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

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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 single-nucleotide polymorphism associations, and the results were later combined using GATES to generate gene-based associations. Our results identified \textit{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 the two separate groups of African Americans with asthma ($n = 1073$, $P = 0.011$ and $n = 1968$, $P = 0.014$), 149 healthy African Americans ($P = 0.003$) and 556 European Americans with asthma ($P = 0.041$). \textit{SPATA13-AS1} was also associated with longitudinal SABA medication usage in the two separate groups of African Americans with asthma ($n = 658$, $P = 0.047$ and $n = 1968$, $P = 0.025$). Future studies are needed to delineate the precise mechanism by which \textit{SPATA13-AS1} may influence SABA response.

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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-A51, was also prospectively assessed for association with clinical measures of SABA use (that is, 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 before 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 aged 12–56 years and had no previous 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 aged 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 confirmed receiving a previous 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 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 medication fills in this covered population. Therefore, we used these data to quantify SABA use in SAPPHIRE individuals (that is, individuals with asthma).

Lung function testing and assessment of BDR

Lung function testing was performed using a Fleisch-type pneumotachometer (KoKo PFT Spirometer, nSpire Health, Louisville, CO, USA) and following 2005 ATS/ERS spirometry recommendations. Patients using inhaled bronchodilators were asked to withhold these medications for the 12 h before lung function tests. To assess response, we administered a 360 microgram (mcg) dose (that is, four puffs) of inhaled albuterol sulfate hydrofluoroalkane (GlaxoSmithKline, Research Triangle Park, NC, USA) from a standard metered dose inhaler using an AeroChamber Plus Flow-Vu spacer (Monahan Medical Corp., Plattsburgh, NY, USA). Pulmonary function was reassessed 15 min after administering albuterol. BDR was measured as the change in FEV1, between the baseline (pre-bronchodilator) measure and post-bronchodilator FEV1, using the following equation:

\[
\text{BDR} = \frac{(\text{FEV}_1\text{post-bronchodilator} - \text{FEV}_1\text{pre-bronchodilator}) \times 100}{\text{FEV}_1\text{pre-bronchodilator}}
\]

Predicted values for pulmonary function parameters, such as FEV1, were obtained using standard equations derived from US population.

Genotying and quality control

Genome-wide genotype data were generated from DNA isolated from blood samples donated by 321 study individuals (that is, a set of healthy individuals and individuals with asthma). Genotyping was performed using the Axiom AFR array (Affymetrix, Santa Clara, CA, USA) and following the quality control (QC) guidelines of the manufacturer. We excluded genotyped samples from the analysis for the following reasons: 11 had a discordance of 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 status in the electronic record as compared with patient’s self-report.

We filtered out single-nucleotide polymorphisms (SNPs) which did not 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 <5%, those with Hardy–Weinberg equilibrium P-value <10⁻⁸ and non-autosomal SNPs. The final set for association testing comprised 586 952 polymorphisms.

We also used principal component (PC) analysis to exclude outliers. First we removed four individuals who clustered closely with the CEU samples of HapMap based on the first and second PCs. PC 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 two PCs was ≥ 6 s.d.s 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 1073 individuals with asthma, respectively.

Statistical analysis

The primary phenotype (that is, quantitative trait) in this study was SABA response as measured by the change in FEV1, before and after use of inhaled albuterol. As a secondary outcome, we assessed SABA use based on number of prescription fills. Supplementary Figure S1 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 the 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 (Supplementary Figure S2).

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 (that is, albuterol-induced change in FEV1). 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. This program used the genome-wide autosomal markers to calculate an identity by state 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 (that is, 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. All SNP association P-values were based on two-tailed tests. We then used the previously described gene-based association test that uses extended Times procedure (GATES) 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. The National Center for Biotechnology Information dbSNP database was used to define principal component (PC) markers to calculate an identity by state kinship matrix. 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 x 10⁻⁶. We then attempted to replicate the two significantly associated genes, SPTA13-A51 and SULT1A1, from the discovery set in SAPPHIRE individuals with asthma who had genome-wide genotype data (n = 1073). The genobased test in individuals with asthma combined the SNP-associations within those genes adjusted for age, sex, BMI, smoking status (that is, current, past, and never), percentage of predicted FEV1, and the first 10 PCs. The gene that continued to be statistically significant after the initial replication was reassessed in the following groups that did not have...
Genes associated with SABA response in healthy African American subjects and replication in African American individuals with asthma

The gene-based test identified two genes SPATA13-AS1 (P = 2.35 × 10⁻⁶) and SULT4A1 (P = 1.49 × 10⁻⁶), which crossed the genome-wide significance threshold (Figure 1 and Table 2). These associations were then reassessed in 1073 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

As SPATA13-AS1 was statistically significant in both the discovery set and initial replication set, we reassessed this gene in four additional groups—149 healthy African American individuals, 178 healthy European American individuals, 556 European American individuals with asthma and 1968 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 × 10⁻⁵), and the statistical association was even stronger when the discovery set was included in the combined analysis (P = 7.38 × 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

