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Gene-based association identifies *SPATA13-AS1* as a pharmacogenomic predictor of inhaled short-acting beta-agonist response in multiple population groups

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Abstract

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CONFLICT OF INTEREST

Authors declare no conflict of interest.

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Inhaled short-acting beta-agonist (SABA) medication is commonly used in asthma patients to rapidly reverse airway obstruction and improve acute symptoms. We performed a genome wide association study of SABA medication response using gene-based association tests. A linear mixed model approach was first used for SNP associations, and results were later combined using GATES to generate gene-based associations. Our results identified *SPATA13-AS1* as being significantly associated with SABA bronchodilator response in 328 healthy African Americans. In replication, this gene was associated with SABA response among 2 separate groups of African Americans with asthma (n=1,073, p=0.011 and n=1,968, p=0.014), 149 healthy African Americans (p=0.003), and 556 European Americans with asthma (p=0.041). *SPATA13-AS1* was also associated with longitudinal SABA medication usage in 2 separate groups of African Americans with asthma (n=658, p=0.047 and n=1,968, p=0.025). Future studies are needed to delineate the precise mechanism by which *SPATA13-AS1* may influence SABA response.

Keywords

pharmacogenomics; bronchodilator agents; asthma; adrenergic beta agonists; lung function tests; genome wide association study

INTRODUCTION

Inhaled short-acting beta-agonist (SABA) medications are among the most commonly used drugs in the treatment of asthma.[1] These medications exert their influence in the lung by binding to β_2-adrenergic receptors located on the smooth muscle cells of the lower respiratory tract, resulting in a decrease in intracellular calcium levels, muscle relaxation, and bronchodilation.[2]

Inhaled beta-agonist medications are capable of producing analogous physiologic effects in both asthmatic and non-asthmatic individuals. The sympathomimetic effects include both a systemic response (e.g., increased in heart rate, tremor, decreased systemic vascular resistance, and decreased plasma potassium levels)[3;4] and pulmonary changes (i.e., bronchodilation and increased lung function).[5;6] The effect of inhaled beta-agonist medicine on healthy individuals is also evinced by their banned use (with the exception of limited amounts of albuterol and formoterol) in competitive sport by the World Anti-Doping Agency and the International Olympic Committee (http://www.wada-ama.org/). Population-based studies of SABA medication have shown that age, body type, and smoking status contribute relatively small amounts to the variation in pulmonary response, even after excluding individuals with obstructive lung disease and heart disease.[7] This suggests that a considerable portion of SABA response in the lung is genetically determined even among healthy individuals.

The benefits of SABA medication to reduce airway resistance and air trapping among individuals with asthma is well known.[8] This response is often quantified as bronchodilator response (BDR), which is measured as the change in lung-function (i.e., the forced expiratory volume in 1 second [FEV_1]) before and shortly after SABA administration. Polygenic estimates give a lower bound of 28.5% for the heritability of BDR,[9] and a recent genome wide association study (GWAS) for BDR in European American individuals with

asthma reported a novel association with *SPATS2L*, a spermatogenesis-associated gene.[10] However, the degree of lung function change in response inhaled SABA medication in asthmatic individuals is also influenced by factors related to asthma severity, such as the degree of neutrophilic inflammation.[11] Since the purpose of the current study was to identify genes involved in SABA pharmacodynamics rather than genes involved in asthma severity, we chose to study healthy individuals (i.e., individuals without an asthma diagnosis) in our discovery set and then attempt to replicate genetic associations in both individuals with and without asthma.

In this article, we report the results of a genome wide association study (GWAS) of inhaled SABA medication response. A diverse set of healthy individuals and individuals with asthma from the Study of Asthma Phenotypes and Pharmacogenomic Interactions by Race-ethnicity (SAPPHIRE) were used to identify genetic predictors of SABA-induced bronchodilation. The resulting candidate gene, *SPATA13-AS1*, was also prospectively assessed for association with clinical measures of SABA use (i.e., number of prescription fills) among individuals with asthma.

