β-Adrenergic Receptor Polymorphisms and Response to Salmeterol

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Rationale: **Several studies suggest that patients with asthma who are homozygous for arginine at the 16th position of the β_2-adrenergic receptor may not benefit from short-acting β-agonists.**

Objectives: **We investigated whether such genotype-specific effects occur when patients are treated with long-acting β-agonists and whether such effects are modified by concurrent inhaled corticosteroid (ICS) use.**

Methods: **We compared salmeterol response in patients with asthma homozygous for arginine at B16 (B16Arg/Arg) with those homozygous for glycine at B16 (B16Gly/Gly) in two separate cohorts. In the first, subjects were randomized to regular therapy with salmeterol while simultaneously discontinuing ICS therapy. In the second, subjects were randomized to regular therapy with salmeterol while continuing concomitant ICS.**

Results: **In both trials, B16Arg/Arg subjects did not benefit compared with B16Gly/Gly subjects after salmeterol was initiated. In the first cohort, compared with placebo, the addition of salmeterol was associated with a 51.4 L/min lower A.M. peak expiratory flow (PEF; $p = 0.005$) in B16Arg/Arg subjects(salmeterol, $n = 12$; placebo, $n = 5$) as compared with B16Gly/Gly subjects (salmeterol, $n = 13$; placebo, $n = 13$). In the second cohort, B16Arg/Arg subjects treated with salmeterol and ICS concurrently ($n = 8$) had a lower A.M. PEF (36.8 L/min difference, $p = 0.048$) than B16Gly/Gly subjects ($n = 22$) treated with the same regimen. In addition, B16 Arg/Arg subjects in the second cohort had lower FEV_1 (0.42 L, $p = 0.003$), increased symptom scores (0.2 units, $p = 0.034$), and increased albuterol rescue use (0.95 puffs/d, $p = 0.004$) compared with B16Gly/Gly subjects.**

Conclusions: **Relative to B16Gly/Gly patients with asthma, B16Arg/Arg patients with asthma may have an impaired therapeutic response to salmeterol in either the absence or presence of concurrent ICS use. Investigation of alternate treatment strategies may benefit this group.**

Keywords: asthma; β-adrenergic receptor; β-agonists; pharmacogenetics; salmeterol

β_2-Adrenergic agonists are the most commonly prescribed medications for asthma due to their quick relief of symptoms of airflow obstruction (1). Whether frequent or regularly scheduled use of these medications actually reduces asthma control is controversial (2–5). Several studies have suggested that short-acting β-agonists such as albuterol may produce adverse outcomes (e.g., decreased peak flow or increased risk of exacerbation) in patients homozygous for arginine (Arg/Arg) at the 16th amino acid position of the β-adrenergic receptor gene (herein referred to as B16) compared with patients homozygous for glycine (Gly/Gly) at B16 (*see* Figure E1 of the online supplement) (6–8).

Large, controlled, prospective studies show that long-acting β-agonists are more effective than albuterol for asthma treatment, resulting in their widespread use, especially with concurrent inhaled corticosteroid (ICS) therapy (9). However, a number of studies have also suggested an association between use of long-acting β-agonists with adverse outcomes such as asthma exacerbation and, possibly, increased risk of death (10–13). Whether this phenomenon with long-acting β-agonists is related to a genotype-specific susceptibility to adverse effects, as observed with regular use of short-acting β-agonists, or whether the bronchodilating and symptom-relieving effects of salmeterol mask increasing inflammation and delay awareness of worsening asthma is unclear (14).

We sought evidence of a genotype-specific difference in the response to long-acting β-agonists by analyzing two studies in which patients were treated with salmeterol. In the first, subjects had received salmeterol or placebo while inhaled steroids were withdrawn as part of the National Heart Lung and Blood Institute's (NHLBI) Salmeterol or Corticosteroid (SOCS) trial (15). In the second, subjects received salmeterol and a constant dose of ICS as part of the Salmeterol ± Inhaled Corticosteroid (SLIC) trial (16). We hypothesized that if a genotype-specific difference between B16Arg/Arg and B16Gly/Gly subjects were to occur in response to regular salmeterol use, independent of concurrent ICS use, a large proportion of patients with asthma could benefit from modification of asthma treatment regimens.

METHODS

We genotyped subjects who provided written, informed consent to participate in two published NHLBI Asthma Clinical Research Network trials: the SOCS (15) and SLIC trials (16). On the basis of the data from our previous studies showing a genotype-specific difference in A.M. PEF between B16Arg/Arg and B16Gly/Gly subjects in response to β-agonist therapy (6, 8), we focused our primary analysis on the effect of salmeterol in A.M. PEF in these two genotypes. We secondarily examined the effect of salmeterol on other outcomes in these studies, as well as the effect of other previously identified β-adrenergic receptor gene polymorphisms. The original studies are reviewed below.

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Trial Overviews

Trial 1: SOCS trial. The SOCS trial was a multicenter, 28-wk, randomized, double-blind, placebo-controlled, parallel-group trial comparing long-acting β-agonist monotherapy with continued therapy with ICS in subjects with persistent asthma (*see* Figure 1) (15). After 6 wk of treatment with open-label triamcinolone acetonide (400 μg twice daily), 164 subjects with well-controlled asthma ($FEV_1 > 80\%$ predicted, and PEF variability $< 20\%$) were randomized in a double-blind manner for 16 wk to the following groups: (*1*) to continue inhaled triamcinolone (400 μg twice daily), (*2*) to substitute ICS with salmeterol xinafoate (42 μg twice daily) via metered-dose inhaler as monotherapy, or (*3*) to substitute ICS with placebo. The trial concluded with a 6-wk, single-blind, placebo run-out. Albuterol sulfate was used as needed as rescue medication. Although no significant differences between the salmeterol and triamcinolone groups were observed with respect to A.M. PEF, P.M. PEF, asthma symptom scores, rescue albuterol use, or quality of life, the salmeterol group had more asthma exacerbations, as well as greater increases in sputum eosinophils, eosinophilic cationic protein, and tryptase. It was concluded that patients with persistent asthma well controlled by low doses of triamcinolone cannot be switched to salmeterol monotherapy without risk of clinically significant loss of asthma control.

To assess the effect of β_2-adrenergic receptor (β2AR) genotype on response to salmeterol as monotherapy, we analyzed the B16 genotype–specific effect of treatment with salmeterol compared with placebo.

