13TLRM0438_Ortega
Articles
LB
Version 2
This version saved: 16:06, 21-Jan-14

THELANCETRM-D-13-00438
S2213-2600(13)70289-3
Embargo:January 27, 2014—00:01 [GMT]
Funding: NIH funded

Effect of rare variants in *ADRB2* on risk of severe exacerbations and symptom control during longacting β agonist treatment in a multiethnic asthma population: a genetic study

Victor E Ortega, Gregory A Hawkins, Wendy C Moore, Annette T Hastie, Elizabeth J Ampleford, William W Busse, Mario Castro, Domingo Chardon, Serpil C Erzurum, Elliot Israel, Federico Montealegre, Sally E Wenzel, Stephen P Peters, Deborah A Meyers, Eugene R Bleecker

Summary

Background Severe adverse life-threatening events associated with longacting β agonist (LABA) use have caused the US Food and Drug Administration (FDA) to review the safety of these drugs, resulting in a boxed warning and a mandatory safety study in 46 800 patients with asthma. Identification of an at-risk, susceptible subpopulation on the basis of predictive biomarkers is crucial for understanding LABA safety. The β_2-adrenergic receptor gene (*ADRB2*) contains a common, non-synonymous single nucleotide polymorphism, Gly16Arg, that is unlikely to account for the rare, life-threatening events seen with LABA use. We hypothesise that rare *ADRB2* variants modulate therapeutic responses to LABA therapy and contribute to rare, severe adverse events.

Methods In this genetic study, *ADRB2* was sequenced in 197 African American, 191 non-Hispanic white, and 73 Puerto Rican patients. Sequencing identified six rare variants, which were genotyped in 1165 patients with asthma. The primary hypothesis was that severe asthma exacerbations requiring hospital admission were associated with rare *ADRB2* variants in patients receiving LABA therapy. This outcome was assessed overall and by ethnic group [A: OK to add?]. Replication was done in 659 non-Hispanic white patients with asthma.

Findings Patients receiving LABA with a rare *ADRB2* variant had increased asthma-related hospital admissions (15 [44%] of 34 patients with rare variant *vs* 121 [22%] of 553 patients with common *ADRB2* alleles admitted to hospital in past 12 months; meta-analysis for all ethnic groups, p=0·0003 [A: the Editor prefers to present the p value in this way for consistency throughout the journal]). Specifically, increases in hospital admission rates were recorded in LABA-treated non-Hispanic white patients with the rare Ile 164 allele compared with non-Hispanic white patients with the common allele (odds ratio [OR] 4·48, 95% CI 1·40–13·96, p=0·01) and African American patients with a 25 bp promoter polynucleotide insertion, –del376, compared with African American patients with the common allele (OR 13·43, 95% CI 2·02–265·42, p=0·006). The subset of non-Hispanic white and African American patients receiving LABAs with these rare variants had increased exacerbations requiring urgent outpatient health-care visits (non-Hispanic white patients with or without the rare Ile 164 allele, 2·6 [SD 3·5] *vs* 1·1 [2·1] visits, p<0·0001 [A: as above for format of p value]; and African American patients with or without the rare insertion, 3·7 [4·6] *vs* 2·4 [3·4] visits, p=0·01), and more frequently were treated with chronic systemic corticosteroids (OR 4·25, 95% CI 1·38–14·41, p=0·01, and 12·83, 1·96–251·93, p=0·006). Non-Hispanic white patients from the primary and replication cohorts with the rare Ile 164 allele were more than twice as likely as Thr 164 homozygotes to have uncontrolled, persistent symptoms during LABA treatment (p=0·008–0·04).

Interpretation The rare *ADRB2* variants Ile164 and –del376 are associated with adverse events during LABA therapy and should be evaluated in large clinical trials including the current FDA-mandated safety study.

Funding US National Institutes of Health.

Introduction

Common β_2-adrenergic receptor gene (*ADRB2*) variation has been studied intensively including a non-synonymous polymorphism at codon 16 encoding either glycine or arginine, Gly16Arg, which has been shown to affect responses to regular use of shortacting β agonists (SABA).[1,2] However, the effects of Gly16Arg genotypes have not been observed with longacting β agonists (LABAs).[3–5]

Adverse, life-threatening responses to regular SABA therapy have also been observed during asthma mortality epidemics in the 1960s in the UK and New Zealand related to high-dose regimens of isoprenaline and fenoterol, but not with salbutamol.[6–8] In two large observational trials, the Serevent Nationwide Surveillance Study[9] and the SMART study,[10] the adverse outcomes of life-threatening asthma exacerbations or asthma-related death associated with the use of LABA were rare. Despite the rarity of these events [A: OK to add?], the findings that severe adverse life-threatening events are associated with LABA use have resulted in a boxed warning from the US Food and Drug Administration (FDA) and a mandatory ongoing LABA

Lancet Respir Med 2014

Published Online
January 27, 2013
http://dx.doi.org/10.1016/S2213-2600(13)70289-3

Center for Genomics and Personalized Medicine, Wake Forest School of Medicine, Winston-Salem, NC, USA (V E Ortega MD, G A Hawkins PhD, W C Moore MD, A T Hastie PhD, E J Ampleford PhD, Prof S P Peters MD, Prof D A Meyers PhD, Prof E R Bleecker MD); **Department of Medicine, University of Wisconsin, Madison, WI, USA** (Prof W W Busse MD); **Department of Medicine, Washington University School of Medicine, St Louis, MO, USA** (Prof M Castro MD); **Hospital Episcopal San Lucas, Ponce School of Medicine, Ponce, Puerto Rico** (D Chardon MD, F Montealegre PhD); **Department of Pathobiology and Respiratory Institute, Lerner Research Institute, Cleveland Clinic Foundation, Cleveland, OH, USA** (Prof S C Erzurum MD); **Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA** (Prof E Israel MD); **and Asthma Institute, University of Pittsburgh, Pittsburgh, PA, USA** (Prof S E Wenzel MD)

Correspondence to:
Prof Eugene R Bleecker, Center for Genomics and Personalized Medicine, Wake Forest School of Medicine, Winston-Salem, NC 27157, USA
ebleeck@wakehealth.edu