MATERIALS AND METHODS

Patients and Setting

This study was approved by the Institutional Review Board at Henry Ford Health System. All participants provided written consent prior to enrollment. *Healthy* patients were recruited from southeastern Michigan. These patients received care from a large, integrated health system serving the greater Detroit metropolitan statistical area, and therefore, had detailed longitudinal clinical records of care received. These individuals were age 12–56 years and had no prior clinical diagnosis of asthma, chronic obstructive pulmonary disease, or congestive heart failure either in the electronic medical record or by self-reports. For our discovery set, we included healthy individuals who self-identified as being African American and who had genome wide genotype data.

For the initial replication we used participants with asthma from the SAPPHIRE cohort (clinicaltrials.gov identifier: NCT01142947). All SAPPHIRE participants received care from the same health system and were age 12–56 years at the time of enrollment. Patients with asthma had both a physician diagnosis of asthma documented in the electronic medical record and they confirmed receiving a prior diagnosis of asthma. Asthma patients denied having chronic obstructive pulmonary disease or congestive heart failure, and they had no record of these conditions in their medical records. We restricted the analysis in this initial replication group to those who identified themselves as African American and who had genome wide genotype data.

For additional replication groups, we used enrolled healthy individuals and individuals with asthma recruited from the same geographic area. These individuals had similar inclusion criteria but included both self-reported African American and self-reported European American individuals; however, they did not have existing genome wide genotype data.

Many SAPPHIRE participants had available electronically recorded information on medication prescription fills by virtue of their membership in the health system and in affiliated health maintenance organization. We have previously shown that these records capture ~99% of all asthma medications fills in this covered population.[12] Therefore, we used these data to quantify SABA use in SAPPHIRE individuals (i.e., individuals with asthma).

Lung Function Testing and Assessment of Bronchodilator Response

Lung function testing was performed using a Fleisch-type pneumotachometer (KoKo PFT Spirometer®, nSpire Health Inc., Louisville, CO) and following 2005 ATS/ERS spirometry recommendations.[27;28] Patients using inhaled bronchodilators were asked to withhold these medications for the 12 hours prior to lung function tests. To assess response we administered a 360 microgram (mcg) 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). Pulmonary function was reassessed 15 minutes after administering albuterol. Bronchodilator response 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, using the following equation:

$$\mathrm{BDR}=((\mathrm{FEV}_{1\,(\mathrm{post-brochodilator})}-\mathrm{FEV}_{1\,(\mathrm{pre-bronchodilator})})\times 100)/\mathrm{FEV}_{1\,(\mathrm{pre-bronchodilator})}.$$

. Predicted values for pulmonary function parameters, such as the forced expiratory volume at one second (FEV_1), were obtained using standard equations derived from U.S. population.[29]

Genotyping and Quality Control

Genome wide genotype data was generated from DNA isolated from blood samples donated by 1,521 study individuals (i.e., a set of healthy individuals and individuals with asthma). Genotyping was performed using the Axiom® AFR array (Affymetrix Inc., Santa Clara, CA) and following the quality control (QC) guidelines of the manufacturer. We excluded genotyped samples from the analysis for the following reasons: 11 had a dish QC < 0.82; 41 samples had an overall call rate < 0.97, and 17 samples had a sex discordance based on X-heterozygosity. The average genotype calling rate was 99.35% among the remaining samples and the average concordance rate among 15 duplicate pairs was 99.64%. Forty-two individuals (16 healthy patients and 26 patients with asthma) were removed due to ambiguities between asthma statuses in the electronic record as compared with patient self-report.

We filtered out SNPs which didn't meet the following QC metrics: overall SNP call rate ≥0.95, Fisher's linear discriminant ≥3.6, and Het strength offset ≥ −0.1. This reduced the number of analyzable SNPs from 893,968 to 862,897. In addition, we removed SNPs with minor allele frequency (MAF) < 5%, those with Hardy-Weinberg equilibrium p-value < 10^{-5}, and non-autosomal SNPs. The final set for association testing comprised 586,952 polymorphisms.

