Salmeterol response is not affected by β_2-adrenergic receptor genotype in subjects with persistent asthma

Eugene R. Bleecker, MD,[a] Steven W. Yancey, MS,[b] Leslie A. Baitinger, MS,[b] Lisa D. Edwards, PhD,[b] Michael Klotsman, PhD,[b] Wayne H. Anderson, PhD,[b] and Paul M. Dorinsky, MD[b] *Winston-Salem and Research Triangle Park, NC*

Background: Recent studies suggest that there might be an association between albuterol use and worsening asthma in patients homozygous for arginine (Arg/Arg) at codon 16 of the β-receptor. However, it is not known whether similar responses occur in Arg/Arg patients receiving long-acting β_2-agonists.
Objective: We sought to evaluate the effects of variation in the β_2-adrenergic receptor gene *(ADRB2)* on clinical response to salmeterol administered with fluticasone propionate.
Methods: Subjects (n = 183) currently receiving short-acting β_2-agonists were randomized to twice-daily therapy with salmeterol, 50 μg, administered with fluticasone propionate, 100 μg, in a single inhaler or daily therapy with montelukast for 12 weeks, followed by a 2- to 4-day run-out period.
Results: There was sustained and significant improvement ($P < .001$) over baseline in all measures of asthma control in subjects receiving salmeterol, regardless of Arg16Gly genotype. Morning peak expiratory flow in subjects with the Arg/Arg genotype showed 89.0 ± 16.1 L/min improvement over baseline compared with 93.7 ± 12.7 L/min for Gly/Gly subjects and 92.5 ± 11.9 L/min for Arg/Gly subjects. Pairwise changes were similar for Arg/Arg compared with Gly/Gly or Arg/Gly genotypes (estimated differences, 4.7 L/min and 3.5 L/min, respectively). Responses did not appear to be modified by haplotype pairs. During the run-out period, all subjects had predictable and similar decreases in measures of asthma control, with no differences between genotypes.
Conclusion: Response to salmeterol does not vary between *ADRB2* genotypes after chronic dosing with an inhaled corticosteroid.
Clinical implications: Analyses from this study indicate that genetic polymorphisms leading to Arg16Gly sequence changes within the β_2-adrenergic receptor do not affect patients' responses to recommended asthma therapy with salmeterol and fluticasone propionate. (J Allergy Clin Immunol 2006;118:809-16.)

Key words: *Asthma, salmeterol xinafoate, genotype, polymorphism (genetics), receptors, β_2, adrenergic, fluticasone propionate*

Genetic studies in asthma have led to advances in our understanding of the association between gene variants and the responses to specific asthma treatments. Recent studies have revealed the presence of single nucleotide polymorphisms (SNPs) within *ADRB2*, the gene coding for the β_2-adrenergic receptor (β_2-AR). Some *ADRB2* SNPs result in changes within the amino acid sequence of the β_2-AR, leading to alterations of its properties, possibly associated with various asthma-related phenotypes, including lower pulmonary function and altered bronchodilator reversibility to short-acting β_2-adrenergic agents.[1,2] Variability in response, attributed to SNPs associated with asthma severity or altered β-agonist pharmacology, might have important implications for asthma clinical therapy and could define subgroups of asthmatic patients with differential responses.

The β_2-AR is a 413-amino-acid G protein–coupled receptor encoded for by an intronless gene (*ADRB2*) located on chromosome 5q31.32.[3] Variation in the activity of this cell-surface receptor mediates the effects of β_2-adrenergic agonists on a number of important cellular responses.[4] Screens of *ADRB2* have revealed at least 19 SNPs within the coding and promoter region, some of which might influence response to β_2-agonists.[1,5,6] The most prominent coding SNP is characterized by substitution of glycine for arginine at codon 16 (Arg16Gly), which occurs commonly in the general population (minor allele frequency in approximately one sixth of the general asthma population and about one fourth of the African American population).[7,8] *In vitro* studies have shown that the Gly-16 receptor exhibits enhanced downregulation after β-agonist exposure, but the data remain inconsistent.[9]

From [a]Wake Forest University School of Medicine, Winston-Salem; and [b]GlaxoSmithKline, Research Triangle Park.

