

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

Melissa L. Spear, BS¹, Donglei Hu, PhD², Maria Pino-Yanes^{3, 4, 5}, PhD, Scott Huntsman, MS², Celeste Eng, BS², Albert M. Levin, PhD⁶, Marquitta J. White, PhD, MS², Meghan E. McGarry, MD, MAS⁷, Neeta Thakur, MD, MPH², Joshua M. Galanter, MD^{1, 2, 8}, Angel C.Y. Mak, PhD², Sam S. Oh, PhD, MPH², Adam Davis, MA, MPH⁹, Rajesh Kumar, MD, MS¹⁰, Harold J. Farber, MD, MSPH¹¹, Kelley Meade, MD¹², Pedro C. Avila, MD¹³, Denise Serebrisky, MD^{14, 15}, Michael A. Lenoir, MD¹⁶, Emerita Brigino-Buenaventura, MD¹⁷, William Rodriguez Cintron, MD¹⁸, Shannon M. Thyne, MD¹⁹, Jose R. Rodriguez-Santana, MD²⁰, Jean G. Ford, MD²¹, Rocio Chapela, MD²², Andrés Moreno Estrada, MD, PhD²³, Karla Sandoval, PhD²³, Max A. Seibold, PhD²⁴, L. Keoki Williams, MD, MPH^{25, 26}, Cheryl A. Winkler, PhD²⁷, Ryan D. Hernandez, PhD^{1, 28, 29}, Dara G. Torgerson, PhD², and Esteban G. Burchard, MD, MPH^{1, 2}

Affiliations:

1. Department of Bioengineering and Therapeutic Sciences, University of California, San Francisco, San Francisco, CA, 94158
2. Department of Medicine, University of California, San Francisco, San Francisco, CA 94158
3. Research Unit, Hospital Universitario N.S. de Candelaria, Universidad de La Laguna, Tenerife, Spain, 38010
4. CIBER de Enfermedades Respiratorias, Instituto de Salud Carlos III, Madrid, Spain, 28029
5. Genomics and Health Group, Department of Biochemistry, Microbiology, Cell Biology and Genetics, Universidad de La Laguna (ULL), La Laguna (Tenerife), Spain, 38010

6. Department of Public Health Sciences, Henry Ford Health System, Detroit, MI
7. Department of Pediatrics, University of California, San Francisco, San Francisco, CA 94143
8. Department of Epidemiology and Biostatistics, University of California, San Francisco, San Francisco, CA, 94158
9. UCSF Benioff Children's Hospital Oakland, Center for Community Health and Engagement
10. Ann & Robert H. Lurie Children's Hospital of Chicago, Pediatrics, Chicago, Illinois, 60614
11. Department of Pediatrics, Section of Pulmonology, Baylor College of Medicine and Texas Children's Hospital, Houston, TX, 77030
12. UCSF Benioff Children's Hospital Oakland, Oakland, California
13. Division of Allergy-Immunology, Northwestern University Feinberg School of Medicine, Chicago, Illinois, 60611
14. Pediatric Pulmonary Division, Jacobi Medical Center, Bronx, NY, 10461
15. Albert Einstein College of Medicine, Pediatrics, Bronx, New York
16. Bay Area Pediatrics, Oakland, CA, 94609
17. Department of Allergy & Immunology, Kaiser Permanente-Vallejo Medical Center, Vallejo, CA, 94589
18. Veterans Caribbean Health System, San Juan, Puerto Rico, 00921
19. Department of Pediatrics, David Geffen School of Medicine at ULCA, Olive View-UCLA Medical Center, Sylmar CA 91342
20. Centro de Neumologia Pediatrica, San Juan, Puerto Rico, 00917
21. Columbia University, New York, NY, 21205
22. Instituto Nacional de Enfermedades Respiratorias, Mexico City, MX, 14080

23. National Laboratory of Genomics for Biodiversity (LANGEBIO), CINVESTAV, Irapuato, Guanajuato 36821, Mexico
24. Department of Pediatrics, National Jewish Health, Denver, CO, 80206
25. Center for Health Policy and Health Services Research, Henry Ford Health System, Detroit, MI, 48202
26. Department of Internal Medicine, Henry Ford Health System, Detroit, MI, 48202
27. Basic Research Laboratory, National Cancer Institute, Leidos Biomedical Research, Frederick National Laboratory, Frederick, MD 21701
28. California Institute for Quantitative Biosciences (QB3), University of California, San Francisco, CA 94158
29. Institute for Human Genetics, University of California, San Francisco, CA, 94158

Corresponding author:

Esteban G. Burchard, MD, MPH

Department of Bioengineering & Therapeutic Sciences and Medicine

University of California, San Francisco

1550 4th St. RM584B

San Francisco, CA 94158

Telephone: (415) 514-9677

Fax: 415-514-4365

Email: esteban.burchard@ucsf.edu

Declarations of all sources of funding: This work was supported in part by the Sandler Family Foundation, the American Asthma Foundation, the RWJF Amos Medical Faculty Development Program, National Institutes of Health 1R01HL117004, R01HL128439, National Institute of Health and Environmental Health Sciences R01 ES015794, R21ES24844, and the National Institute on Minority Health and Health Disparities 1P60 MD006902, U54MD009523, 1R01MD010443. This project has been funded part with federal funds from the National Cancer Institute, National Institutes of Health, under contract HHSN26120080001E. The content of this publication does not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organizations imply endorsement by the U.S. Government. This Research was supported in part by the Intramural Research Program of the NIH, National Cancer Institute, Center for Cancer Research. MLS was supported in part by a National Science Foundation Graduate Research Fellowship under Grant No. 1144247. MP-Y was funded by the Ramón y Cajal Program (RYC-2015-17205) by the Spanish Ministry of Economy and Competitiveness. MP-Y was also supported by award number AC15/00015 by Instituto de Salud Carlos III through AES and EC within AAL framework, and the SysPharmPedia grant awarded from the ERA-CoSysMed 1st Joint Transnational Call from the European Union under the Horizon 2020; DGT was supported in part by the California Institute for Quantitative Biosciences (QB3). JMG was supported in part by NIH Training Grant T32 (5T32GM007546) and career development awards from the NHLBI K23 (5K23HL111636) and NCATS KL2 (5KL2TR000143) as well as the Hewett Fellowship; NT was supported in part by a institutional training grant from the NIGMS (T32GM007546) and career development awards from the NHLBI (5K12HL119997 and K23- HL125551-01A1), Parker B. Francis Fellowship Program, and the American Thoracic Society; RK was supported with a career development

award from the NHLBI (5K23HL093023); HJF was supported in part by the GCRC (RR00188); PCA was supported in part by the Ernest S. Bazley Grant. LKW received grant support from the Fund for Henry Ford Hospital, the American Asthma Foundation, and the following NIH institutes: NHLBI (R01HL118267, R01HL079055), NIAID (R01AI079139), and NIDDK (R01DK064695).