We also used principal component (PC) analysis to exclude outliers.[13] First we removed 4 individuals who clustered closely with the CEU samples of HapMap based on the 1st and 2nd PCs. Principal component analysis was performed using the *prcomp* function in R based on a randomly selected subset of 10,000 SNPs with mean centering of genotypes. Using an iterative algorithm, we then successively removed individuals if any of their top 2 PCs was more than 6 standard deviations from the sample mean. Five additional individuals were removed using this method. Therefore, the analytic samples for the discovery and first replication set consisted of 328 healthy individuals and 1,073 individuals with asthma, respectively.

For replication individuals without existing genome wide genotype data, we used TaqMan® allelic discrimination assays (Applied Biosystems, Foster City, CA) for additional genotyping. For the gene that we carried forward for additional replication, we re-genotyped those SNPs which had a p-value <0.05 (in the discovery set) and for which pairwise linkage disequilibrium (LD) was <0.8.

Statistical Analysis

The primary phenotype (i.e., quantitative trait) in this study was SABA response as measured by the change in FEV_1 before and after use of inhaled albuterol. As a secondary outcome, we assessed SABA use based on number of prescription fills. Figure S1 of the online supplement shows the distribution of the SABA response phenotype in all of the groups analyzed as part of this study. Differences in the characteristics of healthy individuals and individuals with asthma were assessed using chi-squared test for categorical variables and t-test for continuous variables. We also compared the minor allele frequencies of SNPs between healthy African American individuals and African Americans individuals with asthma (Figure S2 of the online supplement).

For the discovery set of healthy individuals, we used an additive genetic model to assess the relationship between genotype (coded as 0, 1 or 2) and SABA response (i.e., the albuterol induced change in FEV_1). In order to account for both relatedness among individuals and the underlying population structure, we used a linear mixed model as implemented in the program EMMAX.[14] This program used the genome wide autosomal markers to calculate an identity by state (IBS) kinship matrix. This matrix was used to adjust the genotype associations, in addition to separate adjustments for age, sex, body mass index (BMI), smoking status (i.e., current, past, and never), and the first 10 PCs. Using a mixed model approach minimizes p-value inflation and spurious associations resulting from population substructure and cryptic relatedness between individuals. Incorporating PCs as covariates also minimizes p-value inflation at unusually differentiated markers.[15] All SNP association p-values were based on 2-tailed tests. We then used the previously described gene-based association test that uses extended Simes procedure (GATES)[16] to generate gene-based associations from the individual SNP associations derived through EMMAX. This approach combines all SNP p-values in a given gene so as to derive an overall level of statistical significance; the procedure also accounts for pairwise LD between variants.[16–18] The National Center for Biotechnology Information dbSNP database was used to define gene sets, whereby all genotyped SNPs within 5 kilobases (kb) of the start and stop site are

ascribed to that gene unit. Given the number of genes assessed (21,037), the conservative Bonferroni significance threshold for the gene-based association was $p < 2.38 \times 10^{-6}$.

We then attempted to replicate the two significantly associated genes, *SPATA13-AS1* and *SULT4A1*, from the discovery set in SAPPHIRE individuals with asthma who had genome wide genotype data (n = 1,073). The gene-based test in individuals with asthma combined the SNP-associations within those genes adjusted for age, sex, BMI, smoking status (i.e., current, past, and never), percent of predicted FEV_1, and the first 10 PCs.

The gene that continued to be statistically significant after the initial replication was reassessed in the following groups which didn't have genome wide genotype data: healthy African American individuals (n=149), healthy European American individuals (n=178), African American individuals with asthma (n=1,968), and European American individuals with asthma (n=556). The SNP-based associations that composed the gene-based test were adjusted for age, sex, BMI, and smoking status and percent of predicted FEV_1. Association testing was performed using the program PLINK[19] and assuming an additive genetic model; SNP based p-values were based on 2-tailed tests. To obtain a combined gene-based association across groups, we meta-analytically combined the SNP-based associations for *SPATA13-AS1* across groups, and then we converted these associations into a gene-based p-value using the GATES procedure.

