapping results

We tested for an association of BDR with local genetic ancestry inferred at 478,441 SNPs in 949 African Americans with asthma (190 from SAGE I and 759 from SAGE II)

(Supplementary Figures 3 & 4). A meta-analysis across both studies yielded no significant associations with ancestry ($p<2.4\times10^{-4}$) (Supplementary Figure 5). The most significant peak was located on chromosome 8p11, where African ancestry was associated with higher BDR ($\beta=1.49$, $p=6.34\times10^{-4}$) (Supplementary Table 6). A meta-analysis across SAGE I, SAGE II and GALA II yielded results consistent with previous findings in the original admixture mapping study of GALA II (see [10]) (Supplementary Figure 6).

DISCUSSION

We performed a genome-wide association study for bronchodilator drug response in African Americans and identified a population-specific association between BDR and rs73650726, located on chromosome 9. Specifically, we discovered that the G (A2) allele of rs73650726 was associated with increased BDR and is more common in African Americans compared to European populations (Figure 3). The variant rs73650726, located on chromosome 9, does not map to any gene, but SNPs in high linkage disequilibrium ($r^2\geq0.8$) with this marker are located in enhancer histone marks in lung tissues [36].

Our results demonstrate that population-specific genetic variation contributes to variation in BDR in African American children with asthma. We further combined our results in a meta-analysis for BDR in African Americans and Latinos and identified multiple intronic variants in *PRKG1* that were associated with BDR in both populations. Overall, our results demonstrate that population-specific and shared genetic factors contribute to variation in BDR among African American children with asthma.

Three of our significantly associated variants fell within the intronic region of an annotated gene, Protein Kinase, CGMP-Dependent, Type I (*PRKG1*). *PRKG1* encodes for a cyclic GMP-dependent protein kinase, which phosphorylates proteins involved in regulating platelet activation and adhesion[51], gene expression[52, 53], vascular smooth muscle cell contraction[54], and feedback of the nitric-oxide (NO) signaling pathway[55]. Notably, the NO pathway is a key pathway in modulating vasodilation in response to beta-agonists such as albuterol via β_2-adrenergic receptors [56], making *PRKG1* a highly plausible BDR candidate gene. The three SNPs are in high linkage disequilibrium ($r^2\geq0.8$) with variants known to be functional[57], and are all associated with the expression of *PRKG1* in the lung – a tissue highly relevant to BDR. From the GTEx project database, the reference allele for all three SNPs was associated with decreased expression of the gene in lung tissue[50]. Thus, additional studies are required to identify the causal underlying variation at this locus, such as direct sequencing of this locus, and how the expression of *PRKG1* may be related to differences in BDR.

We sought to replicate our study findings and candidate SNPs previously found to be associated with BDR. The African American population-specific SNP, rs73650726, replicated in the opposite direction in the SARP cohort which could be due to differences in study design (Supplementary Figure 7). In candidate gene studies of BDR, the gly16arg variant in the Beta-2 adrenergic receptor gene (*ADRB2)* has consistently replicated opposite effects on BDR depending on whether medication exposure was acute or chronic[12, 58–60]. The SARP and SAGE studies administered different albuterol doses, had differences in

medication withholding periods, and SARP individuals were more likely to be treated with long-acting β_2-adrenergic receptor agonists (LABAs) over extended periods for severe disease. Additional factors that may have impacted replication include the presence of population specific differences in genetic contributions to BDR, lack of power due to small populations sizes, and/or varying patterns of linkage disequilibrium between populations. Furthermore, we were limited in sample size in GALA I[25] to evaluate associations at low frequency variants, and note that SAPPHIRE is comprised of mainly adults[31] in comparison to SAGE and GALA II, which are comprised of mainly children.