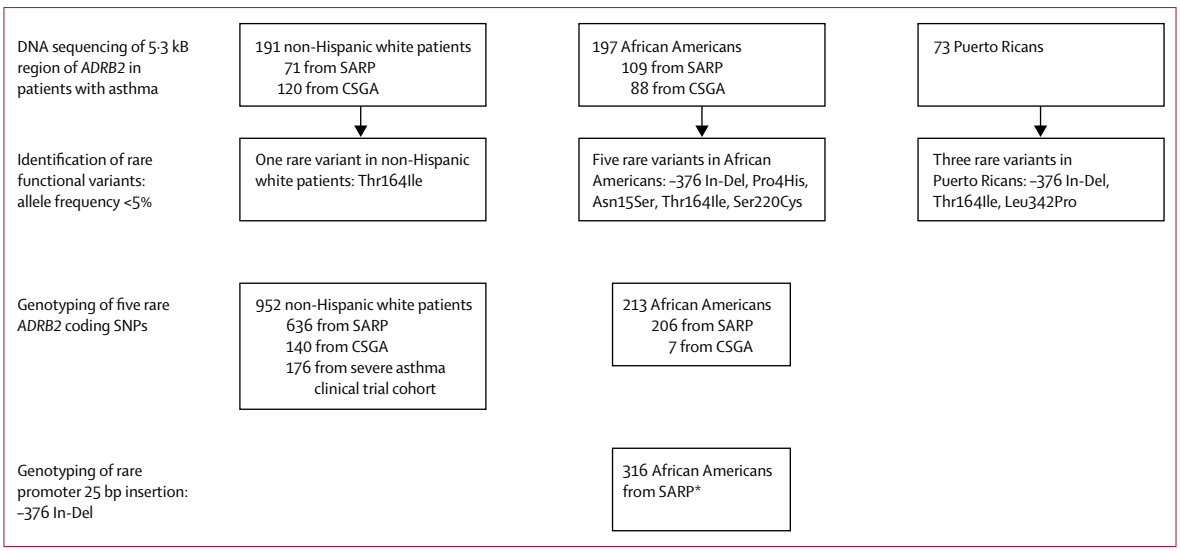

***Figure 1*: Sequencing and genotyping of rare *ADRB2* variants in different ethnic groups from the primary cohort**
Study participants from three ethnic groups were evaluated for rare *ADRB2* variants either with DNA sequencing or targeted genotyping. SARP=Severe Asthma Research Program.[17] CSGA=Collaborative Study on the Genetics of Asthma.[16] SNP=single nucleotide polymorphism. *103 of 316 African American participants genotyped for the –376 In-Del were also evaluated with sequencing of *ADRB2*.

safety study in 46 800 patients with asthma.[11,12] Thus, identification using genetics or other predictive biomarkers of at-risk, susceptible subpopulations is crucial in understanding LABA safety.

We did a genetic study in patients with mild to severe asthma to test the hypothesis that rare *ADRB2* variants are associated with increased hospital admission for asthma exacerbations in patients receiving treatment with LABAs. This primary endpoint is the same as that being used in the FDA-mandated LABA safety study, in which it serves as a surrogate outcome for rare, asthma-related life-threatening exacerbations or death.[12,13] We hypothesise 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, study of patients from different ancestral backgrounds is important.[14,15]

Methods

Study populations

For this genetic study the primary cohort included 355 participants from the National Heart Lung and Blood Institute (NHLBI) Collaborative Study on the Genetics of Asthma (CSGA)[16] and 1022 participants from the NHLBI Severe Asthma Research Program (SARP)[17] in which diagnosis of asthma was based on the presence of either methacholine bronchial hyper-responsiveness or bronchodilator reversibility, less than 5 pack-years of tobacco exposure, and asthma symptoms. Baseline data were also analysed from a clinical trial[18] of 176 white patients with uncontrolled, persistent, severe asthma while receiving high-dose inhaled corticosteroids and LABA therapy. Additionally, 73 Puerto Rican participants with a physician's diagnosis of asthma were recruited during a documented asthma exacerbation at the emergency department of Hospital Episcopal San Lucas (Ponce, Puerto Rico). This primary cohort of 1626 patients was characterised with pulmonary function tests and questionnaires that recorded treatment use, indices of health-care use including hospital admissions during the preceding 12 months, and symptom control.

For replication, in a separate cohort, 3-month questionnaire data were collected from study enrolment up to 24 months of longitudinal evaluation in 659 non-Hispanic white patients with asthma characterised as difficult to treat by their primary care physicians from the TENOR study.[19]

In all study cohorts, questionnaires were standardised across all study sites and administered by centrally trained clinical staff.[20] Questions regarding health-care use specifically queried whether these events occurred because of an asthma attack or breathing problems. Symptom control was recorded for the previous 3 months in the primary and replication cohorts based on National Asthma Education and Prevention Program (NAEPP) guidelines.[21]

These studies were approved by the institutional review boards at all sites and informed consent was obtained from all participants.

Procedures

Figure 1 summarises sequencing and genotyping undertaken in the primary cohort. A 5350 bp region [A: please confirm number (–3470 to 1886 = 5356?)] of *ADRB2* (–3470 bp 5′ of the ATG start site to +1886 bp