Disclosure of potential conflict of interest: E. R. Bleecker has received grant support from Altana, AstraZeneca, Boehringer-Ingelheim, Centocor, Genentech, GlaxoSmithKline, and Novartis; has consultant arrangements with Aerovance, Altana, AstraZeneca, Centocor, Critical Therapeutics, Genentech, GlaxoSmithKline, and Novartis; and is on the speakers' bureau for AstraZeneca, GlaxoSmithKline, Genentech, and Merck. M. Klotsman and S. W. Yancey are both employed by and own stock in GlaxoSmithKline. W. H. Anderson, P. M. Dorinsky, L. A. Baitinger, and L. D. Edwards all are employed by GlaxoSmithKline.

Received for publication December 1, 2005; revised June 25, 2006; accepted for publication June 27, 2006.
Available online August 29, 2006.

Reprint requests: Eugene R. Bleecker, MD, Pulmonary, Critical Care, Allergy and Immunologic Diseases, Center for Human Genomics, Wake Forest University School of Medicine, Medical Center Blvd, Winston-Salem, NC 27157. E-mail: ebleeck@wfubmc.edu.

0091-6749/$32.00

doi:10.1016/j.jaci.2006.06.036

Abbreviations used
ADRB2: β_2-Adrenergic receptor gene
β_2-AR: β_2-Adrenergic receptor
BAGS: Beta-agonist Genotype Study
BARGE: Beta Adrenergic Response by Genotype
BUP: β-upstream peptide
FSC: Fluticasone propionate/salmeterol
HWE: Hardy-Weinberg equilibrium
ICS: Inhaled corticosteroid
LABA: Long-acting β_2-agonist
PEF: Peak expiratory flow
SABA: Short-acting β_2-agonist
SLIC: Salmeterol ± ICSs
SNP: Single nucleotide polymorphism
SOCS: Salmeterol or Corticosteroids

Data analyses of clinical trials suggest that SNPs can influence the response to both short-acting β_2-agonists (SABAs) and long-acting β_2-agonists (LABAs).[1,10,11] However, variations in study designs and differences in sample size have led to inconclusive results.[12] For example, some studies have reported no change in bronchodilator response to either SABAs[13] or LABAs[11,14] in asthmatic subjects with varying β_2-AR genotypes. In contrast, results from the Beta-agonist Genotype Study (BAGS) reported by Israel et al[10] showed that B16-Arg/Arg homozygous subjects had a small decrease in morning peak expiratory flow (PEF) when receiving regular albuterol, but no change was observed with albuterol used on an as-needed basis. Taylor et al[11] have extended these observations in *ADRB2* variations in pulmonary function showing that Arg/Arg homozygous subjects receiving albuterol had a decrease in PEF, as well as an increase in exacerbations. Interestingly, this effect was not found in Arg/Arg homozygous subjects receiving salmeterol.[11] More recently, the Beta Adrenergic Response by Genotype (BARGE) study from the Asthma Clinical Research Network has shown, using a prospective study design, that Arg/Arg homozygous subjects have reduced peak flow and associated responses over time compared with Gly/Gly homozygous subjects.[15] In addition, a recent retrospective analysis of 2 Asthma Clinical Research Network clinical trials has shown decreased pulmonary function with salmeterol in Arg/Arg homozygous individuals.[16]

The clinical implications of these findings could be significant, considering the recent shift toward the use of LABAs administered with inhaled corticosteroids (ICSs) to achieve asthma control in patients with persistent asthma.[17] This approach is recommended by global asthma treatment guidelines in patients who remain symptomatic despite low doses of an ICS.[17,18] In the present study we analyzed data from 2 large, identical randomized trials in which genetic samples were collected.[19,20] The aims of these analyses were to evaluate the effects of variation in *ADRB2* on clinical response to the LABA, salmeterol, administered with the ICS, fluticasone propionate, over a 12-week period and to evaluate clinical asthma stability during withdrawal of therapy.

METHODS

Subjects

Data from 2 identical studies, with results published in 2001 and 2002, in which DNA was collected were used for these analyses.[19,20] The replicate trials were designed to evaluate the efficacy and safety of fluticasone propionate/salmeterol (FSC), 100/50 μg Diskus (GlaxoSmithKline, Research Triangle Park, NC), in adolescent and adult subjects whose asthma was inadequately controlled with SABAs alone. Subjects were eligible for inclusion if they were 15 years of age or older and had a history of persistent asthma of at least 6 months. All subjects were required to have an FEV_1 of between 50% and 80% of predicted value and demonstrate 15% or greater reversibility within 30 minutes after 2 puffs (180 μg) of albuterol (Ventolin; GlaxoSmithKline). Spirometry was performed according to the American Thoracic Society published guidelines.[21] Before study enrollment, the aims of these clinical trials and pharmacogenetic analyses were fully explained, and informed consent was obtained from each participant. The study protocols were reviewed and approved by the appropriate institutional review boards.

