CHEST

Original Research

COPD

ADRB2 Polymorphisms and Budesonide/Formoterol Responses in COPD

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Background: **Effects of β_2-adrenergic receptor gene (*ADRB2*) polymorphism on therapeutic responses to long-acting β_2-adrenergic agonists have not been evaluated in long-term COPD trials. We aimed to investigate the effects of the *ADRB2* Gly16Arg polymorphism on response to formoterol alone or in combination with the inhaled corticosteroid budesonide in patients with COPD.**

Methods: **Patients ≥ 40 years of age with moderate to very severe COPD from the 12-month trial I (NCT00206167) or the 6-month trial II (NCT00206154) were randomly assigned to bid budesonide/formoterol pressurized metered-dose inhaler (pMDI) 320/9 μg or 160/9 μg, budesonide pMDI 320 μg + formoterol dry powder inhaler 9 μg (trial II), budesonide pMDI 320 μg (trial II), formoterol dry powder inhaler 9 μg, or placebo. The effect of Gly16Arg on predose FEV_1 and 1-h postdose FEV_1, exacerbations, diary variables, and adverse events were analyzed.**

Results: **No significant interaction between genotype and treatment response was observed for predose ($P \geq .197$) or postdose FEV_1 ($P \geq .125$) in either pharmacogenetic study (n = 2,866). The number of COPD exacerbations per patient-treatment year was low and similar across genotypes for the active treatment groups (both studies). Percentages of patients with adverse events were similar across Gly16Arg genotype groups for each treatment.**

Conclusion: **Therapeutic response and tolerability to long-term treatment with formoterol alone or in combination with budesonide was not modified by *ADRB2* Gly16Arg genotype in two large independent pharmacogenetic studies in patients with moderate to very severe COPD.**

Trial registry: **ClinicalTrials.gov; Nos.: NCT00206167, NCT00206154; URL: clinicaltrials.gov.**

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Abbreviations: *ADRB2* = β_2-adrenergic receptor gene; AE = adverse event; ATS = American Thoracic Society; BCSS = Breathlessness Cough and Sputum Scale; BUD = budesonide; DPI = dry powder inhaler; FM = formoterol; ICS = inhaled corticosteroid; LABA = long-acting β_2-adrenergic agonist; LS = least squares; PBO = placebo; pMDI = pressurized metered-dose inhaler; SABA = short-acting β_2-adrenergic agonist; SGRQ = St. George Respiratory Questionnaire; SNP = single-nucleotide polymorphism

A single-nucleotide polymorphism (SNP) causing amino acid substitution at position 16 of the β_2-adrenergic receptor gene (*ADRB2*) is associated with asthma and COPD[1] and may affect response to β_2-agonists. Three possible genotypes result from substitution of arginine for glycine at this position. Studies in patients with persistent asthma reported a deleterious effect with regular use of short-acting β_2-adrenergic agonists (SABAs) in patients with the *ADRB2* Arg/Arg genotype.[2-4] A potential Arg/Arg interaction with long-acting β_2-adrenergic agonists (LABAs) was reported in an early asthma study.[5] Larger studies with LABAs in asthma, including two genotype-stratified clinical trials,[6,7] have failed to demonstrate an Arg/Arg or Arg/Gly genotype effect,[6-8] although there may be a loss of protection in methacholine responsiveness in patients with Arg/Arg receiving LABA therapy.[8]

In COPD, LABAs are used as monotherapy and in combination with inhaled corticosteroids (ICSs). Overlap between phenotypes in asthma and COPD exists[9] related to β_2-smooth muscle responses.[10,11] The role of the ADBR2 polymorphisms in treatment response in COPD is unclear. Some studies suggest an interaction of *ADRB2* with acute bronchodilator response in patients with COPD,[12-15] whereas others have found no effect of *ADRB2* polymorphism on acute bronchodilator response in some COPD populations.[15,16]

The only COPD trial assessing response to ICS/LABA based on *ADRB2* was small (n = 104) and only 12 weeks in duration[17]; thus, larger and longer trials are required to determine whether there is an Arg/Arg effect in patients with COPD. The efficacy and tolerability of budesonide/formoterol pressurized metered-dose inhaler (pMDI) (Symbicort Inhalation Aerosol; AstraZeneca LP) in two randomized, active- and placebo-controlled, double-blind trials (6- and 12-month) of > 3,500 patients with moderate to very severe COPD was previously reported.[18,19] The present study assessed pharmacogenetic data from these two trials (n = 2,866) to evaluate the effect of *ADRB2* Gly16Arg polymorphism on the efficacy and tolerability of budesonide/formoterol combination treatment and its monocomponents.

Materials and Methods

Study Design

Methodology for these trials in patients with moderate to very severe COPD has been reported previously.[18,19] A specific consent was used to obtain blood samples for pharmacogenetic analysis. Institutional review board or independent ethics committee ethical approval for the pharmacogenetic component was obtained at each site.

