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Effect of Rare Genetic Variants in the β 2 Adrenergic Receptor Gene on the Risk for Exacerbations and Symptom Control During Long-Acting Beta Agonist Treatment in a Multi-Ethnic Asthma Population

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VEO, DAM, and ERB performed the literature search. VEO, GAH, WCM, SPP, DAM, and ERB designed the study. VEO, WCM, WWB, MC, DC, SCE, EI, FM, SEW, SPP, and ERB enrolled patients in the studies. VEO, GAH, WCM, EJA, WWB, MC, DC, SCE, EI, FM, SEW, SPP, DAM, and ERB were involved in the acquisition of data. VEO, GAH, EJA, SPP, DAM, and ERB analyzed data. VEO, GAH, WCM, WWB, MC, DC, SCE, EI, FM, SEW, SPP, and ERB contributed to oversight of the study. VEO, EJA, DAM, and ERB provided statistical expertise. VEO, GAH, ATH, EJA, SCE, EI, SEW, SPP, DAM, and ERB participated in data interpretation. The report was drafted by VEO, GAH, DAM, and ERB. All authors have provided input to the report and approved the final version.

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The following conflicts of interest were declared:

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Abstract

Background—Severe adverse life-threatening events associated with long-acting beta agonists (LABA) use have caused the FDA to review LABA safety which has resulted in a boxed warning and a mandatory LABA safety study in 46,800 asthmatics. Identification of an at-risk, susceptible subpopulation using predictive biomarkers is critical in understanding LABA safety. The β_2 -adrenergic receptor gene (*ADRB2*) contains a common, nonsynonymous single nucleotide polymorphism, Gly¹⁶Arg, that is unlikely to account for rare, life-threatening events. We hypothesize that rare *ADRB2* variants with strong effects modulate therapeutic responses to long-acting beta agonist (LABA) therapy and contribute to rare, severe adverse events.

Methods—*ADRB2* was sequenced in 197 African Americans, 191 non-Hispanic Whites, and 73 Puerto Ricans. Sequencing identified six rare variants which were genotyped in 1,165 asthmatics (total=1,626). The primary hypothesis was that severe asthma exacerbations requiring hospitalization were associated with rare *ADRB2* variants. Replication was performed in 659 non-Hispanic White asthma subjects.

Findings—Asthmatics receiving LABA with a rare variant had increased asthma-related hospitalizations (meta-analysis for all ethnic groups: $p=2.83 \times 10^{-4}$), specifically LABA-treated non-Hispanic Whites with the rare Ile¹⁶⁴ allele (only rare variant in Whites, OR4.48, 95% CI 1.40–14.0, $p=0.01$) and African Americans with a 25 base-pair promoter polynucleotide insertion (OR 13.43, 95% CI 2.02–265.4, $p=0.006$). The subset of non-Hispanic Whites and African Americans receiving LABAs with these rare variants had increased exacerbations requiring urgent outpatient healthcare visits (non-Hispanic Whites with or without the rare Ile¹⁶⁴ allele: 2.6 visits versus 1.1 visits, $p=8.4 \times 10^{-7}$ and African Americans with or without the rare insertion: 3.7 visits versus 2.4 visits, $p=0.01$), and more frequently were treated with chronic systemic corticosteroids (OR4.2, 95% CI 1.4–14, $p=0.01$ and OR12.83, 95% CI 1.96–251.9, $p=0.006$). Non-Hispanic Whites from the primary and replication cohorts with the rare Ile¹⁶⁴ allele were more than twice as likely to experience uncontrolled, persistent symptoms during LABA treatment ($p=0.008$ – 0.04).

Interpretation—Rare *ADRB2* variants are associated with adverse events during LABA therapy and should be evaluated in large clinical trials including the current FDA-mandated LABA safety study.

Introduction

Common β_2 -adrenergic receptor gene (*ADRB2*) variation has been studied intensively including a nonsynonymous polymorphism at codon 16 encoding for either glycine or arginine, Gly¹⁶Arg, which has been shown to affect responses to regular use of short-acting beta agonists (SABA).^{1, 2} However, these effects of Gly¹⁶Arg genotypes have not been observed with long-acting beta agonists (LABA).^{3–5}

Adverse, life-threatening responses to regular SABA therapy have also been observed during asthma mortality epidemics in the 1960's in the United Kingdom and New Zealand related to high-dose regimens of isoproterenol and fenoterol, but not with salbutamol (albuterol).^{6–8} In two large observational trials, the Salmeterol Asthma Multi-Center Research Trial (SMART) and the Severent Nationwide Surveillance Study, the adverse outcomes of life-threatening asthma exacerbations or asthma-related death associated with

the use of LABA were rare.^{9, 10} Findings that severe adverse life-threatening events are associated with LABA use have resulted in a boxed warning from the Federal Food and Drug Administration (FDA) and a mandatory ongoing LABA safety study in 46,800 asthma patients.^{11, 12} Thus, identification using genetics or other predictive biomarkers of at-risk, susceptible subpopulations is critical in understanding LABA safety.