Study design

Eligible subjects entered a 2-week run-in period during which all participants replaced their oral or inhaled SABAs with albuterol prescribed as needed for the relief of acute asthma symptoms. PEF, albuterol use, asthma symptoms, and nighttime awakenings were recorded daily by the subjects on a diary card. After the run-in period, participants meeting randomization criteria (defined as the best FEV_1 of between 50% and 80% of predicted value but within ±15% of the best predose FEV_1 obtained at screening and 5 or more days requiring albuterol use or a diary card asthma symptom score of ≥2 on 3 or more days by using a 6-point scale [0 = no symptoms, 5 = severe symptoms] during the previous week) entered the double-blind phase of the study and were randomized to receive one of the following treatments for a 12-week period: FSC, 100/50 μg Diskus twice daily plus placebo montelukast once daily, or oral montelukast, 10 mg once daily plus placebo Diskus twice daily. The current analyses were restricted to those participants randomized to therapy with FSC.

Information on the participant's race/ethnicity, medical condition and treatment, medical history, and family medical history was collected and recorded according to a standardized protocol. Baseline data for PEF, albuterol use, asthma symptoms, and nighttime awakenings was defined as the mean value over the 7 days before randomization. Baseline FEV_1 was defined as the randomization visit FEV_1 measurement. During the study, FEV_1 was measured at treatment weeks 1, 4, 8, and 12 and 3 days after treatment.

Genotyping

Genotyping was performed in a blinded manner at a central laboratory (GlaxoSmithKline) using collected blood samples on all subjects for whom a sample was available. Previously characterized coding SNPs in the β_2-AR gene (HUGO nomenclature: *ADRB2*; sequence accession ID: NM_000024)[22] found in the β-upstream peptide (BUP) at nucleotide position −47 from the start codon (BUP-Cys/Arg) at amino acid positions 16 (Arg16Gly), 27 (Gln27Glu), and 164 (Thr164Ile) were genotyped.

Departures from Hardy-Weinberg equilibrium (HWE) were assessed by using a χ^2 goodness-of-fit test. Assuming that each locus is in HWE, haplotype probabilities were estimated among white

TABLE I. Localization of SNPs and identification of haplotypes of the β_2-AR gene (*ADRB2*) among 296 white subjects

		Location				
Haplotype	Corresponding Drysdale haplotype[1]	AA19 BUP Cys/Arg −47	AA16 Gly/Arg +46	AA27 Gln/Glu +79	AA164 Thr/Ile +491	Frequency (%)
A	2	C	G	G	C	42.5
B	1, 4	T	A	C	C	38.9
C	6	T	G	C	C	17.3
D	7	T	G	C	T	<1
E	2	C	G	G	C	<1

TABLE II. Demographic and baseline characteristics of subjects by Arg16Gly genotype

	Genotype		
	B16-Arg/Arg (n = 29)	B16-Arg/Gly (n = 89)	B16-Gly/Gly (n = 65)
Mean age (y [SD])	37.9 (14.4)	37.5 (14.2)	33.8 (13.2)
Range	16-78	16-83	16-72
Sex (n [%])			
Male	15 (52)	35 (39)	31 (48)
Female	14 (48)	54 (61)	34 (52)
Race/ethnicity (n [%])			
White	25 (86)	78 (88)	52 (80)
African American	2 (7)	4 (4)	4 (6)
Hispanic	2 (7)	5 (6)	8 (12)
Asian	0	1 (1)	1 (2)
Other	0	1 (1)	0
FEV_1 % reversibility (SD)	20.2 (6.4)	25.7 (12.2)	25.1 (10.6)
FEV_1 % predicted (SD)	68.4 (6.5)	68.2 (8.6)	67.0 (8.1)
Morning PEF (SD)	363.7 (110)	345.7 (105)	376.6 (99)
Nighttime awakenings (SD)	0.58 (0.8)	0.51 (0.7)	0.51 (0.6)
Albuterol use, total puffs (SD)	4.93 (3.2)	4.80 (2.9)	5.52 (3.7)
Rescue-free days (SD)	9.4 (21.0)	9.0 (19.7)	7.9 (17.7)
Asthma symptom scores (SD)	1.52 (1.0)	1.56 (0.9)	1.61 (0.9)
Symptom-free days (SD)	7.4 (13.6)	6.6 (14.8)	8.8 (18.5)

participants by using an expectations/maximization algorithm.[23] Genetic analyses were performed by using the HelixTree software package (Golden Helix, Bozeman, Mont).