Acknowledgments:

The authors acknowledge the patients, families, recruiters, health care providers, and community clinics for their participation in SAGE and GALA II. In particular, we thank study coordinator Sandra Salazar and the recruiters who obtained the data: Duanny Alva, MD; Gaby Ayala-Rodriguez; Lisa Caine; Elizabeth Castellanos; Jaime Colon; Denise DeJesus; Blanca Lopez; Brenda Lopez, MD; Louis Martos; Vivian Medina; Juana Olivo; Mario Peralta; Esther Pomares, MD; Jihan Quraishi; Johanna Rodriguez; Shahdad Saeedi; Dean Soto; and Ana Taveras.

The contents of this publication are solely the responsibility of the authors and do not necessarily represent the official views of the NIH.

Abstract

Background: Short-acting B₂-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 asthma³.

Objective: To identify genetic variants that may contribute to differences in BDR in African Americans with asthma.

Methods: We performed a genome-wide association study 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 3 genotypes. We used linear regression models adjusting for age, sex, body mass index 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. Two additional populations of 416 Latinos and 1,325 African Americans were used to replicate significant associations.

Results: We identified a population-specific association with an intergenic SNP on chromosome 9q21 that was significantly associated with BDR (rs73650726, $p=7.69 \times 10^{-9}$). A trans-ethnic meta-analysis across African Americans and Latinos identified three additional SNPs within the

intron of *PRKGI* that were significantly associated with BDR (rs7903366, rs7070958, and rs7081864, $p \leq 5 \times 10^{-8}$).

Conclusions: 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.

Key messages:

- A GWAS for BDR in African American children with asthma identified an intergenic population specific variant at 9q21 to be associated with increased bronchodilator drug response (BDR).
- A meta-analysis of GWAS across African Americans and Latinos identified shared genetic variants at 10q21 in the intron of *PRKGI* to be associated with differences in BDR.
- Further genetic studies need to be performed in diverse populations to identify the full set of genetic variants that contribute to BDR.

Key words: genome-wide association study, meta-analysis, admixture mapping, bronchodilator drug response, albuterol, African Americans, Latinos, asthma

Abbreviations used:

BDR: bronchodilator drug response

BMI: body mass Index

FEV₁: forced expiratory volume in one second

GALA I: Genetics of Asthma in Latino Americans

GALA II: Genes-environments & Admixture in Latino Americans

GWAS: genome-wide association study

MAF: minor allele frequency

SABA: short-acting β_2 -agonist

SAGE: Study of African Americans, Asthma, Genes, & Environments

SAPPHIRE: Study of Asthma Phenotypes and Pharmacogenomic Interactions by Race-Ethnicity

SNP: single nucleotide polymorphism

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 (FEV₁) after administration of a SABA. BDR is a complex trait involving interactions among inflammatory cells⁶, airway epithelium⁷, smooth muscle cells⁸, and the autonomic nervous system⁹. Variation in BDR is likely influenced by both population-specific and shared environmental and genetic factors¹⁰⁻¹². 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 severity¹³. 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 overburdened 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 individuals³. 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 BDR¹⁰. 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 (i.e. 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 traits²⁸. 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^{23, 25-27}. 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 1840 Latinos from GALA II (Genes-environments and Admixture in Latino Americans) studies²⁶, respectively (total N=2,789). 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} and 1,325 African Americans from the Study of Asthma Phenotypes and Pharmacogenomic Interactions by Race-Ethnicity (SAPPHIRE)^{30, 31}.

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 California²⁹. 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 recommendations³², to obtain forced expiratory volume in one second (FEV₁) 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 FEV₁ 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<5th), normal (5th≤BMI<85th), overweight (85th≤BMI<95th), and obese (BMI≥95th). For participants older than 20 years old, 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≤24.9), overweight (25≤BMI≤29.9) and obese (BMI≥30).

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

Genotyping and quality control (SAGE)

A total of 1,819 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).

	SAGE I	SAGE II	GALA II
Total (N)	190	759	1830
Age (year)	18 (9.3)	14 (3.6)	13 (3.2)
Sex (%Male)	41%	52%	55%
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=40)
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 1: Descriptive statistics of SAGE I, SAGE II, & GALA II. Values shown are the means, with the standard deviation in parentheses.

Phasing of genotyped SNPs was performed using SHAPE-IT³³, and genotype imputation was performed using IMPUTE2^{34,35} using all populations from 1000 Genomes Project Phase III³⁶ 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

A total of 1,830 Mexican and Puerto Rican asthma cases genotyped with the Axiom LAT1 array (World Array 4, Affymetrix) for the Genes-environments and Admixture in Latino Americans (GALA II) study were included in our analysis (Table 1 above). Specific details about the study are described elsewhere¹⁰. Briefly, 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

For our replication phase, 247 Mexican and 169 Puerto Rican asthma cases genotyped with the Genome-Wide Human SNP Array 6.0 (Affymetrix) for the Genetics of Asthma in Latino Americans (GALA I) study were included. Details of the study are described elsewhere¹¹,
30 .

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

For additional replication, we included 1,325 Africans Americans with asthma from the Study of Asthma Phenotypes and Pharmacogenomic Interactions by Race-Ethnicity (SAPPHIRE) genotyped with the Genome-Wide Human SNP Array 6.0 (Affymetrix). Estimates of local ancestry were obtained using RFMix³⁷. Details of the study are described elsewhere^{25, 31}.

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. Global ancestry was estimated using ADMIXTURE³⁸ in a supervised analysis assuming two ancestral populations for African Americans and three ancestral populations for Latinos. A union set of SNPs was obtained by merging genotyped SNPs in SAGE and the ancestral populations (CEU/YRI). Local ancestry was estimated using the program LAMP-LD³⁹ in the GALA and SAGE studies and with RFMix in SAPPHIRE³⁷.