Study I was 12 months (NCT00206167[18]); genotype results from the 6-month study II (NCT00206154[19]) served to replicate the genotype findings from study I. Eligible patients were randomized to bid budesonide/formoterol pMDI 320/9 μg, budesonide/formoterol pMDI 160/9 μg, budesonide pMDI 320 μg + formoterol dry powder inhaler (DPI) 9 μg (trial II only), budesonide pMDI 320 μg (trial II only), formoterol DPI 9 μg, or placebo.[18,19] The coprimary clinical end points were predose FEV_1 and 1-h postdose FEV_1 (e-Appendix 1). Secondary efficacy variables included morning and evening peak expiratory flow, COPD exacerbations resulting in oral corticosteroid treatment or hospitalization, dyspnea, Breathlessness Cough and Sputum Scale (BCSS[20]) score, health status assessed using the St. George Respiratory Questionnaire (SGRQ[21]), and diary end points. The effect of baseline reversibility to albuterol (based on American Thoracic Society [ATS] criteria[22]) on the relationship between Gly16Arg genotype and FEV_1 also was assessed.

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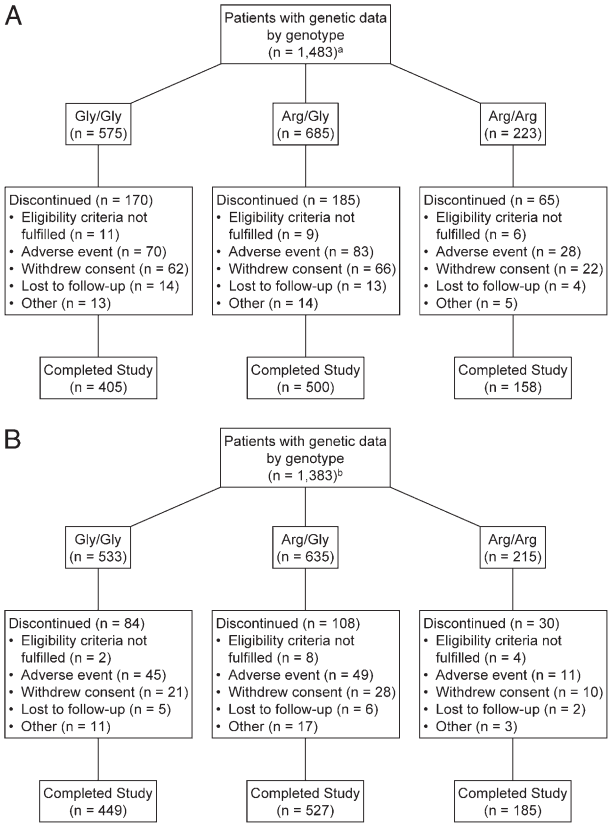

Figure 1. Patient disposition by β_2-adrenergic receptor gene (*ADRB2*) Gly16Arg genotype. A, Study I. B, Study II. [a]Of the 1,490 patients with DNA samples in study I, genotype data were missing for seven patients; n = 1,483 for genotyped patients. [b]Of the 1,393 patients in study II, genotype data were missing for 10 patients; n = 1,383 for genotyped patients.

Genotyping and Haplotype Construction

Blood DNA extraction was done using standard methods by Tepnel Life Sciences PLC. Eleven SNPs from the coding and 5′-flanking regions of the *ADRB2* gene (rs11958940, rs17778257, rs2895795, rs2053044, rs12654778, rs11959427, rs1042711, rs1042713, rs1042714, rs1800888, rs1042718) were successfully genotyped using TaqMan methodology (Applied Biosystems) (e-Appendix 1).[23] Quality control measures included a genotype call rate of 99% for all SNPs and no departure from Hardy-Weinberg Equilibrium for any SNP (exact *P* value > 0.17 for rs1042713 [Gly16Arg]). In addition, 100% concordance was shown in 337 samples directly sequenced across Cys-19Arg (rs1042711), Gly16Arg, and Gln27Glu (rs1042714) SNPs (e-Tables 1, 2). Haplotypes were imput from the SNP genotype data using SNPHAP freeware, version 1.3 (written by David Clayton, Cambridge Institute for Medical Research, and distributed under the terms of the GNU general public license) (e-Appendix 1). Due to the high linkage disequilibrium observed across *ADRB2* ($D' > 0.99$ for all SNP pairs), haplotype pair assignments were unambiguous. Haplotypes were constructed for the white patient population only.

Statistical Analyses

The pharmacogenetic analyses included all patients who gave additional consent for pharmacogenetic testing, regardless of ethnic origin; analyses were repeated for each racial subgroup separately. Changes in FEV_1, SGRQ, BCSS, and diary variables were compared from baseline (e-Appendix 1) to the average during

the treatment period. Analysis of covariance models (with no imputation for missing data) were used to assess the effect of Gly16Arg (rs1042713) genotype on treatment response. Each model included the following factors: country, treatment, genotype, and genotype-by-treatment interaction, plus the corresponding baseline value as a continuous covariate. The interaction between the 3-SNP haplotype (Cys-19Arg_Gly16Arg_Gln27Glu) pairs and treatment response was assessed similarly. Formal statistical analyses were supplemented with a thorough visual inspection of data trends, thus ensuring that more moderate but consistent genetic effects across studies, treatments, and time points were not overlooked (e-Appendix 1).

Results

Patients

DNA samples were obtained from 1,490 of 1,964 randomized patients (75.9%) in trial I and 1,393 of 1,704 randomized patients (81.7%) in trial II (Fig 1). Demographic and baseline clinical characteristics are presented in Table 1. Patients had moderate to very severe COPD, with a mean FEV_1 of 1.05 L. The percentages of patients who met ATS criteria for bronchodilator reversibility were similar across Gly16Arg genotype groups (29%-39%) in both studies. There were no differences in baseline characteristics by Gly16Arg genotype. The genetic diversity within white patients resulted in six haplotype-pair assignments, with the patients with Gly/Gly, Gly/Arg, and Arg/Arg being divided into three, two, and one haplotype-pair subgroups, respectively. Analyses in the white subpopulation were similar to the results found in the entire population, and, therefore, are not reported separately. Results in the nonwhite racial categories were uninformative because of small sample sizes.