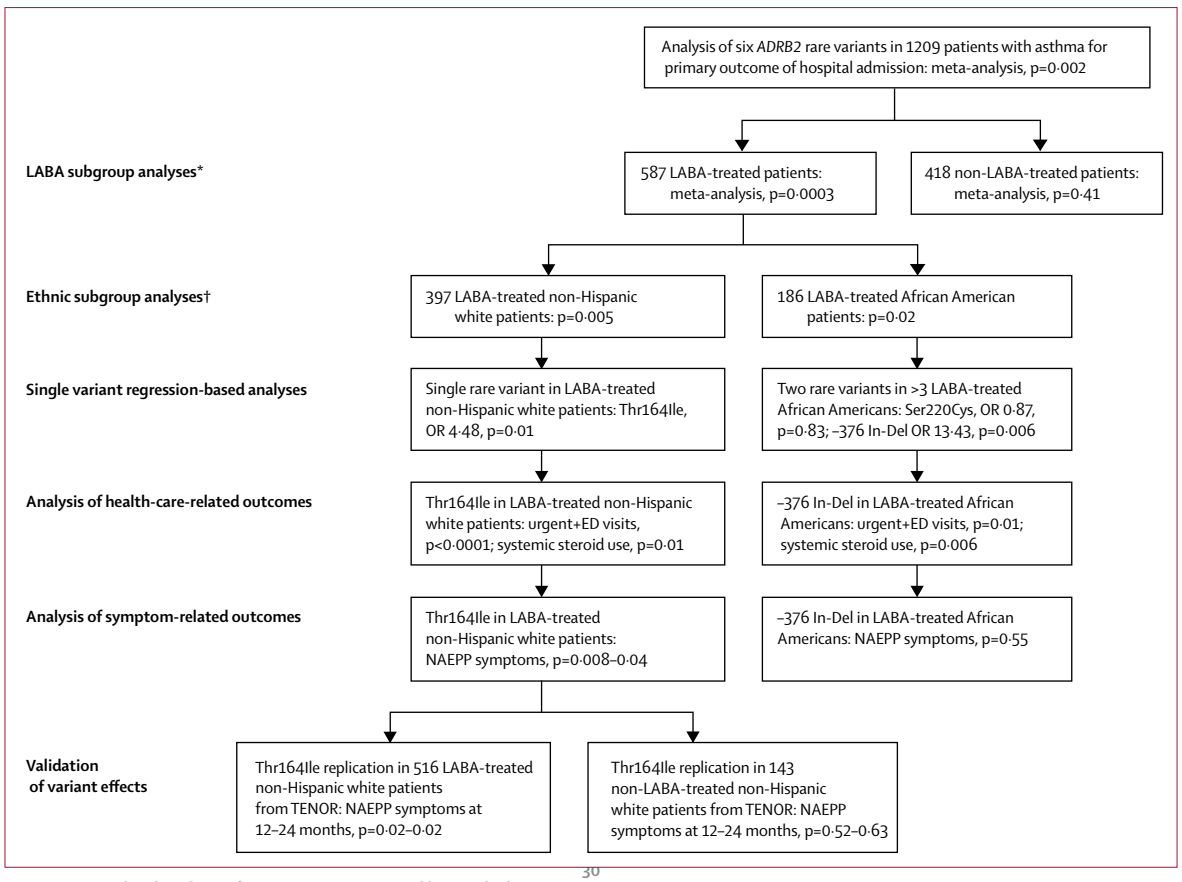

***Figure 2*: Hierarchical analysis of rare *ADRB2* variants and hospital admissions**

A hierarchical analysis of rare *ADRB2* variants and risk of hospital admission. The first step analysed each ethnic group for rare variant effects on risk of hospital admission to obtain a gene-level p value for all rare variants in the multiethnic population (p=0·002); this analysis included only patients for whom data were available for hospital admission (1209 of 1626 in the primary cohort). [A: legend shortened to reduce repetition of data from figure and main text] The first subgroup analysis was stratified by LABA treatment, followed by analyses by ethnic group. Regression-based analysis of single rare variants in each ethnic group further identified variants accounting for increased risk of hospital admission. Subsequent, regression-based analyses assessed effects on additional health-care-related and symptom-related outcomes. The adverse effect of the Thr164Ile variant on symptom control was validated in LABA-treated non-Hispanic white patients from an independent cohort. LABA=longacting β agonist. OR=odds ratio. ED=emergency department. NAEPP=National Asthma Education and Prevention Program. TENOR=The Epidemiology and Natural History of Asthma: Outcomes and Treatment Regimes cohort.[19] *A subgroup of patients from the Collaborative Study on the Genetics of Asthma[16] were recruited before or soon after LABAs were approved for use in the USA for management of asthma; therefore, data for LABA treatment were not obtained in these patients. †Four LABA-treated Puerto Rican patients were not analysed owing to the small number.

after the ATG start site) including the 413-aminoacid (1239 bp) intronless coding region was sequenced in 191 non-Hispanic white and 197 African American patients 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 non-synonymous single nucleotide polymorphisms (SNPs) of the *ADRB2* gene, identified after sequencing and predicted to have functional effects, were genotyped with the MassARRAY genotyping system (Sequenom Inc, San Diego, CA, USA) in the remaining participants from the primary cohort (952 non-Hispanic white and 213 African American patients). We genotyped a 25 bp insertion-deletion within the promoter region in 316 African American patients from SARP with fragment analysis using an ABI3700 DNA Analyzer (Applied Biosystems, Foster City, CA, USA). Genotypes for eight common SNPs were obtained, including Gly16Arg (rs1042713).

772 control participants without asthma from CSGA and SARP were also sequenced or genotyped for rare variants (appendix). Thr164Ile was genotyped in 659 non-Hispanic white patients from the TENOR study for replication. The reference genomic sequence for *ADRB2* in human beings was compared with non-human primates using the University of California, Santa Cruz (CA, USA) genome browser to determine the ancestral alleles of common *ADRB2* variants.

See Online for appendix

Statistical analysis

Rare variants identified with sequencing that had a predicted effect on protein structure and function (based on TFsearch, FastSNP, SIFT, and Polymorphism Phenotyping v2 [Poly-Phen 2]) scores were analysed with

	Non-Hispanic white patients				African American patients			Puerto Rican patients (n=73)
	SARP (n=707)	CSGA (n=260)	Severe asthma (n=176)	Combined (n=1143)	SARP (n=315)	CSGA (n=95)	Combined (n=410)	
Female (%)	445 (63%)	162 (62%)	95 (54%)	702 (61%)	185 (59%)	59 (62%)	244 (60%)	60 (82%)
Age (years)	37 (15)	27 (14)	51 (12)	37 (16)	30 (15)	28 (13)	30 (14)	38 (19)
Pulmonary function								
% predicted baseline FEV_1	74 (23)	82 (18)	60 (12)	74 (21)	77 (20)	74 (21)	76 (21)	NA
FEV_1/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
FEV_1 % reversibility from baseline	13 (15)	12 (16)	17 (16)	13 (15)*	14 (15)	19 (17)	15 (15)*	NA
Methacholine PC_{20}	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
Cases with a rare variant	26 (4%)	7 (3%)	5 (3%)	38 (3%)†	36 (11%)	19 (20%)	55 (13%)†‡	4 (5%)‡

Data are n (%) or mean (SD). SARP=National Heart Lung and Blood Institute Severe Asthma Research Program.[17] CSGA=National Heart Lung and Blood Institute Collaborative Study on the Genetics of Asthma.[16] Severe asthma=Severe Asthma Clinical Trial Cohort.[18] FEV_1=forced expiratory volume in 1 s. NA=not available. FVC=forced vital capacity. PC_{20}=provocation concentration of inhaled methacholine needed to reduce FEV_1 by 20%. *p=0·02 between African American and non-Hispanic white patients. †p<0·0001 between African American and non-Hispanic white patients. ‡p=0·04 between African American and Puerto Rican patients.

***Table 1*: Characteristics of the asthma cohorts**

	Nucleotide position	Non-Hispanic white patients		African American patients		Puerto Rican patients	
		n	MAF	n	MAF	n	MAF
Insertion-deletion	–376	0	0	15	0·02	2	0·01
Pro4His	11	0	0	1	0·001	0	0
Asn15Ser	44	0	0	2	0·002	0	0
Thr164Ile	491	38	0·02	5	0·006	1	0·007
Ser220Cys	659	0	0	32	0·04	0	0
Leu342Pro	1025	0	0	0	0	1	0·007
All rare variants	..	38	0·02	55	0·07	4	0·03

Rare variant frequency is denoted in terms of total number of patients with asthma from the study population with a rare variant and minor allele frequencies (MAF).

***Table 2*: Rare variants identified within *ADRB2* in asthma cases by ethnic group**

the sequence kernel association test (SKAT) for the primary outcome of hospital admission 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, further analyses were done with a stratified, hierarchical analysis (figure 2) to test for individual rare variant effects on the primary outcome of hospital admission and other secondary outcomes related to health-care use and asthma severity including urgent outpatient health care or emergency department visits during the past year, level of corticosteroid use from low-dose to high-dose inhaled corticosteroids or chronic systemic (oral and injectable) corticosteroids, and symptom control based on the NAEPP guidelines.[21,27] We analysed urgent outpatient health care or emergency department visits as a continuous variable using a generalised linear model with a Poisson distribution and a log link function. Remaining outcomes were analysed with logistic regression. This hypothesis-driven stratified, hierarchical analysis (figure 2) tested for gene-by-environment interactions with LABA exposure.[28]

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

Data for the outcome of symptom control based on NAEPP guidelines were collected for the past 3 months and analysed 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[14] and confirmed in a larger population.[15]

Role of the funding source

The sponsors of the study had no role in study design, data collection, analysis, and interpretation, or writing of the report. ERB, DAM, and VEO had full access to all data in the study and had final responsibility for the decision to submit for publication.