Trial 2: SLIC trial. The SLIC trial was a multicenter 24-wk, randomized, controlled, blinded, double-dummy, parallel group trial examining the effects of adding salmeterol to triamcinolone (*see* Figure 2) (16). After a 6-wk run-in period with open-label triamcinolone acetonide (400 μg twice daily), 175 subjects whose asthma was not well controlled ($FEV_1 \leq 80\%$ predicted, or average PEF variability $\geq 20\%$) received add-on therapy with 42 μg of salmeterol xinafoate (two puffs) twice daily via metered-dose inhaler for 2 wk plus 400 μg of inhaled triamcinolone acetonide twice daily. Half the subjects were then randomly assigned to maintain triamcinolone dosage throughout the trial or undergo a blinded, one-step 50% reduction in triamcinolone for 8 wk followed by an 8-wk triamcinolone elimination phase of salmeterol monotherapy. Albuterol sulfate was used throughout as needed as rescue medication. This study demonstrated that, in patients with persistent asthma suboptimally controlled by ICS therapy alone but whose asthma symptoms improve after addition of salmeterol, a substantial reduction (50%) in ICS dose could occur without a significant loss of asthma control. However, total elimination of triamcinolone therapy results in a significant deterioration in asthma control and, therefore, could not be recommended.

To assess the effect of salmeterol in the setting of concomitant ICS use, we analyzed polymorphisms of the β2AR gene in individuals in the SLIC trial randomized to salmeterol and a steady dose of triamcinolone.

Sample Collection and Genotypic Analysis

Blood was drawn and genetic material collected for genotyping from 53 subjects receiving salmeterol and 43 placebo subjects in the SOCS trial, and from 74 individuals randomized to receive salmeterol and a steady dose of triamcinolone in the SLIC trial. Genomic DNA was prepared for genotypic analysis by standard methods (17). The primary polymorphism studied, A+46G, coding for the 16th amino acid position of β2AR, consisted of either glycine (Gly) or arginine (Arg). The three possible genotypes are termed B16Arg/Arg, B16Arg/Gly, and B16Gly/Gly. Genotypes at B16 were assessed by the amplification refractory mutation system (18, 19) and confirmed in all subjects by restriction fragment length polymorphism by individuals unaware of the clinical trial results (6, 20). We also genotyped seven other polymorphic loci on the β-adrenergic receptor gene (the previously reported polymorphisms C-709A, G-654A, C-47T, C+79G, G+252A, C+491T, and C+523A; *see* Table E1 for genotype success rates) (21, 22), and performed haplotype analysis as previously described (23). Additional β-adrenergic receptor gene loci genotypes were assessed by single-base extension followed by matrix-assisted laser desorption/ionization–time of flight mass spectrometry (Sequenom, San Diego, CA) (24). We performed additional secondary analyses for single single nucleotide polymorphism (SNP) associations between A.M. PEF and variants at SNP +79 and +523.

Statistical Analysis

In view of our prior results in B16Arg/Arg as compared with B16Gly/Gly patients (6, 8), we set out to compare A.M. PEF primarily, and the other outcomes secondarily, in these two genotype-defined groups. In both trials, patients who met preassigned criteria for treatment failure or asthma exacerbation received protocol-defined rescue treatment with prednisone, inhaled triamcinolone, or both. Because rescue therapy could affect outcomes, data were analyzed by the intent-to-treat method, and the last observation carried forward method, in which the last value observed before treatment failure, asthma exacerbation, or drop-out was carried forward to the end of the 16-wk randomized treatment period in SOCS (Week 22) and to the end of the 18-wk

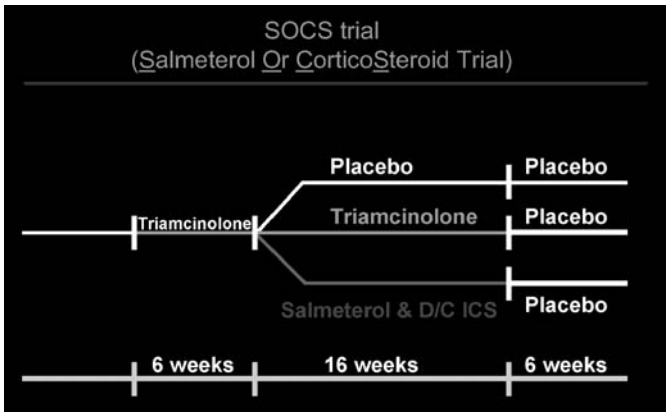

Figure 1. The Salmeterol or Corticosteroid (SOCS) trial. After 6 wk of treatment with open-label inhaled corticosteroid (ICS; triamcinolone acetonide), subjects with asthma were randomized in a double-blind manner for 16 wk to the following: (*1*) to continue inhaled triamcinolone, (*2*) to substitute ICS with salmeterol xinafoate via metered-dose inhaler as monotherapy, or (*3*) to substitute ICS with placebo. The trial concluded with a 6-wk single-blind placebo run-out.

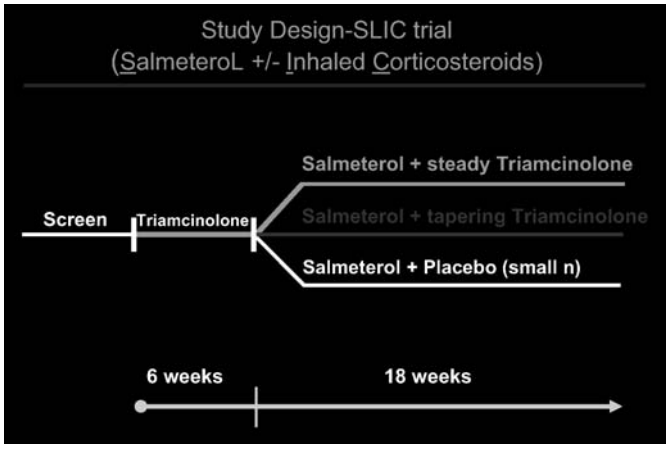

Figure 2. The Salmeterol ± Corticosteroid (SLIC) trial. After a 6-wk run-in period with open-label ICS (triamcinolone acetonide), subjects whose asthma was not well controlled received add-on therapy with 42 μg of salmeterol xinafoate (two puffs) twice daily via metered-dose inhaler for 2 wk plus 400 μg of inhaled triamcinolone acetonide twice daily. Half the subjects were then randomly assigned to maintain triamcinolone dosage throughout the trial or to undergo a blinded, one-step 50% reduction in triamcinolone for 8 wk followed by an 8-wk triamcinolone elimination phase of salmeterol monotherapy. A small group of subjects received salmeterol with placebo ICS.