We performed a genetic study in 1,626 mild to severe asthmatics to test the hypothesis that rare *ADRB2* variants are associated with increased hospitalization for asthma exacerbations. This is the same primary endpoint that is being used in the FDA-mandated LABA safety study where it serves as a surrogate outcome for rare, asthma-related life-threatening exacerbations or death.^{12, 13} We hypothesize that rare variant effects are independent of common gene variation, including the Gly¹⁶Arg locus. Since previous sequencing of *ADRB2* has identified rare variants (allele frequency <5%) with allele frequencies that vary among different ethnic groups, it is important to study asthmatics from different ancestral backgrounds.^{14, 15}

Methods

Study Populations (Primary Cohort N=1,626 and Replication Cohort N=659)

The majority of subjects were from the National Heart Lung and Blood Institute (NHLBI) Severe Asthma Research Program (SARP) and the NHLBI Collaborative Study on the Genetics of Asthma (CSGA) where a diagnosis of asthma was based on the presence of either methacholinebronchial hyper responsiveness or bronchodilator reversibility, less than 5 pack-years of tobacco exposure, and asthma symptoms.^{16, 17} Baseline data were analyzed from a clinical trial of 176 White asthma subjects with uncontrolled, persistent, severe asthma while receiving high-dose inhaled corticosteroids and LABA therapy (Severe Asthma Clinical Trial Cohort, [ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT00207740) identifier: NCT00207740).¹⁸ 73 Puerto Rican subjects with a physician's diagnosis of asthma were recruited during a documented asthma exacerbation at the emergency department of Hospital Episcopal San Lucas in Ponce, Puerto Rico. The "primary cohort" was characterized with pulmonary function and questionnaires that recorded medication use, indices of healthcare utilization including hospitalizations over the preceding 12 months, and symptom control. For replication, three-month questionnaire data collected over 24 months from 659 non-Hispanic White asthma subjects characterized as "difficult-to-treat" by their primary care physicians from The Epidemiology and Natural History of Asthma: Outcomes and Treatment Regimes (TENOR) was analyzed for asthma symptoms.¹⁹ These studies were approved by the Institutional Review Boards at all sites and informed consent was obtained from all subjects.

In all study cohorts, questionnaires were standardized across all study sites and administered by centrally-trained clinical staff.²⁰ Questions regarding healthcare utilization specifically queried whether these events occurred because of an "asthma attack" or "breathing problems." Symptom control was recorded for the past three months in the primary and replication cohorts based on the National Asthma Education and Prevention Program (NAEPP) Guidelines.²¹

DNA Sequencing and Genotyping of *ADRB2* Rare Variants

A 5,350 base-pair (bp) region of *ADRB2* (–3470 bp 5' of the ATG start site to +1886 bp after the ATG start site) including the 413-amino acid (1,239 bp) intronless coding region was sequenced in 197 African American and 191 non-Hispanic White from CSGA and SARP and 73 Puerto Rican asthma cases using previously described methods.^{14, 15} Rare genetic variants were defined by an allele frequency of less than 0.05. Five rare nonsynonymous single nucleotide polymorphisms (SNP's) identified after sequencing and predicted to have functional effects were genotyped using the Mass ARRAY genotyping system (Sequenom, Inc) in the remaining asthma subjects consisting of 213 African American and 952 non-Hispanic Whites. A 25 bp insertion-deletion within the promoter region was genotyped in 316 African American asthmatics with fragment analysis using an ABI3700 DNA Analyzer. Genotypes for eight common SNP's were obtained, including Gly¹⁶Arg [rs1042713]. 772 non-asthmatic controls from CSGA and SARP were also sequenced or genotyped for rare variants (appendix). Thr¹⁶⁴Ile was genotyped in 659 non-Hispanic Whites from TENOR for replication. The reference genomic sequence for *ADRB2* in humans was compared to non-human primates using the University of California, Santa Cruz (UCSC) Genome Browser to determine the ancestral alleles of common *ADRB2* variants.

Statistical Methods

Rare variants identified with sequencing that had a predicted impact on protein structure and function (based on TFsearch, FastSNP, SIFT, and Polymorphism Phenotyping v2 [PolyPhen 2]) scores were analyzed using the Sequence Kernel Association Test (SKAT) for the primary outcome of hospitalization for a severe asthma exacerbation to identify gene-level rare variant associations.^{22–26} All analyses were adjusted for study cohort, sex, and age. Since the primary outcome was significant (figure 1), further analyses were performed with a stratified, hierarchical analysis (figure 1) to test for individual rare variant effects on the primary outcome of hospitalization and other, secondary outcomes related to healthcare utilization and asthma severity including urgent outpatient healthcare or emergency department [ED] visits over the past year, level of corticosteroid use from low to high-dose inhaled corticosteroids (ICS) or chronic systemic (oral and injectable) corticosteroids, and symptom control based on the NAEPP guidelines.^{21, 27} Urgent outpatient healthcare or emergency department [ED] visits was analyzed as a continuous variable using a generalized linear model with a Poisson distribution and a log link function. Remaining outcomes were analyzed with logistic regression. This hypothesis-driven stratified, hierarchical analysis (figure 1) tested for gene-by-environment interactions with LABA exposure.²⁸