Efficacy

There was no indication that the effect of treatment on pulmonary function is influenced by *ADRB2* Gly16Arg genotype. For predose FEV_1 (Fig 2) and postdose FEV_1 (Fig 3), respectively, no significant interaction between treatment and genotype was observed in study I ($P = .674$ and $P = .414$) or study II ($P = .197$ and $P = 0.125$). There was no suggestion in either study of Gly16Arg genotype modifying the effect of treatment as evidenced by the number of exacerbations per patient-treatment year (Fig 4). The number of exacerbations per patient-treatment year resulting in hospitalization was low across all genotype groups in both studies (I: 0.08-0.20; II: 0-0.31) (e-Fig 1).

Diary data analysis showed no clinical or statistical evidence of an effect of Gly16Arg genotype on treatment response. Treatment responses for diary variables of dyspnea, morning and evening peak expiratory

Table 1—*Demographic and Baseline Clinical Characteristics by* ADRB2 *Gly16Arg Genotype*

	Study I			Study II		
Characteristic	Gly/Gly (n = 575)	Arg/Gly (n = 685)	Arg/Arg (n = 223)	Gly/Gly (n = 533)	Arg/Gly (n = 635)	Arg/Arg (n = 215)
Sex						
Male	370 (64)	433 (63)	139 (62)	373 (70)	451 (71)	133 (62)
Female	205 (36)	252 (37)	84 (38)	160 (30)	184 (29)	82 (38)
Race						
White	541 (94)	643 (94)	206 (92)	508 (95)	609 (96)	195 (91)
Black	7 (1)	9 (1)	6 (3)	8 (2)	12 (2)	6 (3)
Other	27 (5)	33 (5)	11 (5)	17 (3)	14 (2)	14 (7)
Mean age (SD), y	63 (9)	63 (9)	63 (10)	64 (9)	63 (9)	63 (9)
Smoking status						
Ex-smoker	319 (56)	396 (58)	130 (58)	309 (58)	373 (59)	117 (54)
Habitual smoker[a]	235 (41)	263 (38)	79 (35)	204 (38)	241 (38)	88 (41)
Occasional smoker[b]	21 (4)	26 (4)	14 (6)	20 (4)	21 (3)	10 (5)
Received ipratropium at randomization	235 (41)	308 (45)	107 (48)	351 (66)	404 (64)	128 (60)
% Reversibility[c] at screening ≥ 12% + ≥ 200 mL FEV_1 improvement	169 (29)	231 (34)	70 (31)	160 (30)	220 (35)	83 (39)
Predose FEV_1						
Mean (SD), L	1.05 (0.35)	1.05 (0.36)	1.04 (0.35)	1.05 (0.35)	1.07 (0.36)	1.03 (0.36)
Mean (SD), % predicted	34.5 (9.4)	34.4 (9.2)	34.4 (9.3)	33.9 (9.1)	34.6 (9.7)	34.1 (9.6)

Data are given as No (%) unless otherwise indicated. Percentages were rounded and may not add up to 100%.

[a]Smokes ≥ 1 cigarette daily and for ≥ 1 year prescreening.

[b]Smokes < 1 cigarette daily, has been smoking for < 1 year prescreening, or stopped smoking ≤ 6 month prescreening.

[c]Reversibility was assessed based on improvements in FEV_1 from the prebronchodilator value to the postbronchodilator value 15-30 min after administration of two inhalations of albuterol pressurized metered-dose inhaler (total dose: 180-200 μg).