TABLE 1. MEAN BASELINE CHARACTERISTICS OF B16Arg/Arg AND B16Gly/Gly SUBJECTS RANDOMIZED TO SALMETEROL AND PLACEBO ARMS OF THE SOCS TRIAL

	Arg/Arg Placebo vs. Salmeterol			Gly/Gly Placebo vs. Salmeterol			Combined Arg/Arg vs. Gly/Gly		
Characteristic	Placebo (*n = 5*)	Salmeterol (*n = 12*)	p Value	Placebo (*n = 13*)	Salmeterol (*n = 13*)	p Value	Arg/Arg (*n = 17*)	Gly/Gly (*n = 26*)	p Value
Male, n (%)	1 (20)	5 (42)	0.60	3 (23)	5 (38)	0.67	6 (35)	8 (31)	0.76
Minority, n (%)	2 (40)	5 (42)	1.00	4 (31)	2 (15)	0.64	7 (41)	6 (23)	0.21
African American, n (%)	1 (20)	1 (8)	0.51	0 (0)	1 (8)	1.00	2 (12)	1 (4)	0.32
Age, mean (SD), yr	33.44 (15.11)	38.47 (11.88)	0.47	33.17 (10.48)	25.10 (9.39)	0.05**	36.99 (12.64)	29.14 (10.59)	0.03**
A.M. PEF, mean (SD), L/min*	446.2 (66)	434.8 (125)	0.85	431.3 (82)	480.2 (121)	0.24	438.2 (109)	455.7 (104)	0.60
PM PEF, mean (SD), L/min*	456.2 (67)	444.4 (119)	0.84	436.9 (82)	496.4 (131)	0.18	447.9 (104)	466.6 (112)	0.58
PEF variability, mean (SD), %*†	14.9 (5.9)	10.1 (4.5)	0.09	10.6 (3.7)	9.7 (5.4)	0.63	11.5 (5.3)	10.2 (4.6)	0.38
FEV_1, mean (SD), L	2.88 (0.71)	2.99 (0.75)	0.79	2.95 (0.61)	3.30 (0.96)	0.28	2.96 (0.71)	3.13 (0.81)	0.49
FEV_1% predicted, mean (SD)	92.8 (9.4)	92.7 (9.5)	0.98	93.5 (8.8)	95.4 (7.9)	0.58	92.7 (9.2)	94.5 (8.2)	0.52
PC_{20}, mean (CV), mg/ml‡	1.01 (1.37)	0.97 (1.97)	0.96	0.42 (1.45)	0.66 (1.61)	0.33	0.98 (1.76)	0.54 (1.54)	0.11
A.M. symptom scores (SD)§	0.5 (0.6)	0.3 (0.5)	0.57	0.3 (0.4)	0.3 (0.3)	0.98	0.36 (0.50)	0.29 (0.33)	0.56
P.M. symptom scores (SD)§	0.5 (0.5)	0.5 (0.5)	0.89	0.4 (0.4)	0.4 (0.4)	0.92	0.46 (0.46)	0.39 (0.40)	0.63
Average rescue puffs β-agonist/d, mean (SD)	1.7 (2.9)	1.9 (2.2)	0.84	2.6 (3.0)	2.4 (2.5)	0.85	1.8 (2.4)	2.5 (2.7)	0.40
Exhaled nitric oxide, mean (SD), ppb	17.5 (7.1)	20.5 (18.2)	0.80	18.4 (11.6)	21.3 (12.3)	0.63	19.5 (14.9)	20.0 (11.7)	0.93
Asthma quality-of-life score‖¶ (Q1, Q3)	2.9 (2.5, 3.0)	1.9 (1.4, 2.6)	0.13	1.9 (1.7, 2.84)	1.9 (1.6, 2.)	0.49	2.13 (1.56, 2.97)	1.90 (1.61, 2.53)	0.71

Definition of abbreviations: CV = coefficient of variation; PEF = peak expiratory flow; Q1, Q3 = interquartile range; SOCS = Salmeterol or Corticosteroid trial.

Sex, minority, and African-American status are summarized with frequencies and percentages and are compared via an exact χ^2 test. A.M. and P.M. PEF, PEF variability, FEV_1, FEV_1% predicted, A.M. and P.M. symptom scores, average puffs of β-agonist, exhaled nitric oxide, and PC_{20} were compared via a two-sample *t* test.

* Values represent averages for the fifth and sixth weeks of the run-in period.

† PEF variability was calculated as ([evening PEF − morning PEF]/evening PEF) × 100.

‡ Geometric mean.

§ Asthma symptoms were graded by the subject each day, from 0 for no symptoms to 3 for incapacitating symptoms.

‖ Median.

¶ Asthma-specific quality-of-life questionnaires were completed by the subjects during clinical-center visits. A score of 1.0 indicates that asthma had no effect on the overall quality of life; a score of 2.0, "a little limited" by asthma; 3.0, "some limitation"; and 7.0, "total limitation."

** $p < 0.05$.

randomized treatment period in SLIC (Week 24). In the SOCS trial, patients were randomized to placebo or salmeterol; the effect was compared with placebo. Statistical analysis included comparison of each response variable to baseline* stratified by genotype at the end of treatment (both SOCS and SLIC trials), and in the case of the SOCS trial, at the end of run-out, on the basis of our findings from our prior trial that suggested that a significant effect occurred after withdrawal of the β-agonists (6). In the SOCS trial, because we performed analysis of our primary outcome, change in A.M. PEF, at two endpoints (Weeks 22 and 28), we applied a Bonferroni correction with a significant p value ≤ 0.025 for the primary endpoint. In the SLIC trial, the primary analysis outcome, change in A.M. PEF, was examined at only one point in time (Week 24). Other outcomes in both trials were examined secondarily in an exploratory manner, and no correction was applied to those outcomes.

In the SOCS trial, for all response variables, except asthma-related quality-of-life (AQOL) score (data available for only select weeks), a two-slope (first six treatment weeks, remaining 10 treatment weeks) segmented model was fitted to the data. A single-slope model was used to evaluate the run-out phase because active therapy was discontinued. In the SLIC trial, slopes were fitted for the first 2 wk of the salmeterol treatment phase, followed by two 8-wk periods of constant triamcinolone therapy. Model estimates of change from baseline for each response variable by genotype were calculated and compared at the end of treatment and run-out. Because AQOL score was not distributed normally, comparisons for change from baseline at the end of treatment and run-out used signed rank and Wilcoxon rank sum tests. All outcomes were adjusted for age and baseline lung function by including them as covariates in the model.