In conclusion, we identified two novel loci with biological plausibility whereby genetic variation is associated with differential response to albuterol, the most commonly prescribed asthma medication. One of these loci contains variation associated with BDR that is common to African Americans, a population that has historically been understudied in genetic studies[61–63]. Further genetic studies in African Americans are essential for identifying a more comprehensive set of genetic variants that contribute to differences in BDR, which in turn will lead to a better understanding of the pharmacogenetic response to asthma therapies. This will provide the foundation for genetic risk profiling and precision medicine, identifying novel genes and pathways associated with BDR, and the development of novel asthma therapies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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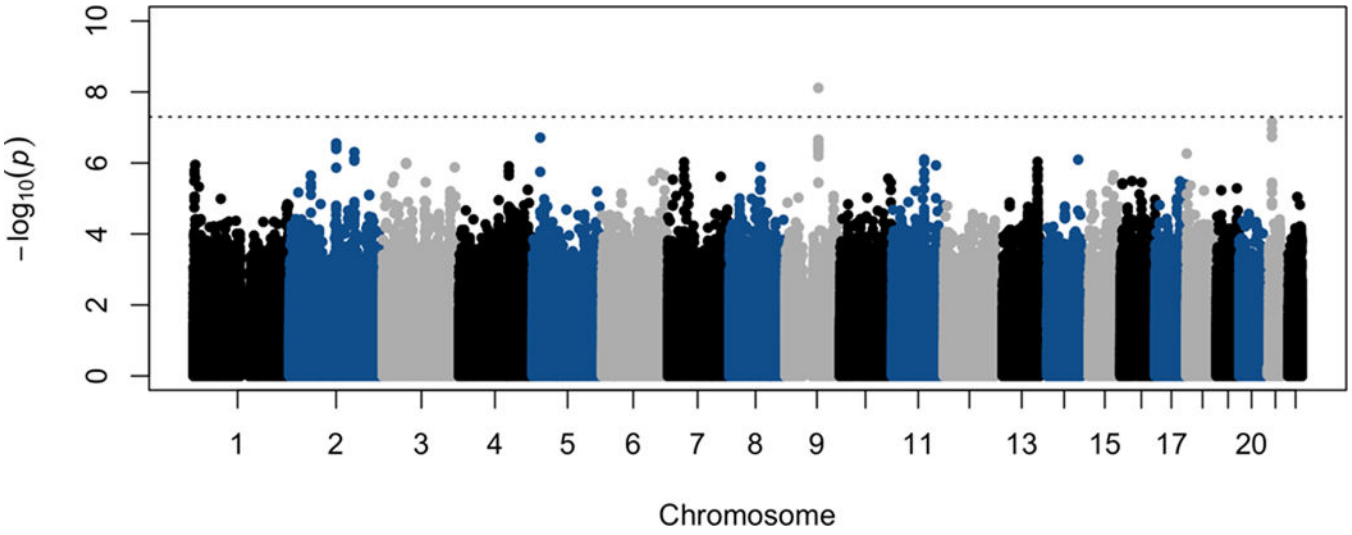

Figure 1: Meta-analysis of genome-wide association studies with BDR in African Americans.

Association testing for BDR was performed using linear regression including age, sex, BMI category, local and global ancestry as covariates separately in SAGE I and II and combined in a meta-analysis. Dotted line indicates the genome-wide significance threshold of 5×10^{-8}.