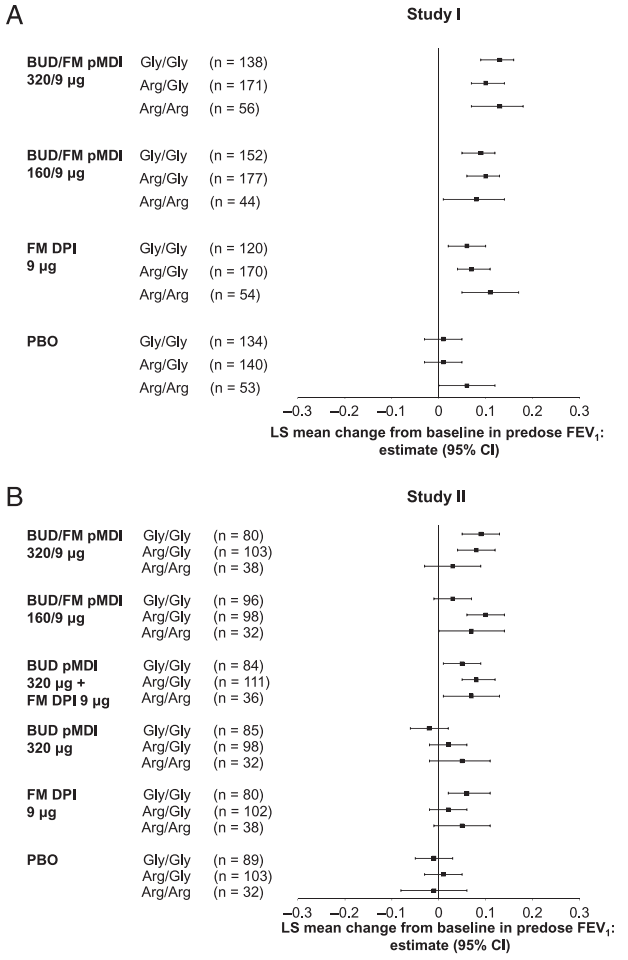

FIGURE 2. Adjusted mean change from baseline (95% CI) in predose FEV_1 by treatment and *ADRB2* Gly16Arg genotype. A, Study I. B, Study II. BUD = budesonide; DPI = dry powder inhaler; FM = formoterol; LS = least squares; PBO = placebo; pMDI = pressurized metered-dose inhaler. See Figure 1 legend for expansion of other abbreviation.

flow, rescue medication use, and BCSS score also were similar across genotype groups, with no significant interaction between treatment and genotype in study I ($P \geq .378$) or study II ($P \geq .133$) (e-Table 3). Assessments of the patterns of clinical response showed no suggestion of any consistent trends. No evidence was found to suggest that the effect of treatment on SGRQ was modified by Gly16Arg genotype in study I ($P = .909$) or study II ($P = 0.648$) (e-Fig 2). Despite small numbers of patients in each subgroup, there was no evidence to suggest the presence of any interaction between genotype, treatment, and baseline FEV_1 reversibility status (e-Figs 3, 4).

Analysis of haplotype data provided no evidence to suggest an effect of Cys-19Arg_Gly16Arg_Gln27Glu haplotype on treatment response in either study. No significant interaction between treatment and haplotype was observed for either the coprimary end points in study I ($P \geq .212$) or study II ($P \geq .170$) or for secondary outcomes in study I ($P \geq .379$) or study II ($P \geq .072$) (e-Table 4).

Safety

The mean duration of exposure to trial medications was similar across genotype groups in both pharmacogenetic studies. The percentages of patients with adverse events (AEs), treatment-related AEs, and serious AEs generally were similar across Gly16Arg genotype groups for each treatment in study I (Table 2) and study II (Table 3). As previously reported, 15 patients died during the randomized treatment period in trial I, and 11 patients died during randomized treatment in trial II. Of these patients, 14 and 10 patients in trials I and II, respectively, were genotyped and included in the pharmacogenetic studies (Tables 2, 3). The incidence of death was similar across genotype groups.

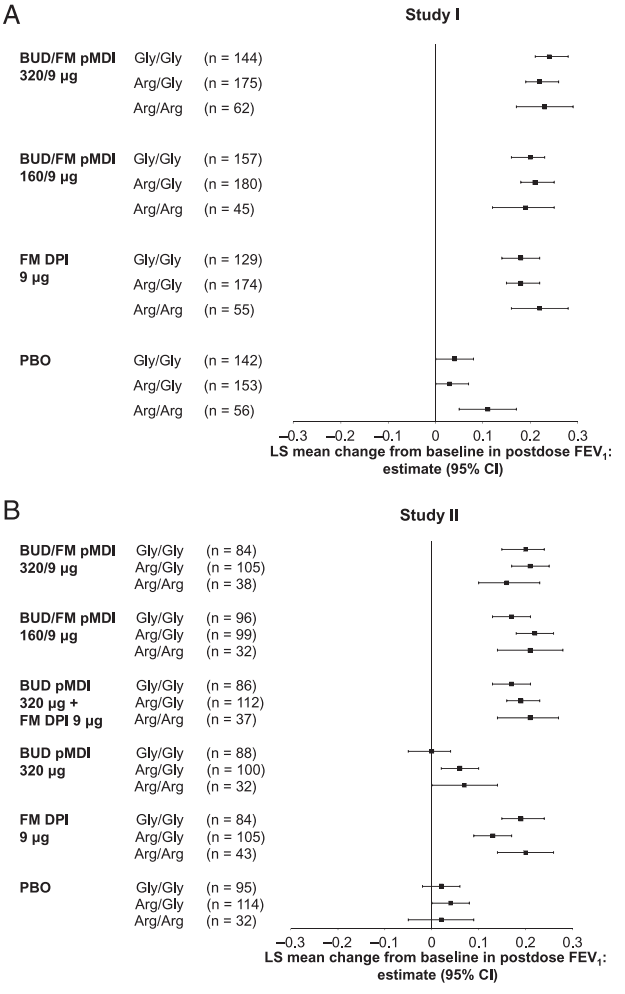

FIGURE 3. Adjusted mean change from baseline (95% CI) in postdose FEV_1 by treatment and *ADRB2* Gly16Arg genotype. A, Study I. B, Study II. See Figure 1 and 2 legends for expansion of abbreviations.