* Baseline was defined as the average of Weeks 5 and 6, the 2 wk before randomization, for those response variables that were measured daily (A.M. and P.M. PEF, PEF variability, average symptom scores, and average puffs of β-agonist). Baseline is defined as the measurement at Week 6, the week of randomization, for the remaining response variables (FEV_1, FEV_1 %predicted, PC_{20}, exhaled nitric oxide, and AQOL score). Change from baseline was calculated for all response variables each available week after baseline by taking the measurement that week and subtracting the baseline value as defined above.

Genetic Analysis

Each of the polymorphic loci was tested for deviations from Hardy-Weinberg equilibrium using the exact test in the computer software Genetic Data Analysis (25). Haplotype blocks were identified using two linkage disequilibrium–based algorithms (26, 27) and one recombination-based algorithm (28) as implemented in the computer program Haploview (27). Association between haplotypes and A.M. PEF was tested using the statistical package haplo.stats (23), which implements a score test to test for statistical significant association between haplotypes and outcome measurements, allowing for adjustment for nongenetic covariates. Adjusting for treatment arms, association between haplotypes formed by all contiguous marker loci (e.g., all adjacent two-marker haplotypes, all adjacent three-marker haplotypes, etc.) and A.M. peak flow changes were tested in both trials. In the SOCS trial, the changes considered were from baseline (Week 6) to Week 22 and from baseline to Week 28. In the SLIC trial, the A.M. peak flow change was from baseline (Week 6) to Week 24. In addition, stratified analyses were also done for several treatment arms, including the SOCS salmeterol arm, the SOCS placebo arm, and the SLIC salmeterol + triamcinolone arm. In the stratified analyses, association between the haplotypes and A.M. peak flow at the end of treatment and run-out (Week 24 in the SLIC trial, Weeks 22 and 28 in the SOCS trial) was tested with adjustment for A.M. peak flow at baseline. Haplotypes formed by all possible marker combinations were considered (e.g., all possible two-marker haplotypes, all possible three-marker haplotypes, etc.).

TABLE 2. CHANGE FROM BASELINE TO THE END OF TREATMENT PERIOD OF THE SOCS TRIAL FOR EACH OUTCOME IN B16Arg/Arg AND B16Gly/Gly SUBJECTS RANDOMIZED TO SALMETEROL TREATMENT ARM, COMPARED WITH PLACEBO

	A/A Sal-Plac (*n = 12, salmeterol; n = 5, placebo*)		G/G Sal-Plac (*n = 13, salmeterol; n = 13, placebo*)		A/A Sal-Plac–G/G Sal-Plac	
Outcomes	Difference	p Value	Difference	p Value	Difference	p Value
A.M. PEF, L/min*	−29.9 (−58.5, −1.3)	0.04¶	21.5 (−0.0, 43.0)	0.05¶	−51.4 (−87.2, −15.6)	0.005¶
P.M. PEF, L/min*	−11.7 (−40.2, 16.9)	0.42	4.2 (−17.8, 26.1)	0.71	−15.8 (−51.8, 20.2)	0.39
PEF variability, %*†	2.4 (−1.5, 6.3)	0.22	−3.0 (−6.0, −0.1)	0.05¶	5.4 (0.6, 10.3)	0.03¶
FEV_1, L*	0.06 (−0.23, 0.34)	0.69	0.11 (−0.11, 0.34)	0.32	−0.05 (−0.42, 0.30)	0.76
A.M. symptom scores*‡	0.1 (−0.1, 0.3)	0.48	0.0 (−0.1 0.2)	0.83	0.1 (−0.2, 0.3)	0.66
P.M. symptom scores*‡	0.2 (−0.1, 0.4)	0.15	0.0 (−0.2, 0.2)	0.99	0.2 (−0.1, 0.5)	0.25
Average rescue puffs β-agonist/d*	0.3 (−0.7, 1.3)	0.58	0.1 (−0.7, 0.9)	0.84	0.2 (−1.1, 1.5)	0.75
Exhaled nitric oxide, ppb*	8.9 (−4.9, 22.6)	0.20	−4.1 (−14.6, 6.4)	0.44	13.0 (−4.3, 30.3)	0.14
Asthma quality-of-life score§‖	0.2 (−0.8, 1.1)	0.75	−0.5 (−1.3, 0.4)	0.26	0.6 (−0.7, 1.9)	0.34

Definition of abbreviations: PEF = peak expiratory flow; SOCS = Salmeterol or Corticosteroid trial.

* Two-slope segmented model, mean (95% confidence interval).

† PEF variability was calculated as ([evening PEF − morning PEF]/evening PEF) × 100.

‡ Asthma symptoms were graded by the subject each day, from 0 for no symptoms to 3 for incapacitating symptoms.

§ Asthma-specific quality-of-life questionnaires were completed by the subjects during clinical-center visits. A score of 1.0 indicates that asthma had no effect on the overall quality of life; a score of 2.0, "a little limited" by asthma; 3.0, "some limitation"; and 7.0, "total limitation."

‖ Signed rank test, Wilcoxon rank sum test, median (Q1, Q3).

¶ $p < 0.05$.

RESULTS

Trial 1: SOCS Trial

Fifty-four subjects in the SOCS trial were randomized to receive salmeterol and simultaneously have their ICS withdrawn, whereas 56 were randomized to receive placebo. Of the subjects who gave blood samples for genetic analysis, 48 salmeterol subjects (Arg/Arg, n = 12; Gly/Gly, n = 13), and 43 placebo patients (Arg/Arg, n = 5; Gly/Gly, n = 13) were successfully genotyped at the B16 locus. The genotypic distribution at this locus was consistent with the Hardy-Weinberg equilibrium (p = 0.918). Table 1 summarizes the prerandomization baseline characteristics of the Arg/Arg and Gly/Gly subjects who received either salmeterol or placebo. Subjects of different genotypes were well matched except that B16Gly/Gly subjects receiving salmeterol were younger than subjects in the other groups.

During the treatment period, compared with those treated with placebo, A.M. PEF increased in salmeterol-treated Gly/Gly subjects (21.5 L/min, p = 0.050; Table 2; Figure 3). In contrast, it declined by 29.9 L/min (p = 0.040) in salmeterol-treated Arg/Arg subjects compared with placebo, producing a difference between genotypes of 51.4 L/min (p = 0.005; Figure 3; Table 2). At the end of the run-out, this genotype-specific difference had decreased to 32.5 L/min (p = 0.179).