Results

Table 1 shows baseline characteristics of the asthma cohorts. Table 2 shows rare variants identified within *ADRB2* in asthma cases in each ethnic group. 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. All

	Non-Hispanic white patients				African American patients			Puerto Rican patients (n=73)
	SARP (n=707)	CSGA (n=260)	Severe asthma (n=176)	Combined (n=1143)	SARP (n=315)	CSGA (n=95)	Combined (n=410)	
Patients with available data	627	151	176	954	314	49	363	73
Asthma-related hospital admission in past 12 months (%)	73 (12%)*	8 (5%)	NA†	81 (10%)‡	76 (24%)*	13 (27%)	89 (25%)‡	18 (25%)*
Urgent visits in past 12 months (mean visits per year)	1·1 (2·2)	0·8 (2·2)	0·6 (1·2)	0·9 (2·1)‡	1·7 (2·9)	1·5 (2·2)	1·7 (2·8)‡	5·1 (12·4)
LABA use (%)	398 (64%)	37 (36%)§	176 (100%)	611 (68%)¶	189 (60%)	6 (13%)§	195 (54%)¶	5 (7%)
High-dose ICS use (%)	246 (40%)	NA	176 (100%)	422 (53%)	143 (46%)	NA	143 (46%)	NA
Regular systemic steroid use (%)	127 (20%)	1 (1%)	NA	128 (16%)	60 (19%)	2 (4%)	62 (17%)	4 (6%)

Data are n (%) or mean (SD). Data are missing for a subset of patients: in SARP, medication use and health-care use data were not obtained for 81 patients; CSGA participants were recruited in different phases during which time the questionnaire data were changed, thus data were not available for 155 patients. SARP=National Heart Lung and Blood Institute Severe Asthma Research Program.[17] CSGA=National Heart Lung and Blood Institute Collaborative Study on the Genetics of Asthma.[16] Severe asthma=Severe Asthma Clinical Trial Cohort.[18] NA=not available. LABA=longacting β agonist. ICS=inhaled corticosteroid. *Data for systemic steroid use and urgent outpatient visits were evaluated in one non-Hispanic white patient and three African Americans from SARP and one Puerto Rican patient, but hospital admission data were not available for these five participants. †In the severe asthma cohort, urgent outpatient visits were assessed whereas hospital admission was not. ‡p<0·0001 between African American and non-Hispanic white patients. §The first LABA, salmeterol, was approved for management of asthma after recruitment for CSGA was started; therefore, only a subgroup of 102 non-Hispanic white and 46 African American patients were evaluated for LABA treatment. ¶p=0·02 between African American and non-Hispanic white patients.

***Table* 3: Asthma-related health-care use**

rare SNPs had FastSNP risk scores ranging from two (low predicted effect on protein function) to four (high predicted effect; appendix) and four SNPs were predicted to be damaging by a SIFT score less than 0·05.[24] The –376 In-Del rare variant is a 25 bp polynucleotide insertion located 5′ upstream of the coding region within a SP1 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 patient (appendix). Rare variants were not associated with asthma susceptibility (appendix). African American patients with asthma had a significantly higher frequency of rare variants compared with non-Hispanic white (p<0·0001) and Puerto Rican patients (p=0·04; table 1). With one exception, all rare variants occurred on a haplotype background containing the ancestral Gly 16 allele (appendix).

Table 3 shows asthma-related health-care use. African American participants had a greater frequency of hospital admissions (p<0·0001, table 3) and urgent health care including emergency room visits for asthma exacerbation (p<0·0001) during the preceding year, but were less likely to be treated with a LABA compared with non-Hispanic white participants (odds ratio [OR] 0·73, 95% CI 0·56–0·96, p=0·02; table 3). Non-Hispanic white patients with asthma selected for sequencing had a higher frequency of urgent physician visits in the past year compared with those who were genotyped (p<0·0001; appendix). African American patients selected for sequencing had significantly lower baseline lung function and a greater degree of airflow obstruction compared with those who were genotyped (p<0·0001 for forced expiratory volume in 1 s [FEV_1] percentage of predicted and p<0·0001 for FEV_1/forced vital capacity ratio; appendix).

In the search for rare variants and rare variant frequencies it was important to evaluate as large a population as possible. Patients with asthma from CSGA and SARP were recruited either at different times or from different study sites; therefore, LABA use and health-care use were not evaluated in a subset of these patients (n=236). Table 3 summarises asthma-related medication and health-care use in these cohorts. Data for hospital admission were not obtained from the 176 participants from the severe asthma trial cohort, a further four participants from SARP, and one Puerto Rican patient; thus, of the primary cohort (n=1626), data for 1209 patients were available for analysis of hospital admission. A meta-analysis of the combined multiethnic asthma populations of non-Hispanic white, African American, and Puerto Rican patients with rare *ADRB2* variants (n=75) showed an increased likelihood of a severe asthma exacerbation requiring hospital admission in the past 12 months compared with those without rare variants (n=1134; p=0·002; table 4). In the subset treated with a LABA, 15 (44%) of 34 patients with rare variants had an asthma-related hospital admission during the past year compared with 121 (22%) of 553 without a rare variant (p=0·0003; table 4). This increased frequency of hospital admission was primarily observed in LABA-treated non-Hispanic white patients (p=0·005; table 4) and LABA-treated African Americans (p=0·02; table 4). These associations were due to the Ile 164 rare variant in LABA-treated non-Hispanic white patients (OR 4·48, 95% CI 1·40–13·96, p=0·01; figure 3A) and the rare –376 promoter insertion variant (OR 13·43, 2·02–265·42, p=0·006; figure 3B) in LABA-treated African Americans. Ser220Cys, the remaining frequent rare variant in the asthma cohort, was not associated with hospital admission in LABA-treated African Americans (OR 0·87, 95% CI 0·22–2·98, p=0·83). In patients not on LABAs, rare *ADRB2* variants were not associated with hospital admission (table 4).

Analysis of haplotypes with common coding variants (Gly16Arg, Gln27Glu) identified only an association of one

	n		Patients admitted to hospital in past 12 months		
	Rare variant	Common alleles	Rare variant, n (%)	Common alleles, n (%)	SKAT p value
All patients with asthma	75	1134	22 (29%)	166 (15%)	0·002
Non-Hispanic white	27	750	7 (26%)	74 (10%)	0·02
African American	44	316	14 (32%)	75 (24%)	0·07
Puerto Rican	4	68	1 (25%)	17 (25%)	0·4
Treatment with LABA*	34	553	15 (44%)	121 (22%)	0·0003
Non-Hispanic white	13	384	6 (46%)	61 (16%)	0·005
African American	21	165	9 (43%)	59 (36%)	0·02
Puerto Rican	0	4	NA†	1 (25%)	NA
No treatment with LABA*	28	390	2 (7%)	29 (7%)	0·41
Non-Hispanic white	10	218	0	6 (3%)	0·51
African American	15	109	1 (7%)	7 (6%)	0·98
Puerto Rican	3	63	1 (33%)	16 (25%)	0·42

SKAT=Sequence Kernel Association Test adjusted for age, sex, and study cohort. LABA=longacting β agonist. NA=not available. *Data for LABA use were not available for a subgroup of patients from the Collaborative Study on the Genetics of Asthma[16] recruited before or soon after LABAs were approved for use in the USA for management of asthma. †Not reported individually for hospital admissions owing to small numbers for comparisons.