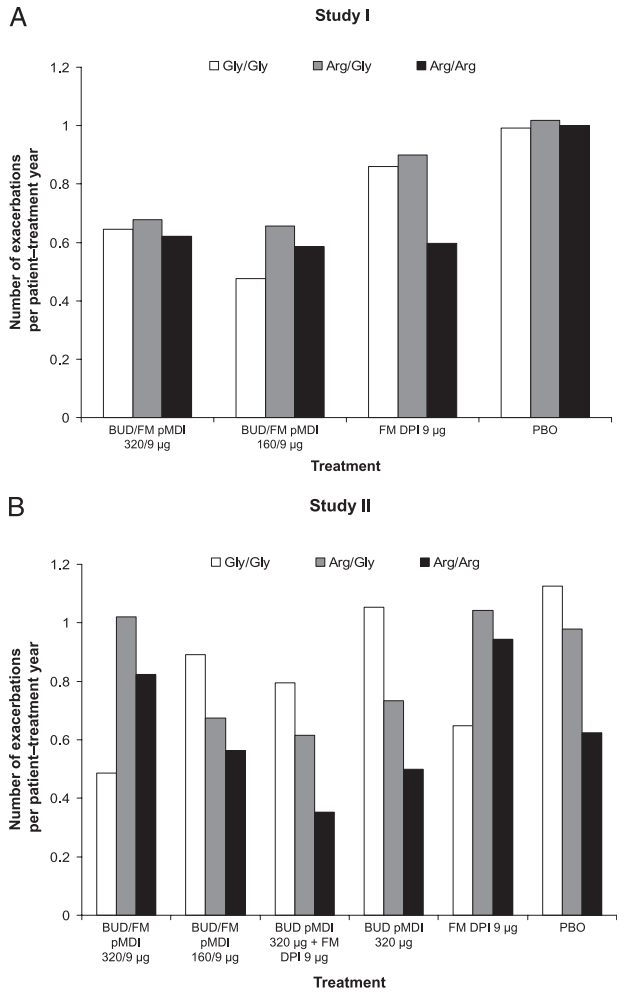

FIGURE 4. Number of exacerbations per patient-treatment year by treatment and *ADRB2* Gly16Arg genotype. A, Study I. B, Study II. See Figure 1 and 2 legends for expansion of abbreviations.

DISCUSSION

Results from this pharmacogenetic research showed no effect of Gly16Arg genotype on the efficacy or tolerability of LABA therapy with formoterol, either alone or in combination with budesonide, in COPD. Improvements from baseline to the treatment average in pulmonary function, exacerbation frequency, dyspnea score, SGRQ total score (in study II), and diary variables were similar across genotype groups. Clinical trial I was designed with sufficient power to detect a treatment effect on exacerbations over 12 months; there is no pharmacogenetic evidence from study I that suggests that the Gly16Arg genotype modified the effectiveness of formoterol in reducing COPD exacerbations. These findings were confirmed in the 6-month pharmacogenetic study II. Overall, similar results from two large independent data sets representing patients with up to 12 months of treatment support the consistency of the findings.

This article presents a thorough and detailed investigation of the effects of *ADRB2* Gly16Arg polymorphism in COPD from two large clinical trials. Patients included in the pharmacogenetic research were representative of the entire clinical trial populations in terms of baseline characteristics and clinical trial efficacy and safety results. The Gly16Arg genotype frequencies in these patients are similar to those reported in previous studies in asthma patients[6,24,25] and normal control populations.[25] Because sample sizes in the nonwhite racial categories were small, the present findings should not be generalized to nonwhite COPD populations.

Few studies have explored the effect of Gly16Arg genotype on treatment response in COPD. A study of 104 Korean patients with COPD reported no effect of Gly16Arg genotype on FEV_1 after 12 weeks of treatment with fluticasone/salmeterol and no lessening of treatment response in Arg/Arg homozygotes.[17] Additionally, that study suggested that there was no effect of Gly16Arg_Gln27Glu haplotype on treatment response.[17] However, their study was limited by a relatively small population sample and short duration of treatment. The data reported here comprised two large independent patient sample sets from long-term trials (6 and 12 months) with no evidence of an effect of *ADRB2* genotype or haplotype on therapeutic responses (e-Appendix 1).

This is the only large study to examine response to ICS/LABA treatment by *ADRB2* genotype in patients with COPD. Early observations of an association between Arg16Gly and adverse effects from regular use of SABAs were in asthma patients.[2-4] However, results of larger, more recent asthma studies have demonstrated a lack of effect of *ADRB2* genotype on ICS/LABA treatment response. A large prospective clinical trial in asthma demonstrated no effect of Gly16Arg genotype on the effectiveness or tolerability of formoterol or salmeterol used in combination with an ICS.[6] More recently, two prospective genotype trials have not shown an effect of *ADRB2* genotype for the LABA salmeterol alone or in combination with an ICS.[7,8] In the Long-Acting Beta Agonist Response by Genotype (LARGE) trial, a genotype-stratified, placebo-controlled, crossover trial, there were similar improvements in lung function and asthma symptoms in patients with the Gly/Gly and Arg/Arg genotypes.[8] In this trial, methacholine responsiveness was at baseline levels 24 h after the last administration of the ICS/LABA (a drug combination with a 12-h duration of activity) in the Arg/Arg homozygotes compared with the patients with Gly/Gly who still showed a small improvement in methacholine responsiveness at this 24-h time point.