We also compared the change in physiologic outcomes, symptoms, exhaled nitric oxide, albuterol rescue use, and AQOL in the B16Arg/Arg and B16Gly/Gly subjects (Table 2). All of these secondary outcomes (Table 2) tended to deteriorate in B16Arg/Arg subjects compared with B16Gly/Gly subjects at the end of active treatment, but the only outcome that approached statistical significance was change in PEF variability (5.43%; p = 0.030).

There was no significant difference in asthma exacerbations or treatment failures when individuals of different genotypes were treated with salmeterol or placebo: 3 of 12 (25%) B16Arg/Arg subjects and 5 of 13 (38%) B16Gly/Gly subjects treated with salmeterol (p = 0.38) and 1 of 5 (20%) B16Arg/Arg subjects and 5 of 13 (38%) B16Gly/Gly subjects treated with placebo (p = 0.62) experienced an exacerbation or treatment failure by the end of the treatment phase. There was no difference between any B16Arg/Arg and all B16Gly/Gly subjects experiencing an exacerbation or treatment failure (p = 0.888).

In a secondary analysis, we examined the response of the heterozygotes in this study. During the treatment period, heterozygotes treated with salmeterol (n = 23) as compared with those treated with placebo (n = 25) experienced an improvement in A.M. PEF compared with baseline (+18.18 L/min increase over baseline, p = 0.025), which was significantly different from the decline observed with in B16Arg/Arg subjects (p = 0.004; *see* Table E2).

We also secondarily examined the association of A.M. PEF at the end of treatment and run-out periods with other polymorphic loci in the β2AR gene and haplotypic combinations of these loci (*see* METHODS). Only one test generated a p value less than 0.05 (p = 0.045 for haplotype formed by c47t–c523a with A.M. peak flow at Week 28 in the SOCS trial).

Trial 2: SLIC Trial

Seventy-four subjects in the SLIC trial were randomized to have salmeterol added to ICS therapy (triamcinolone). A total of 58

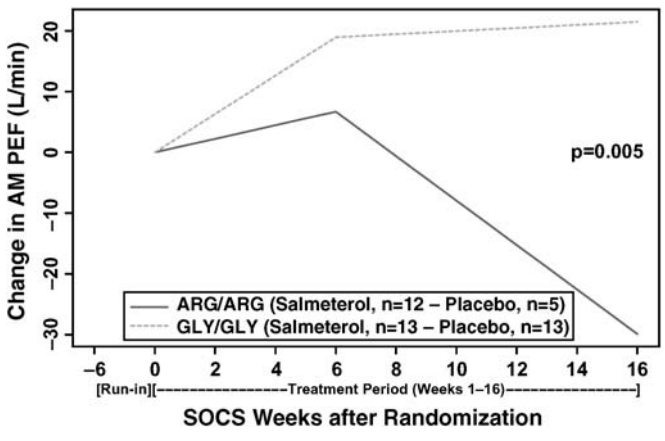

Figure 3. Change from baseline in A.M. peak expiratory flow (PEF) in B16Arg/Arg and B16Gly/Gly subjects from the SOCS trial who were randomized to treatment with salmeterol compared with placebo; p values are for comparisons between genotype groups at the end of the treatment period (Week 16). Each *line* represents linear segmental depictions of mean data.

TABLE 3. MEAN BASELINE CHARACTERISTICS OF B16Arg/Arg AND B16Gly/Gly SUBJECTS RANDOMIZED TO SALMETEROL AND CONSTANT DOSE OF TRIAMCINOLONE ARM OF SLIC TRIAL

Characteristic	Arg/Arg (*n = 8*)	Gly/Gly (*n = 22*)	p Value
Male, n (%)	3 (38)	12 (55)	0.68
Minority, n (%)	3 (38)	5 (23)	0.64
African American, n (%)	2 (25)	2 (9)	0.55
Age, mean (SD), yr	28.6 (9.1)	36.1 (11.4)	0.11
A.M. PEF, mean (SD), L/min*	406.3 (87)	404.5 (140)	0.97
P.M. PEF, mean (SD), L/min*	412.8 (83)	423.1 (124)	0.83
PEF variability, mean (SD), %*†	11.6 (4.2)	13.6 (5.3)	0.34
FEV_1, mean (SD), L	2.46 (0.37)	2.53 (0.63)	0.77
FEV_1% predicted, mean (SD)	71.1 (7.6)	71.1 (8.4)	0.10
PC_{20}, mean (CV), mg/ml‡	1.57 (2.01)	2.10 (1.42)	0.58
A.M. symptom scores, mean (SD)*§	0.2 (0.2)	0.3 (0.3)	0.33
P.M. symptom scores, mean (SD)*§	0.2 (0.2)	0.4 (0.3)	0.08
Average rescue puffs β-agonist/d, mean (SD)*	0.9 (1.3)	1.5 (1.1)	0.25
Exhaled nitric oxide, mean (SD), ppb	14.9 (6.7)	17.1 (8.2)	0.59
Asthma quality-of-life score, median (Q1, Q3)‖	1.8 (1.3, 3.3)	2.5 (1.9, 2.7)	0.25

Definition of abbreviations: CV = coefficient of variation; PEF = peak expiratory flow; Q1, Q3 = interquartile range; SLIC = Salmeterol ± Inhaled Corticosteroid trial.

Sex, minority, and African-American status are summarized with frequencies and percentages and are compared via an exact χ^2 test. A.M. and P.M. PEF, PEF variability, FEV_1, FEV_1% predicted, A.M. and P.M. symptom scores, average puffs of β-agonist, exhaled nitric oxide, and PC_{20} were compared via a two-sample *t* test.

* Values represent averages for the fifth and sixth weeks of the run-in period.

† PEF variability was calculated as ([evening PEF − morning PEF]/evening PEF) × 100.

‡ Geometric mean.

§ Asthma symptoms were graded by the subject each day, from 0 for no symptoms to 3 for incapacitating symptoms.

‖ Asthma-specific quality-of-life questionnaires were completed by the subjects during clinical-center visits. A score of 1.0 indicates that asthma had no effect on the overall quality of life; a score of 2.0, "a little limited" by asthma; 3.0, "some limitation"; and 7.0, "total limitation."

of 70 subjects from whom blood was obtained were successfully genotyped at B16: 8 were Arg/Arg and 22 were Gly/Gly, consistent with Hardy-Weinberg equilibrium (p = 0.95). Table 3 summarizes the prerandomization characteristics of the B16Arg/Arg and B16Gly/Gly subjects.