Lastly, for a number of study participants with asthma, we had longitudinal pharmacy data for the amount of SABA use; this included 658 African American individuals from the initial replication set with genome wide genotype data, 556 European American individuals from the replication set without genome wide genotype data, and 1,968 African American individuals from the replication set without genome wide genotype data. In these groups we assessed the gene-based association between *SPATA13-AS1* and frequency of SABA use. Analyses were performed for each group separately, and gene-based associations were repeated after meta-analytically combining the SNP-based associations for each group.

RESULTS

Study subjects

Genome wide genotype data were available for the 328 healthy African American individuals who composed the discovery set and 1,073 African American individuals with asthma who made up the initial replication set. Additional replication sets included 149 healthy African American individuals, 178 healthy European American individuals, 556 European American individuals with asthma, and 1,968 African American individuals with asthma. The baseline characteristics for all of these groups are shown in Table 1. The distribution of SABA response among the discovery and replication samples is shown in Figure S1 of the online supplement; these distributions appeared to be normally distributed for all of the groups assessed. The allele frequencies between those with and without asthma also appeared to be similar (Figure S2 of the online supplement).

Genes associated with SABA response in healthy African American subjects and replication in African American individuals with asthma

The gene-based test identified 2 genes *SPATA13-AS1* ($p = 2.35 \times 10^{-6}$) and *SULT4A1* ($p = 1.49 \times 10^{-6}$) which crossed the genome wide significance threshold (Figure 1 and Table 2). These associations were then reassessed in 1,073 African American individuals with asthma who had genome wide genotype data. The gene-based association with SABA response was statistically significant for *SPATA13-AS1* (p = 0.011) but was of borderline significance for *SULT4A1* (p = 0.051) (Table 2).

Replication of associations with SABA response in additional groups of African American and European American individuals with and without asthma

Since *SPATA12-AS1* was statistically significant in both the discovery set and initial replication set, we reassessed this gene in 4 additional groups – 149 healthy African American individuals, 178 healthy European American individuals, 556 European American individuals with asthma, and 1,968 African American individuals with asthma. For this replication, we included five SNPs from the *SPATA13-AS1* gene-based test as denoted in Figure 2. *SPATA13-AS1* was significantly associated with SABA response among the healthy African American individuals (p=0.003), the European American individuals with asthma (p=0.041), and the African American individuals with asthma (p=0.014) (Table 2). In only the healthy European American individuals was the gene-based replication of *SPATA13-AS1* not statistically significant (p = 0.357). Combined across all replication groups, the gene-based test for *SPATA13-AS1* was highly significant ($p=8.40 \times 10^{-5}$), and the statistical association was even stronger when the discovery set was included in the combined analysis ($p=7.38 \times 10^{-7}$).

Association between *SPATA13-AS1* and SABA use among individuals with asthma

To look for clinical evidence of a role of *SPATA13-AS1* in SABA response, we examined whether the gene-based test was associated with the number of SABA fills in the year following study enrollment. Data on SABA use were available for the following groups with asthma: 658 of the 1,073 African American individuals with genome wide genotype data, 556 European American individuals without genome wide genotype data, and 1,968 African American without genome wide genotype data. The gene-based association for *SPATA13-AS1* with SABA use in these groups was p = 0.047, p=0.384, and p = 0.025, respectively (Table 3). The combined association in all 3 groups (3,182 individuals) was also statistically significant (p = 0.014).

DISCUSSION

There are multiple unique and important aspects of this pharmacogenomic study of SABA drug response. First, to our knowledge, this is the only genome wide study of asthma treatment response with sufficient numbers of African American individuals to assess this group separately. This is of particular importance because African American individuals are disproportionately affected by asthma and its complications[20;21] and they may possess population-specific risk factors.[22] Next, this is also the only study to date to use healthy individuals to aid in identifying genetic predictors of SABA response. While the degree of

albuterol-induced bronchodilation was less pronounced in these healthy individuals, we believe that the physiologic response to SABA medication was less obscured by underlying lung inflammation in this group, thereby producing a better phenotype for genetic association. Third, we had longitudinal measures of SABA use based on objective records of prescription fills. These latter data allowed us to "cross-validate" genetic associations first identified and replicated using physiologic measures of drug response for their relationship with clinical indicators of SABA usage. These study characteristics combined allowed us to identify a novel and biologically plausible gene, *SPATA13-AS1*, associated with SABA-induced bronchodilation.