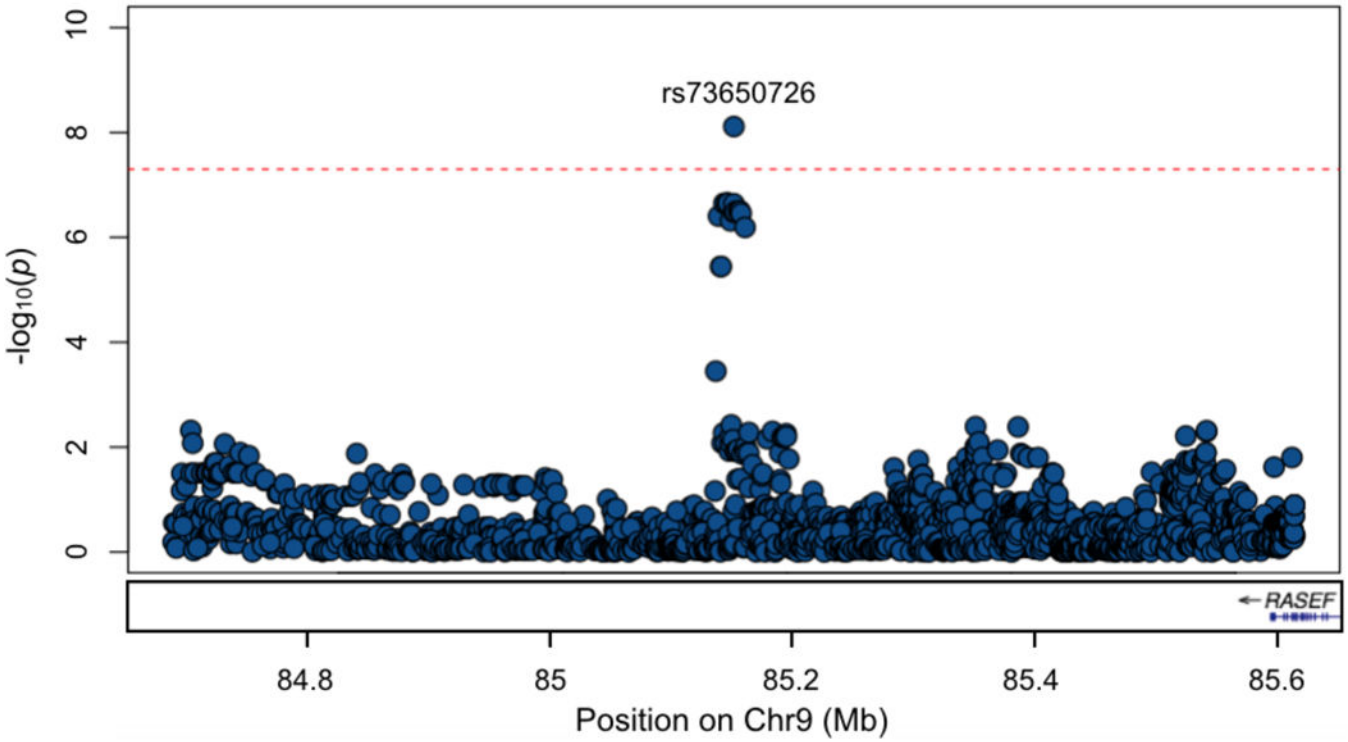

Figure 2: LocusZoom plot of chr9:84653000–85653000.

Region includes genotyped and imputed variants from 1000 Genomes phase 3. Blue = variants common in SAGE I and II. Dotted line indicates the genome-wide significance threshold of 5×10^{-8}.