Statistical analysis

The primary hypothesis is that Arg/Arg homozygotes do not have differing clinical responses to salmeterol in the presence of an ICS during treatment or when treatment is discontinued from subjects with the Arg/Gly or Gly/Gly genotype.

All subjects for whom DNA was obtained were included in the analyses. The original studies were not powered to detect differences among genotypes; rather, the studies were powered to detect clinical outcome differences between FSC and montelukast. However, we performed ANOVAs to test for differences among genotypes (Arg/Arg, Arg/Gly, and Gly/Gly) and haplotypes in white subjects at baseline (Table I). Evaluation of the effect of genotype on each clinical parameter (FEV_1, morning PEF, albuterol use, and asthma symptom scores) during the treatment phase was carried out through the use of repeated-measures analyses adjusted for age, sex, race/ethnicity, baseline FEV_1 percent predicted, and reversibility. SAS PROC MIXED was used to construct least-squares means, their associated SEs, and CIs by week, assuming the observations were correlated within each subject. Similar analyses for daily values of morning PEF, albuterol use, and asthma symptom scores adjusted for age, sex, race/ethnicity, FEV_1 percent predicted, reversibility, and the observed end-of-study value were conducted on the data collected during the run-out phase. In the case of FEV_1, only one posttreatment observation was collected; an analysis of covariance was performed to test for differences in FEV_1 among genotypes during the run-out phase. In addition, analyses of the effects of haplotype on clinical outcome were performed.

RESULTS

Baseline distributions

Genetic samples were available for 183 (43%) FSC recipients from the 2 identical studies. The distribution of the BUP-Cys/Arg, Arg16Gly, Gln27Glu, and Thr164Ile genotypes were all found to be in HWE. *ADRB2* haplotype frequencies from unphased genotypes were estimated, and haplotype pairs were assigned to white individuals. Haplotype frequencies were not estimated in the other ethnic groups because of the small sample size.

There were no significant differences in baseline demographic, clinical, and pulmonary function characteristics across the Arg16Gly genotypes at baseline (Table II).

TABLE III. Changes from baseline in the Arg16Gly genotype at the end of 12 weeks of treatment with FSC

ADRB2 marker	Morning PEF (L/min)	FEV_1 (L)	Albuterol use (puffs/d)	Asthma symptom score
Arg16Gly genotype				
Arg/Arg (n = 29)	89.0 ± 16.1	0.53 ± 0.07	−3.78 ± 0.39	−1.08 ± 0.15
Arg/Gly (n = 89)	92.5 ± 11.9	0.57 ± 0.06	−3.79 ± 0.29	−1.08 ± 0.11
Gly/Gly (n = 65)	93.7 ± 12.7	0.59 ± 0.06	−4.01 ± 0.31	−1.01 ± 0.12