Tests performed in each ethnic group were combined using meta-analysis to account for the genetic heterogeneity and population substructure in subjects from different ancestral backgrounds.²⁹ For SKAT, p-values from each ethnic group were combined using Stouffer's Z score method.³⁰ In order to account for rare variant associations related to ancestry, a subset of 364 African Americans were analyzed for estimates of global African ancestry using previous genome-wide genotyping chip data, and genotypes from 225 HapMap founders with the ADMIXTURE program.³¹

The outcome of symptom control based on NAEPP guidelines was collected for the past 3 months and analyzed in both the primary and replication cohorts to validate the rare variant effects observed in the primary cohort.²¹ Haplotypes, linkage disequilibrium, and haplotype associations with the primary endpoint using logistic regression were calculated with standard methods (PLINK v1.07).³² Haplotypes were designated by a numerical nomenclature initially described by Drysdale and coworkers and confirmed in a larger population.^{14, 15}

Role of the Funding Source

The sponsors of the study had no role in study design; data collection, data analysis, and data interpretation; or writing of the report. Eugene R. Bleecker, MD; Deborah A. Meyers, PhD; and Victor E. Ortega, MD had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Asthma Cohorts and the Identification of Rare ADRB2 Variants

African Americans had a greater frequency of hospitalizations ($p < 0.0001$, table 1: baseline characteristics) and urgent health care including emergency room visits ($p < 0.0001$) over the preceding year for asthma but were less likely to be treated with a LABA compared to non-Hispanic Whites (OR=0.73, 95% CI=0.56–0.96, $p=0.02$, table 1). Non-Hispanic White asthma subjects selected for sequencing experienced a higher frequency of urgent physician visits in the past year compared to those who were genotyped ($p=7.49 \times 10^{-5}$, table 2, appendix). African American asthma subjects selected for sequencing had significantly lower baseline lung function and a greater degree of airflow obstruction ($p=4.51 \times 10^{-8}$ and $p=1.94 \times 10^{-6}$ for FEV1 percentage of predicted and FEV1/FVC ratio, respectively, table 2, appendix). The baseline characteristics of the replication cohort are summarized in table 3 of the appendix.

Six rare *ADRB2* polymorphisms with predicted functional effects were identified: a 25 bp insertion-deletion at nucleotide –376 relative to the ATG start site (–376 In-Del), Pro⁴His (rs148459047), Asn¹⁵Ser (rs33973603), Thr¹⁶⁴Ile (rs1800888), Ser²²⁰Cys (rs3729943), and Leu³⁴²Pro (table 2). All rare SNP's had FastSNP risk scores ranging from two (low predicted effect on protein function) to four (high predicted effect, table 4, appendix) and four SNP's were predicted to be damaging by a SIFT score < 0.05 .²⁴ The –376 In-Del rare variant is a 25bp polynucleotide insertion located 5' upstream of the coding region within a Sp-1 transcription factor binding site (TFsearch score of 87.7).^{23, 26} Thr¹⁶⁴Ile and Ser²²⁰Cys were identified in controls as well as, two novel rare variants, Phe²⁴⁰Leu and Gly³⁸³Arg, each in one subject (table 5 and figure 1, appendix). Rare variants were not associated with asthma susceptibility (table 5, appendix).

African American asthma subjects had a significantly higher frequency of rare variants when compared to non-Hispanic Whites ($p < 0.0001$) and Puerto Ricans ($p=0.04$, table 1). A private rare variant, Pro⁴His, was identified in one African American subject and a novel rare variant, Leu³⁴²Pro, was identified in a Puerto Rican (both confirmed with repeat

sequencing). The rare allele of Ser²²⁰Cys was identified in 32 African Americans (including two Cys²²⁰ homozygotes) with the rare allele of Asn¹⁵Ser was identified only in two African Americans. The -376 In-Del rare variant was found in 15 African Americans and two Puerto Ricans. The rare allele of Thr¹⁶⁴Ile variant was the only rare variant identified in non-Hispanic Whites, but was also identified in five African Americans and one Puerto Rican (table 2). With one exception, all rare variants occurred on a haplotype background containing the ancestral Gly¹⁶ allele (table 6, appendix).