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 published¹⁰, however, this previous work did not include adjustment for BMI; in this study we re-ran the GWAS using a new reference imputation panel and further adjusted by BMI⁴⁰. 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 METAL⁴¹, we performed a meta-analysis of common variants ($MAF \geq 5\%$) across African Americans (SAGE I and SAGE II) and Latinos (GALA II). We selected variants that were common ($MAF \geq 5\%$) 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^{42, 43}. The Bonferroni threshold was calculated as $\alpha=2.4 \times 10^{-4}$ based on 245 ancestral blocks. Admixture mapping was performed separately in SAGE I and SAGE II and combined in a meta-analysis using METAL⁴¹.

Replication in GALA I and SAPPHERE

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 and SAPPHERE 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 3 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 SAPPHERE was also 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. For GALA I and SAPPHERE 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.

RESULTS

Genome-wide association results

After filtering variants with a MAF $\geq 5\%$ 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, 3, and Table 2 below). At this variant, additional copies of the A1 allele (A), was associated with decreased drug response ($\beta=-3.8$, $p=7.69 \times 10^{-9}$) (Table 2 below).

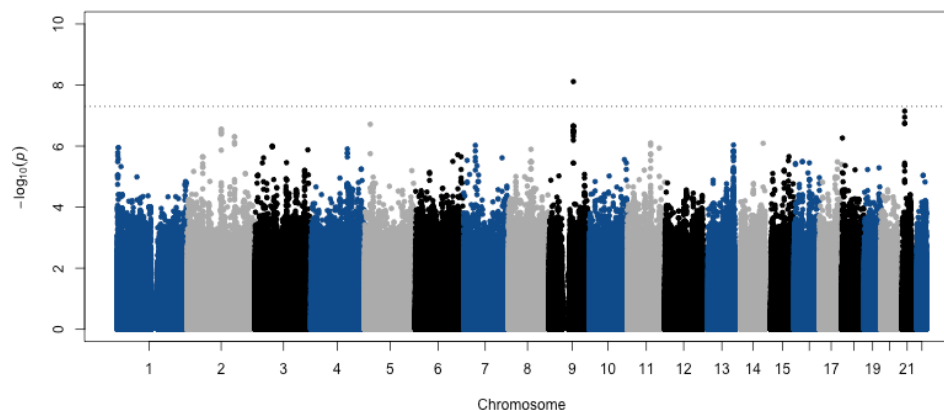


Figure 1: Meta-analysis of genome-wide association studies with BDR in African Americans. Association testing for BDR was performed using linear regression including age, sex, BMI category, local and global ancestry as covariates separately in SAGE I and II and combined in a meta-analysis. Dotted line indicates the genome-wide significance threshold of 5×10^{-8} .

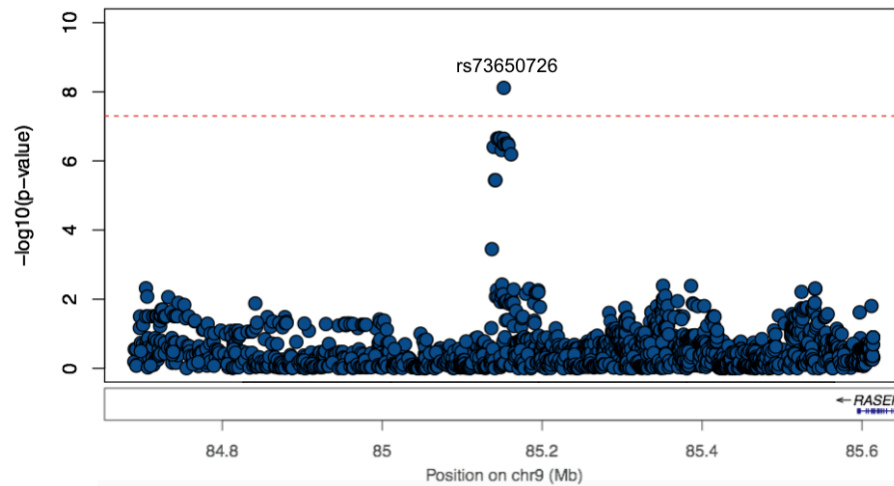


Figure 2: LocusZoom plot of chr9:84653000-85653000. Region includes genotyped and imputed variants from 1000 Genomes phase 3. Blue = variants common in SAGE I and II. Dotted line indicates the genome-wide significance threshold of 5×10^{-8} .

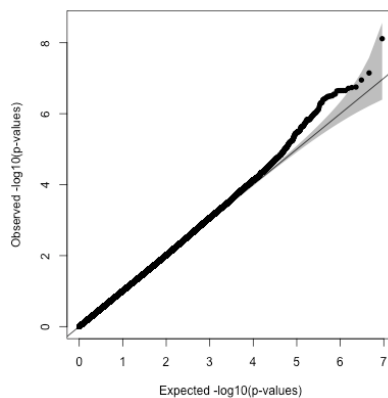


Figure 3: Quantile-quantile plots for genome-wide allelic associations with BDR in a meta-analysis of SAGE I and II (inflation factor: $\lambda = 1.006$ for ~ 10 M common SNPs)

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	7.69x10 ⁻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	3.94x10 ⁻⁸	+++
10q21	rs7070958	53691116	A	G	-1.24	0.23	4.09x10 ⁻⁸	---
10q21	rs7081864	53690331	A	G	1.23	0.22	4.94x10 ⁻⁸	+++

Table II: 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

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 (Table III below). 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 4 below)⁴⁴.

SAGE I								
Chr	SNP	Position (hg19)	A1	A2	Freq (A1)	Effect (A1)	StdErr	Pvalue
9q21	rs73650726	85152666	A	G	0.92	-2.99	1.92	0.12
10q21	rs7903366	53689774	C	T	0.4	-0.54	1.02	0.6
10q21	rs7070958	53691116	A	G	0.39	-0.5	1.03	0.63
10q21	rs7081864	53690331	G	A	0.39	-0.46	1.03	0.65

SAGE II								
Chr	SNP	Position (hg19)	A1	A2	Freq (A1)	Effect (A1)	StdErr	Pvalue
9q21	rs73650726	85152666	A	G	0.92	-3.9	0.7	3.36x10 ⁻⁸
10q21	rs7903366	53689774	C	T	0.41	-1.66	0.39	2.83x10 ⁻⁵
10q21	rs7070958	53691116	A	G	0.41	-1.66	0.4	3.22x10 ⁻⁵
10q21	rs7081864	53690331	G	A	0.41	-1.65	0.4	3.26x10 ⁻⁵

GALA II								
Chr	SNP	Position (hg19)	A1	A2	Freq (A1)	Effect (A1)	StdErr	Pvalue
9q21	rs73650726	85152666	A	G	0.99	0.29	1.25	0.81
10q21	rs7903366	53689774	C	T	0.6	-1.06	0.28	1.74x10 ⁻⁴
10q21	rs7070958	53691116	A	G	0.6	-1.08	0.28	1.57x10 ⁻⁴
10q21	rs7081864	53690331	G	A	0.6	-1.06	0.28	1.79x10 ⁻⁴

Table III: Individual genome-wide association study results from SAGE I, SAGE II, and GALA II.