SPATA13-AS1 is an anti-sense RNA which overlaps the gene *SPATA13* (otherwise known as *ASEF2*). The expression of this product has been recorded in studies in different cell types.[23–29] The specific regulatory mechanism of *SPATA13-AS1* is not known, although it could reasonably be assumed to have *cis*-acting effects on *SPATA13* expression. Many of the variants used in our gene-based test for *SPATA13-AS1* are located just proximal or distal to *SPATA13-AS1* in evolutionarily conserved intronic regions in *SPATA13* (Figure 2). Therefore, at this point we cannot definitively state that *SPATA13-AS1* is causally involved in SABA response, since our gene-based test may also reflect important functional variants in *SPATA13*. Distinguishing these possibilities will likely require sequencing of this region to fully characterize variation within both genes, as well as functional information, such as tissue-specific expression.

Nevertheless, both *SPATA13* and its anti-sense RNA are interesting candidate genes for SABA medication response. Asef2 (the protein expressed by *SPATA13*) has been recently identified to function as a guanine nucleotide exchange factor (GEF).[30] By facilitating the substitution of GTP for GDP, Asef2 appears to specifically activate Rho-family GTPases, such as Rac1[30;31] and Cdc42[30–32] but may inactivate RhoA.[31] Rho-family GTPases, in turn, influence a wide range of cellular functions including smooth muscle contraction.[33;34] Smooth muscle contraction requires myosin light chain (MLC) phosphorylation and the subsequent sliding of actin and myosin filaments over each other. RhoA increases Rho Kinase activity which inhibits myosin phosphatase, and inhibiting myosin phosphatase promotes smooth muscle contraction by keeping MLC phosphorylated.[35] Recent evidence also suggests that RhoA influences airway smooth muscle contraction through its effect on adhesosome signaling and Cdc42 mediated actin polymerization. Therefore, it is plausible that *SPATA13-AS1* and *SPATA13* influence SABA induced bronchodilation through an effect on Rho-family GTPase mediated MLC phosphorylation and actin polymerization.[36] If confirmed via functional studies, these represent a novel target for future asthma therapy in airway smooth muscle.

To identify *SPATA13-AS1* we used a gene-based test of association. This approach is somewhat novel when compared with the more commonly used SNP-based association studies. For example, a SNP-based approach requires many more association tests, and correction for this multiple testing invariably leads to many missed true associations (i.e., many true associations do not meet the stringent genome wide significance thresholds). Multimarker methods,[37;38] such as gene-based association approaches,[16–18] can improve power and reduce the total number of tests. Moreover, as genes are the basic functional units

of the genome, gene-based association is arguably more natural and may be easier to replicate since it may be less likely to be confounded by population-specific allele frequencies or differing patterns of linkage disequilibrium.[16] Thus, by not relying on any one SNP estimate, gene-based tests may provide a more robust measure of genetic association. It is also important to note that while gene-based tests may identify implicated genes, these tests do not imply a direction of effect as is often inferred in SNP-based association analyses (although the direction of individual SNP associations often differs between studies and population groups,[39–41] as shown in Table S1 of the online supplement for *SPATA13-AS1* in our discovery and replication populations). Therefore, gene-based tests may be better suited to identify genes for more focused study.