Effects of Rare ADRB2 Variants and Long-acting Beta Agonist Exposure on Severe Asthma Exacerbations Requiring Hospitalization

A meta-analysis of the combined multi-ethnic asthma populations of non-Hispanic Whites, African Americans, and Puerto Ricans with rare *ADRB2* variants (n=75) showed an increased likelihood of a severe asthma exacerbation requiring hospitalization in the past 12 months when compared to those without rare variants (n=1134) (p=0.002, table 3). In the subset treated with a LABA, 15 (44 percent) of 34 subjects with rare variant had an asthma-related hospitalization during the past year compared to 121 (22 percent) of 553 without a rare variant (p=2.83 × 10⁻⁴, table 3). This increased frequency of hospitalization was primarily observed among LABA-treated non-Hispanic Whites (p=0.005, table 3) and LABA-treated African Americans (p=0.02, table 3). These associations were due to the Ile¹⁶⁴ rare variant in LABA-treated non-Hispanic Whites (OR=4.48, 95% CI=1.40–14.0, p=0.01, figure 2A) and the rare -376 promoter insertion variant (OR=13.43, 95% CI=2.02–265.4, p=0.006, figure 2B) in LABA-treated African Americans. Ser²²⁰Cys, the remaining frequent rare variant in the asthma cohort, was not associated with hospitalization in LABA-treated African Americans (OR=0.87, 95% CI=0.22–2.98, p=0.83). In subjects not on LABA, rare *ADRB2* variants were not associated with hospitalization (table 3). Analysis of haplotypes with common coding variants (Gly¹⁶Arg, Gln²⁷Glu) identified only an association of a single *ADRB2* haplotype containing the rare Ile¹⁶⁴ variant with hospitalization in LABA-treated non-Hispanic Whites (p=0.02, table 7, appendix).^{14, 15} This Ile¹⁶⁴-containing *ADRB2* haplotype number seven (based on Drysdale and coworkers) contains the Gly¹⁶ allele of Gly¹⁶Arg (table 6, appendix).¹⁴

Non-Hispanic Whites on LABAs with the rare Ile¹⁶⁴ variant (n=18 Thr¹⁶⁴Ile heterozygotes) had a greater number of urgent outpatient healthcare or ED visits for asthma exacerbations during the past year compared to homozygotes for the common allele (n=553 Thr¹⁶⁴ homozygotes) (2.6 visits versus 1.1 visits, p=8.4 × 10⁻⁷). A similar association with increased urgent outpatient healthcare or ED visits was observed for African Americans with the rare -376 insertion (n=6) compared to homozygotes for the common allele (n=181) (3.7 visits versus 2.4 visits, p=0.01). Of these six LABA-treated African Americans with the rare insertion, five (83 percent) required two or more urgent outpatient healthcare or ED visits in the past year. In subjects not on LABA, the rare Ile¹⁶⁴ variant was associated with reduced urgent visits in non-Hispanic Whites (nine Thr¹⁶⁴Ile subjects versus 216 Thr¹⁶⁴ homozygotes: 0.1 visits versus 0.5 visits, p=0.01) and not associated in African Americans (five subjects with -376 insertion versus 119 without insertion: 1.2 visits versus 0.7 visits, p=0.18).

Detailed data on the use of inhaled and oral, systemic corticosteroid treatment were not available in some cohorts; however, non-Hispanic Whites on LABA from SARP with the rare Ile¹⁶⁴ variant required higher levels of corticosteroid treatment when compared to those with the common allele ($p=0.003$, figure 2, appendix). For example, eight (62 percent) of 13 non-Hispanic Whites on LABA therapy with the rare Ile¹⁶⁴ variant were treated with chronic systemic corticosteroids compared to 104 (27 percent) of 385 Thr¹⁶⁴ homozygotes ($OR=4.2$, 95% $CI=1.4-14$, $p=0.01$). In addition, five (83 percent) of six African Americans on LABA therapy with the -376 promoter insertion were treated with chronic systemic corticosteroids compared to 52 (29 percent) of 180 homozygotes for the common allele without the insertion ($OR=12.83$, 95% $CI=1.96-251.9$, $p=0.006$). In subjects not on LABA, rare *ADRB2* variants were not associated with systemic corticosteroid treatment (Thr¹⁶⁴Ile in non-Hispanic Whites $p=0.13$, -376 In-Del in African Americans $p=0.69$).

All rare *ADRB2* variants remained significantly associated with hospitalization in African Americans treated with LABA when the covariate ancestry was included ($p=0.01$). In addition, the rare -376 In-Del remained significantly associated with hospitalizations ($p=0.006$), urgent outpatient health care visits ($p=0.02$), and chronic systemic corticosteroid use ($p=0.007$) in African Americans treated with LABA when ancestry was used as a covariate.