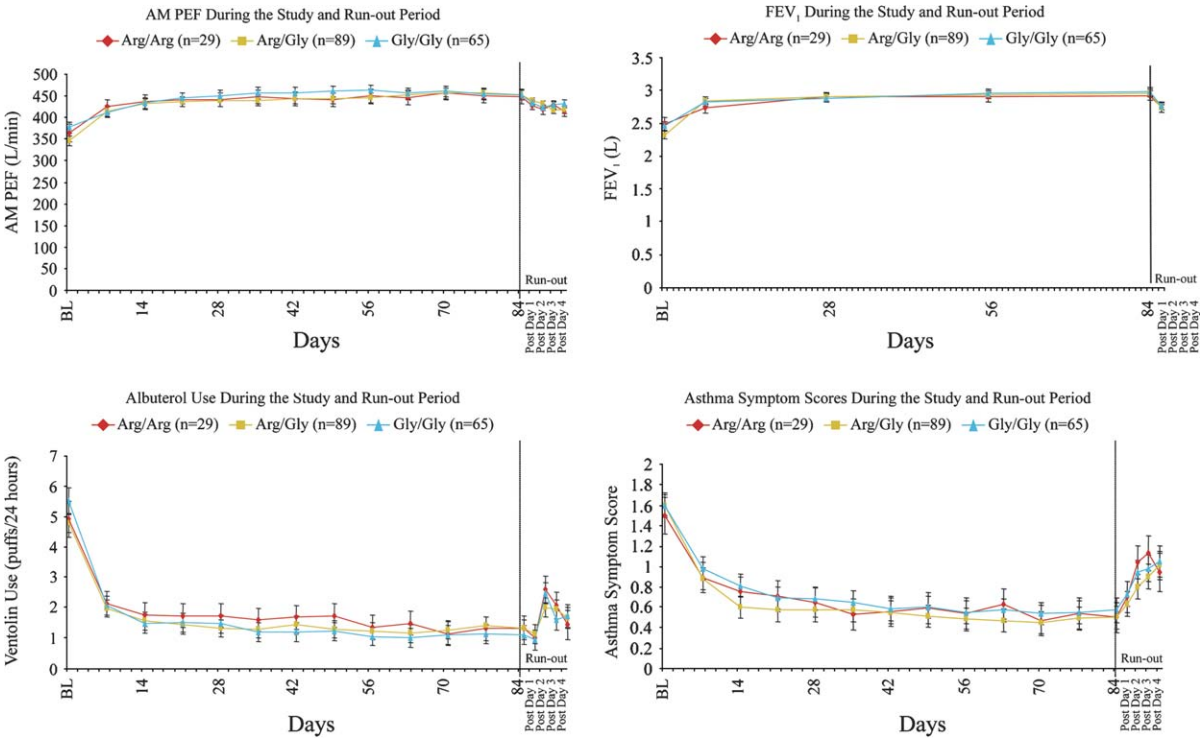

FIG 1. Change from baseline in morning PEF, FEV_1, albuterol use, and asthma symptom scores during the 12-week treatment period and during the run-out period.

Treatment-phase response to FSC by *ADRB2* genotypes and haplotypes

There was sustained and significant improvement ($P < .001$) compared with baseline values in all measures of asthma control in all Arg16Gly genotypes, with no differences observed across genotype subgroups (Table III and Fig 1). Specifically, pairwise change from baseline in morning PEF over the 12 weeks of treatment was similar for the Gly/Gly genotype compared with the Arg/Arg genotype (estimated difference, 4.7 L/min; $P = .774$) and for the Arg/Gly genotype compared with the Arg/Arg genotype (estimated difference, 3.5 L/min; $P = .822$; Table III and Fig 1). The treatment responses to FSC for FEV_1, albuterol use, and asthma symptoms were also similar across all genotype subgroups, and no significant differences across genotype subgroups were observed (Fig 1). The results of the montelukast treatment arm comparison by genotype were similar to those observed in the FSC treatment arm. All observed differences were not significant on adjustment for multiple comparisons (data not shown).

The clinical responses to FSC were also evaluated with respect to other individual *ADRB2* SNPs. Pharmacogenetic associations between response to FSC and *ADRB2* BUP-Cys/Arg, Arg16Gly, Gln27Glu, and Thr164Ile genotypes and haplotype pairs were evaluated, and neither measures of pulmonary function, albuterol use, nor asthma symptom scores were associated with these *ADRB2* genotypes (Table III). With the exception of the rare polymorphism at position 164 and haplotype pairs, no discernable associations were observed.

Because haplotypes might be more informative than individual SNPs, associations between haplotype pairs and outcomes were evaluated (Table IV). Analysis was restricted to the 4 to 5 most frequently observed haplotype pairs. No significant associations were identified. Although it is difficult to definitively rule out a haplotype effect because of sample size, response to regularly

TABLE IV. Changes from baseline at the end of 12 weeks of treatment with FSC stratified by haplotype

Haplotype pair*	Morning PEF (L/min)	FEV_1 (L)	Albuterol use (puffs/d)	Asthma symptom score
AA (n = 27)	101.15 ± 13.21	0.62 ± 0.06	−3.76 ± 0.34	−0.91 ± 0.13
AB (n = 49)	87.19 ± 9.58	0.58 ± 0.05†	−4.05 ± 0.25	−1.17 ± 0.1
AC (n = 18)	93.62 ± 15.64	0.71 ± 0.07	−4.22 ± 0.41	−1.12 ± 0.16
BB (n = 25)	92.03 ± 13.5	0.57 ± 0.07‡	−3.88 ± 0.35	−1.15 ± 0.13
BC (n = 28)	96.6 ± 12.8	0.65 ± 0.06	−3.7 ± 0.33	−1.03 ± 0.13
CC (n = 5)	63.1 ± 29.96	0.92 ± 0.15	−3.75 ± 0.79	−0.7 ± 0.3

*Haplotype pair indicates the haploid genotype pair of each of A, B, and C alleles. For instance, AA indicates an A allele at each of the 2 gene loci on the chromosome, and AB indicates an A allele at the first locus and a B allele at the second.
†P = .033 for AB versus CC.
‡P = .034 for BB versus CC.