Studying diverse non-European populations is valuable and can give us novel insights into the genetic basis of drug response. When compared with studies done in individuals of predominantly European descent, studies such as this one may inform us about genes that are important across groups and which are population-specific. Although the gene-based association for *SPATA13-AS1* did not replicate in healthy European American individuals in this analysis, we may have been underpowered to detect an association in this smaller sized group. However, the association between *SPATA13-AS1* and SABA response did replicate in the larger group of European American individuals with asthma, suggesting that this gene may be part of a pharmacogenetic pathway common to multiple population groups.

While reversible airway disease is considered to be a characteristic of asthma,[42] the effect of SABA medication on lung function is not restricted to individuals with obstructive airway disease.[43][44] This suggests that the variation in SABA response seen in an otherwise healthy population can be used as a phenotype for pharmacogenomics association studies. In fact, the degree and distribution of SABA response observed in our discovery set of healthy individuals is consistent with what has reported in other population-based studies.[7] Nevertheless, the fact that we were able to replicate our associations in individuals with asthma, suggests that the pharmacogenomics association that we observed is generalizable to individuals with asthma, as well.

In summary, we have identified a gene, *SPATA13-AS1*, associated with SABA medication response in African American individuals, and this association replicated in additional samples of both European American and African American individuals. It will be important to assess our findings in additional population groups so as to draw better conclusions about the universal importance of this pharmacogenomic association. Future investigations will also require functional studies to better elucidate the biological mechanisms by which *SPATA13* or its antisense operates in the lung.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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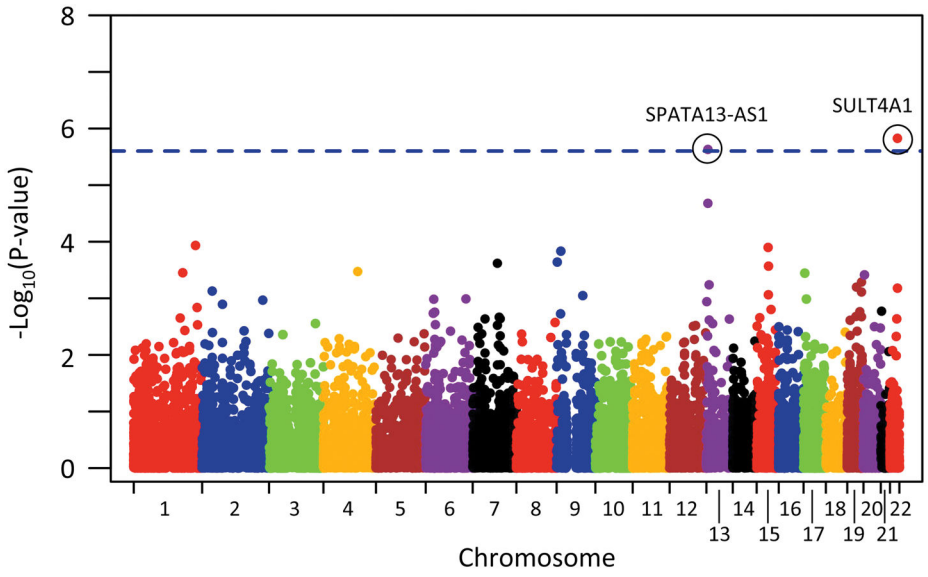

Figure 1.

Manhattan plot of gene-based associations with short-acting beta-agonist response among healthy African American individuals (n = 328). The different autosomal chromosomes are denoted on the x-axis and the observed p-values (−log transformed) are plotted on the y-axis. The blue horizontal dashed line represents the genome wide significance threshold for the gene-based test ($p < 2.38 \times 10^{-6}$). Both *SPATA13-AS1* and *SULT4A1* cross this significance threshold.