Effects of Rare *ADRB2* Variants and Long-acting Beta Agonist Exposure on Symptom Control and Validation in the Replication Cohort

In non-Hispanic Whites treated with LABA from the primary cohort, seven (54 percent) of 13 non-Hispanic Whites on LABA therapy with the rare Ile¹⁶⁴ variant required treatment with a rescue inhaler for uncontrolled symptoms more than twice weekly compared to 100 (26 percent) of 379 Thr¹⁶⁴ homozygotes ($p=0.02$, figure 3, appendix). Significant associations at this locus were also identified for four additional measures of symptom control in non-Hispanic Whites treated with LABA including dyspnea ($p=0.02$, figure 4, appendix), chest tightness ($p=0.03$, figure 5, appendix), wheezing ($p=0.008$, figure 6, appendix), and nocturnal symptoms ($p=0.04$, figure 7, appendix). Significant associations were not identified for any measure of symptom control in non-Hispanic Whites not treated with a LABA (rescue inhaler use $p=0.97$, dyspnea $p=0.82$, chest tightness $p=0.33$, wheezing $p=0.26$, nocturnal symptoms $p=0.75$). In African Americans on LABA therapy, three (50 percent) of six asthmatics with the rare -376 insertion variant required treatment with a rescue inhaler for uncontrolled symptoms more than twice weekly compared to 55 (31 percent) of 179 homozygotes for the common allele, a suggestive trend that did not reach statistical significance ($p=0.55$).

In the replication cohort, symptom severity based on NAEPP guidelines was greater in LABA-treated non-Hispanic Whites ($N=13$) with the rare Ile¹⁶⁴ variant compared to Thr¹⁶⁴ homozygotes ($N=446$, $p=0.02$, figure 8, appendix). Five (38 percent) of 13 non-Hispanic Whites on LABA therapy with the rare Ile¹⁶⁴ variant experienced severe, persistent symptoms compared to 68 (15 percent) of 446 Thr¹⁶⁴ homozygotes at the 12-month follow-up visit (figure 8, appendix). This rare variant association was observed only in subjects treated with LABA at the 12-month and 24-month ($N=430$, 10 subjects with Ile¹⁶⁴) follow-

up visits ($p=0.02$ at each visit, figure 8, appendix). Significant associations were not identified in those not treated with a LABA at the 12-month and 24-month follow-up visits ($p=0.52$ and 0.63).

Discussion

This sequencing and genotyping analysis of *ADRB2* represents the largest collection of well-characterized, multi-ethnic asthma cases studied for rare *ADRB2* variants, many of whom have been treated with LABA. Asthma subjects analyzed with sequencing experienced a greater severity of disease compared to those who were genotyped (table 2, appendix) which provided the potential for identifying rare variants enriched in more severe asthma. The rare variants identified in these asthma cohorts represent all rare nonsynonymous polymorphisms with an allele frequency greater than 0.0006 from the NIH NHLBI GO exome sequencing project (table 1, appendix).³³

Six rare *ADRB2* variants were identified with varying frequencies between ethnic groups. These rare variants were exclusively found (with one exception) on the genetic background of the Gly¹⁶ allele at the Gly¹⁶Arg locus. Findings from sequence analysis in primates indicate that Gly¹⁶ is the sole or ancestral allele at the Gly¹⁶Arg locus. Therefore, altered beta agonist responsiveness previously observed during SABA therapy in Arg¹⁶ homozygotes is not likely to be due to a rare *ADRB2* variant.^{1, 2}

Rare *ADRB2* variants were associated with an increased likelihood of a severe asthma exacerbation requiring hospitalization, specifically among LABA-treated Whites with the Thr¹⁶⁴Ile rare variant and LABA-treated African Americans with the -376 In-Del rare variant. The impact of these rare variants on asthma control in those exposed to LABA is further supported by its association with increased urgent physician or ED visits, systemic corticosteroid use, and poor symptom control. We were able to replicate the genetic effects of the Thr¹⁶⁴Ile variant on symptom control during LABA treatment in an independent, non-Hispanic White asthma cohort. These findings suggest alterations in therapeutic response to LABA in these asthma genetic subpopulations resulting from rare *ADRB2* variants which have rendered asthmatic carriers less responsive to the beneficial bronchodilatory and bronchoprotective effects of LABA therapy. As expected, rare variants differ between different ethnic groups but resulted in similar effects on three different healthcare-related outcomes during LABA treatment, an example of gene-level replication.^{44, 45} The heterogeneity of rare functional variants at specific loci has previously been shown to collectively determine susceptibility for diseases such as breast cancer and idiopathic pulmonary hypertension.^{34, 35} Gene-level replication is critical to understanding rare variant effects in human disease because populations with different ancestries will each have unique rare variants that may impact human disease in a similar way.