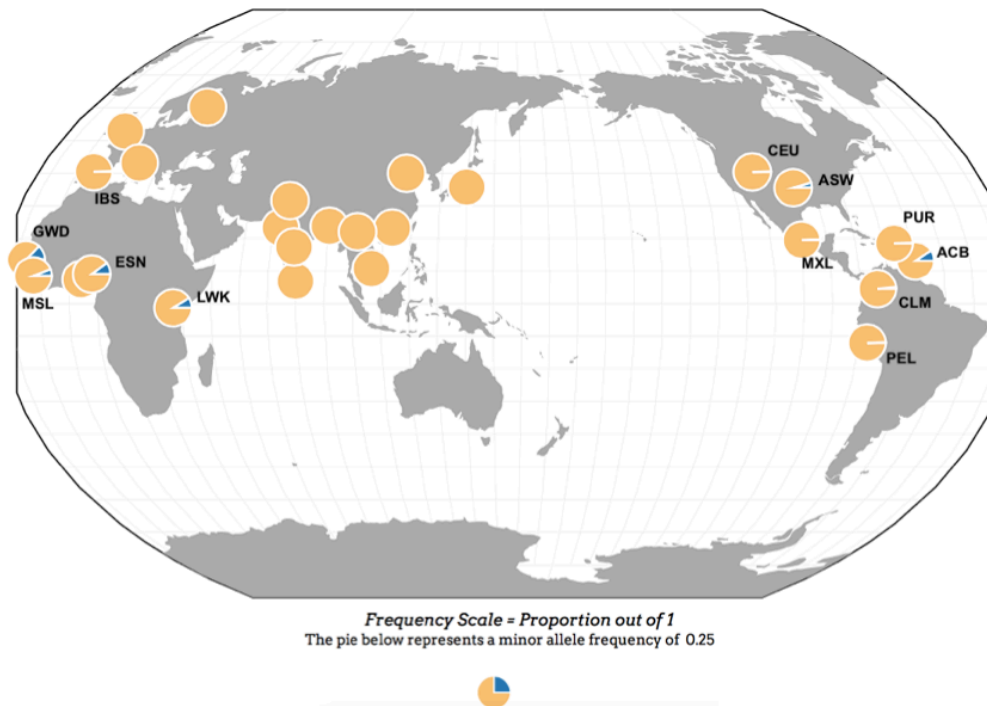


Figure 4: Geographic distribution of allele frequencies of rs73650726. Each pie chart refers to a population from the 1000 Genomes Project phase 3. Yellow= Major allele (A), blue = minor allele (G). rs73650726 is common only in populations with African ancestry.

In order to increase power and identify associations shared between populations we performed a trans-ethnic meta-analysis across African American, Mexican, and Puerto Rican participants from SAGE I, SAGE II, and GALA II, respectively. Following quality control and filtering on variants common in each study ($MAF \geq 5\%$), we took the overlap between the three studies and we performed a meta-analysis on 6,570,864 SNPs ($\lambda = 1.004$). We identified genome-wide significant associations at three SNPs located on chromosome 10 within the intron of *PRKG1*: rs7903366 ($\beta=1.23$, $p=3.94 \times 10^{-8}$), rs7070958 ($\beta=-1.24$, $p=4.09 \times 10^{-8}$), and rs7081864 ($\beta=1.23$, $p=4.94 \times 10^{-8}$) (imputation quality scores > 0.98 , Figures 5, 6 & 7 below, Tables II & III above). All three SNPs are eQTLs for *PRKG1* in lung tissue from the GTEx database (Table IV below)⁴⁵, with the minor allele associated with decreased expression.

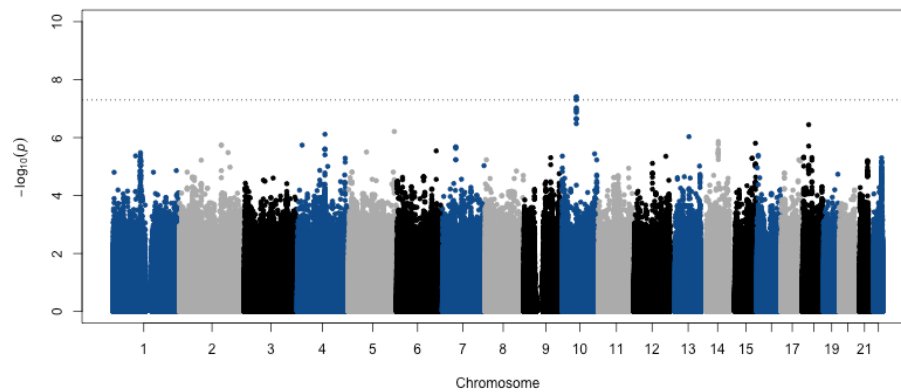


Figure 5: Meta-analysis of genome-wide association studies with BDR in African Americans and Latinos. Association testing for BDR was performed using linear regression including age, sex, BMI category, local and global ancestry as covariates; including ethnicity for GALA II. Dotted line indicates the genome-wide significance threshold of 5×10^{-8} .