***Table 4*: Rare *ADRB2* variant associations with asthma-related hospital admission**

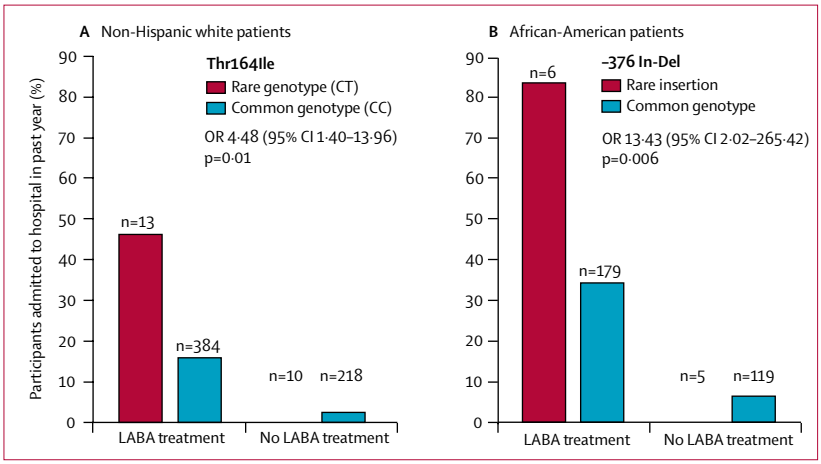

***Figure 3*: Two rare *ADRB2* variants and hospital admission for a severe asthma exacerbation with longacting β agonist (LABA) treatment**

Red bars show the percentage of patients with Thr164Ile variant or the –376 In-Del variant and blue bars show the percentage in those without these rare variants (common genotypes). Thr164Ile was the only rare variant identified in non-Hispanic white patients and a 25 bp promoter insertion deletion (–376 In-Del) was identified in African American but not non-Hispanic white patients.

ADRB2 haplotype containing the rare Ile 164 variant with hospital admission in LABA-treated non-Hispanic white patients (p=0·02; appendix).[14,15] This Ile 164-containing *ADRB2* haplotype number seven (based on Drysdale and coworkers) contains the Gly 16 allele of Gly16Arg (appendix).[14] On the basis of the observed rare variant effects in patients with asthma treated with a LABA in this study, we estimate that 150 non-Hispanic white people would need to be genotyped to identify three people with the rare Ile 164 allele and 100 African Americans genotyped to identify two people with the –376 rare insertion to prevent one hospital admission during LABA treatment in 1 year. Thus, the population attributable risk for hospital admission during LABA treatment due to these rare *ADRB2* variants was 0·07 for Thr164Ile in non-Hispanic white people and 0·09 for the –376 insertion variant in African Americans.

The analysis of urgent outpatient visits included data from the severe asthma clinical trial cohort[18] (n=176, table 3), including five additional LABA-treated patients with the rare Ile 164 variant (table 1). Non-Hispanic white patients on LABAs with the rare Ile 164 variant (n=18 Thr164Ile heterozygotes) had a greater number of urgent outpatient health-care or emergency department visits for asthma exacerbations during the past year compared with homozygotes for the common allele (n=553 Thr 164 homozygotes; 2·6 [SD 3·5] *vs* 1·1 [2·1] visits, p<0·0001). A similar association with increased urgent outpatient health-care or emergency department visits was observed for African Americans with the rare –376 insertion (n=6) compared with homozygotes for the common allele (n=181; 3·7 [SD 4·6] *vs* 2·4 [3·4] visits, p=0·01). Of these six LABA-treated African Americans with the rare insertion, five (83%) needed two or more urgent outpatient health-care or emergency department visits in the past year. In patients not on LABAs, the rare Ile 164 variant was associated with reduced urgent visits in non-Hispanic white patients (nine Thr164Ile patients *vs* 216 Thr 164 homozygotes: 0·1 [SD 0·3] *vs* 0·5 [1·6] visits, p=0·01) and not associated in African Americans (five patients with –376 insertion *vs* 119 without insertion: 1·2 [2·2] *vs* 0·7 [1·2] visits, p=0·18).

Detailed data for the use of inhaled and oral, systemic corticosteroid treatment were not available in some cohorts; however, non-Hispanic white patients on LABAs from SARP with the rare Ile 164 variant needed higher levels of corticosteroid treatment compared with those with the common allele (p=0·003; appendix). For example, eight (62%) of 13 non-Hispanic white patients on LABA therapy with the rare Ile 164 variant were treated with chronic systemic corticosteroids compared with 104 (27%) of 385 Thr 164 homozygotes (OR 4·25, 95% CI 1·38–14·41, p=0·01). Additionally, five (83%) of six African Americans on LABA therapy with the –376 promoter insertion were treated with chronic systemic corticosteroids compared with 52 (29%) of 180 homozygotes for the common allele without the insertion (OR 12·83, 95% CI 1·96–251·93, p=0·006). In patients not on LABAs, rare *ADRB2* variants were not associated with systemic corticosteroid treatment (Thr164Ile in non-Hispanic white patients, p=0·13; –376 In-Del in African Americans, p=0·69).

All rare *ADRB2* variants remained significantly associated with hospital admission in African Americans treated with LABAs when the covariate ancestry was included (p=0·01). Additionally, the rare –376 In-Del remained significantly associated with hospital admission (p=0·006), urgent outpatient health-care visits (p=0·02), and chronic systemic corticosteroid use

(p=0·007) in African Americans treated with LABAs when ancestry was used as a covariate.

In non-Hispanic white patients treated with LABAs from the primary cohort, seven (54%) of 13 with the rare Ile 164 variant needed treatment with a rescue inhaler for uncontrolled symptoms more than twice a week compared with 100 (26%) of 379 Thr 164 homozygotes (p=0·02; appendix). Significant associations at this locus were also identified for four additional measures of symptom control in non-Hispanic white patients treated with LABAs including dyspnoea (p=0·02), chest tightness (p=0·03), wheezing (p=0·008), and nocturnal symptoms (p=0·04; appendix). Significant associations were not identified for any measure of symptom control in non-Hispanic white patients not treated with a LABA (rescue inhaler use, p=0·97; dyspnoea, 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%) of six patients with the rare –376 insertion variant needed treatment with a rescue inhaler for uncontrolled symptoms more than twice a week compared with 55 (31%) of 179 homozygotes for the common allele, although this difference was not significant (p=0·55).

In the replication cohort, symptom severity based on NAEPP guidelines was greater in LABA-treated non-Hispanic white patients (n=13) with the rare Ile 164 variant compared with Thr 164 homozygotes (n=446; p=0·02; appendix). Five (38%) of 13 non-Hispanic white patients on LABA therapy with the rare Ile 164 variant had severe, persistent symptoms compared with 68 (15%) of 446 Thr 164 homozygotes at the 12-month follow-up visit (appendix). This rare variant association was observed only in patients treated with LABAs at the 12-month and 24-month (n=430, ten patients with Ile 164) follow-up visits (p=0·02 at each visit; 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 p=0·63). The baseline characteristics of the replication cohort are summarised in the appendix.

Discussion

This sequencing and genotyping analysis of *ADRB2* represents the largest collection of well characterised, multiethnic patients with asthma studied for rare *ADRB2* variants, many of whom have been treated with LABAs (panel). Patients with asthma analysed with sequencing had more severe disease compared with those who were genotyped, which provided the potential to identify rare variants enriched in severe asthma. The rare variants identified in these asthma cohorts represent all rare non-synonymous polymorphisms with an allele frequency greater than 0·0006 from the US National Institutes of Health (NIH) NHLBI GO exome sequencing project (appendix).[36]

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 Gly 16 allele at the Gly16Arg locus. Findings from sequence analysis in primates indicate that Gly 16 is the sole or ancestral allele at the Gly16Arg locus. Therefore, changes in β agonist responsiveness previously observed during SABA therapy in Arg 16 homozygotes are not likely to be due to a rare *ADRB2* variant.[1,2]

Rare *ADRB2* variants were associated with increased likelihood of a severe asthma exacerbation requiring hospital admission, specifically in LABA-treated white patients with the Thr164Ile rare variant and LABA-treated African Americans with the –376 In-Del rare variant. The effect of these rare variants on asthma control in patients exposed to LABAs is further supported by its association