Bronchial hyperresponsiveness and inflammation are common features of asthma and COPD, although

Table 2—*Percentage of Patients With AEs, Treatment-Related AEs, Discontinuations Due to AEs, and Serious AEs by Treatment Group and* ADRB2 *Gly16Arg Genotype in Study I*

	No. (%) of Patients Experiencing AEs				
Treatment/Genotype	Any AE	Treatment-Related AE	Discontinuations Due to AE	Nonfatal Serious AE	Fatal Serious AE
BUD/FM 320/9 μg					
Gly/Gly (n = 144)	97 (67)	20 (14)	17 (12)	23 (16)	1 (1)
Arg/Gly (n = 175)	117 (67)	24 (14)	17 (10)	27 (15)	2 (1)
Arg/Arg (n = 65)	39 (60)	7(11)	8 (12)	7 (11)	0 (0)
BUD/FM 160/9 μg					
Gly/Gly (n = 157)	96 (61)	18 (12)	10 (6)	18 (12)	0 (0)
Arg/Gly (n = 182)	120 (66)	15 (8)	28 (15)	25 (14)	6 (3)
Arg/Arg (n = 46)	30 (65)	3 (7)	5 (11)	7 (15)	0 (0)
FM 9 μg					
Gly/Gly (n = 130)	80 (62)	11 (9)	21 (16)	32 (25)	1 (1)
Arg/Gly (n = 175)	114 (65)	13 (7)	21 (12)	31 (18)	1 (1)
Arg/Arg (n = 55)	32 (58)	6 (11)	9 (16)	9 (16)	0 (0)
PBO					
Gly/Gly (n = 144)	85 (59)	9 (6)	19 (13)	19 (13)	1 (1)
Arg/Gly (n = 153)	81 (53)	9 (6)	12 (8)	15 (10)	0 (0)
Arg/Arg (n = 57)	33 (58)	4 (7)	10 (18)	5 (9)	2 (4)

AE = adverse event; BUD = budesonide; FM = formoterol; PBO = placebo.

there may be subtle differences in their pathobiology. Hizawa[26] has suggested that the potential mechanism leading to an increased risk of death and serious exacerbations observed with LABA therapy in some asthma trials[27-30] may relate to effects on airway hyperresponsiveness and allergic inflammation rather than agonist-induced receptor downregulation. The findings of the present study showing a lack of genetic effects of Arg16Gly on the treatment responses to LABA in COPD support this hypothesis.

Table 3—*Percentage of Patients With AEs, Treatment-Related AEs, Discontinuations Due to AEs, and Serious AEs by Treatment Group and* ADRB2 *Gly16Arg Genotype in Study II*

	No. (%) of Patients Experiencing AEs				
Treatment/Genotype	Any AE	Treatment-Related AE	Discontinuations Due to AE	Nonfatal Serious AE	Fatal Serious AE
BUD/FM 320/9 μg					
Gly/Gly (n = 84)	38 (45)	8 (10)	6 (7)	7 (8)	1 (1)
Arg/Gly (n = 105)	71 (68)	8 (8)	10 (10)	16 (15)	2 (2)
Arg/Arg (n = 39)	23 (59)	3 (8)	2 (5)	2 (5)	0
BUD/FM 160/9 μg					
Gly/Gly (n = 96)	47 (49)	11 (12)	5 (5)	13 (14)	2 (2)
Arg/Gly (n = 99)	52 (53)	5 (5)	6 (6)	10 (10)	1 (1)
Arg/Arg (n = 32)	15 (47)	2 (6)	0	1 (3)	0
BUD 320 μg + FM 9 μg					
Gly/Gly (n = 86)	51 (59)	6 (7)	4 (5)	9 (11)	0
Arg/Gly (n = 112)	43 (38)	8 (7)	5 (5)	8 (7)	0
Arg/Arg (n = 37)	19 (51)	0	1 (3)	5 (14)	0
BUD 320 μg					
Gly/Gly (n = 88)	50 (57)	6 (7)	12 (14)	12 (14)	2 (2)
Arg/Gly (n = 100)	56 (56)	8 (8)	6 (6)	9 (9)	0
Arg/Arg (n = 32)	22 (69)	4 (13)	1 (3)	1 (3)	0
FM 9 μg					
Gly/Gly (n = 84)	53 (63)	6 (7)	7 (8)	6 (7)	0
Arg/Gly (n = 105)	53 (51)	5 (5)	9 (9)	10 (10)	1 (1)
Arg/Arg (n = 43)	19 (44)	6 (14)	6 (14)	3 (7)	0
PBO					
Gly/Gly (n = 95)	50 (53)	4 (4)	9 (10)	14 (15)	1 (1)
Arg/Gly (n = 114)	58 (51)	7 (6)	8 (7)	7 (6)	0
Arg/Arg (n = 32)	13 (41)	0	0	2 (6)	0

See Table 2 for expansion of abbreviations.

The effects of *ADRB2* gene polymorphism on bronchodilator responsiveness to SABA treatment in COPD vary. Results from a study of 246 Japanese patients with COPD showed an interaction between Gly16Arg genotype and bronchodilator response to salbutamol, with patients with Arg/Arg exhibiting the smallest response, patients with Gly/Arg an intermediate response, and patients with Gly/Gly the largest response.[14] These authors also showed a significant association of haplotype with bronchodilator response. In contrast, Mokry et al[16] reported that *ADRB2* haplotypes may affect ventilatory impairment during acute exacerbations of COPD but not the immediate response to SABA.

Since acute bronchodilator responses are determined partly by baseline lung function, this parameter must be evaluated in these pharmacogenetic studies. SABA response based on ATS reversibility status at baseline was similar across genotype groups in both studies. Regardless of baseline SABA reversibility (ATS criteria), no consistent pattern of response for predose or postdose FEV_1 was observed across Gly16Arg genotype groups, treatments, or studies.