Thr¹⁶⁴Ile is a rare *ADRB2* variant which results in an amino acid coding change within the fourth transmembrane domain of the β_2 -adrenergic receptor. *In vitro*, the rare Ile¹⁶⁴ variant has been shown to decrease agonist-promoted receptor sequestration and reduce receptor ligand binding affinity and coupling to G_s protein in response to different SABAs such as isoproterenol and salbutamol.^{36, 37} These *in vitro* effects have also been observed for

formoterol and salmeterol and also include impaired binding of salmeterol to its “exosite” in receptors expressing this rare variant.³⁷

Based on these *in vitro* observations, there are several possible mechanisms through which the rare Ile¹⁶⁴ variant might alter LABA efficacy: First, the deleterious effects of the Ile¹⁶⁴ variant on receptor ligand binding or coupling to G_s protein results in attenuation of the bronchodilator or bronchoprotective effects of LABA. A second mechanism is that increased airways inflammation occurs in response to LABA treatment among those individuals with the rare Ile¹⁶⁴ variant as a result of decreased receptor sequestration and a subsequent increase in Th₂-type inflammatory cytokine production from continued receptor stimulation.^{38–40} Third, the rare Ile¹⁶⁴ variant might be related to impaired cardiovascular reserve.^{41, 42} This rare variant has been associated with reduced β 2-adrenergic-mediated myocardial contractile response, cardiac inotropic reserve, and chronotropic reserve in controls and an increased relative risk of death or the need for cardiac transplantation among individuals with congestive heart failure from ischemic or dilated cardiomyopathy.^{41–43} Interestingly, the rare Ile¹⁶⁴ variant was associated with reduced baseline lung function and baseline airflow obstruction in a cross-sectional analysis of 62,748 subjects from the Copenhagen City Heart Study and the Copenhagen General Population Study which included a subset with self-reported asthma (N=1,300).⁴⁴

In contrast to non-Hispanic Whites with asthma who primarily have a single rare *ADRB2* variant, African Americans are an admixed ethnic group that had a significantly greater frequency of different rare variants. The relatively higher frequency of different rare variants among African Americans provides a testable hypothesis that could partially account for the differences in asthma morbidity and responses to LABA therapy observed between these ethnic groups.^{10, 45} In African American asthmatics, we identified a rare 25 bp polynucleotide insertion with similar effects on healthcare utilization among those treated with a LABA that was observed with Thr¹⁶⁴Ile in non-Hispanic Whites. The association of the –376 insertion variant with hospitalization and other, healthcare related outcomes in African Americans remained equally significant after adjusting for ancestry. Thus, the effects of rare variants on healthcare utilization in African Americans are not due to specific African or European ancestry. Although there are no *in vitro* studies of the –376 In-Del, this promoter polynucleotide insertion is located within a Sp-1 transcription factor binding site and predicted to impact gene expression.^{23, 46}

The ongoing concern for increased risk of asthma-related life-threatening events and death associated with LABA therapy is based on surveillance trials and meta-analyses in which LABA was not always used with concomitant ICS therapy.^{9, 10} In our multi-ethnic asthma populations, LABA and ICS therapy was the standard of care making it impossible to detect the risk for rare, life-threatening events among subjects treated with a LABA alone.^{47, 48} Asthmatics with severe disease characterized by frequent healthcare utilization were well-represented in this cohort. Recent hospitalization is usually an exclusion criterion for most clinical trials, making replication of these findings more difficult.^{11, 12}

While we only show gene-level replication for two different rare *ADRB2* variants on severe exacerbations requiring hospitalization and two related, secondary outcomes in our primary

cohort, we could not assess for strict replication of each rare variant for hospitalization in an independent, LABA-treated cohort. Further replication of the association between Thr¹⁶⁴Ile in non-Hispanic Whites or the -376 insertion variant in African Americans and this primary outcome remains to be performed in another large multi-ethnic asthma population. Despite this limitation, we replicated associations for a secondary outcome related to asthma severity, symptom control, at the Thr¹⁶⁴Ile locus during LABA treatment in the primary cohort and an independent cohort. Thus, Thr¹⁶⁴Ile genotype could be applied to standard guideline-based approaches for asthma management to determine the best therapeutic option for a small subset of Ile¹⁶⁴ heterozygotes with poor symptom control despite combination therapy with a LABA and ICS.^{47, 48} In coming years, pharmacogenetic data such as that described in this study will become readily available through genetic panels or complete genome sequencing used for personalized medicine.

The occurrence of life-threatening events in the SMART LABA surveillance study was very rare but these rare, serious findings with LABA therapy have caused the FDA to issue a boxed safety warning and initiate a large LABA surveillance study in 46,800 subjects.^{10, 11, 13} Identification of a susceptible asthma subpopulation who is at higher risk for LABA-related adverse events will not only elucidate major pharmacogenetic mechanisms, but could serve as an important predictive biomarker for these rare, serious side effects.