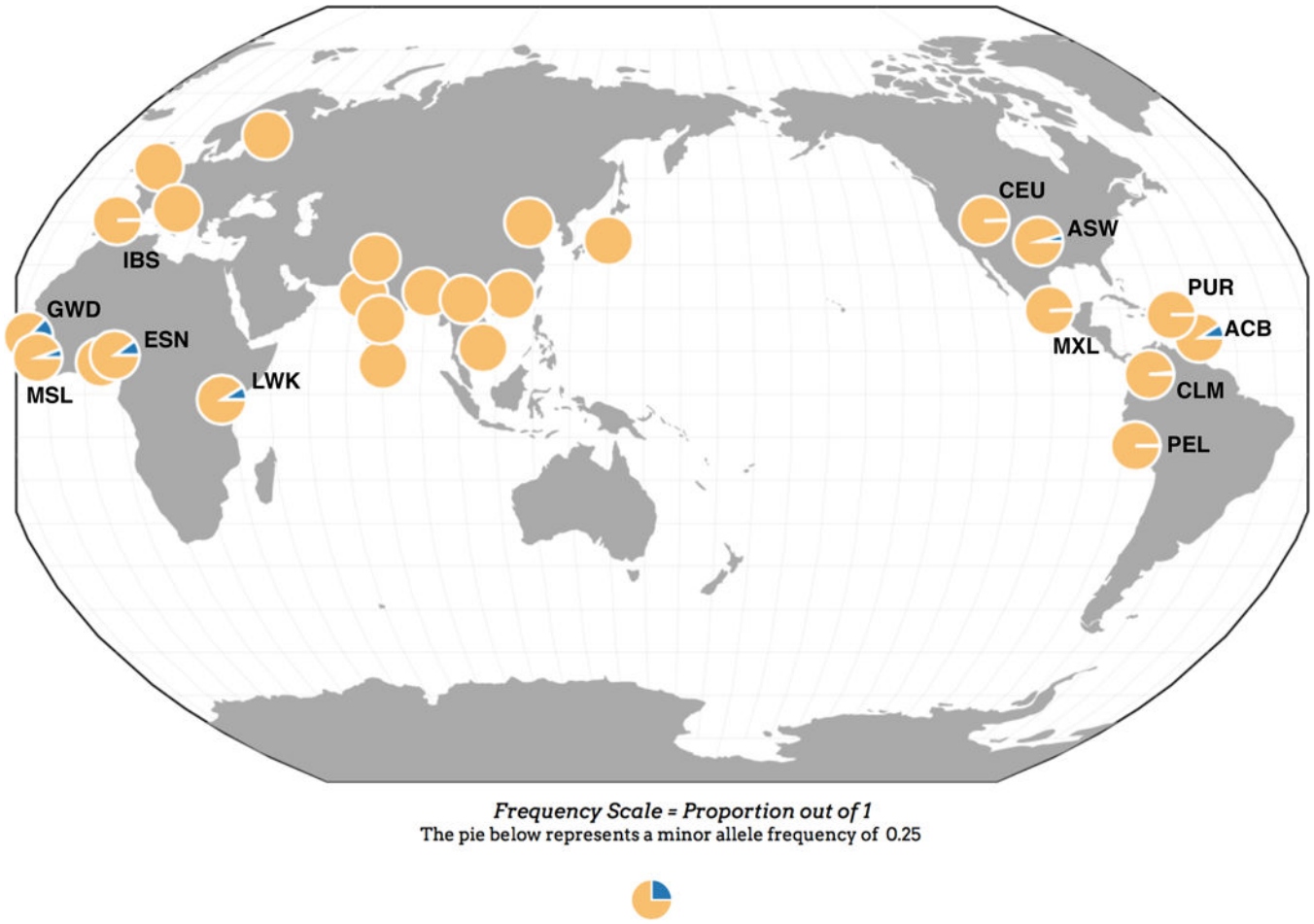

Figure 3: Geographic distribution of allele frequencies of rs73650726.

Each pie chart refers to a population from the 1000 Genomes Project phase 3. Yellow= Major allele (A), blue = minor allele (G). rs73650726 is common only in populations with African ancestry.