TABLE V. Difference between the last 7 days on treatment and the run-out period stratified by genotype for subjects previously treated with FSC

	Genotype*		
	B16-Arg/Arg (n = 29)	B16-Arg/Gly (n = 89)	B16-Gly/Gly (n = 65)
Morning PEF (L/min)	−36.9 ± 11.6	−32.4 ± 8.6	−19.5 ± 9.2
FEV_1 (L)	−0.24 ± 0.07	−0.25 ± 0.06	−0.22 ± 0.06
Total albuterol use	0.25 ± 0.49	0.48 ± 0.34	0.55 ± 0.38
Total asthma symptom score	0.45 ± 0.19	0.51 ± 0.14	0.56 ± 0.15

*No differences between genotypes were noted.

scheduled use of FSC did not appear to be modified by *ADRB2* haplotype pairs. Additional analyses were performed by using software for analyzing haplotypes (haplo.score) for the same phenotypes, and no evidence for a significant association was found.

Exacerbations, defined as any event requiring medication beyond study drug during treatment or the run-out period, were rare. One Arg/Arg subject and 1 Arg/Gly subject experienced an asthma exacerbation during the treatment phase; no subjects experienced exacerbations during the run-out phase.

The number of patients withdrawn was low and similar across groups. Table E1 (in this article's Online Repository at www.jacionline.org) describes the disposition of patients withdrawn during the studies.

Run-out phase response by genotype

After 12 weeks of treatment with FSC, treatment was discontinued for 2 to 4 days while subjects continued to use albuterol as needed and record daily symptoms and PEF on diary cards. Baseline for this run-out period was defined as the average of the last 7 days of the FSC treatment period. During the run-out phase, no differences were noted in any of the clinical responses to FSC withdrawal across the genotypes (Table V). Specifically, subjects had predictable and similar decreases in morning PEF and other measures of asthma control when switched from FSC to treatment with SABAs alone, regardless of genotype (Table V and Fig 1).

Specific responses by B16-Arg/Arg genotype

Because the B16-Arg/Arg genotype is hypothesized to exhibit enhanced downregulation in the presence of chronic β_2-agonist stimulation, morning PEF response for each B16-Arg/Arg subject was examined individually (Fig 2). All but 2 Arg/Arg subjects had improvements over baseline at end point in morning PEF, one of whom had a documented exacerbation on day 71 of the study. Furthermore, the responses to treatment with FSC for individual B16-Gly/Gly and B16-Gly/Arg subjects were similar to those of the B16-Arg/Arg subjects (data not shown).

DISCUSSION

The results of the current study do not indicate a differential effect of *ADRB2* polymorphisms on response to FSC therapy in subjects with asthma in this study. Prior studies suggest that the therapeutic responses to regularly scheduled therapy with short-acting bronchodilators vary as a result of genetic polymorphisms involving a variation at the 16th amino acid position of the β_2-AR. A recent report by Wechsler et al[16] analyzed data from the Salmeterol or Corticosteroids (SOCS) and Salmeterol ± ICSs (SLIC) trials and concluded that a subset of patients in SLIC with B16-Arg/Arg (n = 8) do not improve to the same degree as patients with B16-Gly/Gly (n = 22) receiving salmeterol therapy, either with or without concurrent ICS therapy. In the SOCS trial, including patients with persistent asthma treated with salmeterol after ICS withdrawal, the B16-Arg/Arg patient subset (n = 12) who received salmeterol alone responded significantly worse than the B16-Gly/Gly subset (n = 13), as assessed on the basis of changes in morning PEF, compared with patients who received placebo. However, in contrast to