Panel: Research in Context

Systematic review

We searched PubMed for articles published in any language before September 27, 2013 using the terms “asthma,” “beta agonist,” and “*ADRB2*.” This search identified pharmacogenetic studies of the *ADRB2* locus which reported associations with common variants, particularly Gly¹⁶Arg, on inhaled beta agonist response. These studies consistently reported that Gly¹⁶Arg genotypes were associated with clinical responses to SABA during acute and chronic exposure in asthma subjects, but was not associated with clinical response to LABA treatment. None of these studies genotyped functional rare variants based on sequencing data from asthma subjects in different ethnic groups or tested for rare variant associations.^{1–5, 15} We performed another search of PubMed with the terms “asthma” and “*ADRB2*” to identify previous studies testing the association of rare *ADRB2* variants with asthma severity. This search identified a population-based study of 8,018 subjects from the British 1958 birth cohort in which Thr¹⁶⁴Ile was not associated with asthma, wheezing, or lung function measures; however, medication use and healthcare utilization outcomes were not reported.⁴⁹ Another population-based study of the Copenhagen City Heart Study and the Copenhagen General Population Study cohorts genotyped Thr¹⁶⁴Ile in 62,748 subjects including a subset of 1,300 subjects with self-reported asthma which was not reported individually for lung function measures, medication use, or healthcare utilization outcomes.⁴⁴ A final search of PubMed with the terms “asthma” and “beta2” identified a study of 251 asthma subjects of which 81 had severe, life-threatening asthma. Thr¹⁶⁴Ile was not associated with life-threatening asthma in this cohort; however, LABA use was not evaluated.⁵⁰

Interpretation

To the best of our knowledge, our study is the first to identify a pharmacogenetic interaction between rare genetic variants and asthma severity during LABA therapy. Based on the observed rare variant effects in asthma subjects treated with a LABA in this study, we estimate that 150 non-Hispanic Whites would need to be genotyped to identify three subjects with the rare Ile¹⁶⁴ allele and 100 African Americans genotyped to identify two subjects with the -376 rare insertion in order to prevent a single hospitalization during LABA treatment over a one-year period. Thus, the population attributable risk for hospitalization during LABA treatment due to these rare *ADRB2* variants was 0.07 for Thr¹⁶⁴Ile in non-Hispanic Whites and 0.09 for the -376 insertion variant in African Americans. These population attributable risks reflect a low allele frequency but were three times greater than the frequency of these rare variants in this LABA-treated asthma population. Thus, rare *ADRB2* variants are more likely to account for the rare, life-threatening events reported in the SMART LABA surveillance study than a common variant such as Gly¹⁶Arg. The identification of an asthma subpopulation at risk for rare, adverse events associated with LABA therapy will help to clarify current concerns over LABA safety. The rare Ile¹⁶⁴ variant was also associated with poor symptom control during LABA treatment in two, independent cohorts. Thus, this rare variant is a potential biomarker for more personalized and precise guideline-based management approaches in the small subset of asthmatics that are unresponsive to the combination therapy of a LABA with ICS.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Hierarchal Analysis of Rare *ADRB2* Variants and Hospitalizations

First, a meta-analysis was performed including all cohorts and rare variants with the Sequence Kernel Association Test. Asthma subjects were subsequently divided by long-acting beta agonist (LABA) treatment and then by ethnic group. Each ethnic group was then analyzed to determine which rare variants accounted for the significant association. Logistic regression models were used to analyze individual rare variant effects on hospitalization. For the associated rare variant, analysis of secondary healthcare-related outcomes (outpatient urgent and emergency department [ED] visits and systemic corticosteroid use) and symptom

control based on the National Asthma Education and Prevention Program (NAEPP) was performed in the primary cohort. Validation of rare variant effects was performed in a replication cohort from The Epidemiology and Natural History of Asthma: Outcomes and Treatment Regimes (TENOR) cohort.

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Figure 2. A and B: Two Rare *ADRB2* Variants and Hospitalization for a Severe Asthma Exacerbation with Long-Acting Beta Agonist (LABA) Treatment

Red bars indicate the percentage of asthmatics with the rare Ile¹⁶⁴ variant or the rare -376 polynucleotide insertion variant and blue bars indicate the percentage among those without these rare variants (common genotypes). Thr¹⁶⁴Ile was the only rare variant identified in non-Hispanic Whites (shown in 2A) and a 25bp Promoter Insertion Deletion (-376 In-Del) was identified in African Americans (shown in 2B) but not non-Hispanic Whites.

Table 1

Characteristics of the Asthma Cohorts.