Panel: Research in context

Systematic review

We searched PubMed for articles published in any language before Sept 27, 2013, using the terms "asthma", "beta agonist", and "*ADRB2*." This search identified pharmacogenetic studies of the *ADRB2* locus that reported associations with common variants, particularly Gly16Arg, and inhaled β agonist response. These studies consistently reported that Gly16Arg genotypes were associated with clinical responses to shortacting β agonists (SABA) during acute and chronic exposure in patients with asthma, but was not associated with clinical response to longacting β agonist (LABA) treatment. None of these studies genotyped functional rare variants based on sequencing data from patients with asthma from different ethnic groups or tested for rare variant associations.[1–5,15] We did 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 8018 participants from the British 1958 birth cohort in which Thr164Ile was not associated with asthma, wheezing, or lung function measures; however, treatment use and health-care use outcomes were not reported.[33] Another population-based study[34] of the Copenhagen City Heart Study and the Copenhagen General Population Study cohorts genotyped Thr164Ile in 62 748 participants including a subset of 1300 people with self-reported asthma, which was not reported individually for lung function measures, treatment use, or health-care use outcomes. A final search of PubMed with the terms "asthma" and "beta2" identified a study of 251 patients[35] with asthma of whom 81 had severe, life-threatening asthma. Thr164Ile was not associated with life-threatening asthma in this cohort; however, LABA use was not evaluated.

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. The probable effect of gene variant discoveries at a population level is predicted by the population attributable risk, which depends on the genetic risk and allele frequency observed within a study cohort. The population attributable risks identified for rare variants in this LABA-treated asthma population were low, which reflects low allele frequencies; however, these risks were three times greater than the frequency of these rare variants. 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 of rare, adverse events associated with LABA therapy will help to clarify concerns over LABA safety. The rare Ile 164 variant was also associated with poor symptom control during LABA treatment in two, independent cohorts. Thus, Thr164Ile is a potential biomarker for more personalised and precise guideline-based management approaches in the small subset of patients with asthma who are unresponsive to the combination therapy of a LABA with inhaled corticosteroids.

with increased urgent physician or emergency department 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 changes in therapeutic response to LABAs in these asthma genetic subpopulations resulting from rare *ADRB2* variants that 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 health-care-related outcomes during LABA treatment, which is an example of gene-level replication.[34,37] The heterogeneity of rare functional variants at specific loci has been shown to collectively determine susceptibility to diseases such as breast cancer and idiopathic pulmonary hypertension.[38,39] Gene-level replication is crucial to understanding of rare variant effects in human disease because populations with different ancestries will each have unique rare variants that might affect human disease in a similar way.

Thr164Ile is a rare *ADRB2* variant that results in an aminoacid coding change within the fourth transmembrane domain of the β_2-adrenergic receptor. In vitro, the rare Ile 164 variant decreases agonist-promoted receptor sequestration and reduces receptor ligand binding affinity and coupling to G_s protein in response to different SABAs such as isoprenaline and salbutamol.[40,41] 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.[41]

On the basis of these in-vitro observations, there are several possible mechanisms through which the rare Ile 164 variant might affect LABA efficacy. First, the deleterious effects of the Ile 164 variant on receptor ligand binding or coupling to G_s protein results in attenuation of the bronchodilator or bronchoprotective effects of LABAs. A second mechanism is that increased airway inflammation occurs in response to LABA treatment in individuals with the rare Ile 164 variant as a result of decreased receptor sequestration and a subsequent increase in T-helper-2-type inflammatory cytokine production from continued receptor stimulation.[42–44] Third, the rare Ile 164 variant might be related to impaired cardiovascular reserve.[45,46] 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 in individuals with congestive heart failure from ischaemic or dilated cardiomyopathy.[45–47] Interestingly, the rare Ile 164 variant was associated with reduced baseline lung function and baseline airflow obstruction in a cross-sectional analysis of 62748 participants from the Copenhagen City Heart Study and the Copenhagen General Population Study, which included a subset with self-reported asthma (n=1300).[34]

By contrast with non-Hispanic white patients with asthma who primarily have one rare *ADRB2* variant, African Americans are an admixed ethnic group who had a significantly greater frequency of different rare variants. The relatively higher frequency of different rare variants in African Americans provides a testable hypothesis that could partly account for the differences in asthma morbidity and responses to LABA therapy observed between these ethnic groups.[10,37] In African American patients, we identified a rare 25 bp polynucleotide insertion with similar effects on health-care use in those treated with a LABA to that observed with Thr164Ile in non-Hispanic white patients. The association of the –376 insertion variant with hospital admission and other health-care-related outcomes in African Americans remained equally significant after adjustment for ancestry. Thus, the effects of rare variants on health-care use 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 an SP1 transcription factor binding site and predicted to affect gene expression.[23,48]

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 LABAs were not always used with concomitant inhaled corticosteroid therapy.[9,10] In our multiethnic asthma populations, LABA and inhaled corticosteroid therapy was the standard of care making detection of the risk of rare, life-threatening events in participants treated with a LABA alone impossible.[49,50] Patients with severe asthma characterised by frequent health-care use were well represented in this cohort. Recent hospital admission is usually an exclusion criterion for most clinical trials, making replication of these findings more difficult.[11,12]

We show gene-level replication for two different rare *ADRB2* variants on severe exacerbations requiring hospital admission and two related, secondary outcomes in our primary cohort. We could not replicate the findings for each rare variant [A: edit OK?] for hospital admission in an independent, LABA-treated cohort. Further replication of the association between Thr164Ile in non-Hispanic white patients or the –376 insertion variant in African Americans and this primary outcome remains to be done in another large multiethnic 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, the Thr164Ile genotype could be applied to standard guideline-based approaches for asthma management to determine the best therapeutic option for a small subset

of Ile 164 heterozygotes with poor symptom control despite combination therapy with a LABA and inhaled corticosteroids.[49,50] In coming years, pharmacogenetic data—such as those described in this study—may become readily available through genetic panels or complete genome sequencing and could be used for personalised 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 participants.[10,11,13] Identification of a susceptible asthma subpopulation at increased risk of 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.

Contributors

VEO, DAM, and ERB did 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 analysed 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.