Due to the spectrum of phenotypes investigated, there was a strong likelihood of generating false-positive results based on multiple testing and chance alone. This issue was addressed by having replication populations (through comparison of multiple treatment arms and the use of two studies of similar design). For example, improvements in FEV_1 among patients with the Arg/Arg genotype who received placebo in study I were better than expected for this population of patients with moderate to severe COPD, but this finding was not replicated in study II. Findings from study I suggesting greater improvements in SGRQ total scores in patients with Arg/Arg across all treatments were not replicated in study II. Few differences in baseline and clinical characteristics (eg, ipratropium use) were observed between treatment and genotype groups and could not explain these observations.

Tolerability, assessed by AEs and serious AEs across Gly16Arg genotype groups, was similar. Deaths during randomized treatment were similar across genotype groups in both studies and were low considering the degree of severity of COPD in these large populations. In study I, two patients with the Arg/Arg genotype died of a serious AE while receiving placebo treatment. In neither case was the serious AE related to the patient's *ADRB2* genotype; one patient experienced central circulatory failure and one patient was diagnosed with non-small cell lung cancer.

In conclusion, this study represents the only large pharmacogenetic study to assess the effect of *ADRB2* polymorphism on treatment response to long-term LABA treatment, with or without ICS therapy, in a primarily white patient population with stable COPD. Results of the 12-month study, replicated in the 6-month study, showed no evidence of an effect of Gly16Arg genotype or *ADRB2* haplotype on treatment responses to formoterol, with or without budesonide, based on pulmonary function, exacerbations, symptom control, and quality of life. Tolerance also was independent of *ADRB2* variability. These findings support current COPD guidelines recommending the use of LABAs either as monotherapy for milder disease or in combination with ICS in more severe COPD when there is a higher likelihood of exacerbations.[31] This research shows that LABAs can effectively treat patients with COPD regardless of *ADRB2* genotype.

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Author contributions: Dr Martin had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis, including and especially any adverse effects.

Dr Bleecker: contributed substantially to analysis and interpretation of the data, drafted portions of the article, revised it critically for important intellectual content, and approved the final version for publication.

Dr Meyers: contributed substantially to analysis and interpretation of the data, drafted portions of the article, revised it critically for important intellectual content, and approved the final version for publication.

Dr Bailey: contributed substantially to the analysis and interpretation of the data, revised the manuscript critically for important intellectual content, and approved the final version for publication.

Dr Sims: contributed substantially to the conception and design of the studies; contributed to data acquisition, analysis, and interpretation; reviewed the manuscript critically for important intellectual content; and approved the final version for publication.

Ms Bujac: contributed substantially to the conception and design of the studies; contributed to data acquisition, analysis, and interpretation; reviewed the manuscript critically for important intellectual content; and approved the final version for publication.

Dr Goldman: contributed substantially to the conception and design of the studies, data analysis and interpretation, and critical review for important intellectual content; and approved the final version for publication.

Dr Martin: contributed substantially to the conception and design of the studies, data analysis and interpretation, and critical review for important intellectual content; and approved the final version for publication.

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Additional information: The e-Appendix, e-Figures, and e-Tables can be found in the "Supplemental Materials" section of the online article.