Figure 4 shows the time course of the change in A.M. PEF during combination therapy. Although B16Arg/Arg subjects (n = 8) appeared to initially benefit from the addition of salmeterol, by the end of 18 wk of randomized treatment, A.M. PEF deteriorated. In contrast, B16Gly/Gly individuals (n = 22) had a sustained beneficial effect from salmeterol, resulting in a genotype-specific difference of 36.8 L/min (p = 0.048) between the two groups. There was no significant difference in asthma treatment failures between the genotypes (2 of 8 [25%] B16Arg/Arg subjects and 1 of 22 [5%] B16Gly/Gly subjects; p = 0.17).

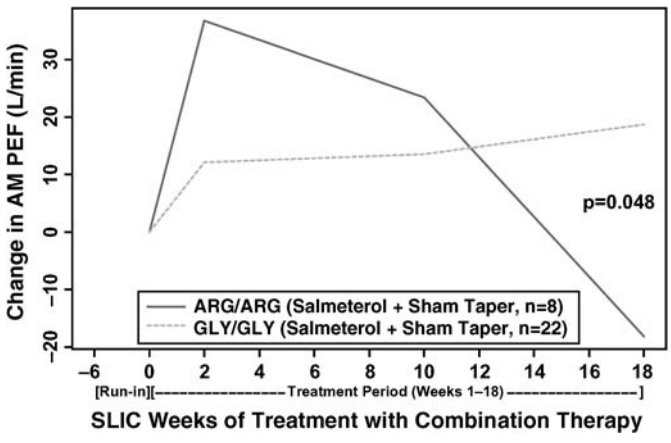

Figure 4. Change from baseline in A.M. PEF in subjects from the SLIC trial who were treated with salmeterol and constant triamcinolone dose throughout entire trial period, in B16Arg/Arg and B16Gly/Gly subjects; p values are for comparisons between genotype groups at the end of the randomized treatment period (Week 18). Each *line* represents linear segmental depictions of mean data.

We also examined the change in physiologic outcomes, symptoms, exhaled nitric oxide, albuterol rescue use, and AQOL in the B16Arg/Arg and B16Gly/Gly subjects (Table 4). The P.M. PEF rates, PEF variability, FEV_1, rescue puffs of β-agonist, and AQOL all improved in B16Gly/Gly subjects when salmeterol was added to their ICS regimen, although nitric oxide, a marker of airway inflammation, increased. There was no improvement in Arg/Arg subjects in these parameters. The difference between genotypes in the change in these outcomes after salmeterol therapy was significant ($p < 0.003$ to $p < 0.037$) for FEV_1, A.M. symptom scores, and average β-agonist rescue use (Table 4). There was also a trend ($p < 0.1$) toward greater PEF variability, and worse AQOL scores in B16Arg/Arg patients compared with B16Gly/Gly patients.

We performed a secondary analysis of the effect of salmeterol on A.M. PEF in heterozygotes in SLIC (*see* Table E2). Heterozygotes who received salmeterol (n = 27) had a 44.3 L/min increase in A.M. PEF ($p < 0.0001$ compared with baseline), which was significantly different from the B16Arg/Arg patient response (p = 0.004). We also found no association between other polymorphisms of the β2AR gene or their haplotypic combination and A.M. PEF.

DISCUSSION

Although the long-acting β-agonist salmeterol improves asthma control in non–genotype-stratified groups (9, 29–31), our current analyses, from two separate trials, suggest that in a subset of

TABLE 4. CHANGE FROM BASELINE TO THE END OF TREATMENT PERIOD OF THE SLIC TRIAL FOR EACH OUTCOME IN SUBJECTS RANDOMIZED TO SALMETEROL AND CONSTANT DOSE OF TRIAMCINOLONE ARM IN B16Arg/Arg AND B16Gly/Gly SUBJECTS

	Arg/Arg (*n* = 8)		Gly/Gly (*n* = 22)		Arg/Arg vs. Gly/Gly	
Outcomes	Difference Compared with Baseline	p Value	Difference Compared with Baseline	p Value	Difference between Genotypes	p Value
A.M. PEF, L/min*	−18.1 (−49.8, 13.6)	0.26	18.7 (0.6, 36.9)	0.04**	−36.8 (−73.4, −0.3)	0.05**
P.M. PEF, L/min*	13.8 (−13.7, 41.1)	0.32	15.3 (−0.2, 30.8)	0.04**	−1.5 (−33.0, 30.0)	0.92
PEF variability, %*†	−0.1 (−3.9, 3.8)	0.97	−4.3 (−6.4, −2.2)	0.0002**	4.2 (−0.2, 8.6)	0.06¶
FEV_1, L*	−0.14 (−0.37, 0.10)	0.24	0.28 (0.14, 0.41)	<0.0001**	−0.42 (−0.68, −0.15)	0.003**
A.M. symptom scores*‡	0.1 (0.0, 0.3)	0.15	−0.1 (−0.2, 0.0)	0.07¶	0.20 (0.0, 0.4)	0.03**
P.M. symptom scores*‡	0.1 (−0.1, 0.2)	0.45	−0.1 (−0.00, 0.01)	0.05¶	0.2 (−0.0, 0.4)	0.11
Average rescue puffs β-agonist/d*	0.46 (−0.08, 1.0)	0.09¶	−0.49 (−0.80, −0.18)	0.003**	0.95 (0.33, 1.58)	0.004**
Exhaled nitric oxide, ppb*	−3.92 (−14.21, 6.36)	0.45	5.58 (0.04, 11.12)	0.05**	−9.50 (−21.19, 2.18)	0.11
Asthma quality-of-life score§‖	−0.04 (−0.47, 1.06)	0.78	−0.41 (−0.72, −0.13)	0.001**	0.37 (0.25, 1.19)	0.08¶

Definition of abbreviation: PEF = peak expiratory flow.

* Three-slope segmented model, mean (95% confidence interval).

† PEF variability was calculated as ([evening PEF − morning PEF]/evening PEF) × 100.

‡ Asthma symptoms were graded by the subject each day, from 0 for no symptoms to 3 for incapacitating symptoms.

‖ Asthma-specific quality-of-life questionnaires were completed by the subjects during clinical-center visits. A score of 1.0 indicates that asthma had no effect on the overall quality of life; 2.0, "a little limited"; 3.0, "some limitation"; and 7.0, "total limitation."