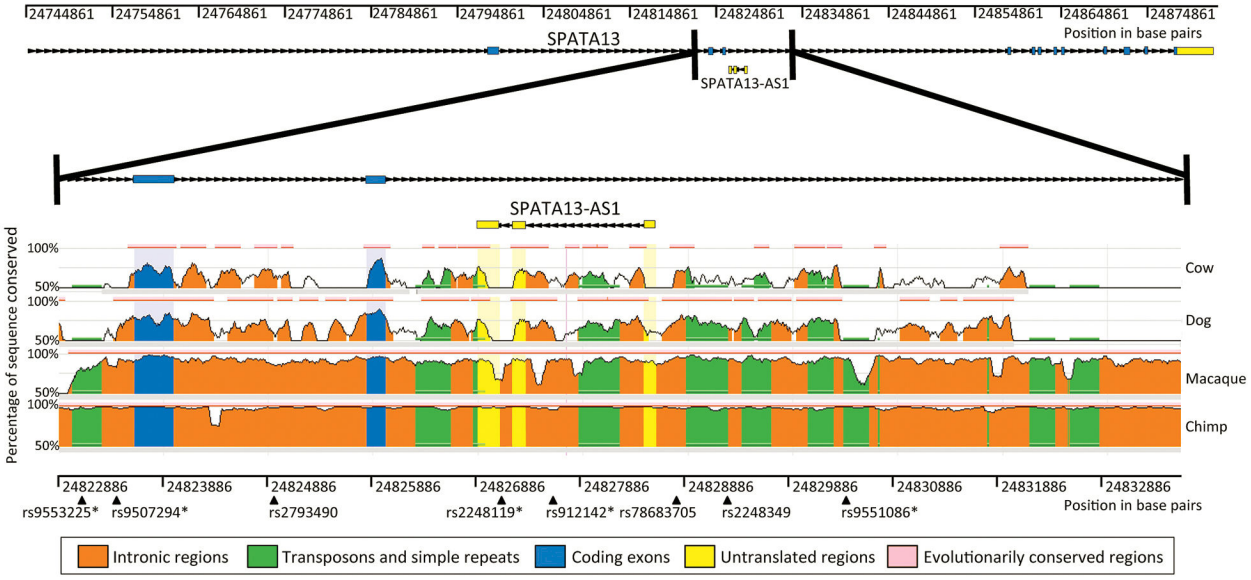

Figure 2.

Evolutionarily conserved regions around the *SPATA13-AS1* gene region. The percentage conservation with humans is plotted along the y-axis for various species. The genetic positions of SNPs used in the gene-based test are shown at the bottom. SNPs that compose the gene-based test in samples without existing genome wide genotype data are denoted with asterisks.

Table 1

Sample characteristics of the discovery and replication sets for the genetic association study of inhaled short-acting beta-agonist response.

Variable	Discovery set with genome wide genotype data	Replication set with existing genome wide genotype data	Replication sets without existing genome wide genotype data			
	Healthy African American individuals (n=328)	African American individuals with asthma (n=1,073)	Healthy African American individuals (n=149)	Healthy European American individuals (n=178)	European American individuals with asthma (n=556)	African American individuals with asthma (n=1,968)
Age (years) – mean ± SD	41.23 ± 13.28	31.65 ± 14.57	33.22 ± 13.52	44.14 ± 12.03	35.07 ± 15.93	33.13 ± 13.73
Female Sex – no. (%)	212 (64.63)	671 (62.53)	102 (68.45)	103 (57.86)	358 (64.38)	1282 (65.14)
Body mass index (kg/m^2) – mean ± SD	32.19 ± 7.58	31.49 ± 9.07	30.39 ± 8.42	28.37 ± 6.95	28.35 ± 8.21	31.87 ± 9.84
Smoking status – no. (%)						
Never	239 (72.8)	893 (83.2)	117 (78.5)	101 (56.7)	449 (80.8)	1200 (61.0)
Past	33 (10.1)	96 (8.9)	17 (11.4)	48 (27.0)	71 (12.8)	177 (9.0)
Current	56 (17.1)	84 (7.8)	15 (10.1)	29 (16.3)	36 (6.4)	591 (30.0)
Asthma age of onset (years) – mean ± SD	--	12.65 ± 13.55	--	--	15.79 ± 14.18	12.77 ± 13.39
FEV_1 (liters) – mean ± SD	2.74 ± 0.71	2.58 ± 0.75	2.89 ± 0.71	3.32 ± 0.82	3.14 ± 0.84	2.54 ± 0.79
Percent of predicted FEV_1 – mean ± SD	97.6 ± 15.3	87.9 ± 18.4	95.6 ± 16.3	95.7 ± 15.5	92.6 ± 16.3	86.7 ± 20.0
SABA response (% change) – mean ± SD*	2.51 ± 7.95	10.53 ± 12.93	2.91 ± 8.22	3.02 ± 5.86	5.76 ± 7.77	8.70 ± 14.21

SD denotes standard deviation; FEV_1, forced expiratory volume at one second; and SABA, short-acting beta-agonist.