Asthma Subject Characteristics	Multi-Ethnic Populations ^a					African Americans			Puerto Ricans
	Non-Hispanic Whites					SARP	CSGA	Combined	
	SARP	CSGA	Severe Asthma	Combined					
Subjects (N)	707	260	176	1143		315	95	410	73
Females (%)	63	62	54	62		59	62	60	82
Mean Age in Years (SD)	37 (15)	27 (14)	51 (12)	37 (16)		30 (15)	28 (13)	30 (14)	38 (19)
Pulmonary Function									
% Predicted Baseline FEV1	74 (23)	82 (18)	60 (12)	74 (21)		77 (20)	74 (21)	76 (21)	NA
FEV1/FVC Ratio	0.70 (0.12)	0.77 (0.12)	0.61 (0.10)	0.70 (0.13)		0.72 (0.12)	0.71 (0.13)	0.72 (0.12)	NA
FEV1 % Reversibility from Baseline	13 (15)	12 (16)	17 (16)	13 (15) ^c		14 (15)	19 (17)	15 (15) ^c	NA
Methacholine PC20	3.8 (6.0)	4.0 (6.2)	NA	3.9 (6.1)		2.5 (4.3)	4.6 (13)	3.0 (7.2)	NA
Asthma-Related Healthcare Utilization									
Urgent Visits Last 12 Months									
(mean visits per year)	1.1 (2.2)	0.8 (2.2)	0.6 (1.2)	0.9 (2.1) ^b		1.7 (2.9)	1.5 (2.2)	1.7 (2.8) ^b	5.1 (12)
Asthma-Related Hospitalization									
Last 12 Months (%)	12	5	NA	10 ^b		24	27	25 ^b	25
LABA Use (%) ^e	64	36	100	68 ^c		60	13	54 ^c	7
High-Dose ICS Use (%) ^e	40	NA	100	53		46	NA	46	NA
Regular Systemic Steroid Use (%)	20	0.66	NA	16		19	4	17	6
Cases with a Rare Variant (N)	26	7	5	38		36	19	55	4
Proportion with Rare Variants	0.04	0.03	0.03	0.03 ^b		0.11	0.2	0.13 ^b	0.05 ^d

^a NHLBI Severe Asthma Research Program=SARP, NHLBI Collaborative Study on the Genetics of Asthma=CSGA, and a Severe Asthma Clinical Trial Cohort=Severe Asthma.

^b p<0.0001 between African Americans and non-Hispanic Whites.

^c p=0.02 between African Americans and non-Hispanic Whites.

^d p=0.04 between African Americans and Puerto Ricans.

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LABA=Long-Acting Beta Agonist or ICS=Inhaled Corticosteroid.

NA=Data not available. Standard deviations for means in parentheses unless otherwise noted.

Table 2Rare Variants Identified within *ADRB2* in Asthma Cases by Ethnic Group.

Nucleotide Position ^a	AA ^{codon} AA ^b	Rare <i>ADRB2</i> Variants by Ethnic Group ^c					
		African Americans		Non-Hispanic Whites		Puerto Ricans	
		N	MAF	N	MAF	N	MAF
-376	Insertion-Deletion	15	0.02	0	0	2	0.01
11	Pro ⁴ His	1	0.001	0	0	0	0
44	Asn ¹⁵ Ser	2	0.002	0	0	0	0
491	Thr ¹⁶⁴ Ile	5	0.006	38	0.02	1	0.007
659	Ser ²²⁰ Cys	32	0.04	0	0	0	0
1025	Leu ³⁴² Pro	0	0	0	0	1	0.007
	All Rare Variants ^d	55	0.07	38	0.02	4	0.03

^aVariants described by nucleotide position relative to the start codon.^bAA^{codon} AA denotes the amino acid (AA) for each allele by amino acid position relative to the start codon. Pro=Proline, His=Histidine, Asn=Asparagine, Ser=Serine, Thr=Threonine, Ile=Isoleucine, Cys=Cysteine, Leu=Leucine, Pro=Proline.^cRare variant frequency is denoted in terms of total number of asthmatics from the study population with a rare variant (N) and minor allele frequencies (MAF).^d“All rare variants” illustrates the frequency of all rare variants combined by ethnic group.

Table 3Rare *ADRB2* Variant Associations with Asthma-Related Hospitalization.

	Rare Variant	Common Alleles	Rare Variant	Common Alleles	p-value
	N	N	N (%) Hospitalized Past 12 Months		SKAT ^b
<u>All Asthma Subjects</u> ^a	75	1134	22 (29)	166 (15)	0.002
Non-Hispanic Whites	27	750	7 (26)	74 (10)	0.02
African Americans	44	316	14 (32)	75 (24)	0.07
Puerto Ricans	4	68	1 (25)	17 (25)	0.4
<u>Treatment with LABA</u>	34	553	15 (44)	121 (22)	2.83×10^{-4}
Non-Hispanic Whites	13	384	6 (46)	61 (16)	0.005
African Americans	21	165	9 (43)	59 (36)	0.02
Puerto Ricans	0	4	NA ^c	25	NA
<u>No Treatment with LABA</u>	28	390	2 (7.1)	29 (7.4)	0.41
Non-Hispanic Whites	10	218	0 (0)	6 (2.75)	0.51
African Americans	15	109	1 (6.7)	7 (6.4)	0.98
Puerto Ricans	3	63	1 (33)	16 (25)	0.42

^a All asthma subjects are from a multi-ethnic population that includes African Americans, Non-Hispanic Whites and Puerto Ricans (PR).

^b Sequence Kernel Association Test (SKAT) adjusted for age, sex, and study cohort.

^c PR not reported individually for hospitalizations due to small numbers for comparisons.