§ Signed rank test, Wilcoxon rank sum test, median (Q1, Q3).

¶ $p < 0.1$.

** $p < 0.05$.

patients with asthma (the one-sixth of patients who are B16Arg/Arg), airway function and indices of asthma control do not improve to the same degree with salmeterol treatment, either with or without concurrent ICS use. These results are similar to those of previous studies that suggested that regular use of the short-acting β-agonist albuterol may produce adverse effects in B16Arg/Arg subjects with asthma (6, 7) and that they may benefit by withdrawal from β-agonists (6, 8).

In the first trial (SOCS), in which subjects with milder asthma had their ICS treatment withdrawn and were treated instead with salmeterol or placebo, B16Arg/Arg subjects who received salmeterol alone, compared with placebo, had a significantly worse response with respect to A.M. PEF compared with B16Gly/Gly individuals. In the second trial (SLIC), in which subjects with more severe asthma received salmeterol while remaining on ICS, A.M. PEF in B16Gly/Gly subjects improved significantly compared with B16Arg/Arg subjects. In this cohort, there were also genotype-specific differences in FEV_1, rescue albuterol use, and symptoms. In both trials, similar to a prior retrospective analysis (6), heterozygotes harboring the B16Arg/Gly genotype responded in a manner similar to that of B16Gly/Gly subjects.

It is clear from our data that B16Arg/Arg subjects do not do as well as B16Gly/Gly subjects when treated with salmeterol. Although we were only able to detect genotype-specific differences in A.M. PEF and a trend toward a difference in PEF variability in our first cohort, the fact that we observed differences in symptoms and medication use in our second cohort suggests that the effect is not restricted to PEF alone. Furthermore, the genotype-specific differences in A.M. PEF in these two trials (51.4 and 36.8 L/min differences in A.M. PEF in the SOCS and SLIC trials, respectively) were substantial and were similar in both magnitude and pattern to those reported with short-acting β-agonists in retrospective studies (6, 7) (*see* Figure E1), as well as in a randomized prospective trial (8). Smaller differences in A.M. PEF (e.g., < 20 L/min) have been associated with clinically important changes in asthma control (32, 33). Of interest, we did not observe a significant genotype-specific difference in P.M. PEF or in some of the other outcomes. This may relate to the sample size or to changes in duration of salmeterol effect with continued use (34).

The reason that B16Arg/Arg patients failed to achieve as salutary a response to salmeterol as B16Gly/Gly subjects is unclear. Some have speculated that it may be due to differences in receptor down-regulation between polymorphic variants of β2AR (35) or to genotype-specific difference in loss of bronchoprotection (36). Others have suggested that persistent β2AR-induced activation of extracellular signal-regulated kinases in airway smooth muscle cells contributes to mitogenesis and exaggerated inflammatory cytokine expression (37). These effects may be affected by polymorphisms of the receptor. A recent report that alterations in the β2AR gene can affect the signaling and function of other receptors that control airway contractility (38) suggests an additional mechanism. These mechanisms might explain the slow onset reported here and in our prior findings with short-acting β-agonists (6, 8) and are consistent with an apparent initial salutary response to salmeterol in B16Arg/Arg patients in the SLIC trial. Furthermore, it is also possible that the B16Arg/Arg polymorphism may be in linkage disequilibrium with the true etiologic polymorphism. In this regard, similar to recent findings that failed to demonstrate an association between acute bronchodilator response and β_2-adrenoceptor haplotype in patients with asthma (39), we were unable to detect stronger associations using combinations of additional polymorphisms (haplotypes) in the gene. Last, we cannot rule out the possibility that the genotype-specific effects associated with salmeterol are related to a secondary increase in albuterol use in B16Arg/Arg patients. However, the fact that B16Arg/Arg subjects receiving salmeterol used more albuterol than B16Gly/Gly subjects suggests that they had sufficient symptoms to warrant the increased use of this rescue medication.

Prior studies with short-acting β-agonists have demonstrated an increased risk of asthma exacerbations in B16Arg/Arg subjects receiving albuterol on a regular basis (7). Although salmeterol appears to have a negative impact in B16Arg/Arg patients not receiving ICS, the question of whether salmeterol is indeed deleterious (with declining lung function or increasing exacerbations, as occurs with short-acting β-agonists) in these subjects when used with ICS is less clear from our data. In the SOCS trial, when ICS were discontinued, salmeterol in and of itself was associated with a decline in A.M. PEF compared with placebo in

B16Arg/Arg subjects. In SLIC, although the outcomes trended in a negative direction, the decline did not reach statistical significance. Whether our failure to demonstrate a deleterious effect in SLIC may have been due to the small number of B16Arg/Arg patients in this arm or due to the concurrent use of ICS is unclear.

This documented decline in B16Arg/Arg subjects treated with salmeterol compared with placebo in SOCS raises the question of whether the reports of a possible small increased rate of death and/or severe deteriorations in asthma symptoms in subjects receiving salmeterol (12, 13) (or other long-acting β-agonists such as formoterol [40]) are related to the effect we observed by genotype. Of note, there were no such severe deteriorations or deaths observed in our studies. Although such potential β-agonist–related adverse effects were reported to occur more frequently in African Americans (13), who have an increased frequency of B16Arg/Arg (41), and although ethnic-specific pharmacogenetic differences have been demonstrated in individuals with different β-adrenergic genotypes (42), it is important to note that inclusion of African Americans did not bias our study finding. There were no more than two African-American subjects of each genotype in any arm of our studies (Tables 1 and 3), and their exclusion had little effect on our results. Furthermore, we did not observe differences between the genotypes in rates of asthma exacerbation as has been previously observed with short-acting β-agonists (7). This may be due to the fact that the rate of exacerbations was not high enough in our cohorts to detect such a difference.

In summary, in two separate trials, including one with concomitant ICS treatment, we demonstrated that B16Arg/Arg patients have a substantially diminished therapeutic response to the regular use of salmeterol, compared with those harboring the B16Gly/Gly genotype. In fact, in the setting of ICS withdrawal, B16Arg/Arg patients may experience adverse effects with salmeterol. These findings suggest that B16Arg/Arg patients ($\sim$ one-sixth of whites and $\sim$ one-fifth of African Americans) might benefit from alternate asthma treatment strategies. Before implementation, our findings would benefit from prospective confirmation and further investigation of concurrent factors that might affect this altered response.