*Short-acting beta-agonist response was measure as the change in FEV_1 after the administration of a 360 mcg dose of albuterol sulfate hydrofluoroalkane. The post-treatment lung function measurement was taken 15 minutes after the administration of albuterol.

Table 2

Gene-based associations for inhaled short-acting beta-agonist response in the discovery and replication sets.*

Gene	Chromosome	Discovery Set†	Replication set with existing genome wide genotype data†	Replication sets without existing genome wide genotype data‡				All replication groups combined (n=3,924)	All groups combined (n=4,252)**
		Healthy African American individuals (n=328)§	African American individuals with asthma (n=1,073)‖	Healthy African American individuals (n=149)¶	Healthy European American individuals (n=178)¶	European American individuals with asthma (n=556)¶	African American individuals with asthma (n=1,968)¶		
SPATA13-AS1	13	2.35×10^{-6}	0.011	0.003	0.357	0.041	0.014	8.40×10^{-5}	7.38×10^{-7}
SULT4A1	22	1.49×10^{-6}	0.051	--	--	--	--	--	--

*Shown are the p-values for gene-based associations generated using the GATES procedure.(16) This procedure combines the single nucleotide polymorphism (SNP) associations around and within a given gene. The outcome variable, short-acting beta-agonist response, was measured as the change in forced expiratory volume at one second (FEV_1) after the administration of a 360 mcg dose of albuterol sulfate hydrofluoroalkane.

†These groups had genome wide genotype data generated using the Affymetrix Axiom AFR array.

‡For these replication groups, we directly genotyped a set of SNPs representing *SPATA13-AS1*. These SNPs included rs9507294, rs912142, rs2248119, rs9551086, and rs9553225.

§For healthy individuals in the discovery set, we accounted for cryptic relatedness and adjusted for patient age, sex, body mass index (BMI), smoking status, and principal components for population substructure. SNP-based associations were combined using the program GATES to generate a gene-based association.

‖For individuals with asthma and genome wide genotype data, we also accounted for cryptic relatedness and used the same covariates as was used in the discovery set; however, we also included baseline percent of predicted FEV_1 as an additional covariate.

¶For individuals without genome wide genotype data, we adjusted for patient age, sex, BMI, percent of predicted FEV_1 and smoking status in all individuals.

**Includes individuals from both the discovery and replication sets.

Table 3

Gene-based association of *SPATA13-AS1* with short-acting beta-agonist use among individuals with asthma.*

Gene	Chromosome	Set with existing genome wide genotype data†	Sets without existing genome wide genotype data‡		All groups with asthma combined (n= 3,182)
		African American individuals with asthma (n=658)	European American individuals with asthma (n=556)	African American individuals with asthma (n=1,968)	
SPATA13-AS1	13	0.047	0.384	0.025	0.014

*Shown are the p-values for gene-based associations generated using the GATES procedure.(16) This procedure combines the single nucleotide polymorphism (SNP) associations around and within a given gene. The outcome variable, short-acting beta-agonist (SABA) use, was based on the number of SABA fills in pharmacy claims data. These data were only available for health plan members with pharmaceutical coverage, hence the smaller sample size.

†Adjusted for age, sex, body mass index (BMI), percent of predicted forced expiratory volume at one second (FEV_1), smoking status, and principal components for population substructure. The association also accounted for cryptic relatedness between subjects.

‡Adjusted for age, sex, BMI, percent of predicted FEV_1, and smoking status.