References

1. Matheson MC, Ellis JA, Raven J, Johns DP, Walters EH, Abramson MJ. Beta2-adrenergic receptor polymorphisms are associated with asthma and COPD in adults. *J Hum Genet*. 2006;51(11):943-951.
2. Israel E, Drazen JM, Liggett SB, et al; National Heart, Lung, and Blood Institute's Asthma Clinical Research Network. 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(1):75-80.
3. Israel E, Chinchilli VM, Ford JG, et al; National Heart, Lung, and Blood Institute's Asthma Clinical Research Network. Use of regularly scheduled albuterol treatment in asthma: genotype-stratified, randomised, placebo-controlled cross-over trial. *Lancet*. 2004;364(9444):1505-1512.
4. Taylor DR, Drazen JM, Herbison GP, Yandava CN, Hancox RJ, Town GI. Asthma exacerbations during long term beta agonist use: influence of beta(2) adrenoceptor polymorphism. *Thorax*. 2000;55(9):762-767.
5. Wechsler ME, Lehman E, Lazarus SC, et al; National Heart, Lung, and Blood Institute's Asthma Clinical Research Network. beta-Adrenergic receptor polymorphisms and response to salmeterol. *Am J Respir Crit Care Med*. 2006;173(5):519-526.
6. Bleecker ER, Postma DS, Lawrance RM, Meyers DA, Ambrose HJ, Goldman M. Effect of ADRB2 polymorphisms on response to long-acting beta2-agonist therapy: a pharmacogenetic analysis of two randomised studies. *Lancet*. 2007;370(9605):2118-2125.
7. 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(7):676-687.
8. Wechsler ME, Kunselman SJ, Chinchilli VM, et al; National Heart, Lung and Blood Institute's Asthma Clinical Research Network. Effect of beta2-adrenergic receptor polymorphism on response to long-acting beta2 agonist in asthma (LARGE trial): a genotype-stratified, randomised, placebo-controlled, crossover trial. *Lancet*. 2009;374(9703):1754-1764.
9. Hizawa N. Genetic backgrounds of asthma and COPD. *Allergol Int*. 2009;58(3):315-322.
10. Celli BR, Tashkin DP, Rennard SI, McElhattan J, Martin UJ. Bronchodilator responsiveness and onset of effect with budesonide/formoterol pMDI in COPD. *Respir Med*. 2011;105(8):1176-1188.
11. Tashkin DP, Altose MD, Bleecker ER, et al. The lung health study: airway responsiveness to inhaled methacholine in smokers with mild to moderate airflow limitation. The Lung Health Study Research Group. *Am Rev Respir Dis*. 1992;145(2 Pt 1):301-310.
12. Konno S, Makita H, Hasegawa M, et al. Beta2-adrenergic receptor polymorphisms as a determinant of preferential bronchodilator responses to β2-agonist and anticholinergic agents in Japanese patients with chronic obstructive pulmonary disease. *Pharmacogenet Genomics*. 2011;21(11):687-693.
13. Umeda N, Yoshikawa T, Kanazawa H, Hirata K, Fujimoto S. Association of beta2-adrenoreceptor genotypes with bronchodilatory effect of tiotropium in COPD. *Respirology*. 2008;13(3):346-352.
14. Hizawa N, Makita H, Nasuhara Y, et al. Beta2-adrenergic receptor genetic polymorphisms and short-term bronchodilator responses in patients with COPD. *Chest*. 2007;132(5):1485-1492.
15. Kim WJ, Hersh CP, DeMeo DL, Reilly JJ, Silverman EK. Genetic association analysis of COPD candidate genes with bronchodilator responsiveness. *Respir Med*. 2009;103(4):552-557.
16. Mokry M, Joppa P, Slaba E, et al. Beta2-adrenergic receptor haplotype and bronchodilator response to salbutamol in patients with acute exacerbations of COPD. *Med Sci Monit*. 2008;14(8):CR392-CR398.
17. Kim WJ, Oh Y-M, Sung J, et al. Lung function response to 12-week treatment with combined inhalation of long-acting beta2 agonist and glucocorticoid according to ADRB2 polymorphism in patients with chronic obstructive pulmonary disease. *Lung*. 2008;186(6):381-386.
18. Rennard SI, Tashkin DP, McElhattan J, et al. Efficacy and tolerability of budesonide/formoterol in one hydrofluoroalkane pressurized metered-dose inhaler in patients with chronic obstructive pulmonary disease: results from a 1-year randomized controlled clinical trial. *Drugs*. 2009;69(5):549-565.
19. Tashkin DP, Rennard SI, Martin P, et al. Efficacy and safety of budesonide and formoterol in one pressurized metered-dose inhaler in patients with moderate to very severe chronic obstructive pulmonary disease: results of a 6-month randomized clinical trial. *Drugs*. 2008;68(14):1975-2000.
20. Leidy NK, Schmier JK, Jones MK, Lloyd J, Rocchiccioli K. Evaluating symptoms in chronic obstructive pulmonary disease: validation of the Breathlessness, Cough and Sputum Scale. *Respir Med*. 2003;97(suppl A):S59-S70.
21. Jones PW, Quirk FH, Baveystock CM, Littlejohns P. A self-complete measure of health status for chronic airflow limitation. The St. George's Respiratory Questionnaire. *Am Rev Respir Dis*. 1992;145(6):1321-1327.
22. Lung function testing: selection of reference values and interpretative strategies. American Thoracic Society. *Am Rev Respir Dis*. 1991;144(5):1202-1218.
23. Livak KJ, Flood SJA, Marmaro J, Giusti W, Deetz K. Oligonucleotides with fluorescent dyes at opposite ends provide a quenched probe system useful for detecting PCR product and nucleic acid hybridization. *PCR Methods Appl*. 1995;4(6):357-362.
24. Bleecker ER, Yancey SW, Baitinger LA, et al. Salmeterol response is not affected by beta2-adrenergic receptor genotype in subjects with persistent asthma. *J Allergy Clin Immunol*. 2006;118(4):809-816.
25. Thakkinstian A, McEvoy M, Minelli C, et al. Systemic review and meta-analysis of the association between {beta}2-adrenoceptor polymorphisms and asthma: a HuGE review. *Am J Epidemiol*. 2005;162(3):201-211.
26. Hizawa N. Pharmacogenetics of β2-agonists. *Allergol Int*. 2011;60(3):239-246.
27. Nelson HS, Weiss ST, Bleecker ER, Yancey SW, Dorinsky PM; SMART Study Group. The Salmeterol Multicenter Asthma Research Trial: a comparison of usual pharmacotherapy for asthma or usual pharmacotherapy plus salmeterol [published correction appears in *Chest*. 2006;129(5):1393]. *Chest*. 2006;129(1):15-26.
28. 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(6884):1034-1037.
29. Mann M, Chowdhury B, Sullivan E, Nicklas R, Anthracite R, Meyer RJ. Serious asthma exacerbations in asthmatics treated with high-dose formoterol. *Chest*. 2003;124(1):70-74.

30. Bensch G, Berger WE, Blokhin BM, et al; International Study Group on Foradil Evaluation in Pediatric Asthma. One-year efficacy and safety of inhaled formoterol dry powder in children with persistent asthma. *Ann Allergy Asthma Immunol*. 2002;89(2):180-190.

31. Global Initiative for Chronic Obstructive Lung Disease (GOLD). Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease: updated 2010. GOLD website. http://www.goldcopd.org/guidelines-global-strategy-for-diagnosis-management.html. Accessed February 22, 2011.