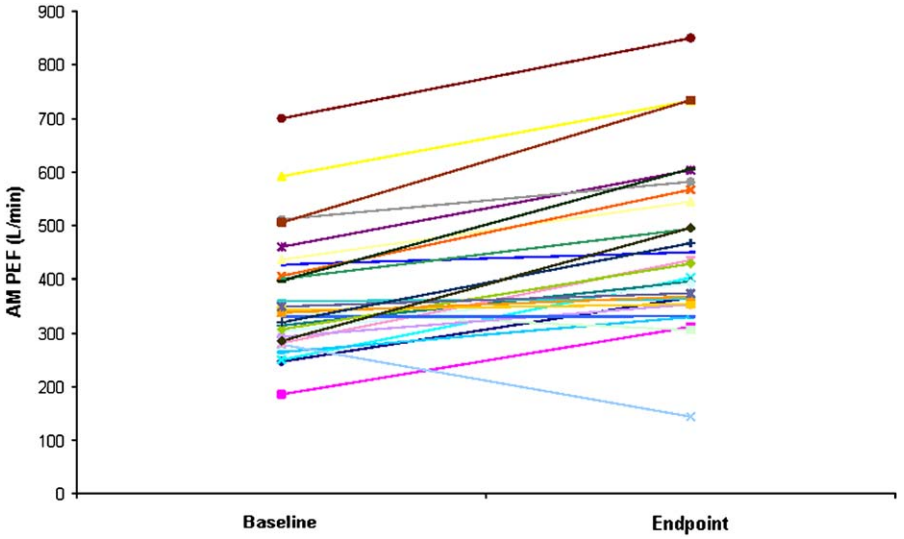

FIG 2. Change from baseline at end point in morning PEF for individual Arg/Arg subjects (n = 29).

the study by Wechsler et al, the findings from this study of 183 subjects, perhaps the largest genetic analysis to date, show that response to the LABA salmeterol is not affected by the *ADRB2* Arg16Gly SNP during chronic dosing in the presence of an ICS. Specifically, there were sustained and quantitatively similar improvements in lung function, symptoms, and albuterol use during chronic treatment, regardless of Arg16Gly genotype. Although not powered to definitively evaluate a haplotype effect, our haplotype analysis supports the individual SNP results. Drysdale et al[1] showed the lowest response in their 4/4 haplotype, corresponding to our BB group, with a total of 14 subjects as opposed to the 25 in our analysis (Table IV). Although we did not measure all 13 possible SNPs, using the SNPs at position −47, 46, 79, and 491, we were able to evaluate the most frequent haplotypes corresponding to those previously reported.

The results of this study are consistent with the findings of Taylor et al,[11] who examined retrospectively the relationship between the frequency of asthma exacerbations and genetic variation at codon 16 of *ADRB2* in subjects receiving placebo, 4-times-daily albuterol, or twice-daily salmeterol for 24 weeks. These investigators demonstrated that although subjects treated with 4-times-daily albuterol had a higher number of exacerbations compared with those receiving placebo, subjects treated with salmeterol had the lowest number of exacerbations. In addition, there was no differential response in exacerbations or lung function in subjects receiving salmeterol, regardless of genotype. Furthermore, a retrospective study by Klotsman et al[24] showed that the serial 12-hour FEV_1 response to salmeterol after 12 weeks of treatment was similar in subjects treated with salmeterol, regardless of concomitant ICS use or Arg16Gly genotype. The current analysis did not evaluate salmeterol as monotherapy, which is not recommended in current asthma guidelines and could have contributed to the failure to detect a polymorphic effect of *ADRB2* on treatment response.

In contrast to this study with salmeterol, a retrospective analysis of BAGS results reported small decreases in morning PEF over time for B16-Arg/Arg subjects (n = 28) who received regularly scheduled albuterol, whereas similar decreases were not observed in either the Arg/Arg homozygous subjects who received as-needed albuterol or in the B16-Gly/Gly subjects (n = 62) who received regularly scheduled albuterol.[10] Interestingly, this study showed a larger decrease in morning PEF after the switch from 4-times-daily albuterol to as-needed albuterol in B16-Arg/Arg but not B16-Gly/Gly subjects. Results from BARGE appear similar to those from BAGS for B16-Arg/Arg subjects receiving regularly scheduled albuterol, in that there was a differential response between the 2 homozygote groups on regular β-agonist therapy, with the Gly/Gly homozygotes showing improvement in PEF and other parameters of asthma control.[15]

The results of the current study evaluating therapeutic responses to salmeterol in patients receiving concomitant corticosteroids do not support an effect of *ADRB2* polymorphisms. The contrasting results between BAGS, BARGE, the retrospective analysis of SOCS and SLIC, and this study can be attributed to various factors, including sample size, differences in study design, asthma severity, intrinsic activity of the β-agonist evaluated, and concomitant use of an ICS.