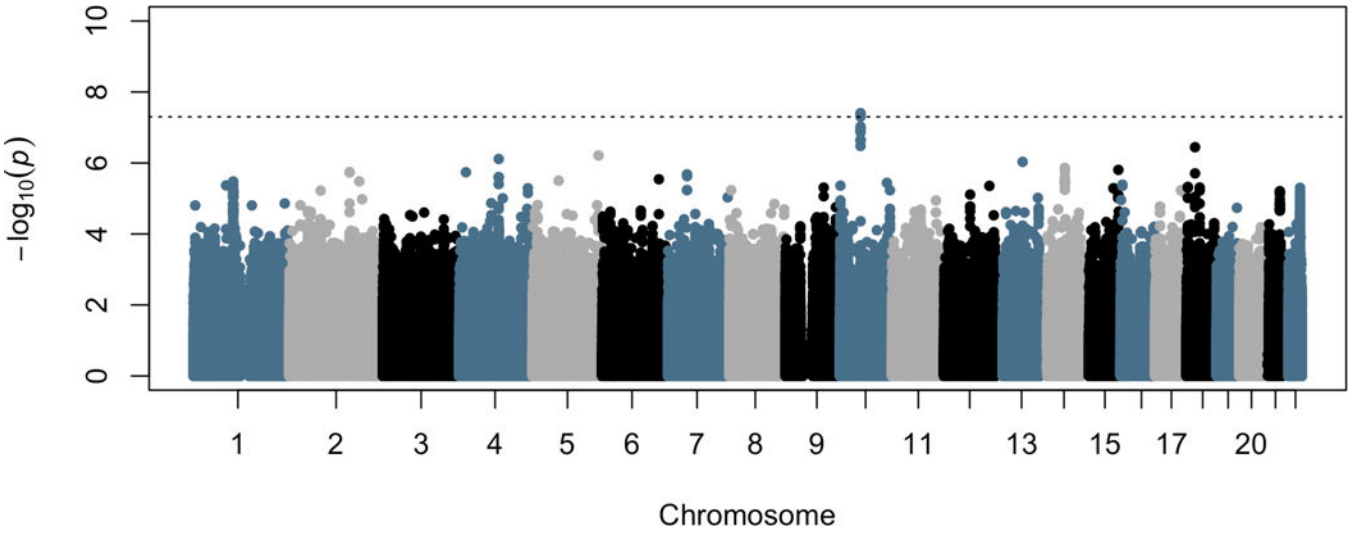

Figure 4: Meta-analysis of genome-wide association studies with BDR in African Americans and Latinos.

Association testing for BDR was performed using linear regression including age, sex, BMI category, local and global ancestry as covariates; including ethnicity for GALA II. Dotted line indicates the genome-wide significance threshold of 5×10^{-8}.