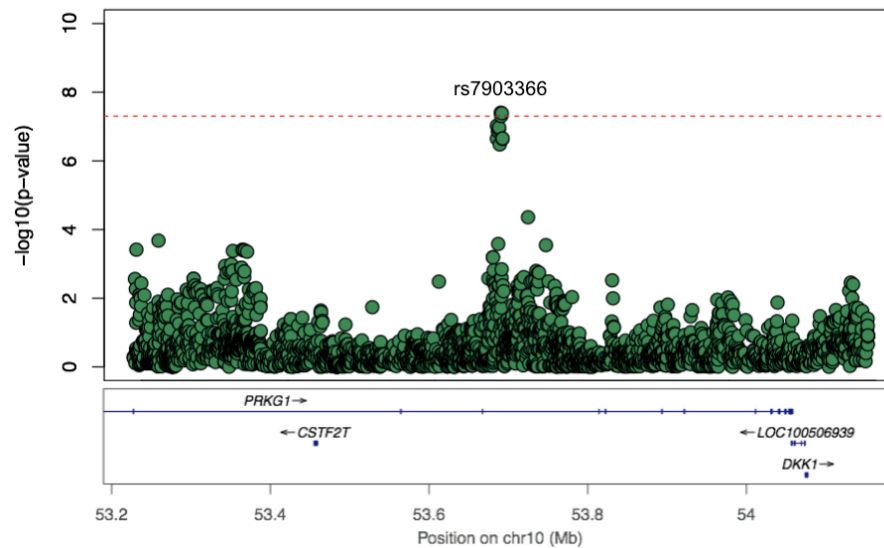


Figure 6: LocusZoom plot of chr10:53200000-54200000. Region includes genotyped and imputed variants from 1000 Genomes phase 3. Green = variants common in SAGE I, SAGE II and GALA II. Dotted line indicates the genome-wide significance threshold of 5×10^{-8} .

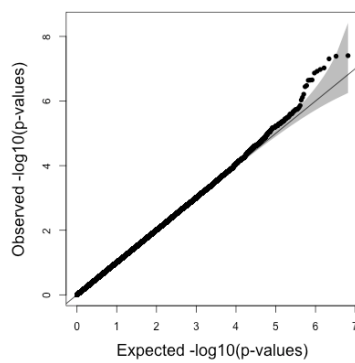


Figure 7: Quantile-quantile plots for genome-wide allelic associations with BDR in a meta-analysis of SAGE I, SAGE II, and GALA II (inflation factor: $\lambda = 1.004$ for all common SNPs)

SNP	Reference Allele	Pvalue	Effect Size	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

Table IV: 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.

Replication of African American population-specific (rs73650726) and shared (rs7903366, rs7070958, rs7081864) was attempted in two independent Latino (GALA I) and African American (SAPPHIRE) studies. Although none of the identified associations replicated in either study population, the African American population-specific association between rs73650726 and BDR, identified in the SAGE studies, displayed a similar trend in direction of effect in Puerto Ricans (GALA I; $\beta = -6.2$) and in African Americans (SAPPHIRE, $\beta = -0.65$) (Table V below). In addition, none of the SNPs within 50 kb of the four original SNPs were significantly associated with BDR following Bonferroni correction (Table VI below). 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 ($p < 3.33 \times 10^{-3}$) (Table VII below); only rs9551086 in *SPATA13* had a p-value below 0.05 ($p = 0.02$).

GALA I								
Population	Chr	SNP	Position (hg19)	A1	A2	Effect (A1)	StdErr	Pvalue
Mexicans	9q21	rs73650726	85152666	A	G	1.71	10.24	0.87
Mexicans	10q21	rs7903366	53689774	C	T	1.02	0.99	0.30
Mexicans	10q21	rs7070958	53691116	A	G	1.04	0.99	0.29
Mexicans	10q21	rs7081864	53690331	G	A	1.03	0.99	0.30
Puerto Ricans	9q21	rs73650726	85152666	A	G	-6.22	3.87	0.11
Puerto Ricans	10q21	rs7903366	53689774	C	T	-0.51	1.01	0.61
Puerto Ricans	10q21	rs7070958	53691116	A	G	-0.53	1.00	0.60
Puerto Ricans	10q21	rs7081864	53690331	G	A	-0.53	1.00	0.60

SAPPHIRE							
Chr	SNP	Position (hg19)	A1	A2	Effect (A1)	StdErr	Pvalue
9q21	rs73650726	85152666	A	G	-0.65	0.94	0.49
10q21	rs7903366	53689774	C	T	0.91	0.55	0.10
10q21	rs7070958	53691116	A	G	0.87	0.55	0.11
10q21	rs7081864	53690331	G	A	0.84	0.55	0.13

Table V: Replication results of candidate SNPs in GALA I and SAPPHIRE.

GALA I									
Population	Chr	SNP	Position (hg19)	A1	A2	Freq (A1)	Effect (A1)	StdErr	Pvalue
Mexicans	9	rs34293766	85126730	T	G	0.94	5.74	2.27	0.01
Mexicans	10	rs59960792	53690251	C	T	0.80	2.97	1.12	0.01
Puerto Ricans	9	rs10780548	85167648	A	G	0.63	2.93	0.99	0.003
Puerto Ricans	10	rs6480581	53672743	T	C	0.74	-2.67	1.08	0.01

SAPPHIRE									
Population	Chr	SNP	Position (hg19)	A1	A2	Freq (A1)	Effect (A1)	StdErr	Pvalue
African Americans	9	rs62576848	85144677	T	C	0.94	3.03	1.23	0.01
African Americans	10	rs35969600	53662118	C	T	0.72	-1.49	0.59	0.01

Table VI: Top replication results (MAF >0.05) +/-50 kb of candidate SNPs in GALA I and SAPPHIRE.

GENE	SNP	Chr	Position	A1	A2	Effect	Pvalue	Direction
<i>SOCS-ASB3</i> region	rs350729 ²³	2	52983773	T	G	0.22	0.53	-+
<i>ADRB2</i>	rs1042713 ¹⁵	5	148206440	A	G	-0.13	0.7	--
<i>ADRB2</i>	rs1042714 ¹⁵	5	148206473	C	G	-0.56	0.22	--
<i>ADCY9</i>	rs2230739 ¹⁸	16	4033436	T	C	-0.14	0.77	+-
<i>CRHR2</i>	rs7793837 ¹⁷	7	30726777	A	T	-0.26	0.58	--
<i>ARG1</i>	rs2781659 ¹⁹	6	131891820	A	G	0.39	0.3	-+
<i>SPATS2L</i>	rs295137 ²⁴	2	201150040	T	C	0.4	0.23	-+
<i>SPATS2L</i>	rs295114 ²⁴	2	201195602	T	C	0.41	0.22	-+
<i>THRB</i>	rs892940 ²⁰	3	24538838	A	G	0.52	0.21	++
<i>CRHR2</i>	rs73294475 ¹⁰	7	30701596	T	C	-0.32	0.51	+-
<i>SPATA13</i>	rs9507294 ³	13	24823347	T	C	0.38	0.48	++
<i>SPATA13</i>	rs912142 ³	13	24827500	A	G	-0.23	0.51	--
<i>SPATA13</i>	rs2248119 ³	13	24827094	A	G	0.49	0.16	++
<i>SPATA13</i>	rs9551086 ³	13	24830330	T	C	-1.36	0.02	--
<i>SPATA13</i>	rs9553225 ³	13	24823006	A	G	-1.17	0.10	--

Table VII. Candidate SNP replication in meta-analysis of SAGE I and SAGE II. Under ‘Direction’ column, 1st symbols refer to SAGE I, second refer to SAGE II.