Compounds with lower intrinsic activity are generally believed to result in a lower potential to induce receptor downregulation, a proposed mechanism of reduced response to β-agonists.[25] High intrinsic activity might also lead to an opposite response in which response to cholinergic tone is enhanced.[26] Salmeterol has the lowest intrinsic activity of the currently available β-agonist bronchodilators.[27]

Because *ADRB2* is a small gene with significant linkage disequilibrium across the 5′ promoter and 3′ untranslated region, functional polymorphisms in partial linkage disequilibrium with the Arg16Gly SNP might also be the

cause of the divergent results in the literature because these would not be equally expressed in trials with small sample sizes.

Previous studies have proposed that some patients might experience poorer asthma control and adverse side effects associated with regular use of the potent SABA fenoterol.[28,29] Because of these results, regular therapy was compared with as-needed use of SABAs and shown to have no adverse effects, but regular SABA use provided no additional therapeutic benefits. Recent pharmacogenetic data raise questions as to whether there might be a subset of subjects, based on β_2-AR genotype, who might not respond well to chronic SABA therapy. The results of the present study suggest that this is not the case for salmeterol when administered with an ICS and that current treatment guidelines are appropriate for a broad population of asthmatic subjects, including those with β_2-AR polymorphisms.

Both clinical trials and observational studies support the therapeutic value of inhaled LABAs in combination with ICSs. Specifically, if β-agonist use were associated with deleterious outcomes in an at-risk genetic subgroup reported to occur in a small but significant percentage of the asthma population (eg, the B16-Arg/Arg genotype is observed in approximately 15% of white subjects), then evaluation of large clinical studies would predictably lead to an increase in adverse outcomes, such as asthma exacerbations, among β-agonist users compared with nonusers. Long-term clinical trials, including OPTIMA[30] and GOAL,[31] have highlighted the advantages of LABA/ICS therapy in terms of improved asthma control and fewer exacerbations. Similarly, Shrewsbury et al,[32] in a meta-analysis of data from 3685 subjects, documented lower frequency of asthma exacerbations and better overall asthma control with a low-dose ICS plus salmeterol compared with higher doses of an ICS alone. Furthermore, a recent pooled analysis of 13 studies including 4020 subjects reported fewer asthma exacerbations and hospitalizations for both white and African American subjects receiving an ICS plus LABA compared with an ICS alone.[33] These observations, although consistent with the current study results, do not exclude the possibility that individual subjects with asthma might be at greater risk with chronic β-agonist therapy. However, examination of results from individual subjects in this study with the various genotypes (B16-Arg/Arg in particular) did not indicate that there was a subset of nonresponders. It could be hypothesized that there might be additional gene-gene interaction linkage disequilibrium with other polymorphisms in *ADRB2* that are associated with exacerbations or as a marker for disease instability.[2] Additionally, observational studies reflecting real-world use of asthma medications support the findings that ICSs plus LABAs resulted in fewer emergency department visits and hospitalizations for asthma, less need for supplemental albuterol, and overall lower costs compared with an ICS alone or an ICS plus leukotriene modifiers.[34-36]

Some important limitations should be considered when interpreting these data. For example, the observations in the present study that there were no associations between genotypes and the treatment responses to FSC or the response to albuterol alone during FSC washout does not eliminate a genetic interaction. In this regard *ADRB2* haplotypes have been described and reported to occur at different frequencies based on ethnicity.[1] The haplotype analysis in this study did not reveal any significant influence on response, but the sample size, the selection criteria of the study population, and the lack of ethnic representation limits these conclusions.

In summary, this study evaluating associations between the polymorphic gene encoding for the β-agonist drug target and responses to therapy with a long-acting β-agonist showed that response to salmeterol does not vary by *ADRB2* genotypes or haplotypes during chronic dosing in the presence of an ICS. However, larger, prospective, clinical pharmacogenetic studies with higher power to evaluate haplotypes across different ethnic/racial groups, as well as genetic epidemiologic studies, are clearly needed to help elucidate this field of great interest.

We thank Laura Sutton, PharmD, for her assistance in preparing the manuscript and Deborah Meyers, PhD, for her assistance with the haplotype analyses.

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