Conflicts of interest

VEO reports receiving funding from the NIH NHLBI in the form of a K12 training award (scholars' programme in the genetics and genomics of lung diseases, NIH HL089992, principal investigator: DAM). WCM reports receiving funding from the NHLBI and grants from Aerovance, Amgen, AstraZeneca, Boehringer, Centocor, Ception, Forest, Genentech, GlaxoSmithKline, MedImmune, Novartis, Pfizer, and Sanofi-Aventis. WWB provides advisory board services to Merck, consulting services to Amgen, Novartis, GlaxoSmithKline, MedImmune, and Genentech, is a member of data monitoring boards and study oversight committees for Boston Scientific, Genentech, and ICON, receives royalties from Elsevier, and receives NIH grant support from NIH NIAID and NHLBI. MC reports receiving university grant monies from NIH and American Lung Association, pharmaceutical grant monies from Asthmatx/Boston Scientific, Amgen, Ception/Cephalon/Teva, Genentech, Medimmune, Merck, Next Bio, Kalobios, Novartis, GlaxoSmithKline, Sanofi-Aventis, Vectura, royalties from Elsevier, consultant fees from Asthmatx/Boston Scientific, Genentech, IPS, Pulmagen, Sanofi-Aventis, and speaking fees from Merck, GlaxoSmithKline, Genentech, Boehringer-Ingelheim, and Asthmatx/Boston Scientific. EI reports receiving consultant fees from Cowen and Co, Infinity Pharmaceuticals, Merck, NKT Therapeutics, Regeneron Pharmaceuticals, Teva Specialty Pharmaceuticals, Gilead Sciences, and Johnson and Johnson, pharmaceutical grant monies paid to his institution from Aerovance, Amgen, i3 Research (Biota), MedImmune, and Novartis, speaking fees from Merck and Novartis, travel grant support from Teva Specialty Pharmaceuticals, and fees for expert testimony from Campbell, Campbell, Edwards and Conroy, Diedrich and Donohue, Ficksman and Conley, Ryan Ryan Deluca LLP, and Sullway and Hollis. SEW chairs the data safety and monitoring board of GlaxoSmithKline for the Long-Acting Beta Agonist Study and serves as the representative to the combined global data safety and monitoring board, and receives fees from GlaxoSmithKline and ICON for these services. SPP is the global principal investigator for AstraZeneca in the Long-Acting Beta Agonist Safety Study and only reported funds received for expenses directly related to this activity; he also reports receiving funding from the NHLBI, consultancies with Aerocrine, AstraZeneca, Airsonnet, Boehringer-Ingelheim, GlaxoSmithKline, Merck, Pfizer, PPD Incorporated, Quintiles, Teva Pharmaceuticals, and Targacept, lecture fees from Integrity and Merck, is a member of speakers' bureaus funded by Integrity CE and Merck, and reports receiving fees from UptoDate for his contribution to a chapter on asthma management. ERB reports receiving funding from NHLBI to support this study (Severe Asthma Research Program U10 HL109164) and consultancies with Aerovance, AstraZeneca, Boehringer, Centocor, GlaxoSmithKline, Genentech, Merck, Novartis, Pfizer, and Roche; he performs clinical trials at Wake Forest University supported by Boehringer, Centocor, GlaxoSmithKline, MedImmune, Genetech, Aerovance, Ception, AstraZeneca, Novartis, Amgen, Pfizer, Forest, and Sanofi-Aventis; and is also supported by grants from Spiromics (HHSN 268200900019C), AsthmaNet (U10 HL098103), and the Pharmacogenetics Network (U01 HL65899). DNA and baseline patient data from Johnson and Johnson (previously known as Centocor) was obtained from patients with severe asthma who participated in a randomised clinical trial of golimumab[18] for which ERB was principal investigator of the Wake Forest clinical site. These patients were genotyped and analysed in a pharmacogenetic study of the tumour necrosis factor α gene that was supported by Johnson and Johnson and presented in abstract form.[51] GAH, ATH, EJA, DC, SCE, FM, and DAM declare that they have no conflicts of interest.

Acknowledgments

This research was supported by research funds from the NIH NHLBI (grants U10 HL109164, RC2 HL 101487, U01 HL65899, R01 HL76285, K12 HL 89992, U10 HL098103, and NR 013700). We thank all the patients who participated in these studies and all faculty and staff; Elliot Barnathan (Johnson and Johnson, Malvern, PA, USA) for providing baseline patient data; Tmirah Haselkorn (Genentech Inc, South San Francisco, CA, USA); and Novartis Pharmaceuticals Corporation, East Hanover, NJ, USA, for providing patient data from the TENOR cohort.

References

1 Israel E, Chinchilli VM, Ford JG, et al. Use of regularly scheduled albuterol treatment in asthma: genotype-stratified, randomised, placebo-controlled cross-over trial. *Lancet* 2004; **364:** 1505–12.

2 Israel E, Drazen JM, Liggett SB, et al. The effect of polymorphisms of the beta(2)-adrenergic receptor on the response to regular use of albuterol in asthma. *Am J Respir Crit Care Med* 2000; **162:** 75–80.

3 Bleecker ER, Nelson HS, Kraft M, et al. Beta2-receptor polymorphisms in patients receiving salmeterol with or without fluticasone propionate. *Am J Respir Crit Care Med* 2010; **181:** 676–87.

4 Bleecker ER, Postma DS, Lawrance RM, Meyers DA, Ambrose HJ, Goldman M. Effect of ADRB2 polymorphisms on response to longacting beta2-agonist therapy: a pharmacogenetic analysis of two randomised studies. *Lancet* 2007; **370:** 2118–25.

5 Wechsler ME, Kunselman SJ, Chinchilli VM, et al. Effect of beta2-adrenergic receptor polymorphism on response to longacting beta2 agonist in asthma (LARGE trial): a genotype-stratified, randomised, placebo-controlled, crossover trial. *Lancet* 2009; **374:** 1754–64.

6 Drazen JM, Israel E, Boushey HA, et al. Comparison of regularly scheduled with as-needed use of albuterol in mild asthma. Asthma Clinical Research Network. *N Engl J Med* 1996; **335:** 841–47.

7 Crane J, Pearce N, Flatt A, et al. Prescribed fenoterol and death from asthma in New Zealand, 1981–83: case-control study. *Lancet* 1989; **1:** 917–22.

8 Pearce N, Burgess C, Crane J, Beasley R. Fenoterol, asthma deaths, and asthma severity. *Chest* 1997; **112:** 1148–50.

9 Castle W, Fuller R, Hall J, Palmer J. Serevent nationwide surveillance study: comparison of salmeterol with salbutamol in asthmatic patients who require regular bronchodilator treatment. *BMJ* 1993; **306:** 1034–37.

10 Nelson HS, Weiss ST, Bleecker ER, Yancey SW, Dorinsky PM. The Salmeterol Multicenter Asthma Research Trial: a comparison of usual pharmacotherapy for asthma or usual pharmacotherapy plus salmeterol. *Chest* 2006; **129:** 15–26.

11 US Food and Drug Administration. FDA Drug Safety Communication: FDA requires post-market safety trials for long-acting beta-agonists (LABAs). 04/15/2011. 2011. http://www.fda.gov/Drugs/DrugSafety/ucm251512.htm (accessed July 10, 2012).

12 Chowdhury BA, Seymour SM, Levenson MS. Assessing the safety of adding LABAs to inhaled corticosteroids for treating asthma. *N Engl J Med* 2011; **364:** 2473–75.

13 Suissa S, Ariel A. US Food and Drug Administration-mandated trials of long-acting beta-agonists safety in asthma: will we know the answer? *Chest* 2013; **143:** 1208–13.

14 Drysdale CM, McGraw DW, Stack CB, et al. Complex promoter and coding region beta 2-adrenergic receptor haplotypes alter receptor expression and predict in vivo responsiveness. *Proc Natl Acad Sci USA* 2000; **97:** 10483–88.

15 Hawkins GA, Tantisira K, Meyers DA, et al. Sequence, haplotype, and association analysis of ADRbeta2 in a multiethnic asthma case-control study. *Am J Respir Crit Care Med* 2006; **174:** 1101–09.

16 Lester LA, Rich SS, Blumenthal MN, et al. Ethnic differences in asthma and associated phenotypes: collaborative study on the genetics of asthma. *J Allergy Clin Immunol* 2001; **108:** 357–62.

17 Moore WC, Meyers DA, Wenzel SE, et al. Identification of asthma phenotypes using cluster analysis in the Severe Asthma Research Program. *Am J Respir Crit Care Med* 2010; **181:** 315–23.