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) (Figures 8 & 9 below). A meta-analysis across both studies yielded no significant associations with ancestry ($p < 2.4 \times 10^{-4}$) (Figure 10 below). The most significant peak was located on chromosome 8p11 where African ancestry was associated with higher BDR ($\beta = 1.49$, $p = 6.34 \times 10^{-4}$) (Table VIII below).

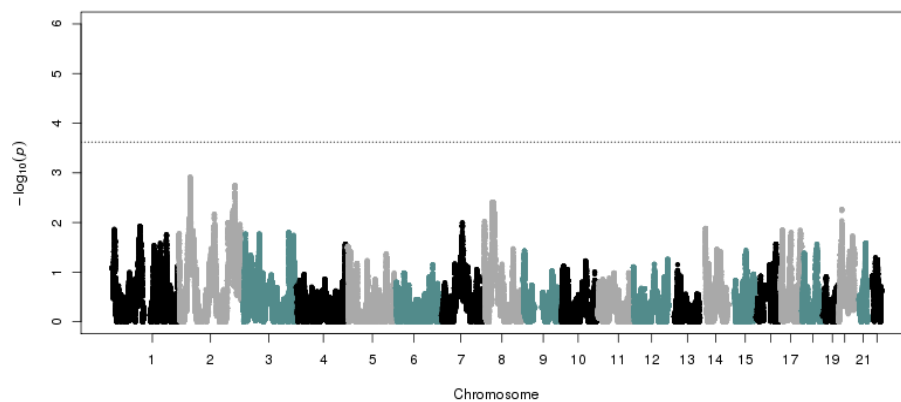


Figure 8: Admixture mapping in SAGE II (n= 759) for African ancestry and BDR. Ancestry association testing was performed at 478,441 markers using linear regression including age, sex, BMI category, and global African ancestry covariates.

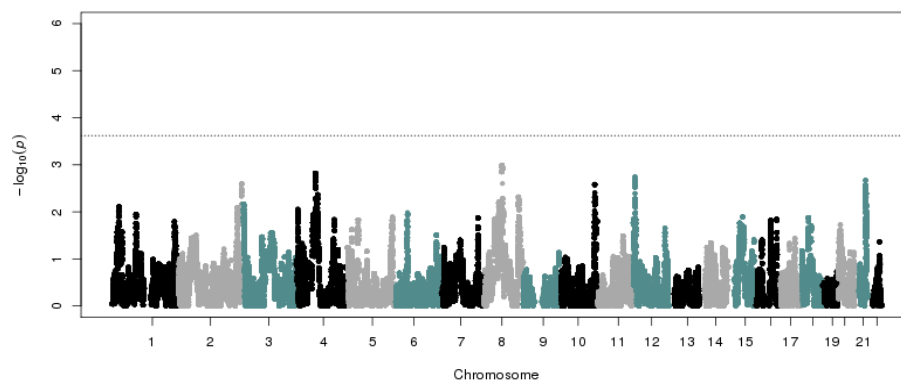


Figure 9. Admixture mapping in SAGE I (n= 190) for African ancestry and BDR. Ancestry association testing was performed at 478,441 markers using linear regression including age, sex, BMI category, and global African ancestry covariates.

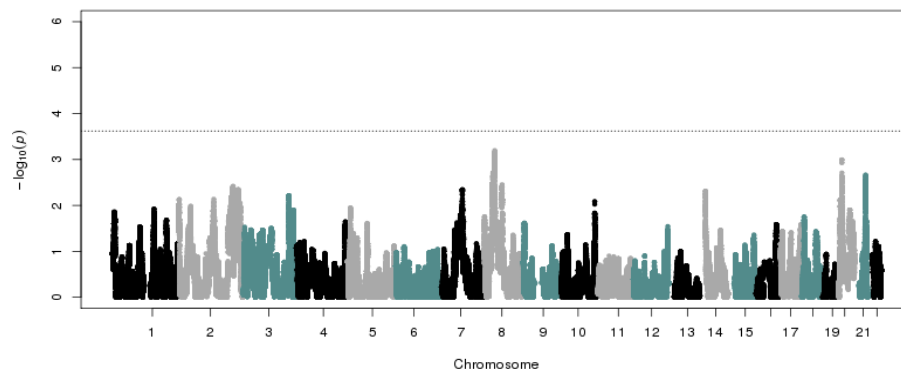


Figure 10. Meta-analysis of admixture mapping in SAGE I and II (n=949) for African ancestry and BDR. Ancestry association testing was performed at 478,441 markers using linear regression including age, sex, BMI category, and global African ancestry covariates.

Chr	SNP	Position (hg19)	Effect	StdErr	Pvalue	Direction
8p11	rs3927941	39805797	1.49	0.44	6.34×10^{-4}	++

Table VIII: Meta-analysis of admixture mapping results within African Americans (SAGE I and II). Under ‘Direction’ the first symbol refers to SAGE I, second to SAGE II

DISCUSSION

We performed a genome-wide association study for bronchodilator drug response in African Americans, and identified a population-specific association between rs73650726, located on chromosome 9, and BDR. Specifically, we discovered that the G allele of rs73650726 was associated with increased BDR and is more common in African Americans compared to European populations (Table II above). The variant rs73650726, located on chromosome 9, does not map to any gene, but SNPs in high linkage disequilibrium ($r^2 \geq 0.8$) with this marker are located in enhancer histone marks in lung tissues⁴⁶.