***Conflict of Interest Statement*:** M.E.W. received less than $5,000/yr in 2003–2005 from Merck, Novartis, and GlaxoSmithKline (GSK) for consultant, advisory board, and lecture fees. E.L. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. S.C.L. received $9,000 in 2003 and $3,000 in 2004 from Merck and $2,000 from Critical Therapeutics for serving on advisory boards. He received $2,500 in 2004 from GSK and $5,500 in 2005 from Merck for participating as a speaker in scientific programs. He received $36,000 in 2003 from Abaris Pharma as a research grant for participating in a clinical trial. R.F.L. received speaker honoraria from GSK, Merck, Aventis, and AstraZeneca in the last 3 yr. In 2002, this totaled $22,000, and in 2003, $12,000. All other yearly amounts for each company were under $10,000. He also received consultant fees from AstraZeneca, Aventis, GSK, and Novartis/Genentech. In 2004, he received $11,000 from AstraZeneca while all the other amounts for all years totaled under $10,000. H.A.B. received payments in 2003, 2004, and 2005 from GSK. He received $19,200 from GSK for service on a GOAL Steering Committee for a multicenter study and for chairing and speaking at conferences, and directs a research project funded by the company at University of California at San Francisco, and in 2005 received $3,000 for the Food and Drug Administration Advisory Committee meeting. He has received payments for honoraria and for consulting from Altana, Sanofi-Aventis, Boehringer-Ingelheim (BI), Novartis, and Sumitomo. A.D. has served on advisory boards for AstraZeneca in October 2002 and for Aerocrine in May 2002. The Brigham and Women's Hospital received a research grant of $55,000 for a clinical trial conducted in January 2005 with A.D. as site investigator. J.V.F. received $800 in 2005 for serving as a consultant for Abgenix. C.A.S. does have a financial relationship that has an interest in the subject of this manuscript. She received $5,000 annually for speaking at conferences sponsored by GSK and AstraZeneca (2002–2005), $5,000 annually (2003–2005) for GSK and AstraZeneca advisory boards, and $50,000 GSK grant support (2002–2004). V.M.C. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. T.J.C. was part of an advisory board with Sanofi-Aventis to discuss ciclesonide and received $5,000 in 2005. He has been a speaker for Merck, GSK, Genentech/Novartis, and Schering Plough and received approximate honoraria of $10,000, $5,000, $8,000, and $7,000, respectively. He has received investigator-initiated research grants from Schering (budget pending), Merck (budget pending), GSK (2003, $50,000), American College of Asthma, Allergy, and Immunology (2005, $100,000), and Methapharm (2002, $5,000). He has performed clinical trials for AstraZeneca (2005, $125,000), BI (2005, $325,000), Novartis (2005, $75,000), Genentech/Novartis (2005, $50,000), Dyax (2002–2005, $85,000), Lev Pharmaceuticals Inc. (2005, $50,000), Protein Design Labs (2002, $25,000), Centacore (2002, $25,000), Sanofi-Aventis (2005, $200,000), and Altana (2004, $150,000). E.D. received $110,000 in 2005 as a research grant for an investigator-initiated study from Genentech/Novartis. M.K. received $6,000 in 2003 and $5,000 in 2004 for speaking at conferences sponsored by Merck; $2,000 in 2004 from GSK for speaking; $8,000 in 2003, $6,000 in 2004, and $3,000 in 2005 for speaking sponsored by Genentech/Novartis; $3,000 in 2005 for speaking sponsored by Sepracor; $3,000 from AstraZeneca consulting in 2004 and 2005; $3,000 from GSK for consulting; research grants from GSK, Genentech, and BI. F.L. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. R.J.M. is one of the Asthma Clinical Research Network (ACRN) investigators holding a patent on use of the β_2-agonist polymorphisms as diagnostic tool; he also served on the Sanofi-Aventis advisory board in 2004–2005 ($<$ $5,000) and on the GSK advisory board in 2003–2005 ($<$ $2,500/yr). S.P.P. has the following involvements: consulting (usually review of scientific grant proposals, review of data concerning drugs used for the treatment asthma, or scientific writing/editing) under the auspices of the National Institutes of Health, Adelphi (*Respiratory Digest*, Associate Editor), American Thoracic Society (*AJRCCM*, Associate Editor), AstraZeneca Pharmaceuticals, Asthma Leadership Council, Discovery, Genentech, Novartis, Omnicare, Respiratory and Allergic Disease (RAD) Foundation, RAND Corporation, *Respiratory Medicine* (Associate Editor), *Respiratory Research* (Associate Editor), Sanofi-Aventis—none of these involved more than $10,000/yr in 2003–2005; participating in physician education programs (including Speaker's Bureaus and CME programs) sponsored by American College of Chest Physicians, American Thoracic Society, American Academy of Allergy, Asthma, and Immunology, American College of Allergy, Asthma, and Immunology, AstraZeneca Pharmaceuticals, Merck Pharmaceuticals, Genentech, Novartis, Practicome, RAD Foundation—none of these involved more than $10,000/yr in 2003–2005 except for Merck (sponsor of Visiting Professorships and educational programs), which involved approximately $12,000 in 2004 and $16,000 (estimated) in 2005; pharmaceutical company clinical trials, as a member of a Wake Forest University clinical trials group, sponsored by Abaris, AstraZeneca, Altana, BI, Centocor, Genentech, GSK, Novartis, Pfizer, and Wyeth. S.J.S. has served as a consultant and member of an advisory board for GSK, AstraZeneca, and Aventis for the last 3 yr and received approximately $6,000/yr from each company, and from Merck for 2 yr at $5,000/yr. He has also received research funds for clinical trial performance from AstraZeneca for $90,000 for 2002–2004 and from Ross Pharmaceuticals for $1,200,000 for 2003–2005. He has no stock ownership or commercial royalties in any of these companies. W.L. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. E.I. served as a consultant for Asthmatx in 2005 and received between $10,000 and $20,000. In addition, a multicenter clinical research project at his institution is currently pending. He receives advisory board fees of less than $10,000 from Merck, and has received speaker fees from Merck in 2003–2005, and participated in a multicenter clinical research project with Merck in 2005. He received speaker's fees from Genentech of between $10,000 and $20,000 in 2005, and serves on a Genentech advisory board, and his institution is conducting a multicenter clinical trial with Genentech. In the past 3 yr, he has participated in multicenter clinical trials with AstraZeneca, BI, Centocor, GSK, and Merck. He received a medical school grant from Merck for support and research for less than $50,000. Some of the ACRN Principal Investigators are inventors on a patent concerning the use of genotype at the β_2-adrenergic receptor and effects of regular albuterol use.

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