18 Wenzel SE, Barnes PJ, Bleecker ER, et al. A randomized, double-blind, placebo-controlled study of tumor necrosis factor-alpha blockade in severe persistent asthma. *Am J Respir Crit Care Med* 2009; **179:** 549–58.

19 Haselkorn T, Fish JE, Zeiger RS, et al. Consistently very poorly controlled asthma, as defined by the impairment domain of the Expert Panel Report 3 guidelines, increases risk for future severe asthma exacerbations in The Epidemiology and Natural History of Asthma: Outcomes and Treatment Regimens (TENOR) study. *J Allergy Clin Immunol* 2009; **124:** 895–902 e1–4.

20 Bergmann MM, Jacobs EJ, Hoffmann K, Boeing H. Agreement of self-reported medical history: comparison of an in-person interview with a self-administered questionnaire. *Eur J Epidemiol* 2004; **19:** 411–16.

21 National Asthma Education and Prevention Program. Expert panel report 3 (EPR-3): guidelines for the diagnosis and management of asthma—summary report 2007. *J Allergy Clin Immunol* 2007; **120** (5 suppl): S94–138.

22 Adzhubei IA, Schmidt S, Peshkin L, et al. A method and server for predicting damaging missense mutations. *Nat Methods* 2010; **7:** 248–49.

23 Heinemeyer T, Wingender E, Reuter I, et al. Databases on transcriptional regulation: TRANSFAC, TRRD and COMPEL. *Nucleic Acids Res* 1998; **26:** 362–67.

24 Ng PC, Henikoff S. Predicting deleterious amino acid substitutions. *Genome Res* 2001; **11:** 863–74.

25 Wu MC, Lee S, Cai T, Li Y, Boehnke M, Lin X. Rare-variant association testing for sequencing data with the sequence kernel association test. *Am J Hum Genet* 2011; **89:** 82–93.

26 Yuan HY, Chiou JJ, Tseng WH, et al. FASTSNP: an always up-to-date and extendable service for SNP function analysis and prioritization. *Nucleic Acids Res* 2006; **34** (web server issue): W635–41.

27 Morris AP, Zeggini E. An evaluation of statistical approaches to rare variant analysis in genetic association studies. *Genet Epidemiol* 2010; **34:** 188–93.

28 Hunter DJ. Gene-environment interactions in human diseases. *Nat Rev Genet* 2005; **6:** 287–98.

29 Morris AP. Transethnic meta-analysis of genomewide association studies. *Genet Epidemiol* 2011; **35:** 809–22.

30 Lee S, Teslovich TM, Boehnke M, Lin X. General framework for meta-analysis of rare variants in sequencing association studies. *Am J Hum Genet* 2013; **93:** 42–53.

31 Alexander DH, Novembre J, Lange K. Fast model-based estimation of ancestry in unrelated individuals. *Genome Res* 2009; **19:** 1655–64.

32 Purcell S, Neale B, Todd-Brown K, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet* 2007; **81:** 559–75.

33 Hall IP, Blakey JD, Al Balushi KA, et al. Beta2-adrenoceptor polymorphisms and asthma from childhood to middle age in the British 1958 birth cohort: a genetic association study. *Lancet* 2006; **368:** 771–79.

34 Thomsen M, Nordestgaard BG, Sethi AA, Tybjaerg-Hansen A, Dahl M. Beta2-adrenergic receptor polymorphisms, asthma and COPD: two large population-based studies. *Eur Respir J* 2012; **39:** 558–66.

35 Weir TD, Mallek N, Sandford AJ, et al. Beta2-Adrenergic receptor haplotypes in mild, moderate and fatal/near fatal asthma. *Am J Respir Crit Care Med* 1998; **158:** 787–91.

36 Exome Variant Server. NHLBI GO exome sequencing project (ESP). http://evs.gs.washington.edu/EVS/ (accessed May 1, 2013).

37 Wechsler ME, Castro M, Lehman E, et al. Impact of race on asthma treatment failures in the asthma clinical research network. *Am J Respir Crit Care Med* 2011; **184:** 1247–53.

38 Ma L, Roman-Campos D, Austin ED, et al. A novel channelopathy in pulmonary arterial hypertension. *N Engl J Med* 2013; **369:** 351–61.

39 Walsh T, King MC. Ten genes for inherited breast cancer. *Cancer Cell* 2007; **11:** 103–05.

40 Green SA, Cole G, Jacinto M, Innis M, Liggett SB. A polymorphism of the human beta 2-adrenergic receptor within the fourth transmembrane domain alters ligand binding and functional properties of the receptor. *J Biol Chem* 1993; **268:** 23116–21.

41 Green SA, Rathz DA, Schuster AJ, Liggett SB. The Ile164 beta(2)-adrenoceptor polymorphism alters salmeterol exosite binding and conventional agonist coupling to G(s). *Eur J Pharmacol* 2001; **421:** 141–47.

42 Agarwal SK, Marshall GD, Jr. Beta-adrenergic modulation of human type-1/type-2 cytokine balance. *J Allergy Clin Immunol* 2000; **105:** 91–98.

43 Lazarus SC, Boushey HA, Fahy JV, et al. Long-acting beta2-agonist monotherapy vs continued therapy with inhaled corticosteroids in patients with persistent asthma: a randomized controlled trial. *JAMA* 2001; **285:** 2583–93.

44 Panina-Bordignon P, Mazzeo D, Lucia PD, et al. Beta2-agonists prevent Th1 development by selective inhibition of interleukin 12. *J Clin Invest* 1997; **100:** 1513–19.

45 Barbato E, Penicka M, Delrue L, et al. Thr164Ile polymorphism of beta2-adrenergic receptor negatively modulates cardiac contractility: implications for prognosis in patients with idiopathic dilated cardiomyopathy. *Heart* 2007; **93:** 856–61.

46 Brodde OE, Buscher R, Tellkamp R, Radke J, Dhein S, Insel PA. Blunted cardiac responses to receptor activation in subjects with Thr164Ile beta(2)-adrenoceptors. *Circulation* 2001; **103:** 1048–50.

47 Liggett SB, Wagoner LE, Craft LL, et al. The Ile164 beta2-adrenergic receptor polymorphism adversely affects the outcome of congestive heart failure. *J Clin Invest* 1998; **102:** 1534–39.

48 Emorine LJ, Marullo S, Delavier-Klutchko C, Kaveri SV, Durieu-Trautmann O, Strosberg AD. Structure of the gene for human beta 2-adrenergic receptor: expression and promoter characterization. *Proc Natl Acad Sci USA* 1987; **84:** 6995–99.

49 Global Initiative for Asthma (GINA). Global strategy for asthma management and prevention. 2012. www.ginasthma.org/local/uploads/files/GINA_Report_2012Feb13.pdf (accessed May 1, 2013).

50 National Heart, Lung, and Blood Institute. National Asthma Education and Prevention Program, expert panel report 3: guidelines for the diagnosis and management of asthma. 2007. www.nhlbi.nih.gov/guidelines/asthma/asthgdln.pdf (accessed May 1, 2013).

51 Meyers DA, Hawkins GA, Wenzel SA, Lo K, Watt R, Bleecker ER. Pharmacogenetic identification of increased responsiveness in severe asthma with anti-TNF (golimumab) therapy. *J Allergy Clin Immunol* 2008; **121:** S798 (abstr).