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. Through investigating the underlying genetics of BDR in diverse populations we have uncovered novel information regarding both the pathophysiology and pharmacogenetics of 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 adhesion⁴⁷, gene expression^{48, 49}, vascular smooth muscle cell contraction⁵⁰, and feedback of the nitric-oxide (NO) signaling pathway⁵¹. Notably, the NO pathway is a key pathway in modulating vasodilation in response to beta-agonists such as albuterol via beta 2-adrenergic receptors⁵², making *PRKG1* a highly plausible BDR candidate gene. The three SNPs are in high linkage disequilibrium ($r^2 \geq 0.8$) with variants known to be functional⁴⁶, and are all associated with the expression of *PRKG1* in the lung – a tissue highly relevant to BDR. From the Genotype-Tissue Expression (GTEx) project database, the reference allele for all three SNPs was associated with decreased expression of the gene in lung tissue⁴⁵. 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 attempted to replicate our study findings and candidate SNPs previously found to be associated with BDR, however we found no significant associations following multiple testing corrections. This could be due to differences in study design, the presence of population specific differences in genetic contributions to BDR, and/or varying patterns of linkage disequilibrium between populations. Furthermore, we were limited in sample size in GALA I²⁵ to evaluate associations at low frequency variants, and note that SAPPHERE is comprised of mainly adults³¹ in comparison to SAGE and GALA II that 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 studies⁵³⁻⁵⁵. Further genetic studies in African Americans is 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.

References:

1. Burchard EG, Ziv E, Coyle N, Gomez SL, Tang H, Karter AJ, et al. The importance of race and ethnic background in biomedical research and clinical practice. *N Engl J Med* 2003; 348:1170-5.
2. Naqvi M, Thyne S, Choudhry S, Tsai HJ, Navarro D, Castro RA, et al. Ethnic-specific differences in bronchodilator responsiveness among African Americans, Puerto Ricans, and Mexicans with asthma. *J Asthma* 2007; 44:639-48.
3. Padhukasahasram B, Yang JJ, Levin AM, Yang M, Burchard EG, Kumar R, et al. Gene-based association identifies SPATA13-AS1 as a pharmacogenomic predictor of inhaled short-acting beta-agonist response in multiple population groups. *Pharmacogenomics J* 2014.
4. Palmer LJ, Silverman ES, Weiss ST, Drazen JM. Pharmacogenetics of Asthma. *American Journal of Respiratory and Critical Care Medicine* 2002; 165:861-6.
5. Nelson HS. Beta-adrenergic bronchodilators. *N Engl J Med* 1995; 333:499-506.
6. Loza MJ, Penn RB. Regulation of T cells in airway disease by beta-agonist. *Front Biosci (Schol Ed)* 2010; 2:969-79.
7. Salathe M. Effects of beta-agonists on airway epithelial cells. *J Allergy Clin Immunol* 2002; 110:S275-81.
8. Shore SA, Moore PE. Regulation of beta-adrenergic responses in airway smooth muscle. *Respir Physiol Neurobiol* 2003; 137:179-95.
9. Jariti T. Asthma, asthma medication and autonomic nervous system dysfunction. *Clin Physiol* 2001; 21:260-9.

10. Drake KA, Torgerson DG, Gignoux CR, Galanter JM, Roth LA, Huntsman S, et al. A genome-wide association study of bronchodilator response in Latinos implicates rare variants. *J Allergy Clin Immunol* 2014; 133:370-8.
11. Burchard EG, Avila PC, Nazario S, Casal J, Torres A, Rodriguez-Santana JR, et al. Lower bronchodilator responsiveness in Puerto Rican than in Mexican subjects with asthma. *Am J Respir Crit Care Med* 2004; 169:386-92.
12. Choudhry S, Ung N, Avila PC, Ziv E, Nazario S, Casal J, et al. Pharmacogenetic differences in response to albuterol between Puerto Ricans and Mexicans with asthma. *Am J Respir Crit Care Med* 2005; 171:563-70.
13. Blake K, Madabushi R, Derendorf H, Lima J. Population pharmacodynamic model of bronchodilator response to inhaled albuterol in children and adults with asthma. *Chest* 2008; 134:981-9.
14. Gorina Y. QuickStats:asthma*death rates, by race and age group - United States, 2007–2009. In (MMWR) *MaMWR* (ed.). Centers for Disease Control and Prevention 2012.
15. Martinez FD, Graves PE, Baldini M, Solomon S, Erickson R. Association between genetic polymorphisms of the beta2-adrenoceptor and response to albuterol in children with and without a history of wheezing. *J Clin Invest* 1997; 100:3184-8.
16. Silverman EK, Kwiatkowski DJ, Sylvia JS, Lazarus R, Drazen JM, Lange C, et al. Family-based association analysis of beta2-adrenergic receptor polymorphisms in the childhood asthma management program. *J Allergy Clin Immunol* 2003; 112:870-6.
17. Poon AH, Tantisira KG, Litonjua AA, Lazarus R, Xu J, Lasky-Su J, et al. Association of corticotropin-releasing hormone receptor-2 genetic variants with acute bronchodilator response in asthma. *Pharmacogenet Genomics* 2008; 18:373-82.

18. Tantisira KG, Small KM, Litonjua AA, Weiss ST, Liggett SB. Molecular properties and pharmacogenetics of a polymorphism of adenylyl cyclase type 9 in asthma: interaction between beta-agonist and corticosteroid pathways. *Hum Mol Genet* 2005; 14:1671-7.
19. Litonjua AA, Lasky-Su J, Schneiter K, Tantisira KG, Lazarus R, Klanderman B, et al. ARG1 is a novel bronchodilator response gene: screening and replication in four asthma cohorts. *Am J Respir Crit Care Med* 2008; 178:688-94.
20. Duan QL, Du R, Lasky-Su J, Klanderman BJ, Partch AB, Peters SP, et al. A polymorphism in the thyroid hormone receptor gene is associated with bronchodilator response in asthmatics. *Pharmacogenomics J* 2013; 13:130-6.
21. Reihsaus E, Innis M, MacIntyre N, Liggett SB. Mutations in the gene encoding for the beta 2-adrenergic receptor in normal and asthmatic subjects. *Am J Respir Cell Mol Biol* 1993; 8:334-9.
22. Duan QL, Lasky-Su J, Himes BE, Qiu W, Litonjua AA, Damask A, et al. A genome-wide association study of bronchodilator response in asthmatics. *Pharmacogenomics J* 2014; 14:41-7.
23. Israel E, Lasky-Su J, Markezich A, Damask A, Szeffler SJ, Schuemann B, et al. Genome-wide association study of short-acting beta2-agonists. A novel genome-wide significant locus on chromosome 2 near ASB3. *Am J Respir Crit Care Med* 2015; 191:530-7.
24. Himes BE, Jiang X, Hu R, Wu AC, Lasky-Su JA, Klanderman BJ, et al. Genome-wide association analysis in asthma subjects identifies SPATS2L as a novel bronchodilator response gene. *PLoS Genet* 2012; 8:e1002824.