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**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 = 1073)   | Healthy African American individuals (n = 149)            | Healthy African American individuals (n = 178)            | Healthy European American individuals (n = 556)           | African American individuals with asthma (n = 1968)      |
| Age (years), mean ± s.d.                      | 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, n (%)                             | 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 ± s.d.        | 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, n (%)                         |                                              |                                                       |                                                         |                                                         |                                                         |                                                         |
| 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 ± s.d.      | —                                           | —                                                     | —                                                        | —                                                        | —                                                        | —                                                        |
| FEV₁ (L) mean ± s.d.                          | 2.74 ± 0.71                                  | 2.58 ± 0.75                                           | 2.89 ± 0.71                                              | 3.32 ± 0.82                                             | 3.14 ± 0.84                                             | 2.54 ± 0.79                                             |
| Percentage of predicted FEV₁, mean ± s.d.     | 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 ± s.d.         | 2.51 ± 7.95                                  | 10.53 ± 12.93                                         | 2.91 ± 8.22                                              | 3.02 ± 5.86                                             | 5.76 ± 7.77                                             | 8.70 ± 14.21                                            |

Abbreviations: FEV₁, forced expiratory volume at 1 s; SABA, short-acting beta-agonist. *Short-acting beta-agonist response was measure as the change in FEV₁, after the administration of a 360 mcg dose of albuterol sulfate hydrofluoroalkane. The post-treatment lung function measurement was taken 15 min after the administration of albuterol.
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 1073 African American individuals with genome-wide genotype data, 556 European American individuals without genome-wide genotype data and 1968 African American individuals 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 the three groups (3182 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. Although the degree of albuterol-induced bronchodilation was less pronounced in these healthy individuals, we believe that the physiological 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 physiological 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 that 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, as 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 tissuespecific 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.\(^ {30} \) 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

**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 (\( P < 2.38 \times 10^{-7} \)). Both SPATA13-AS1 and SULT4A1 cross this significance threshold.

**Table 2.** Gene-based associations for inhaled short-acting beta-agonist response in the discovery and replication sets\(^ a \)

| Gene     | Chromosome | Discovery Set\(^ b \) Replication set with existing genome-wide genotype data\(^ c \) Replication sets without existing genome-wide genotype data\(^ d \) All replication groups combined (n = 3924) | All groups combined (n = 4252)\(^ e \) |
|----------|------------|-------------------------------------------------|----------------------------------|----------------------------------|-----------------------------|
| SPATA13-AS1 | 13 | 2.35 \times 10^{-6} | 0.011 | 0.003 | 0.357 | 0.041 | 0.014 | 8.40 \times 10^{-5} | 7.38 \times 10^{-7} |
| SULT4A1 | 22 | 1.49 \times 10^{-6} | 0.051 | — | — | — | — | — | — |

For individuals without genome-wide genotype data, we adjusted for patient age, sex, body mass index (BMI), percentage of predicted forced expiratory volume at 1 s (FEV\(_1\)) and smoking status in all individuals. \( *\)Shown are the \( P \)-values for gene-based associations generated using the GATES procedure.\(^ {18} \) 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 FEV\(_1\), after the administration of a 360 mcg dose of albuterol sulfate hydrofluoroalkane.\(^ {18} \) These groups had genome-wide genotype data generated using the Affymetrix Axiom AFR array.\(^ {18} \) For these replication groups, we directly genotyped a set of SNPs representing SPATA13-AS1. These SNPs included rs9507294, rs912142, rs2248119, rs9551086 and rs9553225.\(^ {18} \) Includes individuals from both the discovery and replication sets.\(^ {18} \) For healthy individuals in the discovery set, we accounted for cryptic relatedness and adjusted for patient age, sex, BMI, smoking status and principal components for population structure. 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 percentage of predicted FEV\(_1\) as an additional covariate.
may inactivate RhoA. Rho-family GTPases, in turn, influence a wide range of cellular functions, including smooth muscle contraction. 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. 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. 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 (that is, many true associations do not meet the stringent genome-wide significance thresholds). Multimarker methods, such as gene-based association approaches, 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 as it may be less likely to be confounded by population-specific allele frequencies or differing patterns of LD. 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, as shown in Supplementary Table S1 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 that 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. Although reversible airway disease is considered to be a characteristic of asthma, the effect of SABA medication on lung function is not restricted to individuals with obstructive airway disease. This suggests that the variation in SABA response seen in an otherwise healthy population can be used as a phenotype for pharmacogenomic association studies. In fact, the degree and distribution of SABA response observed in our discovery set of healthy individuals is consistent with what has been reported in other population-based studies. Nevertheless, the fact that we were able to replicate our associations in individuals with asthma suggests that the pharmacogenomic 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 and its anti-sense operates in the lung.

CONFLICT OF INTEREST
The authors declare no conflict of interest.

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