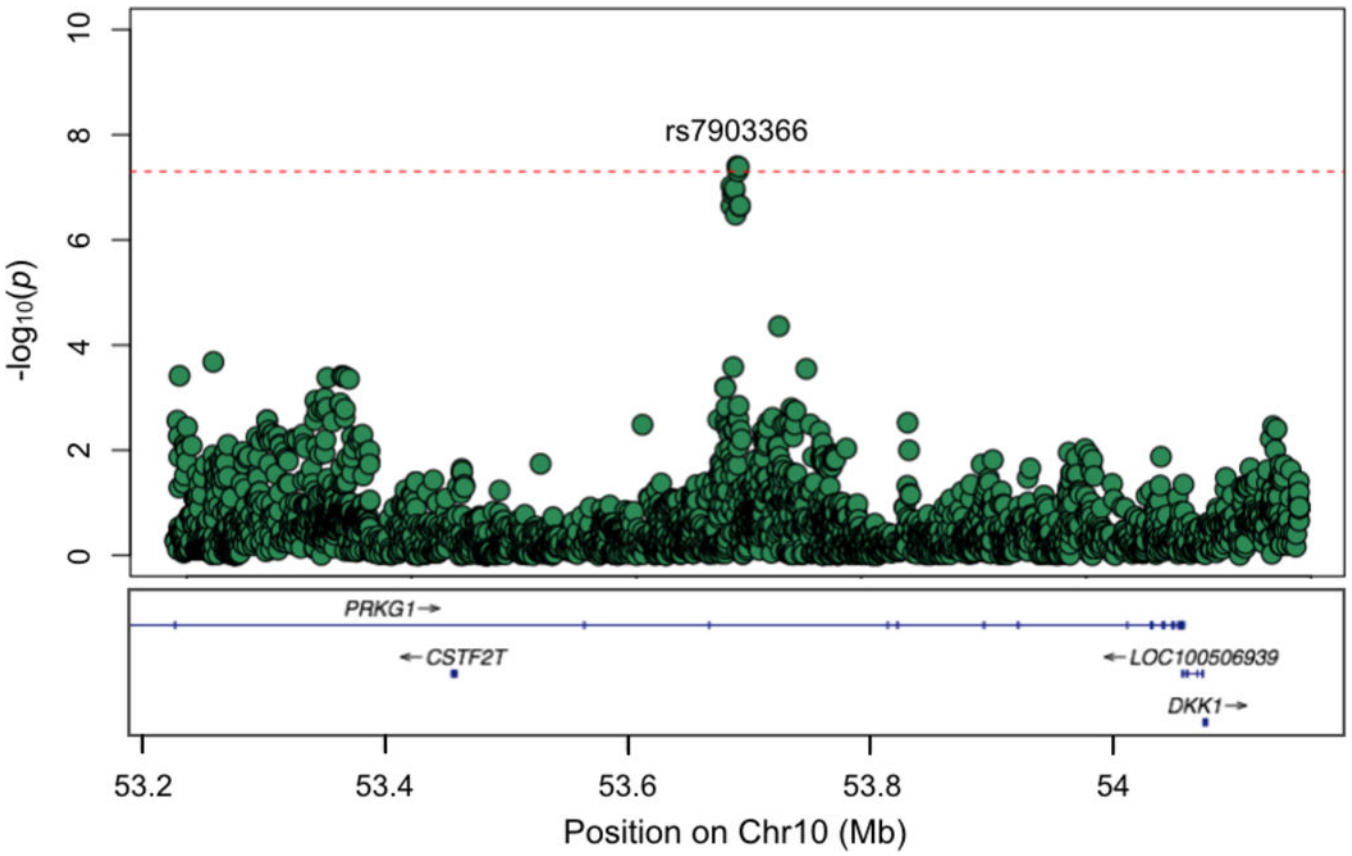

Figure 5: LocusZoom plot of chr10:53200000–54200000.

Region includes genotyped and imputed variants from 1000 Genomes phase 3. Green = variants common in SAGE I, SAGE II and GALA II. Dotted line indicates the genome-wide significance threshold of 5×10^{-8}.

Table 1:
Descriptive statistics of SAGE I, SAGE II, & GALA II asthma cases.

Values shown are the means, with the standard deviation in parentheses.

	SAGE I	SAGE II	GALA II
Total (N)	190	759	1830
Age (year)	18 (9.3)	14 (3.6)	13 (3.2)
<18 years (%)	64%	86%	93%
Sex (%Male)	41%	52%	55%
Race/Ethnicity	African American	African American	Latino
Global African Ancestry	0.81 (0.13)	0.72 (0.12)	0.15 (0.13)
Global Native American Ancestry	-	-	0.30 (0.25)
BMI			
<20 years	25 (7.3) (N=132)	25 (7.2) (N=722)	23 (6.5) (N=1782)
>20 years	31 (7.8) (N=58)	29 (7.0) (N=37)	30 (6.6) (N=48)
Pulmonary Function			
Pre-FEV1 % Predicted	92 (16)	99 (14)	91 (16)
Pre-FVC % Predicted	100 (17)	104 (13)	95 (16)
BDR (%)	9 (9.1)	9.5 (6.9)	11 (8.2)

Table 2:

Genome-wide significant associations identified through a meta-analysis within African Americans (SAGE I and II), and within African Americans and Latinos (SAGE I, SAGE II, and GALA II).

Under ‘Direction’ the first symbol refers to SAGE I, second to SAGE II, and third to GALA II. 0 = absent/rare in study

African Americans (SAGE I and II):								
Chr	**SNP**	**Position (hg19)**	**A1**	**A2**	**Effect (A1)**	**StdErr**	**Pvalue**	**Direction**
9q21	rs73650726	85152666	A	G	-3.8	0.66	7.69×10^{-9}	--0
African Americans + Latinos (SAGE I, SAGE II, GALA II):								
Chr	**SNP**	**Position (hg19)**	**A1**	**A2**	**Effect (A1)**	**StdErr**	**Pvalue**	**Direction**
10q21	rs7903366	53689774	T	C	1.23	0.22	3.94×10^{-8}	+++
10q21	rs7070958	53691116	A	G	-1.24	0.23	4.09×10^{-8}	---
10q21	rs7081864	53690331	A	G	1.23	0.22	4.94×10^{-8}	+++

Table 3:

Correlation between the expression of PRKG1 in the lung and minor alleles at three intronic SNPs associated with BDR (cis-eQTLs).

Data is from the GTEx database.

SNP	Ref Allele	Pvalue	Effect (Ref Allele)	T-Statistic	StdErr	Tissue	Gene
rs7903366	C	0.00051	-0.12	-3.5	0.034	Lung	*PRKG1*
rs7070958	A	0.00046	-0.12	-3.6	0.034	Lung	*PRKG1*
rs7081864	G	0.00052	-0.12	-3.5	0.034	Lung	*PRKG1*