25. Torgerson DG, Ampleford EJ, Chiu GY, Gauderman WJ, Gignoux CR, Graves PE, et al. Meta-analysis of genome-wide association studies of asthma in ethnically diverse North American populations. *Nat Genet* 2011; 43:887-92.
26. Galanter JM, Gignoux CR, Torgerson DG, Roth LA, Eng C, Oh SS, et al. Genome-wide association study and admixture mapping identify different asthma-associated loci in Latinos: the Genes-environments & Admixture in Latino Americans study. *J Allergy Clin Immunol* 2014; 134:295-305.
27. White MJ, Risse-Adams O, Goddard P, Contreras MG, Adams J, Hu D, et al. Novel genetic risk factors for asthma in African American children: Precision Medicine and the SAGE II Study. *Immunogenetics* 2016; 68:391-400.
28. Winkler CA, Nelson GW, Smith MW. Admixture mapping comes of age. *Annu Rev Genomics Hum Genet* 2010; 11:65-89.
29. Nishimura KK, Galanter JM, Roth LA, Oh SS, Thakur N, Nguyen EA, et al. Early-life air pollution and asthma risk in minority children. The GALA II and SAGE II studies. *Am J Respir Crit Care Med* 2013; 188:309-18.
30. Torgerson DG, Gignoux CR, Galanter JM, Drake KA, Roth LA, Eng C, et al. Case-control admixture mapping in Latino populations enriches for known asthma-associated genes. *J Allergy Clin Immunol* 2012; 130:76-82 e12.
31. Gould W, Peterson EL, Karungi G, Zoratti A, Gaggin J, Toma G, et al. Factors predicting inhaled corticosteroid responsiveness in African American patients with asthma. *J Allergy Clin Immunol* 2010; 126:1131-8.
32. Standardization of Spirometry, 1994 Update. American Thoracic Society. *Am J Respir Crit Care Med* 1995; 152:1107-36.

33. Delaneau O, Zagury JF. Haplotype inference. *Methods Mol Biol* 2012; 888:177-96.
34. Howie BN, Donnelly P, Marchini J. A flexible and accurate genotype imputation method for the next generation of genome-wide association studies. *PLoS Genet* 2009; 5:e1000529.
35. Howie B, Marchini J, Stephens M. Genotype imputation with thousands of genomes. *G3 (Bethesda)* 2011; 1:457-70.
36. Genomes Project C, Auton A, Brooks LD, Durbin RM, Garrison EP, Kang HM, et al. A global reference for human genetic variation. *Nature* 2015; 526:68-74.
37. Maples BK, Gravel S, Kenny EE, Bustamante CD. RFMix: a discriminative modeling approach for rapid and robust local-ancestry inference. *Am J Hum Genet* 2013; 93:278-88.
38. Alexander DH, Novembre J, Lange K. Fast model-based estimation of ancestry in unrelated individuals. *Genome Res* 2009; 19:1655-64.
39. Baran Y, Pasaniuc B, Sankararaman S, Torgerson DG, Gignoux C, Eng C, et al. Fast and accurate inference of local ancestry in Latino populations. *Bioinformatics* 2012; 28:1359-67.
40. McGarry ME, Castellanos E, Thakur N, Oh SS, Eng C, Davis A, et al. Obesity and bronchodilator response in black and Hispanic children and adolescents with asthma. *Chest* 2015; 147:1591-8.
41. Willer CJ, Li Y, Abecasis GR. METAL: fast and efficient meta-analysis of genomewide association scans. *Bioinformatics* 2010; 26:2190-1.
42. PLUMMER M BN, COWLES K, VINES K. CODA: Convergence Diagnosis and Output Analysis for MCMC. *R News* 2012:7-11.

43. Sobota RS, Shriner D, Kodaman N, Goodloe R, Zheng W, Gao YT, et al. Addressing population-specific multiple testing burdens in genetic association studies. *Ann Hum Genet* 2015; 79:136-47.
44. Marcus JH, Novembre J. Visualizing the geography of genetic variants. *Bioinformatics* 2016.
45. Consortium GT. The Genotype-Tissue Expression (GTEx) project. *Nat Genet* 2013; 45:580-5.
46. Boyle AP, Hong EL, Hariharan M, Cheng Y, Schaub MA, Kasowski M, et al. Annotation of functional variation in personal genomes using RegulomeDB. *Genome Res* 2012; 22:1790-7.
47. Li Z, Xi X, Gu M, Feil R, Ye RD, Eigenthaler M, et al. A stimulatory role for cGMP-dependent protein kinase in platelet activation. *Cell* 2003; 112:77-86.
48. Tamura N, Itoh H, Ogawa Y, Nakagawa O, Harada M, Chun TH, et al. cDNA cloning and gene expression of human type Ialpha cGMP-dependent protein kinase. *Hypertension* 1996; 27:552-7.
49. Orstavik S, Natarajan V, Tasken K, Jahnsen T, Sandberg M. Characterization of the human gene encoding the type I alpha and type I beta cGMP-dependent protein kinase (PRKG1). *Genomics* 1997; 42:311-8.
50. Burgoyne JR, Madhani M, Cuello F, Charles RL, Brennan JP, Schroder E, et al. Cysteine redox sensor in PKGIa enables oxidant-induced activation. *Science* 2007; 317:1393-7.
51. Pfeifer A, Klatt P, Massberg S, Ny L, Sausbier M, Hirneiss C, et al. Defective smooth muscle regulation in cGMP kinase I-deficient mice. *EMBO J* 1998; 17:3045-51.

52. Dawes M, Chowienczyk PJ, Ritter JM. Effects of inhibition of the L-arginine/nitric oxide pathway on vasodilation caused by beta-adrenergic agonists in human forearm. *Circulation* 1997; 95:2293-7.
53. Bustamante CD, Burchard EG, De la Vega FM. Genomics for the world. *Nature* 2011; 475:163-5.
54. Popejoy AB, Fullerton SM. Genomics is failing on diversity. *Nature* 2016; 538:161-4.
55. Editors PM, Rid A, Johansson MA, Leung G, Valantine H, Burchard EG, et al. Towards Equity in Health: Researchers Take Stock. *PLoS Med* 2016; 13:e1002186.